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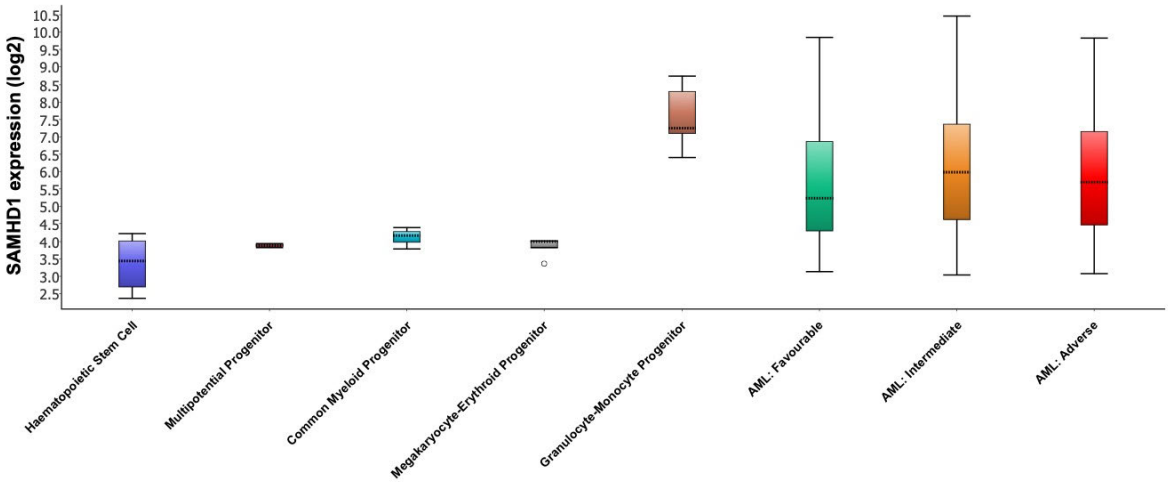
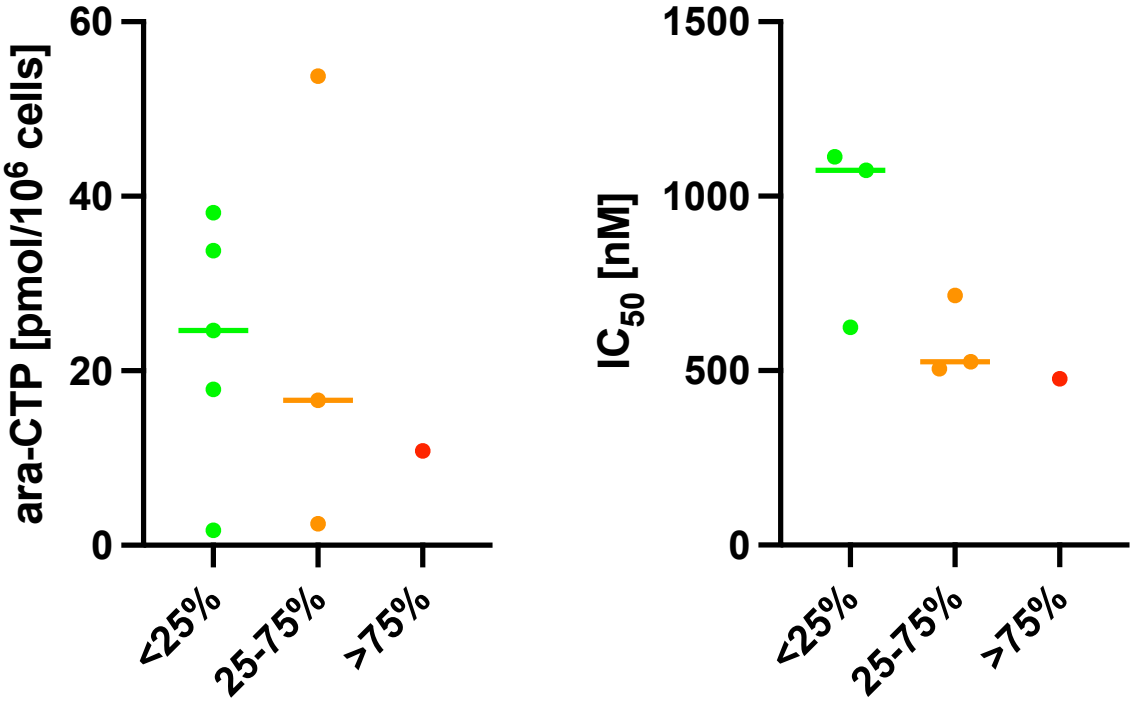
1. Supplementary Figures

1.1 *In silico* analysis of *SAMHD1* mRNA expression in healthy bone marrow progenitors and AML blasts



Gene expression for SAMHD1 (probe 1559883_s_at) was retrieved from the BloodSpot portal (see Methods). Box-whisker plots indicate 25th and 75th percentile (box), the median (dotted band). Whiskers correspond to values within the the 1.5-fold range of the box. Outliers are indicated by empty circles. The number of samples was 6 for ”Haematopoietic Stem Cell”, 2 for ”Multipotential Progenitor”, 3 for ”Common Myeloid Progenitor”, 4 for ”Megakaryocyte-Erythroid Progenitor”, 5 for ”Granulocyte-Monocyte Progenitor”, 262 for ”AML: Favourable”, 1153 for ”AML: Intermediate”, and 292 for ”AML: Adverse”.

1.2 Supplementary Figure S2. Ara-CTP levels in circulating blasts and *ex vivo* sensitivity to ara-C with respect to SAMHD1 expression

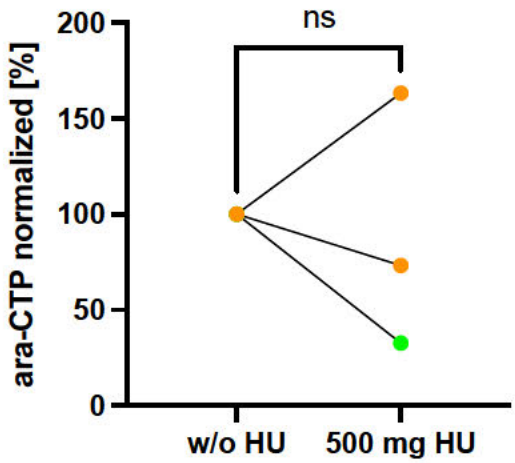


The left panel shows ara-CTP levels in pmol per million cells measured in circulating blasts sampled at the end of ara-C infusions without prior HU.

The right panel shows half-maximal inhibitory concentrations (IC_{50}) of ara-C in diagnostic bone-marrow blasts treated *ex vivo*.

Colors represent levels of SAMHD1 expression at diagnosis (green <25%, orange 25-75%, red >75%).

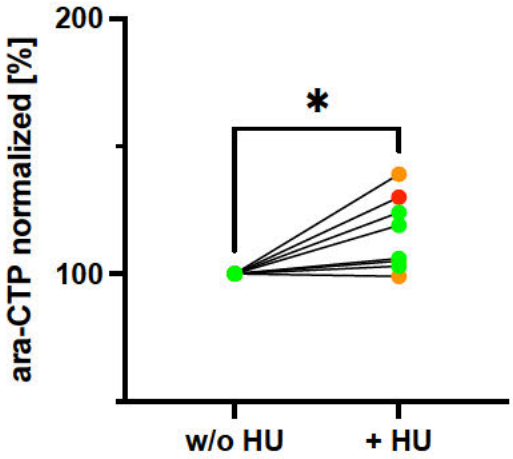
1.3 Supplementary Figure S3. Effect of 500 mg hydroxyurea on peak ara-CTP levels in circulating mononuclear cells.



The left panel shows ara-CTP levels measured in circulating mononuclear cells without hydroxyurea (w/o HU) as compared to ara-CTP with hydroxyurea 500 mg taken p.o. 1 hour prior to start of ara-C infusion, normalized to without hydroxyurea (n=3).

Individual dots correspond to individual patients, color represent levels of SAMHD1 expression at diagnosis (green <25%, orange 25-75%).

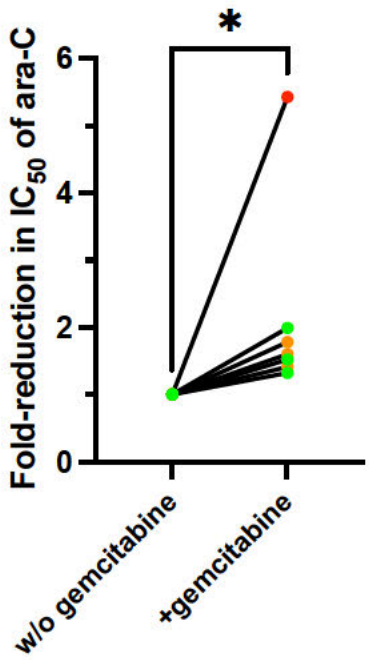
1.4 Supplementary Figure S4. Effect of 60 µM hydroxyurea on ara-CTP levels in diagnostic bone-marrow mononuclear cells *ex vivo*.



Ex vivo cultured mononuclear bone marrow cells were treated for 24 h with 500 nM ara-C in the presence or absence of 60 µM hydroxyurea. The left panel shows ara-CTP levels measured without hydroxyurea (w/o HU) as compared to ara-CTP with hydroxyurea, normalized to without hydroxyurea (n=8). *P*=0.02.

Individual dots correspond to individual patients, color represent levels of SAMHD1 expression at diagnosis (green <25%, orange 25-75%, red >75%).

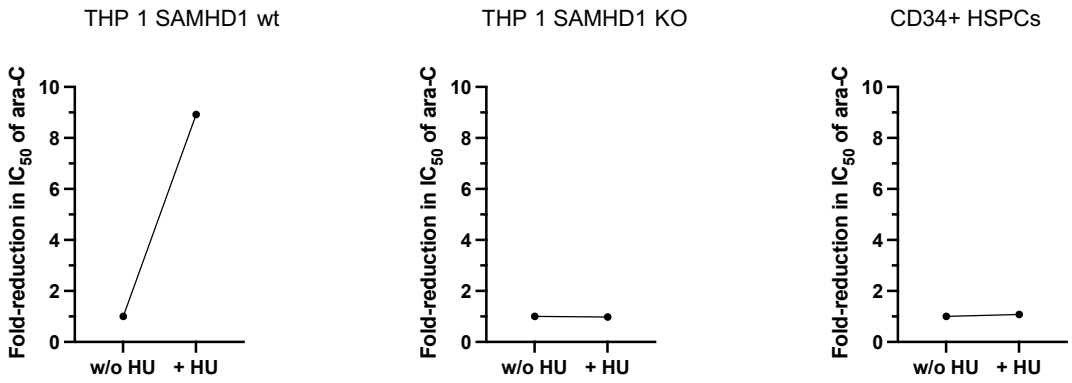
1.5 Supplementary Figure S5. Effect of gemcitabine on *ex vivo* sensitivity to ara-C



Half-maximal inhibitory concentrations (IC_{50}) of ara-C in diagnostic bone-marrow blasts treated *ex vivo* in the presence or absence of 10 nM gemcitabine, normalized to absence of gemcitabine. *P*=0.01.

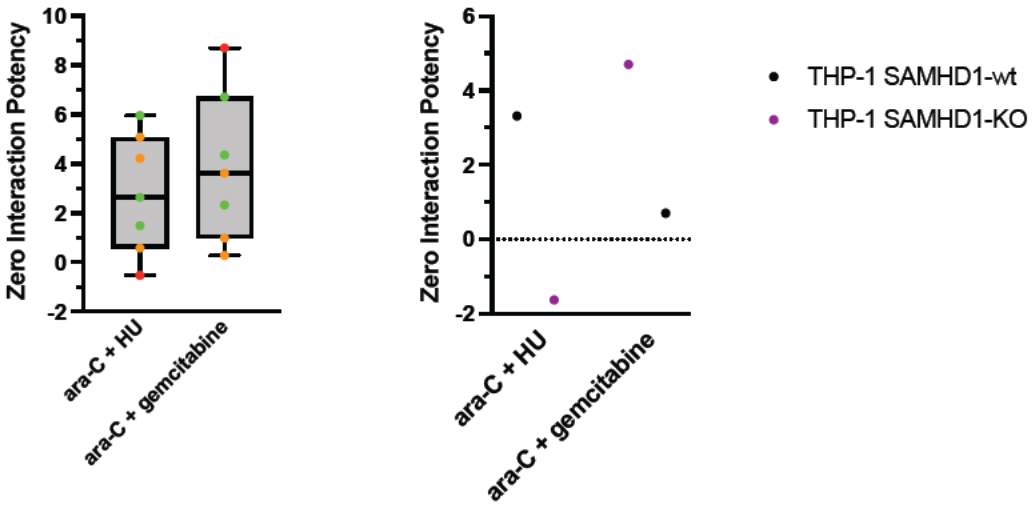
Individual dots correspond to individual patients, color represent levels of SAMHD1 expression at diagnosis (green <25%, orange 25-75%, red >75%).

1.6 Supplementary Figure S6. Effect of hydroxyurea on *ex vivo* sensitivity to ara-C in THP-1 cells and healthy CD34+ HPSCs



Panels show the fold-reduction in IC_{50} values of ara-C in THP-1 SAMHD1-wt, THP-1 SAMHD1-KO, and CD34+ HSCPs treated *ex vivo* in the presence or absence of 60 µM hydroxyurea, normalized to absence of hydroxyurea.

1.7 Supplementary Figure S7. Zero Interaction Potency of ara-C and hydroxyurea in diagnostic bone-marrow mononuclear cells *ex vivo* and THP-1 cells



Zero Interaction Potencies for combinations of ara-C with hydroxyurea (HU) or gemcitabine were calculated (see Methods) for diagnostic bone-marrow mononuclear cells (left panel, n=7) and THP-1 cells (right panel, SAMHD1-wt (black), SAMHD1-KO (purple)).

Individual dots in the left panel correspond to individual patients, color represent levels of SAMHD1 expression at diagnosis (green, <25%, orange 25-75%, red >75%).

2. Supplementary Tables

2.1 Supplementary Table S1: Antibody panels for flow cytometry

EuroFlow panel[1]			**NOPHO AML panel[2]**		
Tube	**Marker**	**Antibody**	**Tube**	**Marker**	**Antibody**
AML1	CD16	Dako	**AML1**	CD56	BD Biosciences
	CD13	BD Biosciences		CD13	BD Biosciences
	CD34	BD Biosciences		CD34	BD Biosciences
	CD117	BD Biosciences		CD117	BD Biosciences
	CD10	BD Biosciences		CD33	BD Biosciences
	CD11b	BD Pharmingen		CD11b	BD Pharmingen
	DR	Biolegend		DR	Biolegend
	CD45	BD Horizon		CD45	BD Horizon
AML2	CD35	BD Pharmingen	**AML2**	CD36	BD Biosciences
	CD64	Invitrogen		CD64	Invitrogen
	CD34	BD Biosciences		CD34	BD Biosciences
	CD117	BD Biosciences		CD117	BD Biosciences
	IREM-2	Immunostep		CD33	BD Biosciences
	CD14	BD Biosciences		CD14	BD Biosciences
	DR	Biolegend		DR	Biolegend
	CD45	BD Horizon		CD45	BD Horizon
AML3	CD36	BD Biosciences	**AML3**	CD15	BD Biosciences
	CD105	BD Pharmingen		NG2	Beckman Coulter
	CD34	BD Biosciences		CD34	BD Biosciences
	CD117	BD Biosciences		CD117	BD Biosciences
	CD33	BD Biosciences		CD2	BD Biosciences
	CD71	BD Biosciences		CD19	BD Biosciences
	DR	Biolegend		DR	Biolegend
	CD45	BD Horizon		CD45	BD Horizon
ALOT	cMPO	Dako	**AML4**	CD7	BD Biosciences
	cCD79a	Dako		CD96	Invitrogen
	CD34	BD Biosciences		CD34	BD Biosciences
	CD19	Beckman Coulter		CD117	BD Biosciences
	CD7	eBioscience		CD123	MACS Miltenyl Biotec
	CD3	BD Biosciences		CD38	BD Biosciences
	cCD3	BD Pharmingen		DR	Biolegend
	CD45	BD Horizon		CD45	BD Horizon
			AML5	CD99	Abd serotec
				CD11a	BD Pharmingen
				CD34	BD Biosciences
				CD117	BD Biosciences
				CD133	MACS Miltenyl Biotec
				CD4	BD Biosciences
				DR	Biolegend
				CD45	BD Horizon

2.2 Supplementary Table S2. Haematologic recovery

Patient number	Cycle number	Day neutrophils > $0.5x10^9$/L	Day neutrophils > $1.0x10^9$/L	Day platelets > $50x10^9$/L	G-CSF
1101	1	18	19	19	Yes
1101	2	16	16	16	Yes
1102	1	16	18	22	Yes
1102	2	17	17	19	Yes
1103	1	22	22	22	Yes
1103	2	18	19	19	Yes
1104	1	23	23	24	Yes
1104	2	20	20	22	Yes
1104	3	26	37	28	Yes
1105	1	20	20	25	Yes
1105	2	17	17	24	Yes
1105	3	20	20	30	Yes
1105	4	24	24	30	Yes
1106	1	21	23	23	Yes
1106	2	17	17	23	Yes
1106	3	20	20	31	Yes
1107	1	16	16	17	Yes
1107	2	18	25	28	Yes
1107	3	22	22	29	Yes
1107	4	28	28	35	Yes
1108	1	16	18	18	Yes
1108	2	18	18	22	Yes
1109	1	19	19	23	Yes
1109	2	17	21	21	Yes
1109	3	20	20	23	Yes
Median (range), n=9	1	19 (16-23)	19 (16-23)	22 (17-25)	NA
Median (range), n=9	2	17 (16-20)	18 (16-25)	22 (16-28)	NA
Median (range), n=5	3	20 (20-26)	20 (20-37)	29 (23-31)	NA
Median (range), n=2	4	24 and 28	24 and 28	30 and 35	NA

2.3 Supplementary Table S3. Mutations assessed with deep sequencing

Patient	Gene	Mutation	Gene-specific forward primer	Gene-specific reverse primer
1101	*RUNX1*	NM_001754.4: c.592G>A, p.(Asp198Asn)	5′-AGGGTGTACCAGCCTGGAG-3′	5′- GGGAAAAGCTTCACTCTGACC-3′
1102	*NRAS*	NM_002524.5: c.G35A, p.(Gly12Asp)	5′-TCACCTCTATGGTGGGATCA- 3′	5′-ACCCTGATTACTGGTTTCCAA-3′
1102	*U2AF1*	NM_001025204.1: c.248G>A, p.(Arg83His)	5′-GAGACATTTACTACCTCGTGTGC-3′	5′-CGGAAAAGGCTGTGATTGA-3′
1103	*FLT3*	NM_004119.3: c.2503G>T, p.(Asp835Tyr)	5′-ACAGTGTGTTCACAGAGACCTG-3′	5′-ATGATAACGACACAACACAAAATAG-3′
1103	*RUNX1*	NM_001754.5: c.328A>C, , p.(Lys110Gln)	5′- CTGCCCTCGCGGATCT -3′	5′-AGCATGGTGGAGGTG-3′
1106	*RUNX1*	NM_001754.5: c.431T>C, p.(Leu144Pro)	5′-CTTCGACCGACAAACCTGAG-3′	5′- GTGTTTAGGTGGTGGCCCTA-3′
1106	*FLT3*	NM_004119.3: c.2521_2522delinsGGCCC, p.(Asn841delinsGlyPro)	5′-CCATGATAACGACACAACACAA-3′	5′- AGGAACGTGCTTGTCACC-3′
1108	*RUNX1*	NM_001754.4: c.835_844dup, p.(Asp282Valfs*321)	5′- GGCACGTCCAGGTGAAAT-3′	5′- AAATCCCACCCCACTTTACA-3′
1108	*CBL*	NM_005188.4: c.1112A>G, p.(Tyr371Cys)	5′-TTTTTCTGTTAACATTTATAATTGCAG-3′	5′- GTGTCCACAGGGCTCAATCT-3′

2.4 Supplementary Table S4. White blood cell composition at time of ara-CTP measurements

Patient ID	Blood cells	Pre treatment	End of cytarabine infusion 1	End of cytarabine infusion 2
1101	WBC, 10E9/L	4.6	5.4	3.8
	MNC, 10E9/L	3.2	3.2	2.5
	Blasts, 10E9/L (% of MNC)	1.5 (46.9)	1.0 (31.3)	1.1 (44.0)
1102	WBC, 10E9/L	3.0	2.3	1.7
	MNC, 10E9/L	2.3	1.2	1.0
	Blasts, 10E9/L (% of MNC)	0.7 (30.4)	0.5 (41.2)	0.4 (40.0)
1103	WBC, 10E9/L	16.0	13.3	7.7
	MNC, 10E9/L	15.6	12.0	6.5
	Blasts, 10E9/L (% of MNC)	8.6 (55.1)	7.2 (60.0)	3.2 (49.2)
1104	WBC, 10E9/L	3.1	2.9	2.7
	MNC, 10E9/L	1.8	0.8	0.4
	Blasts, 10E9/L (% of MNC)	0.2 (11.1)	0.2 (25.0)	0.1 (25.0)
1105	WBC, 10E9/L	28.6	30.0	18.6
	MNC, 10E9/L	18.0	18.2	7.8
	Blasts, 10E9/L (% of MNC)	8.7 (48.3)	9.6 (52.7)	3.3 (41.0)
1106	WBC, 10E9/L	3.6	2.0	NA
	MNC, 10E9/L	3.5	1.8	NA
	Blasts, 10E9/L (% of MNC)	2.2 (62.9)	0.5 (27.8)	NA
1107	WBC, 10E9/L	1.5	1.4	0.9
	MNC, 10E9/L	1.0	0.8	0.4
	Blasts, 10E9/L (% of MNC)	0 (0)	0 (0)	0 (0)
1108	WBC, 10E9/L	5.4	5.3	4.3
	MNC, 10E9/L	3.3	1.6	1.2
	Blasts, 10E9/L (% of MNC)	0.3 (9.1)	0.1 (6.3)	0 (0)
1109	WBC, 10E9/L	76.0	85.2	76.0
	MNC, 10E9/L	41.2	41.0	40.8
	Blasts, 10E9/L (% of MNC)	35.3 (85.7)	37.2 (90.7)	37.6 (92.2)

3. Supplementary References

1. van Dongen JJ, Lhermitte L, Böttcher S, et al. EuroFlow antibody panels for standardized n-dimensional flow cytometric immunophenotyping of normal, reactive and malignant leukocytes. Leukemia 2012;26:1908-75.

2. Tierens A, Bjørklund E, Siitonen S, et al. Residual disease detected by flow cytometry is an independent predictor of survival in childhood acute myeloid leukaemia; results of the NOPHO-AML 2004 study. Br J Haematol 2016;174:600-9.

HEAT-AML (Hydroxyurea-Enhanced Ara-C Treatment of Adult Acute Myeloid Leukaemia)
A phase I/II multi-centre study to assess the tolerability and efficacy of the addition of hydroxyurea to standard ara-C and daunorubicin-based therapy for adults (age ≥ 18 years of age) with newly diagnosed acute myeloid leukaemia (AML)

AUTHORS
Nikolas Herold, Jan-Inge Henter, Stefan Deneberg, Martin Jädersten, Martin Höglund

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DEVELOPMENT PHASE I/II

SPONSOR: Karolinska University Hospital

CHIEF INVESTIGATOR: MD Nikolas Herold

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NO. OF PAGES 59

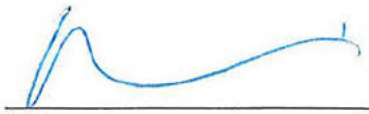
Signature Page

HEAT-AML <version 1.3; 2020-07-06>

Name:
Nikolas Herold
Trial Role: Chief Investigator
Chief Investigator
Signature:

Date:
06 / 07 / 2020

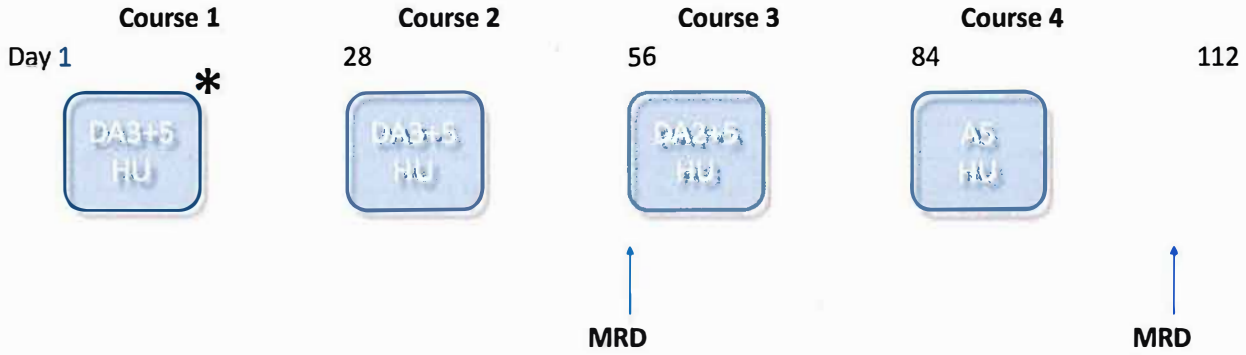
Name:
Nina Perrin
Trial Role:
Sponsor representative; Head of "Medicinsk Enhet Högspecialicerad barnmedicin 1", Karolinska University Hospital
Signature:



Date:
06 / 07 / 2020

This protocol describes the HEAT-AML study and provides information about procedures for patients taking part in the HEAT-AML study. The protocol should not be used as a guide for treatment of patients not taking part in the HEAT-AML study.

1 SCHEME OF STUDY (PHASE II PART)



DA3+5: daunorubicin 60 mg/m², o.d., 8 h IV, day 1, 2, 3; ara-C 1 g/m², b.i.d., 2 h IV, day 1,2,3,4,5
DA2+5: daunorubicin 60 mg/m², o.d., 8 h IV, day 1, 2; ara-C 1 g/m², b.i.d., 2 h IV, day 1,2,3,4,5
A5: ara-C 1 g/m², b.i.d., 2 h IV, day 1,2,3,4,5

HU: hydroxyurea, b.i.d., day 1,2,3,4,5; * during run-in, one dose of HU is omitted on day 1

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3 LIST OF ABBREVIATIONS

AE	Adverse Event
ALT	Alanine Amino Transferase (GPT)
ANC	Absolute Neutrophil Count
AR	Adverse reaction
Ara-C	Cytarabine, cytosine arabinoside
AST	Aspartate Amino Transferase (GOT)
BM	Bone Marrow
CBF	Core binding factor
CI	Confidence interval
CMV	Cytomegalovirus
CR	Complete Remission
CRi	Complete Remission with incomplete blood count
CRF	Case Report Form
CTCAE	Common Terminology Criteria for Adverse Events
DLT	Dose Limiting Toxicity
ECG	Electrocardiogram
EFS	Event Free Survival
EMD	Extra medullary disease
FISH	Fluorescent In Situ Hybridization
FU	Follow up
GCP	Good Clinical Practice
HIV	Human Immunodeficiency Virus
HOVON	Dutch/Belgian Haemato-Oncology Cooperative Group
HRC	Haematocytology Review Committee
HU	Hydroxyurea/hydroxycarbamide
ICH	International Conference on Harmonization of technical requirements for registration of pharmaceuticals for human use
INCA	informationsnätverk för cancervården (Swedish IT platform for research and treatment of cancer patients)
IV	Intravenous
LAP	Leukaemia Associated Phenotype
METC	Medical Ethical review committee
MLFS	Morphologic leukaemia-free state
MK	Monosomal Karyotype
MRD	Minimal Residual Disease
NYHA	New York Heart Association
OS	Overall Survival

4 SYNOPSIS

4.1 Rationale

This trial aims to improve effective treatments for newly diagnosed adult AML patients (≥ 18 years of age), the majority of which (~70%) still succumb to their disease. Among other factors, a reason for treatment failure may be expression of the protein SAMHD1 that limits the efficacy of one of the two major agents used to treat AML, cytarabine (ara-C) by inactivation of its active metabolite ara-CTP. We have discovered that hydroxyurea (HU), a drug that is regularly used in pre-treatment or palliation of AML, but has not systematically been combined with ara-C in AML patients, inhibits SAMHD1's activity towards ara-CTP. Therefore, addition of hydroxyurea to ara-C may improve the efficacy of ara-C. The phase I part of this trial evaluates safety of addition of HU to standard-of-care treatment for AML patients (i.e. combination of ara-C and daunorubicin during induction and consolidation courses) at three different dose levels. The phase II part evaluates whether addition of the maximally tolerated dose of HU as determined during phase I part prior to every infusion of ara-C has an effect on treatment outcome as assessed by measurement of minimal residual disease.

4.2 Study objectives

Primary objectives for phase I part (run-in)

1. To assess the safety and tolerability of HU at three different dose-levels added to standard AML therapy consisting of ara-C and daunorubicin (frequency and severity of toxicities; dose-finding)

Secondary objectives for phase I part (run-in)

1. To assess the time to haematopoietic recovery (ANC 0.5 and 1.0 x 10^9/L; platelets 50 x 10^9/L) after each chemotherapy treatment cycle, defined as the time from the start of the cycle until recovery.
2. To assess the effect of HU added to standard AML therapy consisting of ara-C and daunorubicin on ara-CTP accumulation in peripheral blasts during the first chemotherapy cycle
3. To assess the role of SAMHD1 protein expression for the effect of HU added to standard AML therapy consisting of ara-C and daunorubicin on ara-CTP accumulation in peripheral blasts during the first chemotherapy cycle

Primary objectives for phase II part

1. To assess the effect of HU added to standard AML therapy consisting of ara-C and daunorubicin on the rate of negative minimal residual disease (MRD-negativity) after the second standard chemotherapy cycle

Secondary objectives for phase II part

1. To assess the toxicity of HU at added to standard AML therapy consisting of ara-C and daunorubicin (frequency and severity of toxicities)
2. To assess the effect of HU added to standard AML therapy consisting of ara-C and daunorubicin on remission (CR/CRi/MLFS) after two standard chemotherapy cycles
3. To assess the effect of HU added to standard AML therapy consisting of ara-C and daunorubicin on event-free survival (EFS) two years after diagnosis
4. To assess the effect of HU added to standard AML therapy consisting of ara-C and daunorubicin on relapse-free survival (RFS) two years after diagnosis
5. To assess the effect of HU added to standard AML therapy consisting of ara-C and daunorubicin on overall survival (OS) two years after diagnosis
6. To assess the role of SAMHD1 protein expression on the effect of HU added to standard AML therapy consisting of ara-C and daunorubicin

7. To assess the role of SAMHD1 protein expression for the effect of HU added to standard AML therapy consisting of ara-C and daunorubicin on ara-CTP accumulation in peripheral blasts during the first chemotherapy cycle

4.3 Study design

This is a prospective, open label, multi-centre phase I/II study.
The scheme of this design consists of the standard treatment for AML with the addition of HU prior to ara-C infusions.

4.4 Patient population

Patients with AML (except acute promyelocytic leukaemia), previously untreated, age ≥ 18 years.

4.5 Intervention

Patients in this study are treated with standard treatment for AML with addition of HU prior to every ara-C infusion. The dose of HU will be determined during the phase I run-in phase of the study. HU p.o. will be administered 1 hour before each ara-C infusion. Effects of HU on intracellular ara-CTP concentrations in peripheral AML blasts will be determined.

4.6 Duration of treatment

Expected duration of 3 to 4 cycles of standard AML therapy (consisting of ara-C and daunorubicin) with HU, including evaluation, is about 4-5 months. All patients will be followed until 5 years after registration.

4.7 Target number of patients

Phase I: 9 patients
Phase II: 60 patients

Expected duration of accrual
Planned start of recruitment Q2 2019
Planned end of recruitment Q4 2021

4.8 Main study endpoint

- MRD-negativity after cycle 2

4.9 Benefit and nature and extent of the burden and risks associated with participation

Patients with AML might benefit in terms of MRD-negativity, CR, EFS, RFS and OS from addition of HU as this might improve the efficacy of ara-C.

4.10 Burden and risks associated with participation

Combinations of HU and ara-C have been tested in clinical trials before, and no unexpected excess toxicity was observed. However, an increase in myelosuppression is at least a theoretical concern as ara-C and HU both are myelosuppressive. In addition, specific ara-C toxicities might be exacerbated. An additional amount of 10-20 ml bone marrow may optionally be taken for the Karolinska AML Biobank at entry and after cycle 2, and two additional peripheral blood samples as compared to standard procedure will be drawn at cycle 1.

4.11 Planned interim analyses and DSMB

After phase I of the trial has been finished, analysis of toxicities including duration of neutropenia will be performed for the three different dose-levels of HU. The DSMB will perform independent analyses.
The phase II of the trial will use the maximum HU dose-level with tolerable toxicity. The study will not proceed to phase II if no tolerable dose-level can be determined.
After inclusion of 20 patients in the phase II of the trial, an interim analysis of toxicities will be performed by the DSMB. If unacceptable toxicity is identified, the trial will be halted.

5 INVESTIGATORS AND STUDY ADMINISTRATIVE STRUCTURE

Responsibility	**Name**	**Affiliation/Address**
Sponsor	Karolinska University Hospital	171 76 Stockholm
Representative of sponsor	Nina Perrin	Högspecialiserad Barnkirurgi och Barnmedicin B77 Karolinska University Hospital Huddinge 141 86 Stockholm
Principal Investigator	Nikolas Herold	Karolinska University Hospital; Karolinska Institutet, Stockholm Tomtebodavägen 18A 171 77 Stockholm
Co-investigators	Jan-Inge Henter	Karolinska University Hospital Solna; Karolinska Institutet, Stockholm Tomtebodavägen 18A 171 77 Stockholm
	Stefan Deneberg	Karolinska University Hospital Huddinge; Karolinska Institutet, Stockholm ME Hematologi M64 141 86 Stockholm
	Martin Jädersten	Karolinska University Hospital Huddinge; Karolinska Institutet, Stockholm ME Hematologi M64 141 86 Stockholm
	Martin Höglund	University Hospital Uppsala; Uppsala University VO hematologi, 50C 751 85 Uppsala

	Sören Lehmann	University Hospital Uppsala; Uppsala University; Karolinska Institutet, Stockholm Sjukhusvägen 85 751 85 Uppsala
	Gunnar Juliusson	Skåne University Hospital; Lund University Verksamhetsområde Hematologi och kärl 222 41 Lund
[redacted]		
Statistician		Karolinska Institutet, Stockholm Tomtebodavägen 18A 171 77 Stockholm
ddPCR/NGS-based MRD	Linda Fogelstrand	Sahlgrenska Universitetssjukhuset Avdelningen för Laboratoriemedicin vid Institutionen för biomedicin 413 45 Göteborg
Immunohistochemistry review (SAMHD1)	Georgios Rassidakis	Karolinska Institutet Cancer Centre Karolinska

		171 76 Stockholm
Registration and Data management	Centrum för Kliniska Cancerstudier	Centrum för Kliniska Cancerstudier Tema Cancer Karolinska Universitetssjukhuset 171 76 Stockholm
Monitoring	Centrum för Kliniska Cancerstudier	Centrum för Kliniska Cancerstudier Tema Cancer Karolinska Universitetssjukhuset 171 76 Stockholm
Serious adverse events (SAEs) notification	Centrum för Kliniska Cancerstudier	Centrum för Kliniska Cancerstudier Tema Cancer Karolinska Universitetssjukhuset 171 76 Stockholm

DSMB

6 INTRODUCTION AND RATIONALE

6.1 AML in adults: unsatisfactory survival and quality of life

Worldwide, more than 300,000 patients are diagnosed with acute myeloid leukaemia (AML) every year(*1*). AML is the most frequent acute blood cancer in adults and the second most frequent blood cancer in the paediatric population. AML consists of clinically, genetically and biologically distinct groups of malignant haematological diseases(*2*).

With regard to survival, as compared to children with survival rates approaching 70%, the outcome for adults suffering from AML is much worse with a 5-year overall survival (OS) ~30%, and even poorer for elderly patients with a 2-year OS of <20%(*3*). Apart from allogeneic haematopoietic stem cell transplantation that has almost halved relapse risk in AML as compared to chemotherapy alone(*4*), only a limited number of drugs have been shown to improve survival in AML(*5*).

6.2 SAMHD1 limits clinical efficacy of AML treatments with cytarabine

Ara-C was developed 1959 and approved by the US Food and Drug Administration 10 years later. Being a deoxycytidine analogue, cytarabine is an antimetabolic pro-drug that is intracellularly phosphorylated to its mono-, di- and triphosphate, and cytarabine triphosphate (ara-CTP) is considered the main active metabolite. Ara-CTP inhibits DNA and RNA polymerases, but is also incorporated into DNA during S phase, which can lead to both base pair mismatching and chain termination, leading to DNA damage and cell death(*6, 7*). It has already been identified in the late 1970s that the ability of AML blasts to accumulate ara-CTP correlates with *in vitro* toxicity and clinical response(*8, 9*). Several studies suggested that the difference in several orders of magnitude of ara-CTP between AML blasts from different patients cannot be explained by differences in levels of transporters and enzymes then known to be involved in ara-C metabolism(*10*).
We have recently demonstrated that the deoxynucleoside triphosphate (dNTP) triphosphohydrolase SAM domain and HD domain 1 (SAMHD1) is the long sought-after driver of detoxification of intracellular ara-CTP pools(*11*). Similarly, we showed that SAMHD1 limits the efficacy of decitabine(*12*), used in the treatment of myelodysplastic syndrome (MDS) and palliative care of AML.
SAMHD1 was first described as a guanosine triphosphate hydrolase in myeloid cells (*13*). Later on, loss-of-function mutations in SAMHD1 were implicated in Aicardi-Goutières syndrome (AGS), a neurodegenerative disease frequently associated with symptoms of systemic lupus erythematosus(*14*). In 2011, SAMHD1 was reported to be a major restriction factor that blocks HIV infection in myeloid cells(*15*). Restriction is thought to be mediated by SAMHD1's ability to hydrolyse all four endogenous deoxynucleotide triphosphates (dNTPs), thereby depriving HIV of substrates for replication. SAMHD1 can be targeted for proteasomal degradation by Vpx, a viral accessory protein expressed by HIV-2 and certain SIV strains(*15*).
SAMHD1 contains two allosteric nucleotide binding sites that regulate tetramerisation and activation as well as one catalytic nucleotide binding site(*16*). Importantly, SAMHD1 has also been shown to be able to hydrolyse the synthetic nucleotide triphosphate of clofarabine(*17*). We showed that recombinant SAMHD1 exhibits ara-CTPase activity *in vitro*. Cells in which SAMHD1 expression was transiently reduced by the simian immunodeficiency virus (SIV) protein Vpx were up to 130-fold more sensitive to ara-C-induced cytotoxicity in a SAMHD1-concentration dependent manner. Permanent disruption using CRISPR/Cas9 also sensitised cells to ara-C, and sensitivity could be abrogated by ectopic expression of wild type (WT) but not dNTPase-deficient SAMHD1. Heterotopic as well as orthotopic AML mouse models lacking SAMHD1 were hypersensitive to ara-C, translating into a near-doubling of overall survival (OS) or functional cure, respectively, after only a single cycle of ara-C treatment. *Ex vivo* treatment with Vpx also sensitised primary patient AML blasts to ara-C in a SAMHD1-dependent manner. Finally, we identified SAMHD1 as a strong risk factor for overall and event-free survival in both paediatric and adult *de novo* AML patient cohorts receiving ara-C treatment(*11, 18*). Hence, inhibition of SAMHD1 might improve outcome of AML patients treated with ara-C.

6.3 Hydroxyurea inhibits SAMHD1 ara-CTPase activity

More recently, we established a phenotypic screening assay and screened for small-molecule inhibitors of SAMHD1(*19*). Within approximately 33,000 compounds, we found the clinically used drugs hydroxyurea

(HU) and gemcitabine to inhibit SAMHD1, thereby dramatically increasing cytarabine toxicity in SAMHD1-positive cells. These drugs could completely abolish the SAMHD1 effect on ara-C toxicity in a dose-dependent manner, while leaving knockout cells unaffected, and low micromolar and nanomolar concentrations of HU and gemcitabine, respectively, were sufficient to achieve complete SAMHD1 inhibition in AML cell lines. More importantly, HU and gemcitabine improved the efficacy of ara-C in primary patient AML blasts in a dose-dependent manner. When SAMHD1 was removed from the AML blasts, HU or gemcitabine had no effect on ara-C toxicity, consistent with being SAMHD1 inhibitors (Fig. 1).

HU is an FDA- and EMA-approved substance used in the treatment of AML for which patents have expired, and extensive safety data exists in both adults and children. Using *in vitro* activity together with cellular thermal shift and cross-linking assays we could furthermore show that HU mechanistically results in allosteric inhibition of SAMHD1 ara-CTPase activity at allosteric site AS2. In parallel, we established immunohistochemistry to quantify SAMHD1 levels in diagnostic AML bone marrow biopsies. Fig. 2 shows examples of a diagnostic bone-marrow biopsy of a patient with high (left) and a patient with low (right) SAMHD1-positivity.

Correlating SAMHD1 expression (as assessed by immunohistochemistry, Fig. 2) with overall survival in two pooled patient cohorts from MD Anderson Cancer Center and Singapore University Hospital that underwent high-dose ara-C consolidation showed a significant disadvantage (P=0.011) for patients expressing high levels of SAMHD1 (Fig. 3), with 3-year OS being >70% in SAMHD1-low as compared to <35% for SAMHD1-high patients.

Eventually, addition of HU to ara-C in AML mouse experiments led to significant improvement of survival (P=0.0082) while ara-C or HU alone were not superior to treatment with vehicle (Fig. 4).

HU has a longstanding role in the treatment of patients with AML. Clinically unstable patients with hyperleukocytosis at risk of severe complications of leukostasis, haemorrhages and tumour lysis syndrome are routinely and effectively treated with a cytoreductive pre-phase of HU alone to reduce the tumour burden(*20*). Combinations of HU and ara-C have been tested in patients before, albeit only in relapsed or refractory patients. These studies suggested that these combinations are safe. Howell *et al.* treated 21 patients with combinations of low-dose ara-C and HU, however, only two patients were diagnosed with AML(*21*). Importantly, no delayed myelosuppression or cumulative toxicity was observed when combining HU and ara-C as compared to ara-C alone; with the exception of mild skin rash, no non-bone-marrow toxicity was observed. A similar study by Schilsky *et al.* combined low-dose ara-C with HU in 25 patients with relapsed non-Hodgkin lymphoma yielding very similar toxicity outcomes(*22*). Furthermore, high-dose ara-C was combined with HU in a paediatric study population of 33 leukaemia patients (7 of which were AML); the maximum tolerated dose of ara-C was 2400 mg/m^2 when combined with HU in this study(*23*).

6.4 Purpose and aims of the study

Even though AML treatment is one of the most intensive treatments in haemato oncology, the death tolls of more than 30% in children and around 70% in adults are unacceptably high; the majority of these deaths are due to refractory or relapsing disease. Since the 1970s, resistance to ara-C has been recognised as a major reason for limited therapy outcome of AML patients. SAMHD1 expression limits survival of patients with AML treated with high-dose ara-C. HU might improve ara-C therapy in AML patients, as HU completely abolished SAMHD1-mediated ara-C resistance in primary AML samples and resensitised human AML cells to ara-C in humanised animal models (NOD/SCID).
The main purpose of the study is to contribute to improved treatment efficacy and survival for patients with AML. This study aims at evaluating safety, tolerability, feasibility and efficacy of the addition of HU to standard AML therapy, to pave the way for larger controlled studies.

This proposal is driven by the hypothesis that HU will increase the cytotoxic effects and clinical efficacy of ara-C against AML, thereby reducing the number of patients whose quality of life is decreased during intensive therapy without the benefit of treatment efficacy.

We provide a multicentre phase-I/II clinical trial evaluating the addition of HU (given prior to each ara-C infusion) to standard AML therapy in newly diagnosed AML patients.

Our operational aims are

- registration of possible excess toxicity due to addition of hydroxyurea (in particular duration of neutropenia and specific side effects for ara-C).
- validation of hydroxyurea's inhibitory effect on SAMHD1 in leukemic blasts from AML patients.
- measuring effects of addition of hydroxyurea on minimal residual disease (MRD) in AML patients.
- measuring effects of addition of hydroxyurea on complete response rates and MLFS in AML patients.
- analysing effects of addition of hydroxyurea on event-free, relapse-free and overall survival in AML patients.

7 STUDY OBJECTIVES

7.1 Primary objectives for phase I part (run-in)

1. To assess the safety and tolerability of HU at three different dose-levels added to standard AML therapy consisting of ara-C and daunorubicin (frequency and severity of toxicities)

7.2 Secondary objectives for phase I part (run-in)

1. To assess the time to hematopoietic recovery (ANC 0.5 and 1.0 x 10^9/L; platelets 50 x 10^9/L) after each chemotherapy treatment cycle, defined as the time from the start of the cycle until recovery.
2. To assess the effect of HU added to standard AML therapy consisting of ara-C and daunorubicin on the rate of negative minimal residual disease (MRD-negativity) after the second standard chemotherapy cycle
3. To assess the effect of HU added to standard AML therapy consisting of ara-C and daunorubicin on remission (CR/CRi/MLFS) after two standard chemotherapy cycles
4. To assess the effect of HU added to standard AML therapy consisting of ara-C and daunorubicin on ara-CTP accumulation in peripheral blasts during the first chemotherapy cycle
5. To assess the role of SAMHD1 protein expression for the effect of HU added to standard AML therapy consisting of ara-C and daunorubicin on ara-CTP accumulation in peripheral blasts during the first chemotherapy cycle

7.3 Primary objectives for phase II part

1. To assess the effect of HU added to standard AML therapy consisting of ara-C and daunorubicin on the rate of negative minimal residual disease (MRD-negativity) after the second standard chemotherapy cycle

7.4 Secondary objectives for phase II part

1. To assess the toxicity of HU at added to standard AML therapy consisting of ara-C and daunorubicin (frequency and severity of toxicities)
2. To assess the time to hematopoietic recovery (ANC 0.5 and 1.0 x 10^9/L; platelets 50 x 10^9/L) after each chemotherapy treatment cycle, defined as the time from the start of the cycle until recovery.
3. To assess the effect of HU added to standard AML therapy consisting of ara-C and daunorubicin on remission (CR/CRi/MLFS) after two standard chemotherapy cycles
4. To assess the effect of HU added to standard AML therapy consisting of ara-C and daunorubicin on event-free survival (EFS) two years after diagnosis
5. To assess the effect of HU added to standard AML therapy consisting of ara-C and daunorubicin on relapse-free survival (RFS) two years after diagnosis
6. To assess the effect of HU added to standard AML therapy consisting of ara-C and daunorubicin on overall survival (OS) two years after diagnosis
7. To assess the role of SAMHD1 protein expression on the effect of HU added to standard AML therapy consisting of ara-C and daunorubicin

8 STUDY DESIGN

This is a multi-centre phase I/II clinical study, consisting of standard treatment for AML with experimental addition of HU prior to ara-C infusions.

8.1 Best available treatment

The study will follow the declaration of Helsinki and the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) Guidelines for Good Clinical Practice (GCP). GCP provides scientific and ethical standards for the design, conduction and reporting of clinical trials involving human subjects. Compliance with GCP will ensure protection of the rights, safety and wellbeing of the subjects, and that the trial data are credible. The study contains the standard therapy for AML in Sweden as defined by the Swedish AML group and is thus regarded as the currently best available therapy (version 3.0; ISBN: 978-91-87587-89-4). In brief, after diagnosis is established, patients are given a first chemotherapy course (DA3+5) consisting of daunorubicin, IV infusion 60 mg/m^2/8h daily for 3 days, in combination with ara-C, IV infusion 1 g/m^2/2h b.i.d for 5 days. Approximately four weeks after the first DA3+5, a bone marrow sample is taken and a second DA3+5 is administered if the blast count in the bone marrow sample is below 10%. A blast count above 10% indicates resistant disease, and patients should change to salvage treatment instead. After another four weeks, a bone marrow sample has to be taken, and a course DA2+5 that removes the daunorubicin application on day 3 is administered if patients are in complete remission. If complete remission is not achieved, salvage treatment should be considered. After another four weeks, a bone marrow sample is taken. If complete remission is not maintained, patients should change to salvage treatment. If patients continue to be in complete remission, they can either proceed to allogeneic stem cell transplantation or receive a course A5 that only consists of ara-C, IV infusion 1 g/m^2/2h b.i.d for 5 days. After additional four weeks, another bone marrow sample is taken for final evaluation. Importantly, patients achieving complete remission at any time during chemotherapy might directly go haematopoietic stem cell transplantation (following the above-mentioned guide-lines and an individual assessment by the treating haematologist).
Tyrosine kinase mutations (in particular *FLT3*) are assumed to be tumour drivers in AML. E.g. Midostaurin is approved by the European Medical Agency (EMA) for the treatment of AML with activating FLT3 mutations in combination with chemotherapy(*24*). **Treatment with midostaurin or other tyrosine kinase inhibitors is no exclusion criterion for the HEAT-AML study.** Of note, combining midostaurin with conventional chemotherapy did not lead to prolonged episodes of neutropenia in a phase-III clinical trial(*25*).

8.2 Run-in (phase I part)

This study consists of a safety run-in phase (phase I part) and an efficacy phase (phase II part). The aim of the run-in phase is to determine a dose of HU that can be added to standard AML therapy with tolerable toxicity. In addition, the inhibitory effect of HU on SAMHD1 by determining levels of ara-CTP in patient blasts from peripheral blood directly after the first and after the second IV application of ara-C (day 1 of first DA3+5) will be assessed. To this end, HU will only be given prior to one of the two ara-C infusion on the first day of the first chemotherapy cycle. Peripheral blood will be sampled at the end of the 2 h infusion for subsequent ara-CTP measurements. As only one ara-C infusion will be preceded 1 hour by intake of HU *per os*, the effect of HU can be estimated by comparing ara-CTP levels after the first and after the second ara-C infusion within the same patient. With the exception of the different dose levels of HU, further treatment of patients from the run-in phase is identical to course one, although HU will be given prior to each dose of ara-C. Three patients will be enrolled for each of the dose-levels (3x3 design).

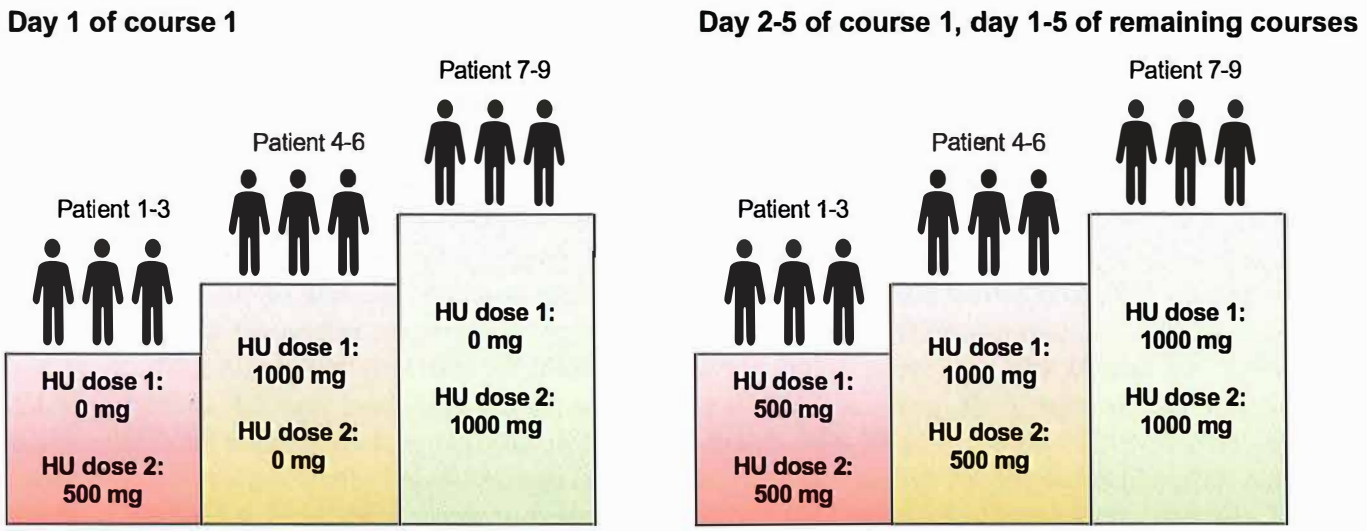

Fig. 5: Hydroxyurea dosing during phase I run-in. 3x3 design.
Graphical representation of the two daily doses HU that are administered 1 hour prior to each ara-C infusion for the first day of course 1 (left panel) and the remaining days and courses (right panel).

8.3 Phase II trial (phase II part)

Backbone of the treatment protocol is the Swedish standard-of-care (see above), consisting of two courses DA3+5, one course DA2+5, and one course A5 or stem cell transplantation. However, HU *per os* 1 hour prior to each infusion of ara-C, i.e. b.i.d. for 5 days for DA3+5, DA2+5, and A5, will be added at the dose-level determined in the phase I part. Stem cell transplantation should be considered depending on patient-related (age, comorbidities) and donor-related risks (immunoincompatibility), as described in the standard of care programme of the Swedish AML group.

Considerations regarding sample size for the primary endpoint MRD-negativity: It is nowadays well established that minimal residual disease (MRD) can be a clinical surrogate marker for survival, in particular relapse-free survival. A recent study of the Belgian-Dutch study consortium HOVON that is actively collaborating with the Swedish AML group has reported retrospective analyses on the power of MRD of patients in clinical remission to predict relapse(*26*). MRD-positivity was defined as $\geq 0.1\%$ leukemic cells in bone marrow samples. The frequency of MRD-positivity declines with the number of chemotherapy courses and is approximately 50%, 30% and 25% after course 1, 2, and 3, respectively.
Hence, standard therapy leads to an MRD-negativity of approximately 70% following course 2 in historical controls. With 60 patients in the phase II part, the study is able to show an increase of MRD-negativity to 85% ($1-\beta = 0.8$; $\alpha = 0.05$) when compared with historical cases (matched for age, sex, cytogenetic/molecular risk-group) at a ratio of 1:4.

Two additional control cohorts will be utilised, one being data from the AML INCA ("informationsnätverk för cancervården") registry, selecting patients having received intensive chemotherapy according to the national guidelines (the same as in the current study) and determining the rate of MRD-negativity after cycle 2. The INCA registry contains a large number of patients with the strength of being population-based. However, selection effects may affect which patients that have data on MRD after cycle 2, which will be hard to account for. Therefore, a second control cohort will consist of consecutive AML patients from the Karolinska University Hospital being treated according to the same protocol between 2016-2018, where data on MRD should be available or else chart reviews will determine the reason for the lack of MRD data, most often due to refractory disease or terminally ill patients. In patients where MRD analysis was not performed, we have the option to perform genetic MRD analysis from biobanked DNA extracted from bone marrow smears taken after cycle 2.

9.1 Eligibility for inclusion

All patients must be registered before start of treatment.

9.1.1 Inclusion criteria:

- Patients with:
  - a diagnosis of AML and related myeloid precursor neoplasia according to the 2016 revised WHO classification (excluding acute promyelocytic leukaemia) including secondary AML (after an antecedent haematological disease (e.g. MDS) and therapy-related AML, ***or***
  - acute leukaemia of ambiguous lineage according to WHO 2016

- Patients 18 years and older.

- Adequate renal and hepatic functions unless clearly disease related as indicated by the following laboratory values:
  - Serum bilirubin ≤ 2.5 x upper limit of normal (ULN, i.e. ≤65 µmol/L), unless considered AML-related or due to Gilbert's syndrome
  - Alanine aminotransferase (ALAT) ≤ 2.5 x ULN, i.e. ≤ 1.9 µcat/L and ≤ 2.9 for females and males, respectively, unless considered AML-related

- Male patients must use an effective contraceptive method during the study and for a minimum of 6 months after study treatment.

- Written informed consent.

- Patient is capable of giving informed consent.

9.1.2 Exclusion criteria

- Acute promyelocytic leukaemia.

- CNS leukaemia.

- Ongoing treatment with gemtuzumab-ozogamicin.

- Major organ failure precluding administration of planned chemotherapy.

- Glomerular filtration rate (GFR) < 30 ml/(min*1.73 m^2)

- Cardiac dysfunction as defined by:
  - Myocardial infarction within the last 3 months of study entry, ***or***
  - Reduced left ventricular function with an ejection fraction < 40% as measured by echocardiogram (will only be performed when clinically suspected) ***or***
  - Unstable angina ***or***
  - New York Heart Association (NYHA) grade IV congestive heart failure (see Appendix I) *or*
  - Unstable cardiac arrhythmias

- Known intolerance to any of the chemotherapeutic drugs in the protocol.

- Positive pregnancy test. Lactating female or female of childbearing potential not using adequate contraception.

- Fanconi anaemia.

- Patients with a history of non-compliance to medical regimens or who are considered unreliable with respect to compliance.

9.1.3 Dose reductions and treatment with midostaurin

Dose-reductions of ara-C to as low as 500 mg/m² decided upon by the treating haematologist are **no** exclusion criterion.
Preceding, concurrent, or subsequent treatment with midostaurin (or similar inhibitors of FLT3) are **no** exclusion criterion.

9.2 Concomitant medication

Concomitant medication is allowed with the exception of other study drugs, AML-directed chemotherapy or other AML-directed disease modifying drugs other than those specified in the protocol (the investigational medicinal products cytarabine, daunorubicin and hydroxyurea as well as midostaurin (or similar inhibitors of FLT3)). Concomitant medication has to be registered (see 10.4).

9.3 Discontinuation criteria

Study participation can be discontinued by the investigator, the sponsor, or the study patient according to the criteria described below.

9.3.1 Sponsor-initiated discontinuation

The sponsor may decide to terminate the study prematurely based on the following criteria:

- If part A fails to demonstrate an increase in ara-CTP concentrations by addition of HU;
- There is evidence of an unacceptable risk for study patients (i.e. safety issue);
- There is reason to conclude that continuation of the study cannot serve a scientific purpose following confirmation of the DSMB.
- The DSMB recommends to end the trial based on viable arguments other than described above.

The sponsor will promptly notify all concerned investigators, the Ethics Committee(s) and the regulatory authorities of the decision to terminate the study. The sponsor will provide information regarding the time lines of study termination and instructions regarding treatment and data collection of enrolled patients.

9.3.2 Investigator-initiated discontinuation

Patients should be withdrawn from protocol treatment if any of the following criteria for withdrawal are met:

- Death
- Patient not eligible in hindsight
- Adverse event preventing further treatment
- Major protocol violation

The investigator can also decide to withdraw a patient from protocol treatment for other reasons than the criteria described above. Examples of such reasons for withdrawal from protocol treatment are:

- Excessive extramedullary drug toxicity preventing continuation of treatment
- Discontinuation of therapy because of change to salvage therapy
- Discontinuation of therapy because of change to azacytidine therapy
- Refusal of patient to continue protocol treatment
- No compliance of the patient: patient is unable or unwilling to adhere to the treatment schedule and/or procedures required by the protocol

9.3.3 Patient-initiated discontinuation

Patients can leave the study at any time for any reason if they wish to do so without any consequences.

If a patient states he or she withdraws their consent to participate in the trial, the investigator should attempt to verify the patient's intent and record this in the patient's medical file:

- The patient can refuse further treatment and/or procedures according to protocol, while still consenting with further follow up data collection.
- The patient can refuse further treatment and/or procedures according to protocol, and withdraw consent for further follow up data collection.

Any data acquired up until withdrawal of consent may still be used.

Patients who are withdrawn from protocol treatment will receive medical care according to local practice.

Patients withdrawn from the study before evaluation following treatment cycle 2 will be substituted by newly enrolled patients. Patients withdrawn from the study after evaluation of treatment cycle 2 will not be substituted.

10 TREATMENT

10.1 Pre-treatment

Patients who have a WBC > 30 x 10^9/L can be pre-treated with **hydroxyurea (HU)** prior to inclusion in the trial, to control WBC count. HU monotherapy can be given for a maximum of 10 days, and can be given until the start of the first treatment cycle. Patients that undergo pre-treatment with HU cannot be enrolled for the run-in phase I of the study.

10.2 Treatment

10.2.1 Treatment cycle 1

Criteria for starting cycle 1
Day 1 of cycle 1 should start as soon as possible from entry.

Treatment schedule:

Agent	**Dose/day**	**Route of administration**	**Days**
Ara-C	1 g/m^2 twice daily	2 hr infusion	1-5
Daunorubicin	60 mg/m^2	8 hr infusion	1, 2, 3
HU	twice daily; dose determined in phase I part	p.o. 1 hr prior to ara-C infusion	1-5

Treatment evaluation of cycle 1

Bone-marrow evaluation on day +25 (± 3 days) from start of cycle 1. Evaluation can be performed earlier if refractory AML is suspected, and the treating haematologist can decide to give course 2 as an early re-induction. MRD assessment after cycle 1 is optional according to local routines.

For response criteria please check appendix B.
Of note:
- Morphologic blast count, and not flowcytometric blast count, is leading

10.2.2 Treatment cycle 2

Criteria for starting cycle 2
The second cycle should be started between 4-5 weeks after the start of the first cycle if the patient is fit for treatment (can also be given earlier, see above). If more than 10% of malignant blasts are present at bone-marrow aspiration on day 25, change to salvage-treatment should be considered which then will exclude the patient from the study.

Treatment schedule cycle 2 (identical to cycle 1):

Agent	**Dose/day**	**Route of administration**	**Days**
Ara-C	1 g/m^2 twice daily	2 hr infusion	1-5
Daunorubicin	60 mg/m^2	8 hr infusion	1, 2, 3
HU	twice daily; dose determined in phase I part	p.o. 1hr prior to ara-C infusion	1-5

Treatment evaluation of cycle 2

Bone marrow evaluation on day +28 (± 3 days) from start of cycle 2. Evaluation can be performed earlier, in particular for patient with high-risk AML. MRD assessment (by flow cytometry and/or sensitive genetic analysis in accordance with national guidelines) after cycle 2 is mandatory.

For response criteria please check appendix B.

10.2.3 Treatment cycle 3

Criteria for starting cycle 3

The cycle should be started as soon as the clinical status and bone-marrow regeneration allow.

Treatment schedule cycle 3:

Agent	**Dose/day**	**Route of administration**	**Days**
Ara-C	1 g/m^2 twice daily	2 hr infusion	1-5
Daunorubicin	60 mg/m^2	8 hr infusion	1, 2
HU	twice daily; dose determined in phase I part	p.o. 1 hr prior to ara-C infusion	1-5

Treatment evaluation of cycle 3

Bone marrow evaluation on day +28 (± 3 days) from start of cycle 3. Evaluation can be performed earlier, in particular for patient with high-risk AML. MRD assessment after cycle 3 is optional according to local routines.

For response criteria please check appendix B.

10.2.4 Treatment cycle 4

Criteria for starting cycle 4

The cycle should be started as soon as the clinical status and bone marrow regeneration allow.

Treatment schedule cycle 4:

Agent	Dose/day	Route of administration	Days
Ara-C	1 g/m^2 twice daily	2 hr infusion	1-5
HU	twice daily; dose determined in phase I part	p.o. 1 hr prior to ara-C infusion	1-5

Treatment evaluation of cycle 4

Bone marrow evaluation on day +28 (± 3 days) from start of cycle 4. Evaluation can be performed earlier, in particular for patient with high-risk AML. MRD assessment after cycle 4 is mandatory.

For response criteria please check appendix B.

10.3 Special precautions and supportive care

Supportive measures for optimal medical care according to local guidelines should be provided during participation in this clinical trial.
Anti-emetics and tumour-lysis syndrome prophylaxis can be given according to institutional practice.

10.3.1 Antimicrobials

If indicated and consistent with institutional guidelines, prophylactic anti-infective drugs can be given.

10.3.2 Decreased glomerular filtration rate (GFR)

Patients with GFR < 30 ml/(min*1.73 m^2) will be excluded from the study.

10.3.3 Missed doses

If a patient missed a HU dose prior to ara-C infusion, the dose **will not** be replaced.

10.3.4 Vomited doses

If a dose is vomited within one hour of ingestion, it will be replaced. If vomiting occurs more than 1 hour after dosing, it will still be considered a complete dose.

10.4 Prevention of pregnancy

The drugs in this study can affect an unborn child. Female patients of childbearing potential in this trial must apply highly effective birth control measures:

- combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation:
  - -oral
  - -intravaginal

-transdermal

- progestogen-only hormonal contraception associated with inhibition of ovulation:
  - -oral
  - -injectable
  - -implantable
- intrauterine device (IUD)
- intrauterine hormone-releasing system (IUS)
- bilateral tubal occlusion
- vasectomised partner
- sexual abstinence (if refraining from heterosexual intercourse during the entire period of risk-associated with the study treatments. The reliability of sexual abstinence will be evaluated in relation to the preferred and usual lifestyle of the subject).

Male patients should not father a child while on this study. It is important that patients understand the need to use birth control while on this study. Male patients must use an effective contraception if sexually active with a female of child-bearing potential. Acceptable methods of contraception are condoms with contraceptive foam, oral, implantable or injectable contraceptives, contraceptive patch, intrauterine device, diaphragm with spermicidal gel, or a sexual partner who is surgically sterilised or post-menopausal. For male patients, effective methods of contraception must be used throughout the study and for 6 months following the last dose.
Female partners of males taking investigational product should be advised to call their healthcare provider immediately if they get pregnant. The male subject should notify the investigator of this partner's pregnancy and her healthcare provider information. The investigator will then provide this information to the sponsor for follow-up as necessary. All details should be documented on the pregnancy form.

10.5 Investigational medicinal product: cytarabine (ara-C)

Ara-C is a deoxycytidine analogue. It must be tri-phosphorylated to its active form, ara-CTP, by deoxycytidine kinase and other nucleotide kinases. Ara-CTP inhibits DNA polymerase. In addition, ara-CTP is incorporated into DNA as a false base, causing inhibition of DNA synthesis. It is S-phase specific. Ara-C penetrates the blood brain barrier (approx. 40% of plasma concentration). It is converted to its inactive form, uracil arabinoside, by cytidine deaminase. Approximately 80% of the dose is recovered in the urine, mostly as uracil arabinoside (ara-U).

Formulation: Cytarabine is available in 20 mg/ml or 100 mg/ml vials. Intact vials can be stored at room temperature.
For IV use, the solution can be diluted with 0.9% sodium chloride or glucose 50 mg/ml.
Dosing and Administration: see section Treatment Details.
Intravenous administration.
Toxicity: Myelosuppression is the dose limiting adverse effect, with leukopenia and thrombocytopenia being predominant. Other common adverse effects include nausea and vomiting (may be severe at high doses), diarrhoea, mucositis, anorexia, alopecia, skin rash, and liver dysfunction. A flu-like syndrome (ara-C syndrome) characterized by fever, rash, and aches in muscle and bone is common. Less common side effects include allergic reactions, palmar-plantar erythrodysesthesia, and cellulitis at the injection site. High doses of ara-C can cause conjunctivitis, hepatitis, and (cerebellar) CNS symptoms including somnolence, peripheral neuropathy, ataxia, and personality changes. CNS symptoms are usually reversible and are more common in the elderly and in patients with renal impairment.
Fever during systemic ara-C: Fever, myalgia, rash, and increased CRP are not uncommon after the start of ara-C treatment. In very severe cases systemic corticosteroids (e.g. 2 mg/kg/day prednisolone) can be considered, as it has been shown to be beneficial in treating or preventing this syndrome. Ara-C treatment usually doesn't need to be discontinued. It is however important to consider infection as a differential diagnosis.
Precautions: Keratitis/conjunctivitis can be caused by high-dose ara-C treatment and prophylactic treatment with ophthalmic corticosteroids is recommended, starting before the first cytarabine dose and continuing until 48 hours after the last one.

10.6 Investigational medicinal product: daunorubicin (Cerubidin)

Formulation: Cerubidin® (Sanofi AB). Daunorubicin is available as red-orange lyophilized powder for injection in 20 mg single dose vials and a preservative free 5 mg/ml solution in 20 mg (4ml) and 50 mg (10 ml) vials. Reconstitute with sterile water for injection to a final concentration of 5 mg/ml. After adding the diluent, the vial should be shaken gently and the contents allowed to dissolve. The reconstituted solution is stable for 24 hours at room temperature and 48 hours refrigerated. Protect from exposure to sunlight. Aqueous Solution: Store refrigerated 2° to 8°C. Protect from light. Retain in package, until contents are used. Powder for injection: Store unreconstituted vial at room temperature 15° to 30°C. Protect from light. Retain in package, until contents are used.
Dosing and Administration: see section Treatment Details.
Intravenously as an 8-hour infusion. Daunorubicin may be diluted in saline or dextrose containing solutions.
Toxicity: Bone-marrow suppression. Myocardial damage that can result in irreversible heart failure: cumulative dose of 450-550 mg/m^2 should not be exceeded. Mucositis, GI toxicity, liver function test abnormalities; and severe subcutaneous necrosis if extravasation should occur.
Interactions: No significant interactions.

10.7 Investigational medicinal product: hydroxyurea (HU)

Formulation: Hydrea® (Bristol-Myers Squibb). HU is available as 500 mg hard capsules for oral administration. Store light-protected at room temperature 15° to 30°C. Retain in package, until contents are used.
Dosing and Administration: see section Treatment Details.
Perorally as tablets 1 hour prior to start of ara-C infusion.
Toxicity: Bone-marrow suppression with leukopenia and thrombocytopenia. Skin rash, skin lesions, alopecia. Azoospermia. GI toxicity, anorexia, liver function test abnormalities. Peripheral neuropathy.

10.8 Acquisition of investigational medicinal products

Cytarabine, daunorubicin and hydroxyurea are registered medicinal products and will be acquired through the local hospital pharmacies. As such, the medicinal products are unambiguously labelled according to the regulatory requirements for medicinal products.

10.9 Discontinuation of investigational medicinal products

The following serious adverse events (SAEs) during AML therapy are well known and will be registered (see 11.4.1):

- Need of and days at intensive care (transfer to an ICU) including days of assisted ventilation
- Infections requiring intravenous antimicrobial treatment (including documentation of infectious agent and clinical signs of sepsis)
- Congestive heart failure
- Cardiac arrhythmia
- Hypoxia (CTC grade 3 or 4)
- Anaphylaxis (CTC grade 3 or 4)
- Cutaneous reactions (e.g. Palmar-plantar erythrodysaesthesia syndrome, rash, ulceration; CTC grade 3 or 4)
- Abdominal pain
- Abdominal symptoms leading to laparotomy
- Neutropenic enterocolitis (CTC grade 3 or 4)
- Multi-organ failure (CTC grade 3 or 4)
- Increased bilirubin levels bilirubin >5x upper normal limit (i.e.> 130 µmol/L)
- Severe thrombosis causing organ dysfunction and/or requiring systemic anticoagulation (CTC grade 3 or 4)
- Renal dysfunction with an increase in creatinine > 3 UNL (i.e. > 300 µmol/L)
- Catastrophic bleeding with documentation of organ involved

- Disseminated intravascular coagulation
- Neurotoxicity
- Mucositis

The treating haematologist may choose to temporarily or permanently discontinue treatment with the investigational medicinal products.

In case of suspected unexpected serious adverse reaction (SUSAR, see 11.3.3), treatment will be discontinued and the national medicine agency in Sweden and the ethics committee in Stockholm, Sweden will be contacted immediately.

10.10 Treatment following study participation

Following completion of the study or discontinuation, the study patients will be treated by the treating haematologist according to local and national guidelines.

11 STUDY PROCEDURES

11.1 Time of clinical evaluations

Chemotherapy cycles are administered in hospital.

11.2 Clinical evaluations and follow-up

After treatment all patients will be followed until 5 years after registration at least every 6 months, according to local practice. Except for SAMHD1 immunohistochemistry that is carried out centrally in Stockholm, all analyses are performed at the local centres.

Hospital visit
Patients will present at the hospital for assessment as indicated.

Medical history
Standard medical history, with special attention for:
WHO performance status

Only at entry:
Previous chemotherapy or radiotherapy
Antecedent haematological diseases (≥ 3 months duration prior to diagnosis AML or high risk MDS, record AML, subtype and type of prior other antecedent haematological disease)
– Antecedent oncological diseases

Check of inclusion/exclusion
Assessment according to criteria listed under 9.1.1 and 9.1.2.

Collection of informed consent
This includes informed consent to participation in the study and to biobanking of research material in the AML biobank at Karolinska

Registration of concomitant medication
Patients will be asked whether they have taken any concomitant medication since the last visit/treatment. If yes, medication will be documented.

Physical examination
Standard physical examination including body weight and height, with special attention for:
Vital signs
Splenomegaly
– Hepatomegaly
Signs of extramedullary leukaemia (if signs, record site, and level of evidence (physical examination, radiologically confirmed, pathologically confirmed))

Haematology
Haemoglobin
Platelets
WBC
WBC differential (either automatically or manually)

Blood chemistry
– Creatinine

Table 1

	Baseline (at diagnosis)	Treatment cycle 1	Treatment evaluation after cycle 1	Treatment cycle 2	Treatment evaluation after cycle 2	Treatment cycle 3	Treatment evaluation after cycle 3	Treatment cycle 4	Treatment evaluation after cycle 4	End of treatment	Follow-up year 1	Follow-up year 2	Follow-up year 3	Follow-up year 4	Follow-up year 5	End of study
Hospital visit	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Medical history	X															
Check of inclusion/exclusion	X															
Collection of informed consent	X															
AE / SAE			X		X		X		X	X	X	X	X	X	X	X
Registration of concomitant medication			X		X		X		X	X						
Physical examination	X		X		X		X		X	X	X	X	X	X	X	X
Haematology	X		X		X		X		X							
Blood chemistry	X		X		X		X		X							
Bone marrow aspirate	X		X		X		X		X							
Morphology	X		X		X		X		X							
Immunophenotyping (flow cytometry)*	X				X (MRD)				X (MRD)							
Cytogenetics	X															
Molecular diagnostics (NGS panel)	X				X (MRD for *NPM1* mutation or CBF-AML)				X (MRD for *NPM1* mutation or CBF-AML)							
Bone marrow biopsy (or bone marrow aspiration clot)	X															
Histopathology	X															
SAMHD1 immunohistochemistry	X (centrally at pathology lab in Stockholm)															
Differential blood count after first and second cytarabine infusion		X														
ara-CTP measurements after first and second cytarabine infusion (contact Nikolas Herold)		X														
Biobanking	X				X											
Coagulation	X															
Serology	X															
Chest X-ray	X															
Echocardiogram	If heart-disease suspected clinically															
ECG	X															
Parameters for response evaluation			X		X		X		X							
Timing and dosing of protocol treatment		X		X		X		X		X						
Survival status											X	X	X	X	X	X

*: *not necessary if NPM1 mutated or CBF-AML*

Albumin
Bilirubin (direct +indirect)
ALAT
LDH
CRP

Bone marrow aspirate, Histopathology
Bone marrow aspirate with bone marrow clot or biopsy at entry, according to local practice for histopathology. Later time points on indication: i.e. persistent aplasia.

Morphology, Immunophenotyping, Cytogenetics, Molecular diagnostics

Standard work up in accordance with national guidelines (analysed at local centre):

- Bone marrow aspirate at entry for:
    - Morphology and cytochemistry to establish WHO subtype of AML
    - Cytogenetics (cell culture and banding analysis)
    - Flow cytometry for immunological phenotyping and for determination of a leukaemia associated immune phenotype (LAIP)
    - Molecular analysis for FLT3-ITD, NPM1, CEBPA according to the Swedish AML Guidelines. In addition, the Illumina TruSight myeloid panel or equivalent can be run to get a comprehensive assessment of myeloid genes according to local practice (http://www.illumina.com/content/dam/illumina-marketing/documents/products/datasheets/datasheet-trusight-myeloid.pdf).

SAMHD1 immunohistochemistry

Sections from bone marrow clot/biopsy for SAMHD1 immunohistochemistry (results are not accessible to the treating physician prior to completion of the study) should be sent for analysis at central pathology laboratory in Stockholm.
Cell clots and/or bone marrow biopsies should be fixed in paraformaldehyde. A polyclonal rabbit anti-SAMHD1 antibody (Bethyl Laboratories, San Antonio, TX, A303-690A) and an automated detection system (Ventana Medical Systems, Roche, Basel, Switzerland) will be used for quantitative immunohistochemistry of SAMHD1. Both percentage of SAMHD1-positive AML blasts (0-100%) as well as staining intensity (1-3) will be assessed.
If bone marrow sections are not available or of insufficient quality, bone marrow smears will be requested for SAMHD1 staining.
As SAMHD1 staining is performed retrospectively, results will not be reported back to the treating haematologist.

Biobanking

All patients will be asked to send 10-20 ml bone marrow to the AML Biobank at Karolinska Institutet at time of diagnosis/study entry and after cycle 2. The biobank part of the study is optional. The patients should receive a separate patient information, and biobanking will be agreed upon based on a separate informed consent form. Biobanked material not used for the purpose of this study will be available for other ethically approved studies in AML, in accordance with regulations of the Karolinska Biobank for AML. The Karolinska Biobank for AML fulfills all criteria for biobanking according to Swedish laws and regulation.

The biobanked marrow can be used for detailed next-generation sequencing based MRD analysis as wells as in-depth *in vitro* studies assessing the level of MRD and SAMHD1-expression in hematopoietic stem and progenitor cell subsets as well as potentially performing functional experiments. As these investigations are performed retrospectively, results will not be reported back to the treating haematologist

End of treatment

All AE/SAE will be summarised. All treatment details, in particular deviations from the study protocol (e.g. dose reductions, reasons for premature termination) will be summarised.
(see also 12.1)

Additional/Specific investigations
- Chest X-ray
- Cardiac ejection fraction, measured by echocardiogram if clinically indicated
- ECG
- Serology for cytomegalovirus (CMV) infection, EBV, HIV (human immunodeficiency virus), hepatitis A, B and C
- Coagulation studies including: fibrinogen, APTT, PTT
- Haematopoietic cell transplantation co-morbidity index (HCT-CI) according to local practice.

11.3 Toxicity assessment

During and following each cycle, toxicity has to be carefully examined and evaluated. To comply with EU-Directive 2001/20/EC and ICH Guidelines for GCP a standardised toxicity registration will be established including reporting of severe adverse events (SAEs) and suspected unexpected severe adverse reactions (SUSARs).
The study will be monitored by a systematic and independent examination of trial-related activities and documents to determine, whether the evaluated trial-related activities were conducted, and the data were recorded, analysed, and accurately reported according to the protocol, Good Clinical Practice (GCP), and the applicable regulatory requirements. This comprises (1) documentation of informed consent, (2) documentation and validity of the inclusion criteria of each patient, (3) documentation that the treatment was given according to the allocated treatment schedule, (4) documentation of data regarding study endpoints in particular toxicities as specified below, date and type of events, MRD results, SAMHD1 expression levels in diagnostic bone marrow samples, ara-CTP measurements where applicable, patient-reported outcome measures, (5) Documentation and reporting of SAE.

Standard AML therapy is very intensive, and a high level of toxicity is expected. Toxicity registration has to early detect unacceptably high and/or unexpected toxicity from the interventional therapy (addition of HU) in order to ensure the safety of the trial subjects. In compliance with EU-Directive 2001/20/EC and ICH Guidelines for Good Clinical Practice (GCP), deaths and suspected unexpected severe adverse reactions (SUSARs) must be documented (safety report) and reported immediately.

The toxicity assessment includes the following:
- Complete history of symptoms and complaints
- Complete physical examination
- Laboratory examination of CBC, ANC, ALAT, creatinine, LDH; other parameters as clinically indicated
- Chest X-ray as clinically indicated
- Electrocardiography when indicated
- Echocardiography when indicated

Toxicities will be reported as described in 11.3.1.
Toxicities will be scored according to the most recent version of the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5
(https://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_50).

11.3.1 Adverse Events (AEs)

Adverse events will be scored according to the most recent version of the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5
(https://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_50). All adverse events will be registered in the electronic case registration form (eCRF).

11.3.2 Severe Adverse Events (SAEs)

Severe adverse events (SAEs) during AML therapy are well known. After each course, the following SAEs will be reported:

- Need of and days at intensive care (transfer to an ICU) including days of assisted ventilation
- Infections requiring intravenous antimicrobial treatment (including documentation of infectious agent and clinical signs of sepsis)
- Congestive heart failure
- Cardiac arrhythmia
- Hypoxia (CTC grade 3 or 4)
- Anaphylaxis (CTC grade 3 or 4)
- Cutaneous reactions (e.g. Palmar-plantar erythrodysesthesia syndrome, rash, ulceration; CTC grade 3 or 4)
- Abdominal pain
- Abdominal symptoms leading to laparotomy
- Neutropenic enterocolitis (CTC grade 3 or 4)
- Multi-organ failure (CTC grade 3 or 4)
- Increased bilirubin levels bilirubin >5x upper normal limit (i.e.> 130 µmol/L)
- Severe thrombosis causing organ dysfunction and/or requiring systemic anticoagulation (CTC grade 3 or 4)
- Renal dysfunction with an increase in creatinine > 3 UNL (i.e. > 300 µmol/L)
- Catastrophic bleeding with documentation of organ involved
- Disseminated intravascular coagulation
- Neurotoxicity
- Mucositis

In addition, the number of days until hematopoietic recovery (ANC 0.5 and 1.0 x 10^9/L; platelets 50 and 100 x 10^9/L) after each chemotherapy treatment cycle, defined as the time from the start of the cycle until recovery for each course will be reported, as well as days of intravenous antibiotic treatment and days in hospital. Some adverse events that are common in AML and expected to occur in the majority of patients, e.g. anaemia, cytopenia, alopecia and nausea, will not be reported.

11.3.3 Causality assessment of Serious Adverse Events

The investigator will decide whether the serious adverse event is related to trial medication, i.e. any of the products from the protocol treatment schedule. The decision will be recorded on the serious adverse event report. The assessment of causality is made by the investigator using the following:

RELATIONSHIP	**DESCRIPTION**
UNRELATED	There is no evidence of any causal relationship.
UNLIKELY	There is little evidence of causal relationship (e.g. lack of temporal association). There is another reasonable explanation for the event.
POSSIBLE	There is some evidence to suggest a causal relationship (e.g. temporal association). However, the influence of other factors may have contributed to the event (e.g. the

	patient's clinical condition, other concomitant treatments).
PROBABLE	There is evidence to suggest a causal relationship and the influence of other factors is unlikely.
DEFINITELY	There is clear evidence to suggest a causal relationship and other possible contributing factors can be ruled out.
NOT ASSESSABLE	There is insufficient or incomplete evidence to make a clinical judgment of the causal relationship.

11.3.4 Suspected unexpected serious adverse reaction (SUSARs)

A SUSAR is defined as a serious adverse reaction which is not consistent with the product information and either results in death, is life threatening or requires hospital admittance or prolongation of existing hospitalisation, causes persistent or significant disability, causes congenital malformation. SAEs that are well-known side effects of the anti-leukemic therapy are not to be registered as SUSARs. The investigator must report SAEs to the sponsor within 24 hours after having been made aware. The sponsor has to evaluate the SAE. If the sponsor suspects a case of SUSAR, the national medicine agency in Sweden and the ethics committee in Stockholm, Sweden will be contacted as soon as possible, but not later than 7 days after having been made aware. SUSARs will also be documented in the safety report.
All serious adverse events will be followed clinically until they are resolved or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist.
Follow up information on SAE's should be reported monthly until recovery or until a stable situation has been reached. The final outcome of the SAE should be reported on a final SAE report.

11.3.5 Pregnancies

Pregnancies of a female subject or the female partner of a male subject, occurring while the subject is on protocol treatment, should be reported to the sponsor.
The investigator will follow the female subject until completion of the pregnancy, and must notify the sponsor of the outcome of the pregnancy within 5 days or as specified below. The investigator will provide this information as a follow-up to the initial pregnancy report. If the outcome of the pregnancy meets the criteria for classification as a SAE (i.e., spontaneous or therapeutic abortion, stillbirth, neonatal death, or congenital anomaly - including that in an aborted foetus), the investigator should follow the procedures for reporting SAEs. In the case of a live "normal" birth, the sponsor should be informed as soon as the information is available. All neonatal deaths that occur within 30 days of birth should be reported, without regard to causality, as SAEs. In addition, any infant death after 30 days that the investigator suspects is related to exposure *in utero* to the investigational medicinal product(s) should also be reported.
The investigator is encouraged to provide outcome information of the pregnancy of the female partner of a male subject, if this information is available to the investigator and the female partner gives her permission.

11.3.6 Reporting of safety issues

The sponsor will promptly notify all concerned investigators, the Ethics Committee(s) and the regulatory authorities of findings that could affect adversely the safety of patients, impact the conduct of the trial, increase the risk of participation or otherwise alter the EC's approval to continue the trial.
In the occurrence of such an event the sponsor and the investigators will take appropriate urgent safety measures to protect the patients against any immediate hazard. The local investigator will inform the patients

and local ethics or review committees according to hospital policy. The sponsor will inform any other parties that are involved in the trial.

11.3.7 Annual safety report

The sponsor will submit once a year a safety report to the Ethics Committees and Competent Authorities of the concerned Member States. The first report is sent one year after the first approval date of the trial. The last report is sent one year after the last patient has completed protocol treatment. The content of the annual safety report will be according to the EU guidance document *Detailed guidance on the collection, verification and presentation of adverse event/reaction reports arising from clinical trials on medicinal products for human use.*

11.3.8 Data Safety and Monitoring Board (DSMB)

The DSMB will advise the Principal Investigator, co-investigators and the chair of the working group in writing about the continuation of the trial. One of the DSMB members is appointed as DSMB Chair. The DSMB will review the general progress and feasibility of the trial, the quality and completeness of the data, adverse events, and safety. The DSMB will consider if there is any concern.

The DSMB will be provided detailed data on outcome and toxicities
a) After Phase I (i.e. after 9 patients),
b) After the Interim report, i.e. after 20 patients, and
c) every 6 month after start of Phase 2, during the recruitment phase
d) every 12 month after start of Phase 2, after the recruitment phase
e) after the end of the study.

DSMB is expected to give a short report (within 4 weeks after receiving the respective data)
a) After Phase I (i.e. after 9 patients),
b) After the Interim report, i.e. after 20 patients,
d) every 12 month after start of Phase 2, after the recruitment phase
e) after the end of the study.

In case of extraordinary events, additional DSMB meetings may be necessary.

11.4 Response evaluation

Following each cycle, at cycle 1 day 25 (± 3 days) and at cycle 2-4 day 28 (± 3 days), the response will be assessed by bone marrow aspiration, blood evaluation and extramedullary disease status evaluation (see Appendix B). If and as long as the marrow is not conclusive a new marrow will be taken as clinically indicated. If the marrow shows no complete remission after cycle 1, the next cycle (i.e. cycle 2) should be started as soon as possible without waiting for recovery of peripheral blood counts; under these circumstances, salvage therapy and withdrawal from the study should be considered by the treating haematologist. Similarly, if the marrow shows evidence of resistant disease after cycle 1 (i.e. presence of >10% malignant blasts as defined by the Swedish AML group guidelines), salvage therapy and withdrawal from the study should be considered by the treating haematologist.

11.4.1 MRD assessment

Definition of MRD: malignant blasts as a percentage of total bone-marrow cells and as a percentage of the whole white blood cell compartment. These percentages are calculated based on the frequency of cells with an aberrant phenotype.

It is nowadays well established that minimal residual disease (MRD) can be a clinical surrogate marker for survival, in particular relapse-free survival. A recent study of the Belgian-Dutch study consortium HOVON that is actively collaborating with the Swedish AML group has reported retrospective analyses on the power of MRD of patients in clinical remission to predict relapse(*26*). MRD-positivity was defined as ≥ 0.1% leukemic cells in bone marrow samples. The frequency of MRD-positivity declines with the number of chemotherapy courses and is approximately 50%, 30% and 25% after course 1, 2, and 3, respectively.
MRD is assessed as described in the standard of care by the Swedish AML group. For the purpose of the study, MRD will be assessed around four weeks after chemotherapy cycle 2. MRD is patient-specific, and will be performed by flow cytometry that follows a specific leukaemia-associated immunophenotype (LAIP) that is established at diagnosis using a panel of 8-10 cell surface markers. A threshold of MRD-positivity is defined by the presence of more than 0.1% leukemic blasts in the sample.
If a mutation in *NPM1* or core binding factor (CBF) fusion genes have been identified, PCR-based MRD-assessment standardised local routine will be performed instead of flow cytometry
In patients for whom a LAIP is not available and that do not have mutations in *NPM1* or CBF fusions, other genetic aberrations detected in the routine genetic testing of diagnostic AML samples can be used for validated (digital droplet) polymerase-chain reaction ((dd)PCR) or next-generation sequencing (NGS) based MRD diagnostics. If no MRD values were determined at the local treating centre, ddPCR or NGS-based MRD analyses will be performed retrospectively on biobanked samples. If biobanked material is not available, material will be requested from routine diagnostic samples.

Immunological and/or molecular examination for MRD detection at diagnosis, after cycle 2, is mandatory, and optional after cycles 1,2 and 4 (a laboratory manual with details will be provided to the participating centres).
Of note: Comprehensive molecular characterisation of AML and minimal residual disease by flow cytometry and/or molecular biology are considered to be essential for standard work up of AML. These assays are performed in appropriate routine laboratories at the individual centre.

11.4.2 Assessment of Complete Response (CR)

Assessment of complete remission:
Complete remission, as defined by the Swedish AML group, requires
- less than 5% malignant blasts in the bone marrow
- absence of malignant blasts in the peripheral blood or other extramedullary sites
- neutrophil count above 1 x 10^9/L
- thrombocyte count above 100 x 10^9/L
- no continued need of erythrocyte transfusions

CR with incomplete blood count recovery (CRi) is defined as CR with the exception that neutrophil count is below 1 x 10^9/L or thrombocyte count below 100 x 10^9/L.

11.4.3 Survival analyses

Event-free survival is defined as time from diagnosis to no response to intensive induction therapy, relapse, or death of any cause, whichever comes first. Overall survival is defined as time from diagnosis to death of any cause. Survival will be visualised using Kaplan-Meier curves, and compared with matched historical controls using the log-rank test. In addition, Cox proportional hazards models will be fitted in to evaluate the percentage of SAMHD1 positive blasts as a continuous predictor, and to adjust for other covariates (age, gender, genetic risk grouping). Alternatively, patients will be grouped into three discrete groups according to SAMHD1 expression with cut-offs of 25% and 75%, and compared using the log-rank test. All statistical analyses will be performed using R version 3.3.2 (The R Foundation for Statistical Computing, Vienna, Austria).
Definition of event:
An event is defined as either relapse, resistant disease or early death. Relapse is defined by the presence of ≥ 5% leukemic cells in the bone marrow after complete remission had been achieved. Extramedullary relapse is diagnosed when a tumour mass is detected with similar histopathological features (morphology, immunophenotype and cytogenetic analysis) as the original AML. Resistant disease (RD) is, defined as ≥ 5%

leukemic cells in the bone marrow after the second chemotherapy course. Early death is defined as any death occurring in patients before achieving remission unless they have been classified as having resistant disease.

11.4.4 ara-CTP measurements

During the phase I part of the trial, 10 ml full blood samples from patients are drawn via phlebotomy or a central-venous access directly after the first and second two-hour ara-C infusion at day 1 of the first course. For patients enrolled and treated at Karolinska University Hospital, during the phase II of the trial, 10 ml full blood samples from patients are drawn via phlebotomy or a central-venous access directly after the first two-hour ara-C infusion at day 1 of the first course. Mononuclear leukocytes are purified using a Ficoll gradient according to standard procedures. Amounts of ara-CTP are quantified centrally using high-performance liquid chromatography as described before(*27*).

12 WITHDRAWAL OF PATIENTS OR PREMATURE TERMINATION OF THE STUDY

12.1 Withdrawal of individual patients from protocol treatment

Patients should be withdrawn from protocol treatment if any of the following criteria for withdrawal are met:

- Death
- Patient not eligible in hindsight
- Adverse event preventing further treatment
- Major protocol violation

Patients can leave the study at any time for any reason if they wish to do so without any consequences. The investigator can also decide to withdraw a patient from protocol treatment for other reasons than the criteria described above. Examples of such reasons for withdrawal from protocol treatment are:

- Excessive extramedullary drug toxicity preventing continuation of treatment
- Discontinuation of therapy because of change to salvage therapy
- Discontinuation of therapy because of change to azacytidine therapy
- Refusal of patient to continue protocol treatment
- No compliance of the patient: patient is unable or unwilling to adhere to the treatment schedule and/or procedures required by the protocol

Patients who are withdrawn from protocol treatment will receive medical care according to local practice.

12.2 Follow up of patients withdrawn from protocol treatment

Patients who are withdrawn from treatment for other reasons than death will be followed as described in 11.1.1 for follow up; this includes patients that discontinued therapy because of switching to salvage or azacytidine therapy. SAE information will be collected as described in 11.3.
However, for patients who are withdrawn from treatment because in hindsight they did not fulfil the eligibility criteria (see 9.1) at time of enrolment, data will be collected until 30 days after the last protocol treatment given. SAE information will be collected as described in 11.3.

12.3 Withdrawal of informed consent

If a patient states he or she withdraws their consent to participate in the trial, the investigator should attempt to verify the patient's intent and record this in the patient's medical file:

- The patient can refuse further treatment and/or procedures according to protocol, while still consenting with further follow up data collection.
- The patient can refuse further treatment and/or procedures according to protocol, and withdraw consent for further follow up data collection.

- The patient can refuse further treatment and procedures according to protocol, withdraw consent for further follow up data collection and withdraw consent to use any data in the trial.

If the patient's intent is to withdraw consent for further data collection or to withdraw consent to use his or her data in the trial, appropriate actions should be taken.
If the patient's intent cannot be verified, further follow up data will be collected for this patient as described in 11.1.1

12.4 Premature termination of the study

The sponsor may decide to terminate the study prematurely based on the following criteria:

- If part A fails to demonstrate an increase in ara-CTP concentrations by addition of HU;
- There is evidence of an unacceptable risk for study patients (i.e. safety issue);
- There is reason to conclude that continuation of the study cannot serve a scientific purpose following confirmation of the DSMB.
- The DSMB recommends to end the trial based on viable arguments other than described above.

The sponsor will promptly notify all concerned investigators, the Ethics Committee(s) and the regulatory authorities of the decision to terminate the study. The sponsor will provide information regarding the time lines of study termination and instructions regarding treatment and data collection of enrolled patients.

13 ENDPOINTS

13.1 Primary endpoint

- MRD-negativity after cycle 2

13.2 Secondary endpoints

- Safety and tolerability (frequency and severity of non-hematological toxicities).
- Time to hematopoietic recovery (ANC 0.5 and 1.0 x 109/L; platelets 50 x 109/L) after each chemotherapy treatment cycle, defined as the time from the start of the cycle until recovery.
- Efficacy profile (response rate (CR, CRi, MLFS), event free survival (EFS), relapse-free survival (RFS), and overall survival (OS))

13.3 Translational endpoints

- Assessing intracellular accumulation of ara-CTP in circulating blasts as a biomarker for efficacy of added hydroxyurea
- Assessing SAMHD1 protein expression in blasts at diagnosis as a biomarker for efficacy of added hydroxyurea

13.4 DEFINITIONS

13.4.1 Overall survival (OS)

OS is defined as the time from the date of entry to the date of death, whatever the cause. The follow-up of patients still alive will be censored at the moment of last visit/contact.

13.4.2 Event-free survival (EFS)

EFS is defined as the time from registration to induction failure, death or relapse whichever occurs first.

13.4.3 Relapse-free survival (RFS)

RFS is defined as time from the date of achievement of CR until relapse or death from any cause, whichever comes first. Patients still in first CR and alive or lost to follow up will be censored at the date of last clinical assessment.

13.4.4 Induction failure

Induction failure is defined as not having achieved at least a CRi or CR after induction cycles 2

14 STATISTICAL CONSIDERATIONS

The aim of this study is to decide whether the addition of HU to standard AML treatment is feasible without causing intolerable excess toxicity and might lead to increased therapy efficacy. This could warrant a continuation to a phase III efficacy study. The final analysis of the primary endpoint will be done when validated data relevant for the primary endpoint for all patients are available.

14.1 Phase I run-in: HU dose-finding

The aim of the run-in phase I is to determine a dose of HU that can be added to standard AML therapy with tolerable toxicity (see 8.2 and 11.4). This will be done with a 3x3 design, with 3 patients per each of the 3 dose-levels. frequency and severity of toxicities will be tabulated.

14.2 Part B: Effect of added hydroxyurea on the rate of MRD-negativity

MRD is a clinical surrogate marker for survival. Standard therapy leads to an MRD-negativity of approximately 70% in historical controls. With 60 patients in the phase II part, the study is able to show an increase of MRD-negativity to 85% ($\beta = 0.8$; $\alpha = 0.05$) when compared with historical cases (matched for age, sex, cytogenetic/molecular risk-group) at a ratio of 1:4.

14.3 Secondary endpoints

Secondary endpoints compare several efficacy measures with historical matched controls:

- the different types of response (CR, CRi, MLFS) will be tabulated and time to response will be calculated
- the actuarial curves for EFS, RFS and OS will be computed using the Kaplan-Meier method and 90% CIs will be constructed
- the mean amounts of ara-CTP in circulating malignant blasts will be compared with and without addition of HU by student's t test
- the effect of SAMHD1 protein expression at diagnosis on MRD, CR, MFLS, EFS and OS will be determined

14.4 Toxicity analysis

The analysis of toxicity will be done primarily by tabulation of the incidence, nature and severity of adverse events with CTCAE grade 2 or more.

14.5 Interim analysis

In the phase II part of the study, an interim analysis of total toxicities, including duration of neutropenia will be performed after the first 20 patients are evaluable and discussed with the DSMB. If unacceptable toxicities are identified, discontinuation of study will be considered.

14.6 Statistical analysis of primary endpoint

To analyse the primary endpoint MRD-negativity (as defined under 11.4), the rate of MRD-negativity of the study patients will be compared to the rate of MRD-negativity of matched historical controls (as defined under 8.3) using conditional logistic regression.

15 REGISTRATION

15.1 Regulatory Documentation

Required regulatory and administrative documents must be provided to the study centre before enrolment of the first patient. This will always include an Ethics Committee approval for the investigational site. The study centre will supply the investigator with an overview of the required documents. Each investigational site will be notified when all requirements are met and enrolment can start

15.2 Registration

Eligible patients should be registered before start of treatment. Patients need to be registered at the Data Centre by registering the patient in the online ALEA Clinical registration matrix (FormsVision).

The following information will be requested at registration:

- Protocol number
- Institution name
- Name of caller/responsible investigator
- Local patient code (optional)
- Sex
- Date of birth or partial date of birth, e.g. year of birth,
- Age at date of inclusion
- Date written informed consent
- Specific items patient gives consent for (see ICF)
- Eligibility criteria
- Stratification factors

All eligibility criteria will be checked with a checklist.

Each patient will be given a unique patient study number (a sequence number by order of enrolment in the trial). Patient study number will be given immediately personally or by phone and confirmed by fax or email.

Local Patient Code is a code that may be assigned to the patient by the investigational site for local administrative purposes. The code may be up to 8 characters long (letters and numbers allowed). The code should follow privacy regulations. It should not contain identifying data, such as patient initials or the complete hospital record number. The local code will be visible in the confirmation messages sent by the study centre to local participants after registration of the patient. The key to this local patient code should only be accessible by the local investigator and the local hospital staff. Using or entering a local patient code is not obligatory.

16 SUPPORTIVE CARE

16.1 Granulocyte-colony stimulating factor (G-CSF)

G-CSF should not be used as it interferes with the primary endpoint. However, in exceptional cases such as invasive fungal infections, it may be used at the discretion of the investigator.

16.2 Antimicrobials, anti-emetic drugs and tumour-lysis syndrome prophylaxis

Antimicrobial prophylaxis, antimicrobial treatment, anti-emetic drugs and tumour-lysis syndrome prophylaxis (allopurinol, rasburicase) can be given according to local guidelines (see also 10.3).

17 DATA COLLECTION AND QUALITY ASSURANCE

17.1 Electronic Case Report Forms (eCRF)

Data will be collected on electronic Case Report Forms (eCRF) to document eligibility, safety and efficacy parameters, compliance to treatment schedules and parameters necessary to evaluate the study endpoints. Data collected on the CRF are derived from the protocol and will include at least:

- Inclusion and exclusion criteria;
- Baseline status of patient including medical history and stage of disease;
- Timing and dosage of protocol treatment;
- Baseline concomitant diseases and adverse events;
- Parameters for response evaluation;
- Any other parameters necessary to evaluate the study endpoints;
- Survival status of patient;
- Reason for end of protocol treatment.

The eCRF will be completed on site by the local investigator or sub-investigator or an authorised staff member. The eCRF must be signed by the local investigator or sub-investigator.
Written instructions for completing the CRF will be provided by the Data Centre.

17.2 Data quality assurance

Steps to be taken to ensure the accuracy and reliability of data include the selection of qualified investigators and appropriate study centres, review of protocol procedures with the investigator before the study, and site visits by the sponsor.
Data collected on the CRF will be verified for accuracy. If necessary, queries will be sent to the investigational site to clarify the data on the CRF. The investigator should answer data queries within the specified time line.

17.3 Monitoring

Monitoring will be performed by The Centre for Clinical Cancer Studies (CTO) at Karolinska University Hospital. Site evaluation visits will be performed to review the quality of data registration and consistency with the source data at the individual site. It will enable CTO to collect quality data and facilitate improvement of the participating sites. Data cleaning or monitoring of the performance of specific trials is not the goal of the site evaluation visits. Site evaluation visits will be performed according to the site evaluation visit plan.
Direct access to source documentation (medical records) must be allowed for the purpose of verifying that the data recorded in the CRF are consistent with the original source data. The sponsor expects that during site visits the relevant investigational staff will be available, the source documentation will be available and a suitable environment will be provided for review of study-related documents.

17.4 Audits and inspections

In accordance with regulatory guidelines, audits may be carried out for this study. The investigator is required to facilitate an audit by means of a site visit.
These audits will require access to all study records, including source documents, for inspection and comparison with the CRFs. Patient privacy must, however, be respected.
Similar auditing procedures may also be conducted by agents of any regulatory body reviewing the results of this study. The investigator should immediately notify the sponsor if they have been contacted by a regulatory agency concerning an upcoming inspection.

18 ETHICS

18.1 Accredited ethics committee

An accredited Ethics Committee will approve the study protocol and any substantial amendment.

18.2 Ethical conduct of the study

The study will be conducted in accordance with the ethical principles of the Declaration of Helsinki, the ICH-GCP Guidelines, the EU Clinical Trial Directive (2001/20/EG), and the Swedish regulatory requirements (Swedish Medicinal Products Act LVFS 2011:19). The local investigator is responsible for the proper conduct of the study at the study site.

18.3 Patient information and consent

Written informed consent of patients is required before enrolment in the trial and before any study related procedure takes place.
The investigator will follow ICH-GCP and other applicable regulations in informing the patient and obtaining consent. The investigator should take into consideration if the patient is capable of giving informed consent. Before informed consent may be obtained, the investigator should provide the patient ample time and opportunity to inquire about details of the trial and to decide whether or not to participate in the trial. All questions about the trial should be answered to the satisfaction of the patient.
There is no set time limit for the patient to make a decision. The investigator should inform each patient if there is a specific reason why he/she must decide within a limited time frame, for example if patients condition necessitates start of treatment or if the trial is scheduled to close for enrolment.

The content of the patient information letter, informed consent form and any other written information to be provided to patients will be in compliance with ICH-GCP and other applicable regulations and should be approved by the Ethics Committee in advance of use.
The patient information letter, informed consent form and any other written information to be provided to patients will be revised whenever important new information becomes available that may be relevant to the patient's consent. Any substantially revised informed consent form and written information should be approved by the Ethics Committee in advance of use. The patient should be informed in a timely manner if new information becomes available that might be relevant to the patient's willingness to continue participation in the trial. The communication of this information should be documented.

18.4 Benefits and risks assessment

The backbone of the study is the best available treatment for AML. As SAMHD1 critically limits therapy outcome by reducing the efficacy of ara-C, addition of HU might alleviate SAMHD1's negative effects. Furthermore, HU in itself has proven anti-AML activity.

The burden and risks associated with participation:

1) Addition of HU might increase the risk for myelosuppression leading to prolonged neutropenia which increases the risk for invasive bacterial and fungal infections as well as increased need of blood and thrombocyte transfusions.
2) Some extra bone-marrow (10-20 ml) may be taken for the Karolinska AML Biobank at the diagnostic bone-marrow sampling and after cycle 2; additional blood samples can be taken on day 1 of cycle 1
3) HU is a cytotoxic drug and might cause adverse reactions (e.g. skin rashes).

In the perspective of the poor outcome of AML patients, the risks of participating seem to be acceptable.

18.4 Trial insurance

Prior to the start of the trial, the sponsor will ensure that adequate insurance for patients is in place covering losses due to death or injury resulting from the trial, in accordance with applicable laws and regulations in each country where the trial is conducted. The sponsor will take out an insurance policy or delegate this responsibility to a national co sponsor. Proof of insurance will be submitted to the Ethics Committee.
In addition, the sponsor will ensure that adequate insurance is in place for both investigator(s) and sponsor to cover liability pertaining to death or injury resulting from the trial.

19 ADMINISTRATIVE ASPECTS AND PUBLICATION

19.1 Handling and storage of data and documents

19.1.1 Patient confidentiality

Each patient is assigned a unique patient study number at enrolment. In trial documents the patient's identity is coded by patient study number as assigned at enrolment. In some cases, <date of birth> is also listed.
The local investigator will keep a subject enrolment and identification log that contains the key to the code, i.e. a record of the personal identification data linked to each patient study number. This record is filed at the investigational site and should only be accessed by the investigator and the supporting hospital staff, and by representatives of the sponsor or a regulatory agency for the purpose of monitoring visits or audits and inspections.

19.1.2 Filing of essential documents

Essential Documents are those documents that permit evaluation of the conduct of a trial and the quality of the data produced. The essential documents may be subject to, and should be available for, audit by the sponsor's auditor and inspection by the regulatory authority(ies)
The investigator should file all essential documents relevant to the conduct of the trial on site. The sponsor will file all essential documents relevant to the overall conduct of the trial. Essential documents should be filed in such a manner that they are protected from accidental loss and can be easily retrieved for review.

19.1.3 Record retention

Essential documents should be retained for 15 years after the end of the trial. They should be destroyed after this time, unless a longer record retention period is required by site specific regulations.
Source documents (i.e. medical records) of patients should be retained for at least 15 years after the end of the trial described in section 18.4. Record retention and destruction after this time is subject to the site's guidelines regarding medical records.
In accordance with the European General Data Protection Regulation (GDPR), Karolinska University Hospital is responsible for storage and use of patient-related information. Each study individual is entitled to apply for access to and, in case of erroneous data, correction of the stored patient-specific information. The study individual is furthermore entitled to make complaints to the regulatory body ("Datainspektionen") and has the right to get records deleted or limited, and can contact the Data Protection Officer via e-mail [redacted] or telephone

19.1.4 Storage of samples

Biological samples should only be stored for the purpose of research if the patient has given consent. If no informed consent was obtained, samples should be destroyed after the patient has completed all protocol treatment and procedures.
Storage of biological samples on site is subject to the site's guidelines; samples may be labelled with the patients identifying information (e.g. name, hospital record number).
Samples that are shipped to another facility (e.g. a central laboratory) for a purpose as described in this protocol or for additional scientific research, should be stripped from any identifying information and labelled with a code (trial name or number and patient study number as assigned at enrolment).

19.2 Amendments

A 'substantial amendment' is defined as an amendment to the terms of the Ethics Committee application, or to the protocol or any other supporting documentation, that is likely to affect to a significant degree:

- the safety or physical or mental integrity of the patients of the trial;
- the scientific value of the trial;
- the conduct or management of the trial; or
- the quality or safety of any intervention used in the trial.

All substantial amendments will be submitted to the Ethics Committee and to the Competent Authority for approval. The amendment will not be implemented in the protocol before approval has been given by the concerned authorities.

Non substantial amendments will be submitted if required according to country specific guidelines, or otherwise will only be recorded and filed by the sponsor.

19.3 Annual progress report

The sponsor will submit a summary of the progress of the trial to the accredited Ethics Committee once a year. The first report is sent one year after the first approval date of the trial. The last report is sent one year after the last patient has completed protocol treatment. Information will be provided on the date of inclusion of the first patient, numbers of patients included and numbers of patients that have completed the trial, serious adverse events/ serious adverse reactions, other problems, and amendments.

19.4 End of trial report

The sponsor will notify the accredited Ethics Committee and the Competent Authority of the end of the trial within a period of 90 days. The end of the study is defined as the last patient's last visit.

In case the study is ended prematurely, the sponsor will notify the accredited Ethics Committee and the competent authority within 15 days, including the reasons for the premature termination.

Within one year after the primary endpoint analysis of the trial, the sponsor will submit an end of study report with the results of the study, including any publications/abstracts of the study, to the accredited Ethics Committee. In addition, all results will be reported in the European online database of the European Union Drug Regulating Authorities Clinical Trials (EudraCT). Upon request of the accredited Ethics Committee or the Competent Authority the sponsor will submit an updated version of the end of study report within one year after the last patient's last visit.

19.5 Publication policy

Trial results will always be submitted for publication in a peer-reviewed scientific journal regardless of the outcome of the trial unless the trial was terminated prematurely and did not yield sufficient data for a publication.

APPENDIX

This summary is based on the following publication:
Arber et al. **The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukaemia**
Blood. 2016 May 19;127(20):2391-405. doi: 10.1182/blood-2016-03-643544.

WHO 2016 classification for Acute Myeloid Leukaemias (AML) and related precursor neoplasms

- Definition AML: ≥ 20% myeloblasts in blood or bone marrow
- Abnormal promyelocytes in acute promyelocytic leukaemia, promonocytes in AML with monocytic differentiation and megakaryoblasts in acute megakaryoblastic leukaemia are considered blast equivalents

AML with recurrent genetic abnormalities

AML with t(8;21)(q22;q22.1);RUNX1-RUNX1T1
AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22);CBFB-MYH11 APL with PML-RARA
AML with t(9;11)(p21.3;q23.3);MLLT3-KMT2A
AML with t(6;9)(p23;q34.1);DEK-NUP214
AML with inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); GATA2, MECOM AML (megakaryoblastic) with t(1;22)(p13.3;q13.3);RBM15-MKL1 Provisional entity: AML with BCR ABL1
AML with mutated NPM1
AML with biallelic mutations of CEBPA
Provisional entity: AML with mutated RUNX1

AML with myelodysplasia-related changes
Therapy-related myeloid neoplasms
AML, NOS

AML with minimal differentiation
AML without maturation
AML with maturation
Acute myelomonocytic leukaemia
Acute monoblastic/monocytic leukaemia
Pure erythroid leukaemia
Acute megakaryoblastic leukaemia
Acute basophilic leukaemia
Acute panmyelosis with myelofibrosis

Myeloid sarcoma
Myeloid proliferations related to Down syndrome

Transient abnormal myelopoiesis (TAM)
Myeloid leukaemia associated with Down syndrome

Acute leukaemias of ambiguous lineage

Acute undifferentiated leukaemia
Mixed phenotype acute leukaemia (MPAL) with t(9;22)(q34.1;q11.2); BCR-ABL1 MPAL with t(v;11q23.3); KMT2A rearranged
MPAL, B/myeloid, NOS
MPAL, T/myeloid, NOS

*Rare cases show < 20% myeloblasts; these should be classified as AML

B Response criteria for AML

These response criteria were published in the 2009 paper, "Diagnosis and management of acute myeloid leukaemia in adults: recommendations from an international expert panel, on behalf of the European LeukemiaNet" [12], and are based on International Working Group recommendations published in 2003

CATEGORY	DEFINITION
Complete remission (CR) [1]	Bone marrow blasts < 5%; absence of blasts with Auer rods; absence of extramedullary disease; absolute neutrophil count >1.0 x 10^9/L; platelet count >100 x 10^9/L; independence of red cell transfusions
CR with incomplete recovery (CRi) [2]	All CR criteria except for residual neutropenia (<1.0 x 10^9/L) or thrombocytopenia (<100 x 10^9/L)
Morphologic leukaemia-free state [3]	Bone marrow blasts <5%; absence of blasts with Auer rods; absence of extramedullary disease; no hematologic recovery required
Partial remission (PR)	Relevant in the setting of phase I and II clinical trials only; all hematologic criteria of CR; decrease of bone marrow blast percentage to 5% to 25%; and decrease of pre-treatment bone marrow blast percentage by at least 50%
Cytogenetic CR (CRc) [4]	Reversion to a normal karyotype at the time of morphologic CR (or CRi) in cases with an abnormal karyotype at the time of diagnosis; based on the evaluation of 20 metaphase cells from bone marrow
Molecular CR (CRm) [5]	No standard definition; depends on molecular target
Resistant disease (RD)	Failure to achieve CR or CRi (general practice; phase II/III trials), or failure to achieve CR, CRi or PR (phase I trials); only includes patients surviving > 7 days following completion of initial treatment, with evidence of persistent leukaemia by blood and/or bone marrow examination
Death in aplasia	Deaths occurring > 7 days following completion of initial treatment while cytopenic; with an aplastic or hypoplastic bone marrow obtained within 7 days of death, without evidence of persistent leukaemia
Death from indeterminate cause	Deaths occurring before completion of therapy, or <7 days following its completion; or deaths occurring > 7 days following completion of initial therapy with no blasts in the blood, but no bone marrow examination available
Progressive disease during the first cycle	<u>During the first cycle, PD is defined as:</u> >25% absolute increase in the bone marrow blast count from baseline to the present assessment (e.g. from 20% to 46%) on bone marrow aspirate (or biopsy in case of dry tap) *or* Clinically indicated (overt leukostasis, uncontrollable coagulopathy or new extramedullary leukaemia requiring immediate action)
Progressive disease	<u>During cycle 2 and subsequent cycles, PD is defined as:</u>

during cycle 2 and subsequent cycles	>25% absolute increase in the bone marrow blast count on bone marrow aspirate (or biopsy in case of dry tap) compared with the last bone marrow performed (e.g. from 20% to 46%) *and/or* An absolute increase in WBC by >50x10^9/L compared to the WBC count prior to cycle 1 (e.g. from 15x10^9/L at entry to 66x10^9/L) *or* Clinically indicated (overt leukostasis, uncontrollable coagulopathy or new extramedullary leukaemia requiring immediate action)
Relapse [6]	Bone marrow blasts > 5%; or reappearance of blasts in the blood; or development of extramedullary disease

[1] All criteria need to be fulfilled; marrow evaluation should be based on a count of 200 nucleated cells in an aspirate with spicules; if ambiguous, consider repeat exam after 5-7 days; flow cytometric evaluation may help to distinguish between persistent leukaemia and regenerating normal marrow; a marrow biopsy should be performed in cases of dry tap, or if no spicules are obtained; no minimum duration of response required.

[2] The criterion of CRi is of value in protocols using intensified induction or double induction strategies, in which hematologic recovery is not awaited, but intensive therapy will be continued. In such protocols, CR may even not be achieved in the course of the entire treatment plan. In these instances, the overall remission rate should include CR and CRi patients. Some patients may not achieve complete hematologic recovery upon longer observation times.

[3] This category may be useful in the clinical development of novel agents within phase I clinical trials, in which a transient morphologic leukaemia-free state may be achieved at the time of early response assessment.

[4] Four studies showed that failure to convert to a normal karyotype at the time of CR predicts inferior outcome.

[5] As an example, in CBF AML low-level PCR-positivity can be detected in patients even in long-term remission. Normalizing to 104 copies of ABL1 in accordance with standardized criteria, transcript levels below 12 to 10 copies appear to be predictive for long-term remission.

[6] In cases with low blast percentages (5-10%), a repeat marrow should be performed to confirm relapse. Appearance of new dysplastic changes should be closely monitored for emerging relapse. In a patient who has been recently treated, dysplasia or a transient increase in blasts may reflect a chemotherapy effect and recovery of haematopoiesis. Cytogenetics should be tested to distinguish true relapse from therapy-related MDS/AML

C Risk group definitions for AML and MDS

AML patients are classified in 4 risk groups according to the table below.

Risk Group	**Criteria at diagnosis and early/late CR**
Good	- inv16(p13q22)/t(16;16)(p13;q22) or CBFB-MYH11 fusion/CBFB-rearrangement - t(8;21)(q22;q22) or RUNX1-RUNX1T1-fusion - *NPM1* mutation without *FLT3*-ITD or with low allele freaquency of *FLT3*-ITD - Biallelic *CEBPA* mutation (normal karyotype)
Intermediate	- *NPM1*-mutation with high allele frequency of *FLT3*-ITD - *NPM1* wildtype and *FLT3* wildtype - *NPM1* mutation with low allele frequency of *FLT3*-ITD in the absence of high-risk karyotype - t(9;11)(p21;q23) or *MLLT3-KTM2A* - cytogenetic aberrations other than low or high risk
Poor	- *NPM1* mutation with high allele frequency of *FLT3* ITD - inv(3)(q21q26) or t(3;3)(q21;q26); *GATA2-MECOM* - t(6;9)(p22;q34); *DEK-NUP214* - t(v;11)(v;q23); *KMT2A* rearrangement - del(5q) or monosomy 5 - monosomy 7 - del(17p) or monosomy 17 - complex karyotype in the absence of t(15;17)(q22;q21), t(8;21)(q22;q22), inv(16)(p13q22)/t(16;16)(p13;q22) or t(9;11)(p21;q23) - monosomal karyotype - t(9;22)(q34.1;q11.2); *BCR ABL1* - mutations in *RUNX1*, *ASXL1*, *TP53* in the absence of low-risk features

- Monosomal karyotype (MK) refers to AML with two or more autosomal monosomies or a single autosomal monosomy in the presence of one or more structural cytogenetic abnormalities

D ***ZUBROD-ECOG-WHO Performance Status Scale***

0	Normal activity
1	Symptoms, but nearly ambulatory
2	Some bed time, but to be in bed less than 50% of normal daytime
3	Needs to be in bed more than 50% of normal daytime
4	Unable to get out of bed
5	Death

E NYHA scoring

The New York Heart Association functional and therapeutic classification applied to dyspnoea

Grade 1	No breathlessness
Grade 2	Breathlessness on severe exertion
Grade 3	Breathlessness on mild exertion
Grade 4	Breathlessness at rest

F Allo-HCT – co morbidity index (according to Sorror)

AlloHCT-Comorbidity Index (HCT-CI) as developed in Seattle. This is will be used at diagnosis. For specific instruction, please refer to Guidelines for assessment of comorbidities in the HCT-CI (Sorror ML, Blood. 2013). Scores are assigned if one of the specific conditions as defined were met at any time in the past medical history of the patient, unless specified otherwise in the definition/footnotes. We greatly encourage the investigators to use the HCT Comorbidity Index Calculator: www.hctci.org

Comorbidity	**Definitions of comorbidities included in the new HCT-CI**	**Score**
Arrhythmia	Any type of arrhythmia that has necessitated the delivery of a specific antiarrhythmic treatment at any time in the patient's past medical history. Examples include atrial fibrillation or flutter, sick sinus syndrome, or ventricular arrhythmias. A score is assigned even if the patient was in normal sinus rhythm at the time of data acquisition or at the landmark date. No score is assigned to transient arrhythmias that never required treatment	1
Cardiovascular	Presence of 1 or more of the following 3 clinical presentations:) Coronary artery disease, based on presence of a documented diagnosis of chronic exertional angina, unstable angina or myocardial infarction at any point in the patient's past medical history (e.g. coronary stent, a coronary artery bypass);) Congestive heart failure, based on statement about the development of symptoms/signs of congestive heart failure (e.g. an exertional or paroxysmal nocturnal dyspnoea) that later responded to diuretics, afterload-reducing agents, beta blocker or digitalis at any time in the past medical history;[1]) Ejection fraction (EF) of 50% or lower as determined by echocardiogram (ECG) or multigated acquisition (MUGA) scan.[1]	1
Inflammatory bowel disease	Presence of a documented prior diagnosis (history of an endoscopic examination of the mucosa with or without confirmatory histology and radiologic findings) of Crohn's disease or ulcerative colitis requiring treatment at any time in the patient's past medical history. If the patient has never received a treatment of this comorbidity, no score is assigned.	1
Diabetes	Diagnosis of diabetes or steroid-induced hyperglycaemia requiring continuous treatment with insulin or oral hypoglycaemic drugs during the instantaneous period of 4 weeks before the landmark date. No score is assigned for this comorbidity if diabetes could be controlled with diet alone or if a previous treatment of diabetes or steroid-induced hyperglycaemia was stopped 4 weeks before the landmark date.	1
Cerebrovascular disease	Prior to diagnosis of transient ischemic attack, subarachnoid haemorrhage, or cerebral thrombosis, embolism, or haemorrhage at any time in the past medical history. No details on treatment are required for assigning a score for this comorbidity.	1
Psychiatric disturbance	Presence of any mood, anxiety, or other psychiatric disorder requiring continuous treatment during the instantaneous period of 4 weeks before landmark date. Patients who are receiving only "as-needed" medications for any of the above disorders are not assigned a score for this comorbidity.	1

HEAT-AML

Mild hepatic[2]	Presence of 1 or more of the following 3 clinical presentations:) Elevated total bilirubin to a value higher than the ULN and up to 1.5 times the upper limit normal (ULN);) Elevated values of ALAT or ASAT, to values higher than the ULN and up to 2.5 times the ULN;) A prior diagnosis of infection with hepatitis B or C at any time in the past medical history before the landmark date.	1
Obesity[3]	Obesity is based on a body mass index (BMI) higher than 35.00 kg/m^2	1

Comorbidity	**Definitions of comorbidities included in the new HCT-CI**	**Score**
Infection	Presence of 1 or more of the following 4 clinical presentations: (1) A documented infection (e.g. by culture or biopsy); (2) Fever of unknown origin; (3) Pulmonary nodules suspicious for fungal pneumonia; (4) A positive purified protein derivative test requiring prophylaxis against tuberculosis. Patients must have started a specific antimicrobial treatment before the landmark date with a recommendation to continue the same antimicrobial therapy (or a similar agent) during the days of administration of a conditioning regimen and beyond start of treatment and beyond day 0 of HCT.	1
Rheumatologic	Presence of a documented prior diagnosis of rheumatologic disease that has required administration of a specific treatment at any time in the patient's past medical history. Diagnoses include systemic rheumatologic and connective tissue disorders such as systemic lupus erythematosus, rheumatoid arthritis, Sjögren's syndrome, scleroderma, polymyositis, dermatomyositis, mixed connective tissues disease, polymyalgia rheumatica, polychondritis and vasculitis syndromes. Patients with undiagnosed polyarthritis, degenerative joint disease, or osteoarthritis are not scored for this comorbidity.	2
Peptic ulcer	Presence of a prior endoscopic or radiologic diagnosis of gastric or duodenal ulcer, noted in the medical record, at any point in the patient's past medical history. Patients with quiescent peptic ulcer who are receiving no treatment in the immediate period before the landmark date are assigned a score for this comorbidity if they have met the prior criteria.	2
Renal	Presence of 1 or more of the following 3 clinical presentations:) Elevated values of serum creatinine to more than 2 mg/dL or more than 176.8 mmol/L;[2]) Chronic renal disease requiring weekly dialysis within the instantaneous period of 4 weeks before landmark date;) A documented prior history of renal transplantation at any point in the patient's past medical history.	2
Moderate pulmonary	Presence of 1 or more of the following 3 clinical presentations:) A percentage of the corrected measured-to-predicted diffusion capacity of carbon monoxide (DLco)[3] in the range of 66%-80%;[1]) A percentage of the measured-to-predicted forced expiratory volume in one second (FEV1) in the range of 66% to 80% (most recent measurements before landmark date);[1]) shortness of breath on slight activity that is attributed to a pulmonary disease and cannot be corrected by blood transfusion for a noticeable anaemia, as assessed during a clinic visit within 2 weeks before landmark date.	2

HEAT-AML

Prior malignancy	Presence of a prior diagnosis of any malignancy that required receiving a specific treatment at any point in the patient's past medical history, regardless of the type of treatment (surgery, radiotherapy, and/or drug therapy). Lymphomas or myelomas that preceded the diagnosis acute myeloid leukaemia [AML], myelodysplastic syndromes, or chronic myeloid leukaemia) are assigned a score for this comorbidity. Similarly, myeloid malignancies that preceded the diagnosis of lymphomas or myelomas are assigned a score for this comorbidity. Patients with a prior diagnosis malignancy from the same lineage of cells of the current malignancy should not be assigned a score for this comorbidity (e.g. patient had diagnosis of AML that was preceded by MDS). Melanoma, but not basal or squamous cell carcinoma of skin, should be assigned a score for this comorbidity. Patients with a prior malignancy that never required a specific treatment. Tumours of a benign nature are not scored for this comorbidity.	3
Heart valve disease[1]	Presence of 1 or more of the following clinical presentations:) At least a moderate or severe degree of valve stenosis or insufficiency, as determined by ECG, whether the valve was mitral, aortic, tricuspid, or pulmonary;) Prosthetic mitral or aortic valve;) Symptomatic mitral valve prolapse.	3

Comorbidity	**Definitions of comorbidities included in the new HCT-CI**	**Score**
Severe pulmonary	Presence of at least 1 of the following 4 clinical presentations:) A percentage of DLco[3] of 65% or less (most recent measurements before landmark date);[1]) A percentage of FEV1 of 65% or less (most recent measurements before landmark date);[1]) Shortness of breath at rest that is attributed to a pulmonary disease and cannot be corrected by blood transfusion for a noticeable anaemia, as assessed during a clinic visit within 2 weeks before the landmark date;) The need for intermittent or continuous oxygen supplementation during 4 weeks before the landmark date.	3
Moderate/severe hepatic[2]	Presence of 1 or more of the following 3 clinical presentations:) Elevated values of total bilirubin to a level higher than 1.5 times the ULN;) Elevated values of any or both ALAT and ASAT to levels higher than 2.5 times the ULN;) A documented diagnosis of liver cirrhosis at any time in the past medical history before the landmark date.	3

1 Most recent measurement before the landmark date
2 Assessment of the laboratory tests (a and/or b) has to include at least 2 values per test on 2 different days. The laboratory value closest to the landmark date should be the value used in defining the severity of comorbidity
3 Dinakara equation: Corrected DLco = uncorrected DLco/(0.6965 X haemoglobin g/dL)

For the Sponsor:

Received (date, sign): SAE-nr:

HEAT-AML	**SAE**	

Patient number ☐☐☐☐ **Centre** _____ Type of report: Initial ☐ Follow up ☐

[redacted]

Patient's age (years) ☐☐	Patient's sex Male ☐ Female ☐	Cycle: .....
Number of days until haematopoietic recovery:	of intravenous antibiotic treatment:	of hospital stay: .....
Date of first Daunorubicin infusion ☐☐/☐☐/☐☐☐☐	Date of last Daunorubicin infusion ☐☐/☐☐/☐☐☐☐	
Date of first ARA C infusion ☐☐/☐☐/☐☐☐☐	Date of last ARA-C infusion ☐☐/☐☐/☐☐☐☐	
Date of first Hydroxyurea dose ☐☐/☐☐/☐☐☐☐	Date of last Hydroxyurea dose ☐☐/☐☐/☐☐☐☐	
Hydroxyurea given dose per day (mg):	Date event became serious ☐☐/☐☐/☐☐☐☐	

Reason for considering the event serious: Death ☐ Life-threatening ☐
Hospitalisation/prolonged hospital stay ☐ Permanent disabling/incapacitating ☐
Resulted in congenital abnormality ☐ Need of ICU care or assisted ventilation ☐
Specify:.....

Description of event
.....
.....
.....

Primary adverse event: ..... CTC Grade1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐

Relation to Daunorubicin:	Definitely ☐	Probable ☐	Possible ☐	Unlikely ☐	Unrelated ☐	Not assessible ☐
Relation to ARA C:	Definitely ☐	Probable ☐	Possible ☐	Unlikely ☐	Unrelated ☐	Not assessible ☐
Relation to Hydroxyurea:	Definitely ☐	Probable ☐	Possible ☐	Unlikely ☐	Unrelated ☐	Not assessible ☐

Action taken regarding Daunorubicin:	None ☐	Dose reduction ☐	Treatment delayed ☐	Stopped ☐
Action taken regarding ARA-C:	None ☐	Dose reduction ☐	Treatment delayed ☐	Stopped ☐
Action taken regarding Hydroxyurea:	None ☐	Dose reduction ☐	Treatment delayed ☐	Stopped ☐

Outcome: ☐ Recovered ☐ Ongoing ☐ Unknown ☐ Dead, Cause of death: .....

Date event no longer serious ☐☐/☐☐/☐☐☐☐ If dead, date of death: ☐☐/☐☐/☐☐☐☐

Name and signature of person completing the form Date ☐☐/☐☐/☐☐☐☐

Investigator's name and signature Date ☐☐/☐☐/☐☐☐☐

All dates as ddmmyyyy *HEAT SAE form Version 1.0*

1 (1)

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