Donated Chemical Probe

Chemical Probe BAY-707
MTH1 Inhibitor

March, 2018

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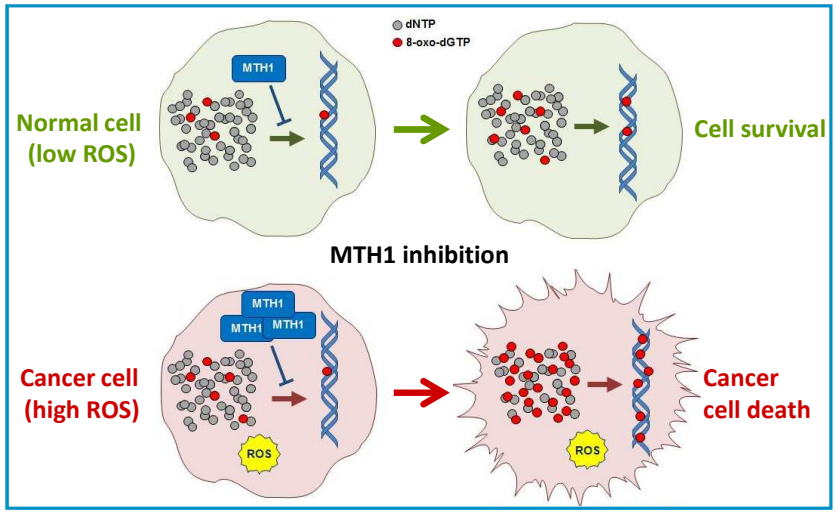
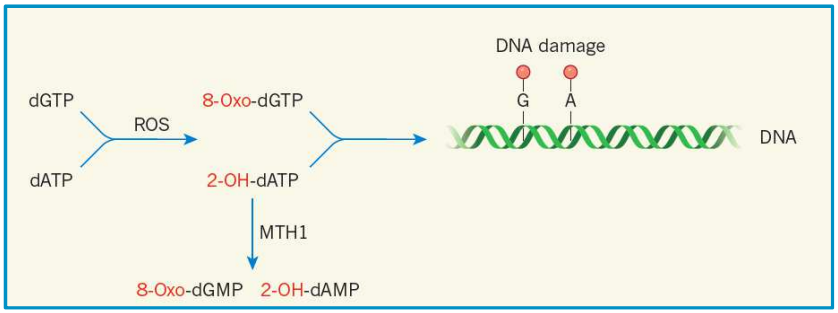
MTH1 probe BAY-707:

Scientific rationale: MTH1 as an anti-cancer target





// Cancer cells are characterized by oxidative stress, which can damage dNTPs/DNA

// **MTH1** (MutT homolog 1, NUDT1) prevents oxidized dNTP incorporation into DNA by hydrolyzing **8-oxo-dGTP** and **2-OH-dATP** to **8-oxo-dGMP** and **2-OH-dAMP**

// MTH1 expression and activity is up-regulated in many cancers compared to normal tissue

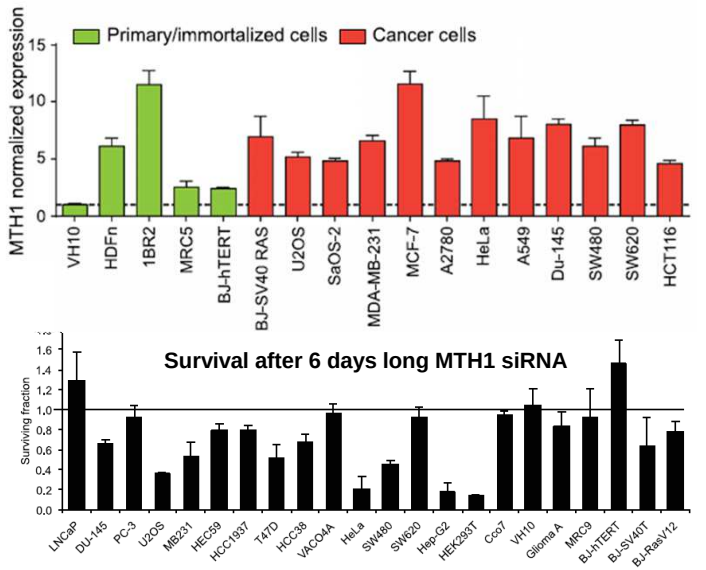
// MTH1 is **non-essential in normal cells** (MTH1 knockout mice show only mild symptoms, Tsuzuki, 2001)

// **Initial disease hypothesis**: inhibition of MTH1 will result in aberrant incorporation of oxidized nucleotides into DNA, subsequently leading to DNA damage, mutations, genomic instability and cancer cell death at excellent tolerability

MTH1 probe BAY-707:

Initial supporting literature for MTH1

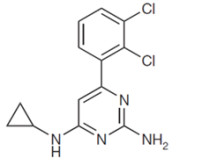
RNAi



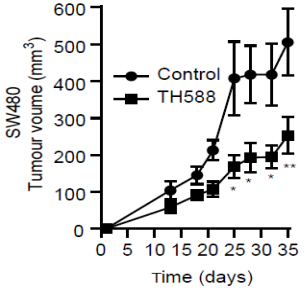
Gad et al., 2014, Nature

Tool compounds

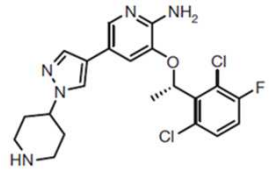
Gad et al., 2014, Nature



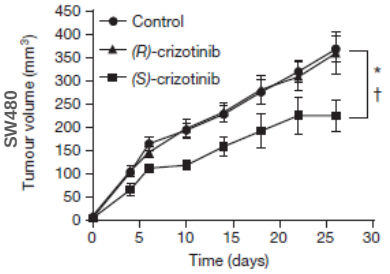
TH588
IC50 biochem= 5nM
IC50 proli (SW480)= 5.3µM



Huber et al., 2014, Nature



(S)-crizotinib
IC50 biochem= 72nM
IC50 proli (SW480)= 2.6µM



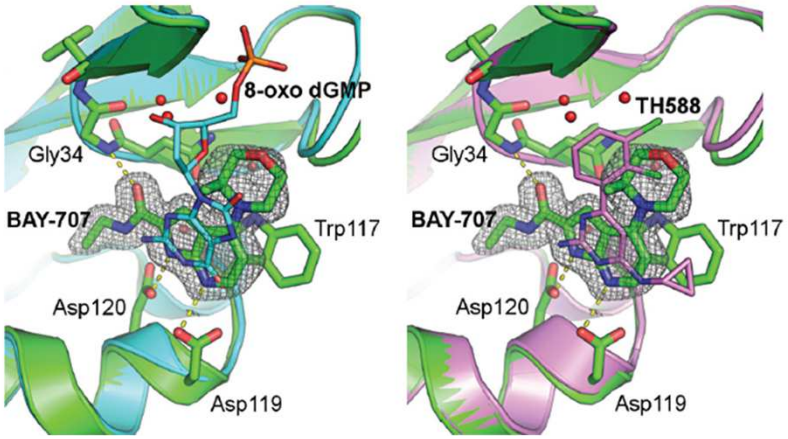
// MTH1 is overexpressed in cancer cells to oppose the DNA damage effects of high ROS levels
// "Cancer-specific lethality" was described upon RNAi-mediated knockdown of MTH1 *&* upon treatment with small molecular weight MTH1 inhibitory tool compounds (e.g. TH588, (S)-crizotinib)
// *In vivo* evidences also supported the assumption that MTH1 is required for cancer cell survival

MTH1 probe BAY-707:

Development of novel MTH1 inhibitors

Property	TH588	BAY-707
IC_{50} MTH1, nM	12	2.3
logD @ pH 7.5	2.5	1.6
Sw pH 6.5, mg/L	38	288
Caco-2 A–B, nm/s (efflux ratio)	307 (0.5)	148 (1.4)
Rat hepatocyte CL, L/h/kg (Fmax)	1.92 (54%)	0.54 (87%)
Human LM CL, L/h/kg (Fmax)	0.64 (51%)	0.29 (78%)

CL, clearance; IC_{50}, half-maximal inhibitory concentration; logD, distribution coefficient; LM, liver microsomes; Sw, aqueous solubility.



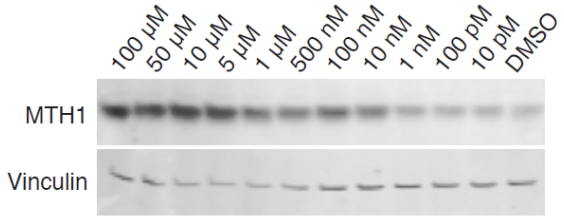
Ellermann et al., 2017; ASC Chem Biol

// Fragment-based screening and structure-based drug design led to discovery of a novel and selective MTH1 inhibitor with low nanomolar enzymatic activity (IC_{50}= 2.3+/-0.8 nM, n=6)
// High selectivity in an in-house kinase panel, favourable physicochemical profile and promising *in vitro* pharmacokinetic properties with high metabolic stability and good cell permeability
// Substrate competitive binding to the active site of MTH1

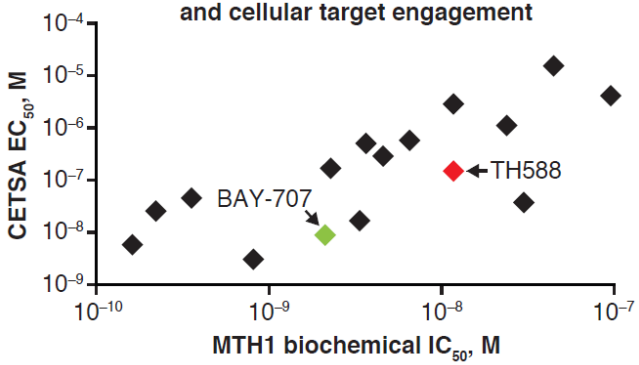
MTH1 probe BAY-707:

Cellular target engagement with CETSA

Cellular Thermal Shift Assay (CETSA)



Correlation between biochemical potency and cellular target engagement



Ellermann et al., 2017; ASC Chem Biol

// Cellular Thermal Shift Assay (CETSA) used to demonstrate on-target cellular activity of BAY-707 and additional structurally related MTH1 inhibitors from the same compound class
// BAY-707 demonstrate a superior cellular target engagement (EC_{50}= 7.6 nM) over the tool compound TH588 (EC_{50}= 133 nM)
// Good correlation between he biochemical potency and cellular target engagement of Bayer's MTH1 compound class
// BAY-707 is a potent, selective and cellularly active MTH1 inhibitor with good PK properties; therefore it is suitable to validate the cellular functions of MTH1, e.g. the MTH1 cancer dependency

MTH1 probe BAY-707:

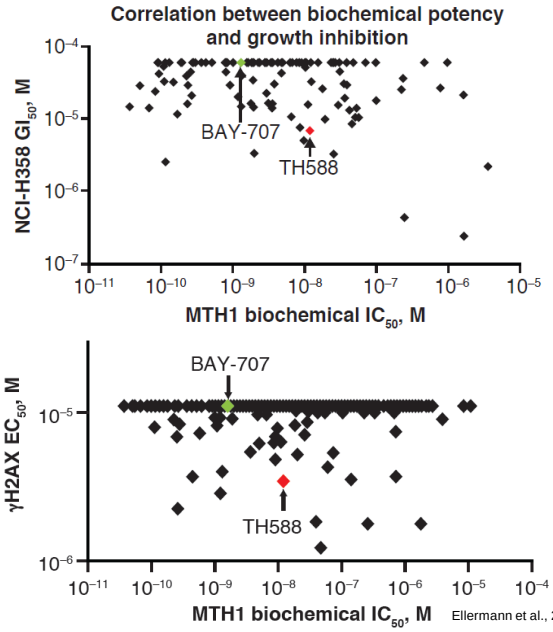
MTH1 is not required for cancer cell survival

Cell line[a]	Indication	TH588 GI_{50}, µM[b]	BAY-707 GI_{50}, µM[b]
HMEC	Normal breast	3.2	>30
NCI-H358	Lung cancer	4.9	>30
NCI-H460	Lung cancer	7.1	>30
A549	Lung cancer	4.0	>30
MCF7	Breast cancer	3.5	>30
MDA-MB-231	Breast cancer	6.3	>30
U2OS	Bone cancer	2.6	>30
HeLa	Cervical cancer	4.2	>30
SW480	Colon cancer	5.3	>30

[a]In total, more than 20 different cancer cell lines from varying indications and scientific rationales were tested.

[b]Growth inhibition with the indicated compounds was performed in 6 day long assays. Both compounds were tested at concentrations of up to 30µM.

GI_{50}, half-maximal growth inhibitory concentration; HMEC, human mammary epithelial cells.

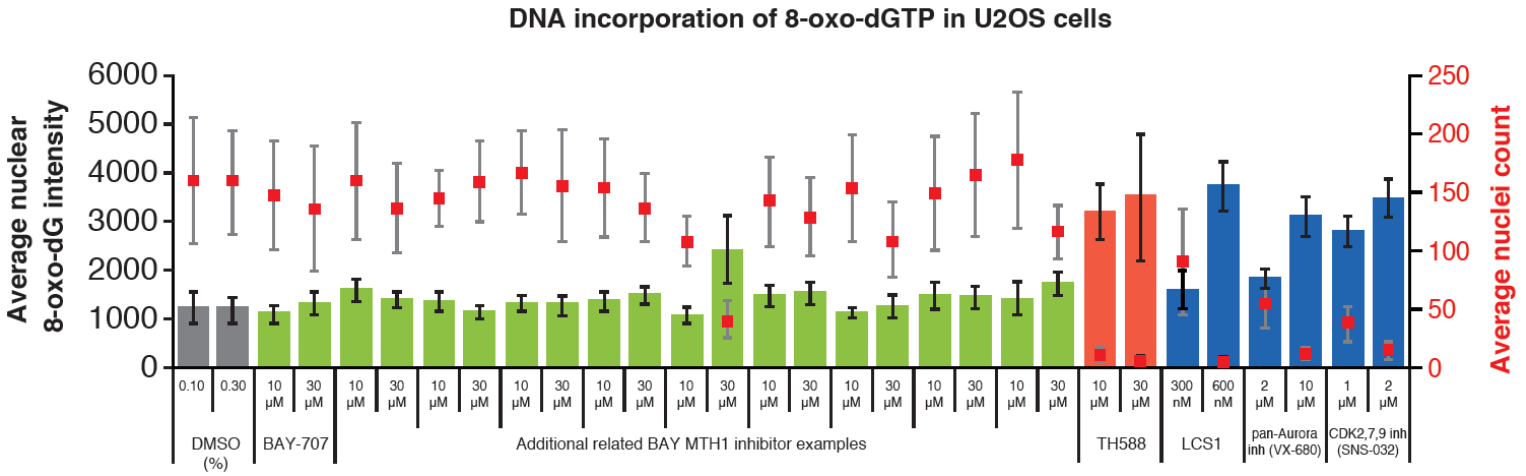


Ellermann et al., 2017; ASC Chem Biol

// TH588 tool compound demonstrate equal cytotoxicity in normal and cancer cells
// BAY-707 demonstrate no cytotoxicity (neither in 2D nor 3D, independently of ROS levels, MTH1 expr.)
// No correlation between the biochemical potency and cytotoxicity of Bayer's MTH1 inhibitors
// No induction of double strand DNA breaks (DSBs) with BAY-707 and no correlation between γ-H2AX EC50 and biochemical potency of Bayer's MTH1 inhibitors
// DSBs observed with some MTH1 inhibitors are independent of their enzymatic activity and likely due to off-target effects

MTH1 probe BAY-707:

MTH1 is not essential for sanitization of oxidized dNTPs

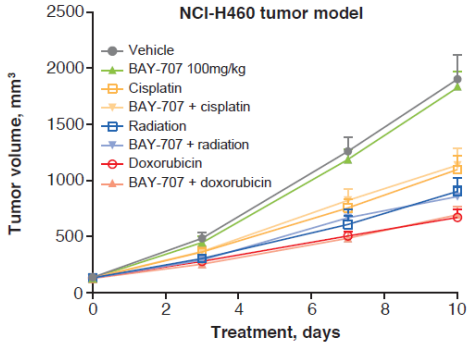


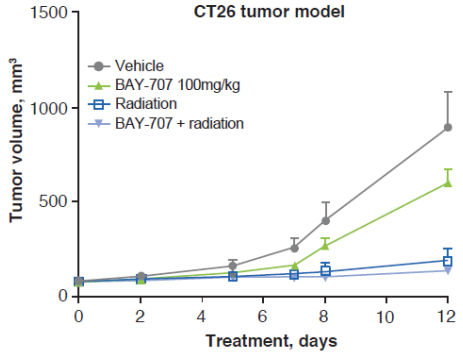
Ellermann et al., 2017; ASC Chem Biol

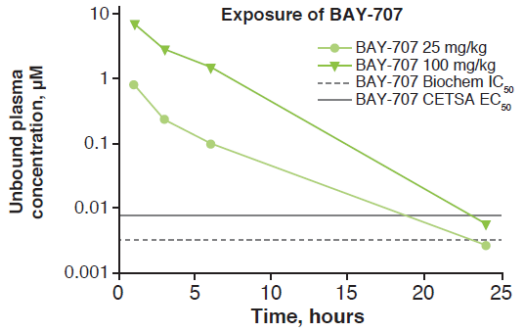
// Genomic incorporation of 8-oxo-dGTP was measure in HCA of immunostained samples
// BAY-707 and structurally related MTH1 inhibitors do not result in increased nuclear incorporation of damaged nucleotides (measured in γ-H2AX and 8-oxo-dGTP assays)
// Off-target cytotoxicity of TH588 and MTH1 siRNAs are the primary reason for the increased nuclear incorporation of 8-oxo-dGTP
// In living cells MTH1 is not essential for sanitization of oxidized nucleotides

MTH1 probe BAY-707:

MTH1 inhibition in mono- or combination-therapies has no effect on in vivo tumor growth







Ellermann et al., 2017; ASC Chem Biol

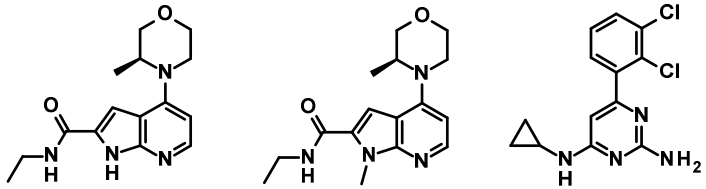
// No significant body weight loss observed with BAY-707 and other Bayer MTH1 inhibitors

// Exposure for both BAY-707 covered biochemical IC_{50} and CETSA EC_{50} for ~24h

// MTH1 inhibition in mono- or combination-therapies has no effect on *in vivo* tumor growth

MTH1 probe BAY-707:

Compound Comparison to MTH1 Negative Control BAY-604



Property	BAY-707	BAY-604	TH588
IC_{50} MTH1 [nM]	2.3	>20000	12
logD@ pH 7.5	1.6	1.9	2.5
Sw pH 6.5 [mg/L]	288	2170	38
Caco-2 A-B [nm/s] (efflux ratio)	148 (1.4)	68 (3.2)	307 (0.5)
Rat hep. CL [L/h/kg] (Fmax)	0.54 (87%)	0.41 (90%)	1.92 (54%)
Hum. LM CL [L/h/kg] (Fmax)	0.29 (78%)	0.015 (99%)	0.64 (51%)
GI_{50} [µM] SW480	>30	>30	5.3
GI_{50} [µM] NCI-H358	>30	>30	4.8

// BAY-707 is a low nanomolar MTH1 inhibitor with IC_{50}= 2.3+/-0.8 nM enzymatic activity
// BAY-604 is structurally related compound to BAY-707 with similar PhysChem and PK properties
// BAY-604 is the negative control of BAY-707 and demonstrates no enzymatic activity until the highest does tested (20 µM)
// BAY-604 demonstrate no cancer cell growth inhibition up to the highest concentration tested (30 µM)

MTH1 probe BAY-707:

Summary / conclusion

// BAY-707 is an MTH1 inhibitor fulfilling all criteria for a chemical probe:

- // Low nanomolar biochemical potency (IC_{50}= 2.3 nM)
- // Substrate-competitive binding
- // Good membrane permeability and single-digit cellular target engagement (CETSA assay EC_{50}= 7.6 nM)
- // Selective against an in-house kinase panel. In contrast to the currently widely used MTH1 tool compound TH588, BAY-707 demonstrate no off-target-related cytotoxicity

// BAY-707 de-validated MTH1 as a broad-spectrum non-oncogenic cancer dependency

BAY-707 will allow to further study the biology of MTH1 in cell cultures and living organisms without a limitation of off-target-related cytotoxicity

BAY-604 is a negative control of BAY-707

We ask for acceptance of MTH1 inhibitor BAY-707 as donated chemical probe

MTH1 probe BAY-707:

Acknowledgement

*Matyas Gorjanacz**
*Manuel Ellermann**
*Ashley Eheim**
Maria Quanz
Andrea Glasauer
Anja Richter
Stefanie Bunse
Katrin Nowak-Reppel
Horst Irlbacher
Benjamin Bader
Judith Guenther
Hanna Meyer
Roland Neuhaus
Jörg Weiske

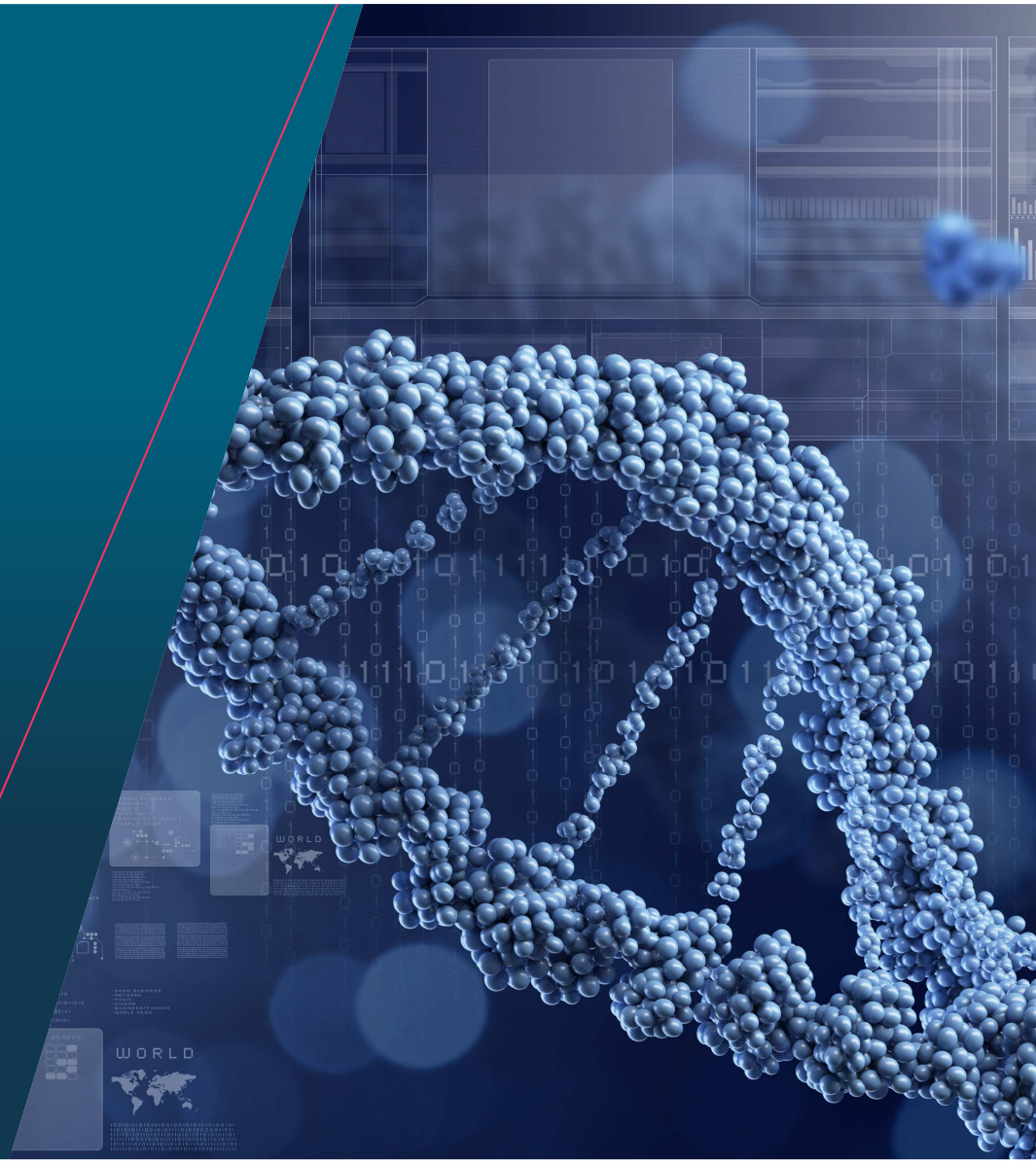
Andrea Haegebarth
Ingo Hartung
Marcus Bauser
Anke Mueller-Fahrnow
Ursula Egner

Jan Huebner

*Fredrik Rahm**
Jenny Viklund
Tobias Ginman
Johan Lindström
Martin Andersson

** Core Team*

Thank You



MTH1 probe BAY-707:

Further cellular validation of MTH1