Mutant IDH Promotes Biliary Cancer Through Altering Histone Methylation Critical for Liver Differentiation

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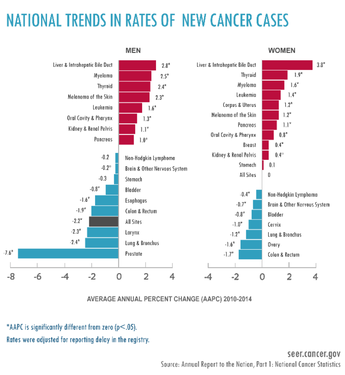
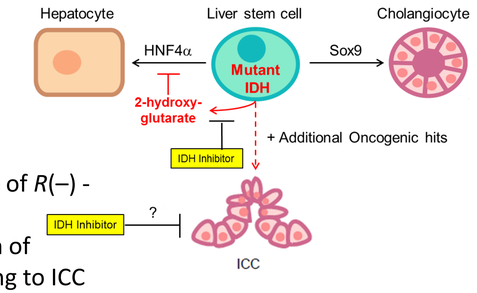
Intrahepatic cholangiocarcinoma (ICC)



- Liver cancer is the most rapidly rising cancer in the US
- Intrahepatic cholangiocarcinoma (ICC) is second most common type of primary liver cancer
- Often at the time of diagnosis, ICC is already in the advanced stages of the disease
- Unresectable ICC with the current standard of care of gemcitabine/cisplatin has a median survival < 1 year
- Scarcity of biopsy tissue and relevant model systems as a major hurdle in ICC translational research

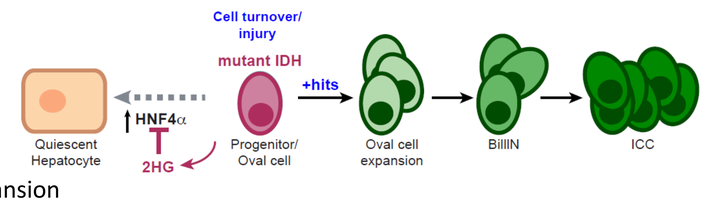
Isocitrate dehydrogenase mutations

- Most frequent genetic lesions in ICC (20-40% of cases)
- Mutations in isocitrate binding site *IDH1* (R132) result in production of *R*(–) - 2-hydroxyglutarate (2-HG), a proposed 'oncometabolite'
- 2-HG blocks hepatocyte differentiation, leading to the accumulation of undifferentiated cells susceptible to additional oncogenic hits leading to ICC (Saha, et al. Nature 2014)

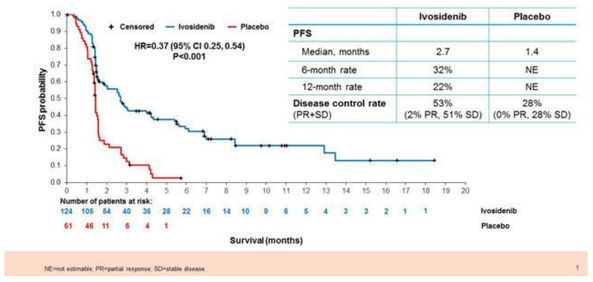


Model for IDH mutant ICC Pathogenesis

- 2HG inhibits hepatocyte differentiation, which involves the decrease in HNF4a.
- Additional oncogenic hits lead to oval cell expansion and progression to ICC.



Clinical Activity of IDHm Inhibitor Ivosidenib (AG-120) - FDA approved in August 2021

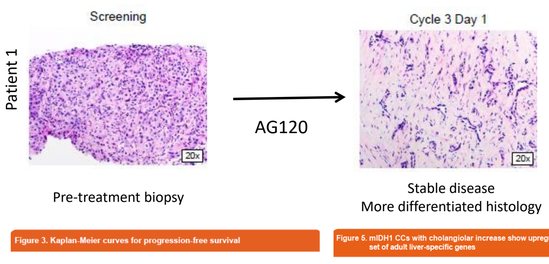


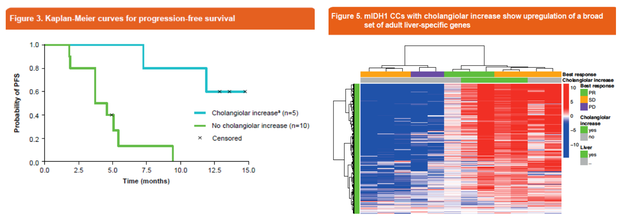
	Ivosidenib	Placebo
PFS		
Median, months	2.7	1.4
6-month rate	32%	NE
12-month rate	22%	NE
Disease control rate (PR+SD)	53% (2% PR, 51% SD)	28% (0% PR, 28% SD)

Ghassan K. Abou-Alfa, MSKCC. Presented in ESMO congress in Spain. 30 Sep 2019

- Ivosidenib demonstrated significant improvement of progression free survival in phase III ClarIDHy trial
- Response is relatively subtle. Good responders showed more differentiated morphology and increased liver specific gene transcriptions but no tumor shrinkage and apoptosis.
- Mechanism of action of AG120 activity remains unclear
- Ivosidenib received FDA approval for treating IDH1 mutant cholangiocarcinoma (August 2021)

Phase I: Good responders show increased cholangiolar histology

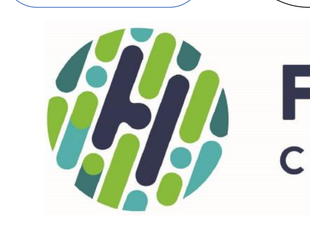




Ishii, et al. AACR-EORTC 2017

Study Objectives

1. **Characterize the effects of mIDH inhibition on chromatin marks in IDHm ICC models**
2. **Identify the downstream target(s) of mIDH1 within the aKG dependent dioxygenase family that promote ICC pathogenesis**
3. **Identify the mechanism of Ivosidenib in inducing differentiation in ICC tumor**



Development of Preclinical ICC Model Systems

In vitro

Cell lines

Human ICC cells:
Established:
SNU-1079
RBE
RBE IDHwt KI
HuCCT1
CCLP
SSP25

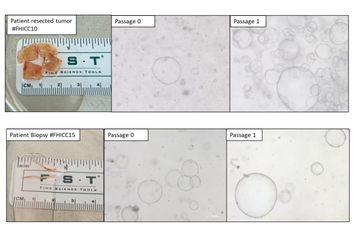
Primary:
ICC1 – ICC12
ICC5

Mouse ICC cell line
SS49 – IDH2*
AL112 – IDH1*
TH199.2
Hepatoblast
(Liver progenitor cells)

IDHm – red
IDH WT - black

Patient-derived models

3D Organoid Cultures



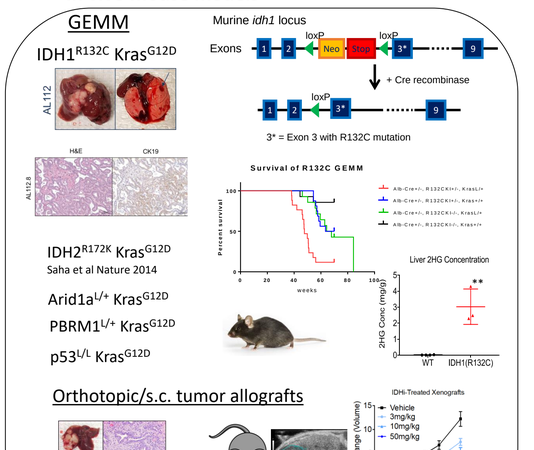
PDX

Tumor Slice Cultures

Saha, et al. Cancer Discovery, 2016

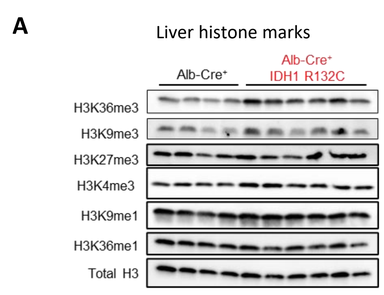
In vivo mouse models

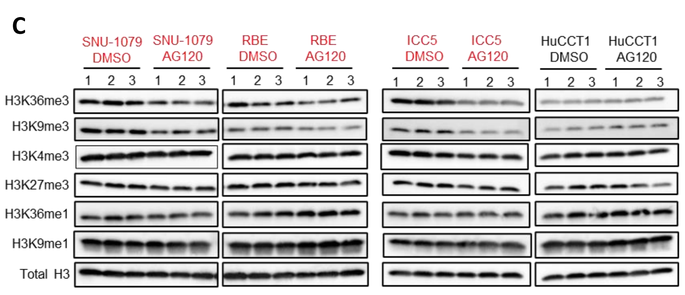
GEMM

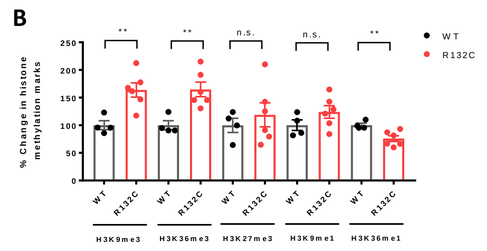


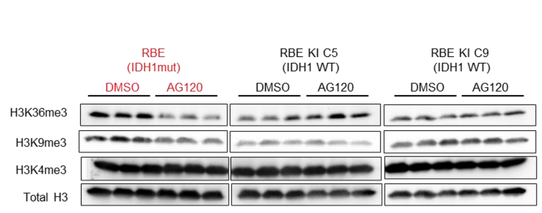
Human Biology Department Retreat 2021 September

Mutant IDH ICC cells Show Global Increase in H3K9me3 and H3K36me3



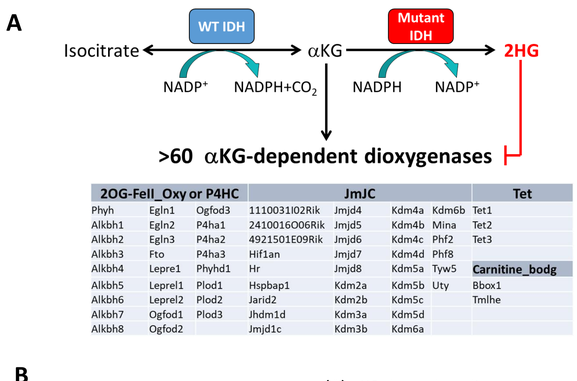




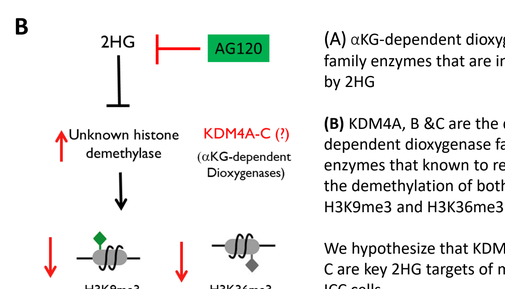


(A) Global changes in histone marks for R132C GEMM livers. IDH1 R132C mutant livers have elevated H3K36me3 and H3K9me3 than IDH WT controls. **(B)** Quantification of protein expressions normalized with total histone. **(C)** Western blot analysis IDH WT (HuCCT1 in black) and IDH1m (RBE, ICC5 & SNU 1079, in red) cell lines treated AG-120 (5 uM) for 5 days. Note that histone marks H3K36me3 and H3K9me3 are reduced with AG-120 treatment in IDHm cell lines. **(D)** Comparison of relevant histone marks change after AG120 in RBE (IDH1 mutant) and its isogenic IDH WT lines KIc5 and KI c9 generated by CRISPR knock-in IDH1 WT further demonstrated that the phenotype is IDH1m specific.

KDM4 family as The Potential Direct Molecular Target of mIDH



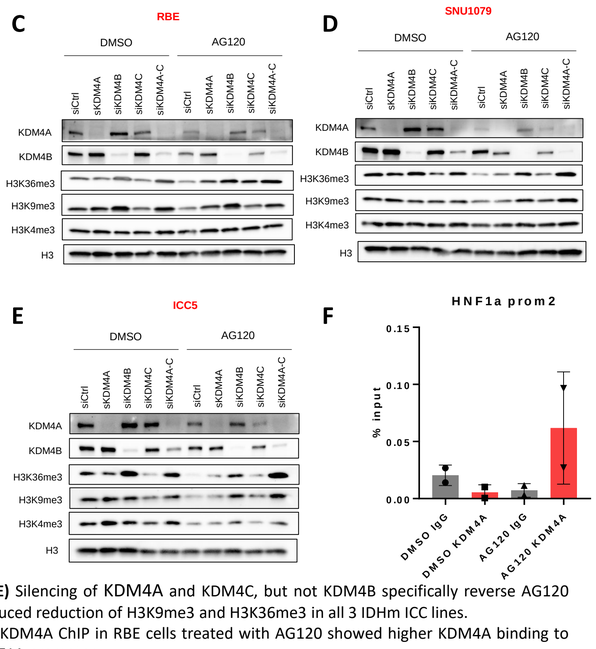
2OG-FeII_Oxy or P4HC			JmJC				Tet
Phyh	Egln1	Ogfod3	1110031I02Rik	Jmjd4	Kdm4a	Kdm6b	Tet1
Alkbh1	Egln2	P4ha1	2410016O06Rik	Jmjd5	Kdm4b	Mina	Tet2
Alkbh2	Egln3	P4ha2	4921501E09Rik	Jmjd6	Kdm4c	Phf2	Tet3
Alkbh3	Fto	P4ha3	Hif1an	Jmjd7	Kdm4d	Phf8	
Alkbh4	Lepre1	Phyhd1	Hr	Jmjd8	Kdm5a	Tyw5	Carnitine_bodg
Alkbh5	Leprel1	Plod1	Hspbap1	Kdm2a	Kdm5b	Uty	Bbox1
Alkbh6	Leprel2	Plod2	Jarid2	Kdm2b	Kdm5c		Tmlhe
Alkbh7	Ogfod1	Plod3	Jhdm1d	Kdm3a	Kdm5d		
Alkbh8	Ogfod2		Jmjd1c	Kdm3b	Kdm6a		



(A) αKG-dependent dioxygenase family enzymes that are inhibited by 2HG

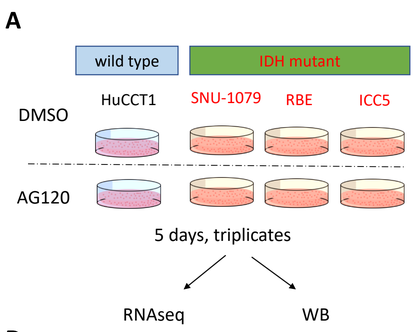
(B) KDM4A, B &C are the αKG-dependent dioxygenase family enzymes that known to regulate the demethylation of both H3K9me3 and H3K36me3

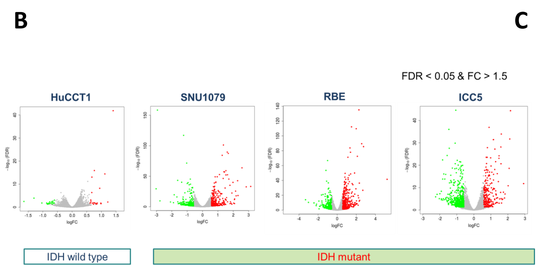
We hypothesize that KDM4A, B or C are key 2HG targets of mIDH in ICC cells

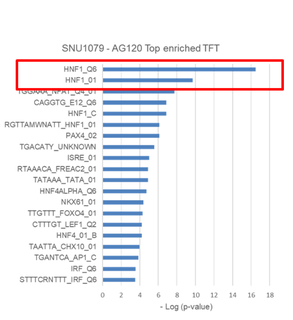


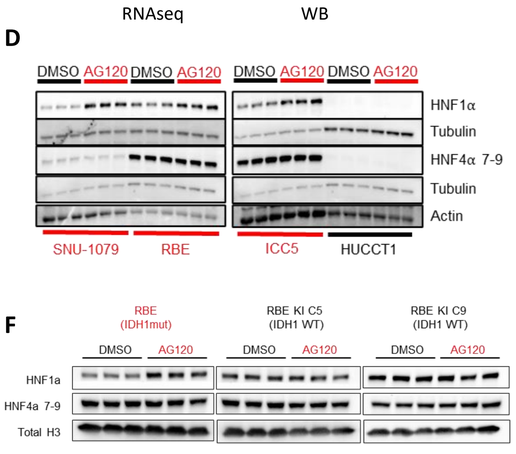
(C-E) Silencing of KDM4A and KDM4C, but not KDM4B specifically reverse AG120 induced reduction of H3K9me3 and H3K36me3 in all 3 IDHm ICC lines.
(F) KDM4A ChIP in RBE cells treated with AG120 showed higher KDM4A binding to HNF1A promoter

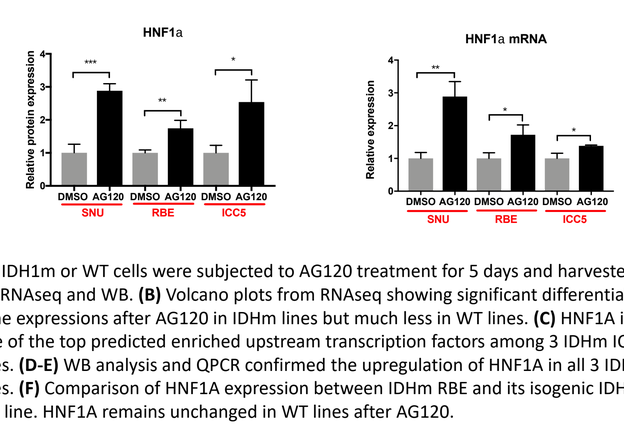
Mutant IDH inhibition Induces Hepatic Nuclear Factor 1A Expression





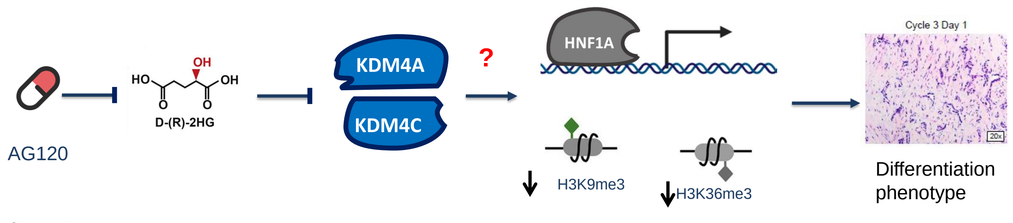






(A) IDH1m or WT cells were subjected to AG120 treatment for 5 days and harvested for RNAseq and WB. **(B)** Volcano plots from RNAseq showing significant differential gene expressions after AG120 in IDHm lines but much less in WT lines. **(C)** HNF1A is one of the top predicted enriched upstream transcription factors among 3 IDHm ICC lines. **(D-E)** WB analysis and QPCR confirmed the upregulation of HNF1A in all 3 IDHm lines. **(F)** Comparison of HNF1A expression between IDHm RBE and its isogenic IDH KI WT line. HNF1A remains unchanged in WT lines after AG120.

Summary and Future Directions



Future directions:

- To validate the effect of mIDH1 inhibition of KDM4A and KDM4C in promoting the pathogenesis of ICC using human IDH1m ICC organoid models
- To examine the effect of AG120 in promoting differentiation phenotype through upregulation of HNF1A by CUT&RUN HNF1A

Acknowledgements

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