Anti-tumour Activity of SGI-110, a novel DNA Hypomethylating Agent, in preclinical Models of Hepatocellular Carcinoma

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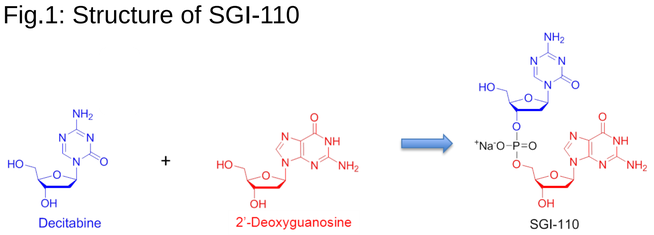
Introduction

SGI-110, a second generation hypomethylating agent (HMA), is currently being tested in a Phase 2 clinical study for the treatment of advanced hepatocellular carcinoma (HCC) in subjects who failed prior treatment with sorafenib (ClinicalTrials.gov Identifier NCT01752933). Epigenetic alterations play an important role in liver carcinogenesis. SGI-110 might offer a promising alternative to current treatment options for HCC. In preclinical experiments SGI-110 affects the clonogenic survival of HCC cells, induces gene-specific as well as global DNA demethylation (LINE-1) in human HCC cell lines and xenografts. In mice bearing human HCC xenografts (HepG2, HuH-7) SGI-110 is well tolerated as a single agent and also in combination with sorafenib. We have now profiled and identified DNA methylation patterns in sorafenib resistant and sensitive HCC patient-derived xenograft (PDX) models associated with tumour progression.

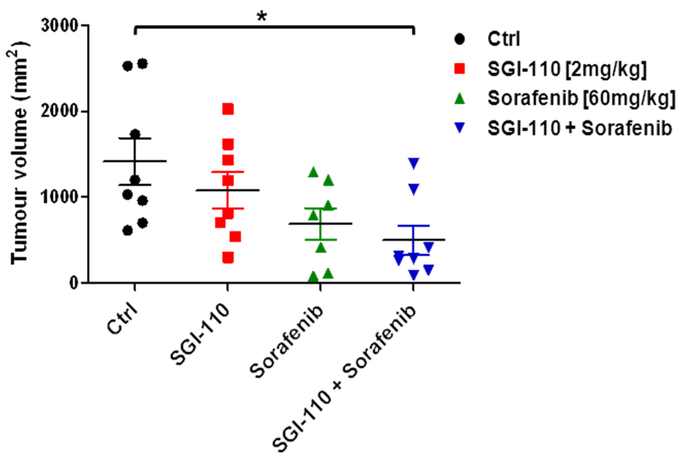
SGI-110 is a dinucleotide of decitabine and 2'-deoxyguanosine being developed as second-generation decitabine with better biological stability"

Fig.1: Structure of SGI-110



Establishment of *in vivo* HCC models to show the combinatory effect of SGI-110 and sorafenib

Fig. 2: HuH-7 xenograft model in balb/c nude mice: SGI-110 primed mice (s.c. day 1-3) showed an increased tumour growth reduction when combined with sorafenib (orally administered daily day 8-15).



We obtained a significant tumour size reduction in both HuH-7 and HepG2 HCC in vivo models when primed with SGI-110 followed by sorafenib, *p<0.05, using 1-way ANOVA with Bonferroni's Multiple Comparison Test, (shown in fig.2 as box plot 95% CI).

LINE-1 demethylation in blood and HuH-7 HCC tumour tissue samples from balb/c nude mice

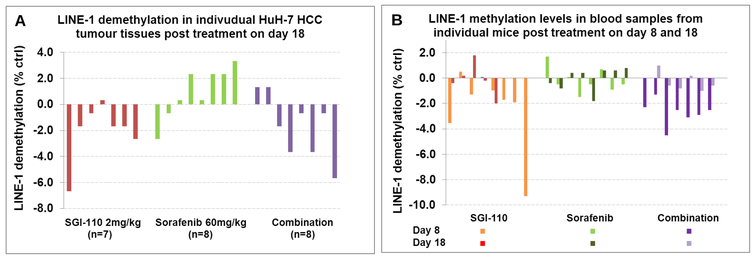


Fig. 3: We observed LINE-1 demethylation in blood samples from mice treated qd x3 with SGI-110 at 2mg/kg (A). LINE-1 methylation was reduced in the HCC tumour tissue collected on day 18 (B). The demethylating effects were seen in both SGI-110 alone and the combination group of SGI-110 followed by sorafenib. The same effects in blood and tumour tissue was seen in the HepG2 HCC *in vivo* model with SGI-110 treatment at qd x5.

Methylation signature from HuPrime® sorafenib resistant PDX models analyzed for selected genes relevant in liver cancer

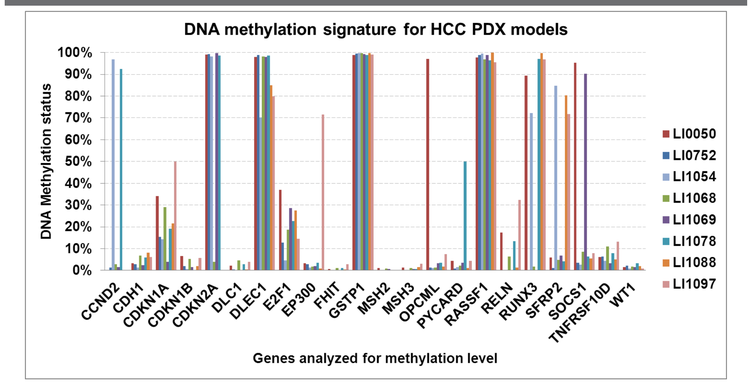


Fig. 4: Eight sorafenib resistant HuPrime® PDX models (Crown Biosciences) have been screened for their DNA methylation profile in 22 selected genes using EpiTect Methyl II Liver Cancer PCR Arrays (Qiagen). The most frequently detected hypermethylated genes were CDKN2A, DLEC1, GSTP1, RASSF1, CCND2 and RUNX3.

Methylation signature from HuPrime® sorafenib sensitive HCC PDX models in comparison to sorafenib resistant PDX HCC tissue samples

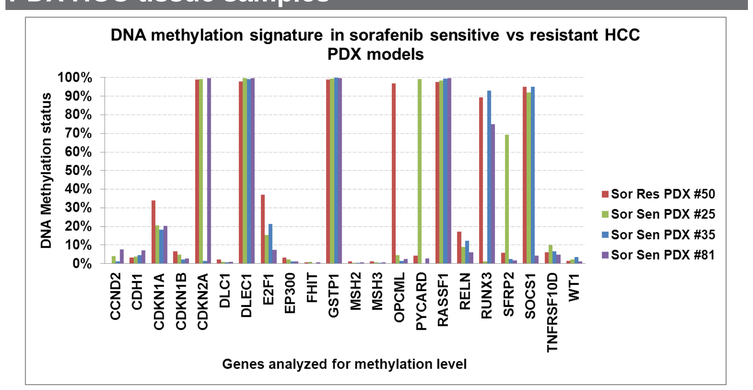


Fig. 5: The methylation profile of 3 sorafenib sensitive HuPrime® PDX models (Crown Biosciences) compared to their distinctive DNA methylation profile in sorafenib resistant HCC PDX. Six of the evaluated genes showed differential methylation profiles in sorafenib-sensitive v. resistant HCC lines and are worthy of further study as possible markers of sorafenib resistance.

Healthy Human Liver Tissue compared to Human Liver Cancer Tissue: A side by side Comparison of Basal Methylation Levels in 22 HCC Marker Genes

Genes:	Normal Liver Tissue			Sorafenib Res PDX #50		Sensitive PDX #25		Sensitive PDX #35		Sensitive PDX #81	
	UM	IM	HM	UM	HM	UM	HM	UM	HM	UM	HM
CCND2	99.6%	0.0%	0.4%			96.0%	4.0%	98.8%	1.2%	92.3%	7.7%
CDH1	99.6%	0.0%	0.4%	96.8%	3.2%	96.3%	3.7%	95.4%	4.6%	93.0%	7.0%
CDKN1A	96.3%	0.0%	3.7%	65.9%	34.1%	79.5%	20.5%	81.9%	18.1%	79.7%	20.3%
CDKN1B	99.8%	0.0%	0.2%	93.3%	6.7%	95.2%	4.8%	97.8%	2.2%	97.3%	2.7%
CDKN2A	99.8%	0.0%	0.2%	0.9%	99.1%	0.7%	99.3%	98.6%	1.4%	0.2%	99.8%
DLC1	99.9%	0.0%	0.1%	97.8%	2.2%	99.2%	0.8%	99.4%	0.6%	99.1%	0.9%
DLEC1	26.2%	73.4%	0.5%	2.0%	98.0%	0.3%	99.7%	0.9%	99.1%	0.2%	99.8%
E2F1	99.8%	0.0%	0.2%	63.0%	37.0%	84.5%	15.5%	78.6%	21.4%	92.5%	7.5%
EP300	99.9%	0.0%	0.1%	96.8%	3.2%	97.8%	2.2%	98.9%	1.1%	98.7%	1.3%
FHIT	99.9%	0.0%	0.1%	99.3%	0.7%	99.0%	1.0%	99.7%	0.3%	99.4%	0.6%
GSTP1	9.0%	90.6%	0.4%	1.1%	98.9%	0.6%	99.4%	0.1%	99.9%	0.2%	99.8%
MSH2	99.8%	0.0%	0.2%	98.9%	1.1%	99.6%	0.4%	99.7%	0.3%	99.3%	0.7%
MSH3	99.9%	0.0%	0.1%	98.7%	1.3%	99.2%	0.8%	99.7%	0.3%	99.2%	0.8%
OPCML	99.8%	0.0%	0.2%	3.0%	97.0%	95.4%	4.6%	98.5%	1.5%	97.6%	2.4%
PYCARD	100.0%	0.0%	0.0%	95.6%	4.4%	0.7%	99.3%	100.0%	0.0%	97.3%	2.7%
RASSF1	26.5%	73.3%	0.2%	2.2%	97.8%	1.5%	98.5%	0.5%	99.5%	0.4%	99.6%
RELN	99.9%	0.0%	0.1%	82.7%	17.3%	91.0%	9.0%	87.8%	12.2%	94.0%	6.0%
RUNX3	93.4%	0.0%	6.6%	10.6%	89.4%	98.9%	1.1%	7.0%	93.0%	25.0%	75.0%
SFRP2	100.0%	0.0%	0.0%	94.1%	5.9%	30.7%	69.3%	97.4%	2.6%	98.3%	1.7%
SOCS1	22.9%	64.3%	12.7%	4.8%	95.2%	7.9%	92.1%	5.0%	95.0%	95.7%	4.3%
TNFRSF10D	99.9%	0.0%	0.1%	93.9%	6.1%	90.1%	9.9%	93.4%	6.6%	95.1%	4.9%
WT1	99.8%	0.0%	0.2%	98.5%	1.5%	97.8%	2.2%	96.5%	3.5%	98.8%	1.2%

Table 1: HuPrime® PDX HCC sorafenib resistant and sensitive tumour DNA was compared with normal liver tissue from a healthy donor for possible differences in their DNA methylation signature shown as UM: unmethylated and HM: hypermethylated levels for each marker gene. Nine genes were identified as gene candidates that showed differences in the methylation signature of healthy liver tissue and malignant tissue (as highlighted in yellow). Similarities between normal liver tissue DNA methylation pattern were mostly in the sorafenib sensitive liver PDX samples (as marked in red). These 9 genes might prove useful candidate markers to further investigate if different DNA methylation level in these genes might play a role in the sensitivity to sorafenib treatment in hepatocellular carcinoma and in the development of novel HCC markers.

Conclusions

- Epigenetic priming with SGI-110 results in an at least additive effect on HuH-7 and HepG2 liver cancer xenograft models in balb/c nude mice and beige SCID HCC mouse models
- Priming with SGI-110 is well tolerated *in vivo* and may help improve the anti-tumour activity of sorafenib.
- Distinct hypomethylation patterns were observed in sorafenib resistant versus sorafenib sensitive human HCC PDX models. Sorafenib resistant and normal healthy liver tissue resulted in differently methylated genes.
- The observed differences in methylation profiles between sensitive and resistant HCC models when compared to healthy liver tissue DNA underscore the important role that can be played by HMAs in this context
- These preclinical data serve as a scientific rationale for the clinical investigation of SGI-110 in HCC.

Poster presented at the Cell Symposia: Cancer Epigenomics Oct 6-8, 2013, Melia, Sitges, Spain.

Poster available to download from Astex's website at www.astx.com