MTL-CEBPA, a drug candidate for hepatocellular-carcinoma enhances efficacy of Sorafenib

#3586

Nagy A Habib*[1], Kai-Wen Huang[2*], Vikash Reebye[1], Mikael Sodergren[1], John Rossi[3]

[1]Imperial College, London, United Kingdom; [2]National Taiwan University Hospital, Taipei, Taiwan; [3] Beckman Research Institute of City of Hope, Duarte, CA, * joint first authors

Background

- MTL-CEBPA is the clinical candidate comprising of CEBPA-51, a small RNA duplex designed to activate endogenous CCAAT/Enhancer-Binding Protein alpha (CEBPA) expression, encapsulated in a NOV340 liposome. The CEBPA-51 short activating RNA (saRNA) specifically targets the endogenous CEBPA gene by Ago-mediated transcriptional activation of CEBPA messenger RNA (mRNA) expression[1].
- C/EBP-a is a master liver-specific transcription factor, and upregulation of CEBPA by saRNA has been shown to be beneficial in a DEN-induced rat model of HCC[2]. As a critical transcriptional regulator of liver-specific genes, CEBPA activation is indicated to improve liver function in refractory diseases, such as HCC, non-alcoholic steatohepatitis, and cirrhosis.
- Sorafenib, a multi-kinase inhibitor is currently used for treatment of patients with advanced HCC. However the low tumour response rates and the side effects associated with this mono-therapy indicates the need to investigate other new therapeutic options. Since the use of a single molecular targeted agent would unlikely achieve a long-lasting remission or cure in late stage HCC, it seems reasonable to speculate that a combination of two or more agents will increase therapeutic gain.

Here we demonstrate the outcome of MTL-CEBPA and Sorafenib combination treatment in DEN induced cirrhotic HCC rats (male Wistar rats) exposed to DEN for 6 weeks to induce HCC.

Methods

Male Wistar rats were exposed to DEN for 6 weeks to induce cirrhosis and HCC[3]. After a 2 week wash-out period, animals were randomised into 5 groups:

1. PBS control
2. MTL-CEBPA for one week
3. MTL-CEBPA for two weeks
4. Sorafenib for two weeks
5. MTL-CEBPA for one week followed by Sorafenib for one week

Treatment groups

Table 1. Experimental group and treatment

	PBS	MTL-CEBPA1	MTL-CEBPA2	Sorafenib	MTL-CEBPA + Sorafenib
Treatment Week 1	PBS	MTL-CEBPA	MTL-CEBPA	Sorafenib	MTL-CEBPA
Treatment Week 2	PBS	--	MTL-CEBPA	Sorafenib	Sorafenib
Route of Administration	i.v.	i.v.	i.v.	P.O.	i.v. (MTL-CEBPA), P.O. (Sorafenib)
Dosage		3 mg/kg	3 mg/kg	10 mg/kg	3 mg/kg (MTL-CEBPA), 10 mg/kg (Sorafenib)
Treatment points	Day 1, 3, 5, 8, 10, 12	Day 1, 3, 5	Day 1, 3, 5, 8, 10, 12	Day 1, 3, 5, 8, 10, 12	Day 1, 3, 5 (MTL-CEBPA) 8, 10, 12 (Sorafenib)
Termination	Day 15	Day 15	Day 15	Day 15	Day 15

Results

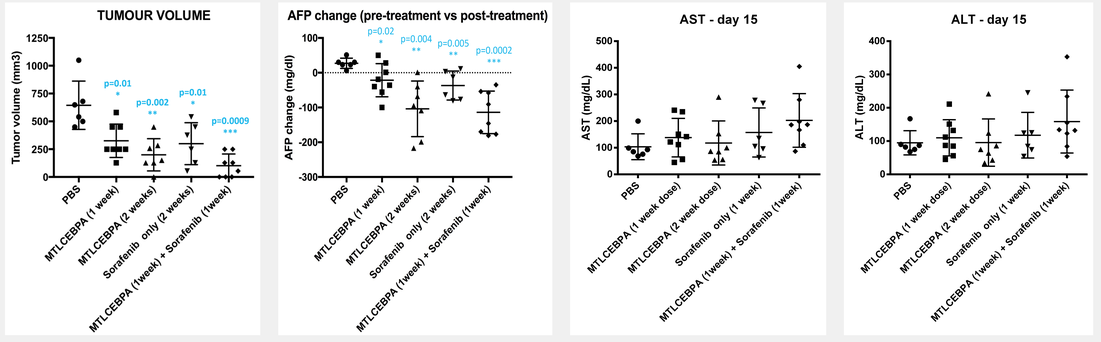

Figure 1. Liver lobes were removed and weighed. The diameters of all macroscopically visible nodules on the liver surface and within the sliced sections were measured. Tumour volume was determined by measuring the total tumour nodules with a diameter of more than 3mm. Serum AFP, AST and ALT were measured in each group. AFP change represents the difference in value of pre vs post treatment. Statistics are treatment vs PBS.

Results

Table 2. Serum and physical parameters of treated animals (mean ± S.D.)

	PBS	MTL-CEBPA1	MTL-CEBPA2	Sorafenib	MTL-CEBPA + Sorafenib
End body weight (g)	339.83 ± 32.87	307.25 ± 61.77	331.87 ± 32.40	334.00 ± 24.26	357.75 ± 29.16
Body weight change (g)	10.67 ± 7.12	8.88 ± 6.92	12.75 ± 7.50	1.33 ± 3.67	13.75 ± 14.12
Liver weight (g)	18.70 ± 1.28	14.24 ± 2.40	14.50 ± 1.73	14.73 ± 2.15	13.72 ± 1.59
Liver/end body weight	0.055 ± 0.005	0.047 ± 0.004	0.043 ± 0.001	0.044 ± 0.005	0.038 ± 0.002
Tumour volume (mm^3)	644.67 ± 216.9	326.00 ± 150.0	300.2 ± 210.1	299.50 ± 188.9	101.25 ± 106
AFP change (mg/dL)	27.17 ± 14.61	-21.50 ± 47.78	-103.86 ± 79.91	-36.83 ± 41.82	-114.00 ± 61.09
ALT (mg/dL)	94.8 ± 36.3	109.9 ± 54.3	95.4 ± 71.1	117.7 ± 68.4	158.6 ± 94.5
AST (mg/dL)	103.7 ± 48.4	138.0 ± 72.2	117.9 ± 82.8	157.3 ± 92.2	202.5 ± 100.5
Bilirubin (mg/dL)	0.81 ± 0.41	0.59 ± 0.41	0.84 ± 0.39	0.53 ± 0.36	0.71 ± 0.25

Results

- The tumour size in PBS control group averaged at 645mm^3. Tumour size in animals with single MTL-CEBPA treatment after one week averaged at 326 mm^3. Tumour size in animals treated with MTL-CEBPA for two weeks averaged at 300mm^3. Tumour size in animals treated with Sorafenib for two weeks averaged at 300 mm^3.
- **Animals treated with MTL-CEBPA + Sorafenib for two weeks showed tumour sizes averaging at 101 mm^3. 3/8 animals had no tumour nodules >3 mm^3.**
- We observed significant AFP changes across all treated animals when compared to PBS control. The best mean reduction was observed in animals treated with MTL-CEBPA + Sorafenib.
- Levels of ALT and AST over the course of the 2-week period suggested that combination treatment did not mediate hepatotoxic effects compared to Sorafenib alone.

Discussion

The results here indicate that Sorafenib combined with MTL-CEBPA significantly inhibits tumour growth when compared to single agent treatment. Serum AFP change was more prominent in the MTL-CEBPA + Sorafenib combination group. Serum levels of AST and ALT and total bilirubin suggested MTL-CEBPA did not contribute additional liver toxicity to Sorafenib treatment.

These results suggest that MTL-CEBPA improved the efficacy of Sorafenib in this DEN induced HCC model, indicating a promising therapeutic option for advanced HCC patients.

References

1. Voutila J, Reebye V, Roberts TC, et al. Development and mechanism of small activating RNA targeting CEBPA, a novel therapeutic in clinical trials for liver cancer. **Molecular Therapy 2017; 25(12)**
2. Reebye V, Huang KW, Lin V, et al. Gene activation of CEBPA using saRNA: preclinical studies of the first in human saRNA drug candidate for liver cancer. **Oncogene 2018; 37(24): 3216-28**.
3. Huang, K. W., Y. C. Huang, K. F. Tai, B. H. Chen, P. H. Lee and L. H. Hwang. "Dual therapeutic effects of interferon-alpha gene therapy in a rat hepatocellular carcinoma model with liver cirrhosis." **Mol Ther 2008; 16(10): 1681-1687**.