Small activating RNA therapeutic MTL-CEBPA in patients with advanced liver cancer: a first-in-human, multi-centre, open-label, phase I trial

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Declaration of Interests:

Employee / paid consultant of MiNA therapeutics: DC, RN, JV, JV, RH, DB, JN, HG, SF, SD, PL, HH, CW

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Abstract

Purpose: Transcription factor C/EBP-α (CCAAT/enhancer-binding protein alpha) acts as a master regulator of hepatic and myeloid functions and multiple oncogenic processes. MTL-CEBPA is a first-in-class small activating RNA oligonucleotide drug which up-regulates C/EBP-α.

Experimental Design: We conducted a phase I, open label, dose escalation trial of MTL-CEBPA in adults with advanced HCC with cirrhosis, or resulting from non-alcoholic steatohepatitis (NASH) or with liver metastases. Patients received intravenous MTL-CEBPA once a week for 3 weeks followed by a rest period of 1 week per treatment cycle in the dose escalation phase (3+3 design).

Results: 38 participants have been treated across 6 dose levels (28-160 mg/m2) and 3 dosing schedules. 34 patients were evaluable for safety endpoints at 28 days. MTL-CEBPA treatment-related adverse events were not associated with dose and no maximum dose was reached across the 3 schedules evaluated. Grade 3 treatment related adverse events occurred in 9 (24%) patients. In 24 HCC patients evaluable for efficacy, an objective tumour response was achieved in 1 patient [4%; partial response (PR) for over 2 years] and stable disease (SD) in 12 (50%). After discontinuation of MTL-CEBPA, seven patients were treated with tyrosine kinase inhibitors (TKI); 3 patients had a complete response with one further PR and two with SD.

Conclusions: MTL-CEBPA is the first saRNA in clinical trials and demonstrates an acceptable safety profile and potential synergistic efficacy with TKIs in HCC. These encouraging Phase I data validate targeting of C/EBP-α and have prompted MTL-CEBPA + sorafenib combination studies in HCC.

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Statement of translational relevance

Preclinical data have emerged suggesting C/EBP-a effects on the tumour microenvironment through myeloid derived suppressor cells could enhance response to sorafenib. The data from this trial provide preliminary validation for targeting C/EBP-a in patients with advanced HCC, particularly in context of sequential administration with TKIs and provide a rationale for combining MTL-CEBPA with TKIs.

Introduction

Primary liver cancer is the seventh most common cancer in terms of incidence and fourth in terms of cancer related mortality, globally accounting for more than 850,000 new cases annually and 9.1% of all cancer deaths[1]. The majority (70-90%) of patients with hepatocellular carcinoma (HCC) have a background of liver cirrhosis. Unfortunately most patients are diagnosed with advanced disease as less than 20% of all cirrhotic patients undergo screening[2].

Sorafenib, a multikinase inhibitor has been the first-line systemic treatment for HCC. However, the overall survival benefit with sorafenib in previously untreated patients with preserved liver function, good performance status and advanced disease although statistically significant, is disappointing (10.7 vs 7.9 months)[3]. In addition, lenvatinib was approved by the FDA as first-line treatment based on the REFLECT trial which showed non-inferiority to sorafenib[4]. Regorafenib, ramicirumab and cabozantanib have demonstrated a further modest survival benefit in the second line setting[5] . The programmed cell death protein-1 (PD-1) immune checkpoint inhibitors nivolumab and pembrolizumab although granted accelerated approval by the FDA in the second line setting, have recently failed to show superiority over sorafenib and best supportive care in phase III clinical trials[6]. Recently the IMBrave150 study demonstrated that combination treatment with atezolizumab in combination with bevacizumab were associated with improved overall and progression-free survival compared with sorafenib in patients with unresectable HCC who have not received prior systemic therapy. Despite this, there is a significant unmet need for novel therapeutics for HCC.

The transcription factor C/EBPα (CCAAT/enhancer-binding protein alpha) is a leucine zipper protein which acts as a master regulator of liver homeostasis, multiple oncogenic processes (including cell cycle control, proliferation and angiogenesis) and the haematopoietic myeloid cell lineage, in which it primes and activates the myeloid gene expression program by binding to promoters or enhancers of myeloid-related genes[7,8]. Deregulation of C/EBPα has been reported in several solid tumours including liver, breast and lung[9]. Additionally C/EBPα is down-regulated in myeloid-derived suppressor cells from tumour bearing mice and C/EBPα knock out mice display greater myeloid-derived suppressor cell tumour infiltration, vascularization and growth[10]. Up-regulation of C/EBPα in rodent models of liver cancer inhibited tumour growth[11-13]. The main mechanism of action of MTL-CEBPA is therefore on myeloid cell differentiation and their effect on the tumour microenvironment.

MTL-CEBPA is a first in class small activating RNA therapeutic comprising SMARTICLES® liposomal nanoparticle encapsulating CEBPA-51, a 21-mer small activating 2'O-Me RNA oligonucleotide duplex designed to specifically target and up-regulate transcription of the CEBPA gene[14]. Transfection of CEBPA-51 in hepatic cell lines, increased levels of C/EBP-a and inhibited cell proliferation[14,15]. Administration of MTL-CEBPA in rodent models of liver cancer increased levels of C/EBP-a and inhibited tumour growth.

In this first-in-human, first-in-class phase I dose and dose-frequency escalation study we evaluate the safety, pharmacokinetics, pharmacodynamics and clinical outcome of MTL-CEBPA in patients with advanced liver cancer.

Materials and Methods

Study design and participants

We report an international multi-centre, non-comparative, open-label, phase I study in patients with advanced HCC to evaluate the safety of dose escalation and dose frequency escalation. The original trial protocol included patients with any liver cancer, however following recruitment of the first 6 patients (4 colorectal liver metastases, 1 ampullary carcinoma metastasis & 1 HCC with cirrhosis) the protocol was amended to recruit only patients with HCC, with this being the intended target population of the subsequent dose expansion phase. This study was conducted at 10 tertiary centres and university hospitals in 3 countries (Singapore, Taiwan and United Kingdom).

Eligible patients were at least 16 years old with histologically confirmed advanced HCC with cirrhosis, or resulting from NASH, with or without cirrhosis, and unsuitable for liver tumour surgery and/or refractory to radiotherapy and other therapies. Patients were required to have a Child-Pugh score of B8 or less and ECOG performance status of 0-1. Full inclusion and exclusion criteria are described in appendix A. All patients provided written informed consent, and the study protocol and amendments were approved by the relevant regulatory authority and each site's institutional review board or independent ethics committee. The study was conducted in accordance with the Declaration of Helsinki.

Procedures

MTL-CEBPA was administered by intravenous infusion over 60 minutes once a week for 3 weeks followed by a rest period of 1 week; this defines a 4-week cycle. MTL-CEBPA dosing was preceded by prednisolone/hydrocortisone and anti-histamine administration to minimise the risk of infusion reactions. The determination of the starting dose of MTL-CEBPA was based on GLP toxicology studies in Sprague Dawley rats and cynomolgus monkeys. Based on these data, a starting dose of MTL-CEBPA 28 mg/m^2 was considered safe in humans.

The dose escalation phase of the study followed a standard 3+3 design (Supplementary Figure 1) with the intention of determining the maximum tolerated dose. Six cohorts (cohorts 1 – 6) of 3 eligible participants were planned at the following doses: 28, 47, 70, 98, 130, 160 mg/m2 weekly (QW). The dose was based on body surface area (BSA) calculation on day 1 of each cycle. After a protocol amendment, three further cohorts (7-9) were evaluated for dose frequency escalation at 70 mg/m2 (BIW d1,2, BIW d1,3 & TIW d1,2,3). Steroid and antihistamine re-dosing was only administered before the first dose of each week.

The dose-limiting toxicities (DLT) were determined on the basis of the incidence and severity of AEs occurring in the first cycle (28 days). Patients were treated until disease progression or unacceptable toxicity. A Safety Review Committee (SRC) was convened to oversee safety, scientific integrity and validity of the study. Safety and tolerability of MTL-CEBPA was evaluated in terms of frequency of AEs graded according to toxicity criteria (NCI Common Terminology Criteria for Adverse Events, CTCAE v 4.03). Patients off treatment were followed up for survival every 3 months. Tumour response and progression was evaluated using the revised Response Evaluation Criteria in Solid Tumours (RECIST1.1).

Outcomes

The primary endpoint was DLT defined as any drug related toxicity grade ≥3 according to the (CTCAE v4.03) with the only exception of aspartate transaminase (AST)/ alanine transaminase (ALT) related DLT defined as Grade 4 AST and/ or ALT abnormal laboratory value >20.0 x upper limit of normal (ULN).

Secondary endpoints included incidence of toxicity as measured by AEs & serious adverse events (SAEs), determination of pharmacokinetic & pharmacodynamic parameters, tumour response and progression-free survival.

Pharmacokinetics

Because of the stability of SMARTICLES® liposomal nanoparticles in plasma and the rapid degradation and elimination of free CEBPA-51 (the active pharmaceutical ingredient) in plasma, it is expected that the plasma concentration measurements of CEBPA-51 reflect the concentration of CEBPA-51 encapsulated in intact MTL-CEBPA nanoparticles. A fluorescently labelled peptide nucleic acid (PNA)-probe, designed against the guide strand of CEBPA-51, was used to extract the single-stranded parent compound. RNA species are quantitated using anion-exchange HPLC and fluorescence detection. Plasma CEBPA-51 is expressed as μg/mL of double-stranded RNA and the lower limit of quantitation is 0.001 μg/mL.

Plasma samples for analysis of CEBPA were collected over the first dosing interval for each Q1wk regimen and for 72h after administration of the second dose. After more frequent dosing with 70 mg/m2 MTL-CEBPA, either twice-weekly (D1, D2 or D1, D3) or three-times weekly (D1, D2, D3) plasma CEBPA concentrations were measured over the first dosing interval (i.e. between the first and the second dose), at trough (prior to the next dose), 24h after the last dose and at 168h after the first dose (i.e. prior to the next cycle).

Pharmacodynamics

10ml of blood was collected in EDTA vacutainers (BD) and captured in a LeukoLOCK™ filter system (Ambion) modified for use for the OUTREACH study. Briefly, the filter captured WBC from whole blood whilst all remaining blood component were flushed out. The filter content was then preserved with RNALater solution and stored at -80°C for total RNA extraction. Total RNA was then isolated from the captured WBC by using a modified trizol extraction method. The captured RNA was then analysed for concentration (Nanodrop) and RNA integrity (Qbit) before proceeding to cDNA synthesis using Quantitect reverse transcription (Qiagen), kit. Transcript levels were measured by qPCR (QuantStudio 5). qPCR was used to record WBC mRNA levels of CEBPA, adenosine, CXCR4, CD274 (PD-1) for select samples of individual patients. Samples were collected at pre-treatment (before start of infusion/Day 1) and then 24hr post treatment (Day 2) as well as 7 days later (Day 8) and 7 days after second cycle (Day 15). All enrolled patients were considered for pharmacodynamic evaluation.

Complement factor such as C3b and Bb as well as a cytokine panel (IL-2, IL-4, IL-6, IL-10, IL-17a, TNF-a and IFN-g) were studied in plasma as part of the safety monitoring of the patients using evidence Investigator Biochip Array technology and ELISA assays.

Statistical analysis

Descriptive statistics were used to characterise safety analyses. Kaplan-Meier methodology was used to determine means and 95% CIs for progression-free survival. Sample sizes for each dose were determined on the basis of observed toxicities, not statistical considerations. Plasma CEBPA concentrations over the first dosing interval, after once-weekly dosing with MTL-CEBPA, were used to derive non-compartmental PK parameters using Phoenix WinNonlin version 7.0 (Certara).

Results

Patient characteristics

Between May 2016 and September 2018, 38 patients were enrolled in the trial, of which 34 were evaluable for safety endpoints at 28 days. Twenty four patients were enrolled in the dose escalation phase at once weekly doses of 28mg/m2 (n=5), 47 mg/m2 (n=4), 70 mg/m2 (n=6), 98 mg/m2 (n=3), 130 mg/m2 (n=3) & 160 mg/m2 (n=3) and 14 patients in the dose frequency escalation at 70mg/m2 on weekly days 1 & 2 (n=6), days 1 & 3 (n=5), and days 1, 2 & 3 (n=3) per week.

Patient demographics and baseline characteristics including previous treatments are presented in Table 1. Overall, 35 patients have discontinued treatment as of the cut-off date. (29 disease progression, 4 study drug toxicity, 1 unrelated adverse event, 1 patient decision)

The majority of patients were of Caucasian ethnicity (n=20) followed by Chinese (n=13), Asian (n=2) and Other (n=3). The median number of lines of systemic treatment that patients received before study enrolment was 1 (range 1-5). Of the overall patient cohort the mean index platelet count was 189.1 x10^9/L (+/- 84), mean index bilirubin 17.7 umol/L (+/- 12.5) and mean index INR was 1.056 (+/- 0.28).

Please insert Tables 1 & 2 here

Dose escalation and safety

34 patients were evaluable for safety endpoints at 28 days. A maximum tolerated dose was not reached. Grade 3 treatment-related AEs occurred in 9 (24%) patients. Treatment-related AEs (all grades) that occurred in more than 10% of patients were fatigue (23.7%), thrombocytopaenia (13.2%), anaemia(13.2%), elevated AST(13.2%), elevated ALP(10.5%), hypoalbuminaemia(10.5%), increased ALT(10.5%) and increased bilirubin(10.5%). The changes in liver function tests were generally transient such that overall there were no significant changes in LFTs at the end of the first and second cycles of treatment compared to baseline. Treatment-related SAEs were reported in 4 (11%) patients. Two of these patients are described below under treatment withdrawal (acute coronary syndrome & hyperbilirubinaemia). Of the two patients who were not withdrawn, one experienced haemorrhage from a stoma and the other an upper respiratory tract infection. Three (7.9%) patients died whilst on study (2 from disease progression and one related to upper GI bleeding from a duodenal ulcer on background of NSAID therapy), and there are no treatment-related deaths.

Two patients were withdrawn with suspected drug-related toxicity which were subsequently deemed by the SRC to be not likely drug-related and therefore the relevant cohorts were not expanded (acute coronary syndrome on background of pre-morbid atherosclerotic disease and self-limiting back pain following drug infusion). One patient was withdrawn from the study due to a drug-related toxicity (hyperbilirubinaemia on background of ultrasound suggestive of acute cholecystitis). One patient was withdrawn outside the 28-day primary end point window. This was a 52-year-old with HCC, previously treated with surgery and sorafenib, who was found to have an elevated GGT following 2 units of alcohol consumption and was withdrawn on day 8 of the third cycle.

Pharmacokinetics

Mean plasma CEBPA-51 concentration vs time profiles for each Q1wk cohort are shown in Figure 1A. Overall, there was an increase in exposure with increasing dose and the plasma CEBPA-51 concentration vs time profiles were similar for the first and the second dose – indicating little drug accumulation over this time period. Although the mean plasma terminal half-life of CEBPA-51 was reasonably consistent across the dose cohorts after once-weekly treatment with MTL-CEBPA, total

plasma clearance and the apparent volume of distribution decrease with increasing dose (Supplementary Table 1). The net effect is a supra-proportional increase in exposure across the dose range 28-160 mg/m2 (Figure 1B).

As observed with the Q1wk regimen, when MTL-CEBPA is dosed either twice- or three-times weekly, the initial rapid decrease in plasma CEBPA-51 concentration after the end of the infusion is the dominant decay phase for the first 6h after dosing. Thereafter, the decay is much slower, although PK parameters and an accurate terminal half-life cannot be estimated over these shorter dosing intervals (Figure 1C). Plasma CEBPA concentration at 24h after each dose is consistent across all the dosing regimens, showing little accumulation of CEBPA-51 even when dosed once-daily (D1, D2, D3) at 70 mg/m2.

Within the overall study there was no effect of age (range 27-80 yrs), gender (9F/29M) and concomitant medication on the pharmacokinetics of MTL-CEBPA (data not shown).

The analysis of complement and cytokine assays as stipulated by MHRA as biomarkers for oligonucleotide safety was performed on a subset of 24 patients. The vast majority of results were within normal ranges or below detection limit. No drug related toxicity emerged from the study of these safety parameters (data not shown).

Please Insert Figure 1 here

Pharmacodynamics

CEBPA mRNA levels were measured from WBCs of patients treated with MTL-CEBPA 24 hours after treatment (post treatment) by quantitative real time PCR and presented as relative expression to baseline at Day 2, 8 and 15 following treatment (see Supplementary Figure 2). CEBPA mRNA levels increased by 1.5-fold consistently across all cohorts treated. When grouped at each time point, CEBPA expression levels showed a significant 1.68-fold increase at Day 2; a 1.4 fold increase at Day 8 and 15. Changes in WCC and neutrophils following drug administration are demonstrated in Figure 2. There were incremental decreases in expression of WBC adenosine, PD-1 and CXCR4 mRNA following drug administration to day 15 (see Supplementary Figure 3).

Please insert Figures 2 here

Efficacy Analysis

Twenty-nine patients who received at least two cycles of treatment were evaluable for response according to RECIST (Supplementary Table 2). The median follow-up was 2 months (range 0.5-36 months). In the 28mg/m^2 QW cohort a 78-year-old female with HCC and cirrhosis on a background of hepatitis B (treated), Child Pugh A5, previously treated with RFA, TACE, surgery, sorafenib, enzalutamide/ placebo in a randomised clinical trial (RCT) and an experimental anti-FGFR4 antibody achieved a confirmed partial response associated with rapid and dramatic decrease of AFP level. This partial response was maintained up to 24 months on treatment and her AFP levels remain within normal range (see Supplementary Figure 4). No objective responses were observed at the 47, 70, 98, 130 and 160 mg/m^2 QW, 70 mg/m^2 BIW or 70 mg/m^2 TIW DLs. In addition, 12 patients at different dose levels achieved stable disease as the best RECIST response at 2 months with 4 maintaining stable disease at 6 months.

The mean progression-free survival for the entire patient cohort was 4.6 months (95% CI 2.2-6.9, SE 1.21) and 4.9 months (95% CI 2.3-7.5, SE1.33) when excluding the patients who did not have HCC as primary pathology.

Follow-up

After discontinuation of MTL-CEBPA 7 patients were treated with TKI as illustrated in Table 3. Of these, one patient who was previously treated with ablative therapy, TACE and anti-CTLA4 with anti-PDL1 was challenged with lenvatinib after MTL-CEBPA therapy and had a partial response but progressed at six months post treatment and passed away 9 months following treatment. Three TKI naïve-patients were found to have a complete radiological response to TKIs following treatment with MTL-CEBPA. One was a 61-year-old male with hepatitis B-related cirrhosis and HCC who was previously treated with ablative therapy, TACE and doxorubicin who had complete radiological resolution of his liver lesions following treatment with sorafenib which is sustained at 9 months. A second patient was a 67-year-old male with HCC related to hepatitis C, previously treated with TACE and anti-PDL1 who had metastatic lesions in the lungs. Following progression on MTL-CEBPA and subsequent treatment with sorafenib experienced a complete radiological response in both liver and lungs 4 months after treatment was started. This response is sustained at 12 months follow-up and the longitudinal cross-sectional imaging is illustrated in Figure 3. The third patient is a 61-year-old male with hepatitis B and C previously treated with ablative therapy, TACE and doxorubicin who progressed after 2 cycles of MTL-CEBPA (during which time he developed lung metastases) was treated with sorafenib. He has shown a complete radiological response to both lung and liver lesions one month after TKI treatment which is sustained on follow-up for 7 months. CT images of the lung lesions are shown in Supplementary Figure 5. Of the remaining patients, one who had previously been treated with lenvatinib had disease progression 2 months after treatment with sorafenib and two patients (one treated with sorafenib prior to MTL-CEBPA) treated with regorafenib have stable disease at 3 months follow-up, however regorafenib was then discontinued due to toxicity.

Please insert Figure 3, Table 3 here

Discussion

This first-in-human and first-in-class multicentre phase I dose and dose escalation study of the RNA oligonucleotide MTL-CEBPA has shown the drug to be well tolerated with no maximum tolerated dose reached. Based on a combination of safety, pharmacokinetics and pharmacodynamics the recommended dose of MTL-CEPBA for further evaluation is 130mg/m2 QW. The toxicity profile was favourable and comparable to the other drugs used in this patient population including sorafenib[3], regorafenib[5] and nivolumab[16]. The non-linear PK behaviour of MTL-CEBPA is suggestive of a saturable capacity-limited tissue/cellular uptake process, dominant in the first 6h after dosing over this dose range, and a slower linear first-order process thereafter. The pharmacodynamic analysis demonstrated target engagement and a reversible and consistent increase of neutrophil count in peripheral blood following drug administration.

Although this trial was not powered to evaluate efficacy, there was evidence of anti-tumour activity with a mean progression-free survival of 4.6 months in pre-treated patients, despite a relatively modest ORR of 4% as monotherapy. The patient who sustained a partial response for 24 months and remains on treatment has been found to have a KRAS mutation in the tumour. KRAS mutations are known to be associated with a protumour inflammatory microenvironment through activation of NFκB and IL22[17] as well as IL6[18] signalling, which may explain this response given the known role of CEBPA in immune function.

The clinical activity that we have observed in patients who have progressed on MTL-CEBPA and were subsequently challenged with TKI has been unusual. A recent literature review has documented 15 published cases of complete response to sorafenib in advanced HCC since the drug was introduced in 2007, including 5 patients with lung metastases[19]. In the original trial that lead to the approval of sorafenib, out of 299 patients randomised to the drug there were no complete responses and only 2 partial responses[3]. A further report has suggested that complete responders may have a specific immune/inflammatory profile with an associated early dermatologic reaction seen in some of this patient group[20]. Of the seven cases in this trial that were treated with MTL-CEBPA and then TKIs we have observed three complete radiological responses and one partial response; two of the patients with a complete response showing complete resolution of multiple lung metastases. The response has been fast following drug administration and durable with no subsequent treatment with MTL-CEBPA for any of the patients who responded. This signal is therefore unlikely to be attributed to the activity of the TKI on its own. Additionally, the significant interval between MTL-CEBPA and TKI treatment suggests potential immune modulatory effects of MTL-CEBPA.

There is evidence that modifying the phenotype of specific sub-populations of WBC results in a tumour microenvironment which is less immune evasive and may be more responsive to conventional therapies. Myeloid-derived suppressor cells are associated with poor response to therapy in multiple solid tumours, including liver cancer with radiotherapy and sorafenib. Patel and colleagues described the dynamic changes that neutrophils undergo in cancer and demonstrated the mechanism of neutrophils' contribution to early tumour dissemination[21] highlighting the importance and plasticity of these cells in cancer progression.

In an HCC pre-clinical mouse model, Zhou and colleagues have shown that TANs recruit macrophages and Treg cells to HCCs to promote their growth, progression, and resistance to sorafenib[22]. Chang et al[23] observed that tumour-infiltrating Ly6G+ MDSCs and other immune suppressors were increased in orthotopic liver tumours using a syngeneic mouse liver cancer cell line. They found that tumour-infiltrating Ly6G+ MDSCs of sorafenib-treated tumours significantly induced IL-10 and TGF-β expressing CD4+ T cells, and downregulated the cytotoxic activity of CD8+ T cells. The combination of anti-Ly6G antibody or anti-IL-6 antibody with sorafenib significantly

reduced the cell proportion of Ly6G+ MDSCs in orthotopic liver tumours, enhanced T cell proliferation and improved the therapeutic effect of sorafenib. They concluded that modulating the tumour microenvironment through targeting tumour-infiltrating Ly6G+ MDSCs represents a strategy to improve the oncological efficacy of sorafenib[23].

The C/EBP-a transcription factor is known to regulate multiple cellular pathways relevant to HCC. Deregulation of C/EBP-a expression has been reported in a variety of human cancers and in HCC, C/EBP-a is reported to inhibit cell proliferation, cell motility and metastasis. This is supported by the observations that CEBPA knock-in mice have reduced susceptibility to HCC and CEBPA up-regulation by saRNA inhibits tumour growth in multiple tumour models[9,11,15]. It is well described that C/EBP-a regulates haematopoiesis by inducing myeloid differentiation. It has been observed that myeloid lineage specific deletion of C/EBP-a results in significantly enhanced myeloid-derived suppressor cell (MDSC) proliferation and expansion, as well as an increase of myeloid progenitors and a decrease of mature cells. Deletion of C/EBP-a in MDSCs enhanced the pro-angiogenic, immune suppressive and pro-tumorigenic behaviour of these cells by upregulating the production of iNOS and arginase, as well as MMP-9 and VEGF[10]. In this study we found a consistent and reversible increase in WCC and neutrophils in keeping with the hypothesis that PBMC upregulation of CEBPA is associated with emergency granulopoiesis and significant increases in the populations of immature monocytes[24]. We also observed downregulation of CXCR4 mRNA in white blood cells following injection of MTL-CEBPA. Chen and colleagues have observed in an orthotopic HCC mouse model that CXCR4 inhibition prevents polarization towards immunosuppressive HCC microenvironment during Sorafenib treatment and that it is also associated with anti-vascular and anti-metastatic effects and HCC progression delay[25].

We hypothesise that pre-treatment of the HCC tumour microenvironment with MTL-CEBPA renders it more susceptible to the effect of TKIs and based on the proposed mechanism and pre-clinical studies (unpublished data) we believe may have synergism with immune checkpoint blockade. This is aligned with current developments, as following the reporting of IMBrave 150 the focus on innovation in systemic HCC treatment is clearly through combination treatment. The clinical activity of MTL-CEBPA in combination with sorafenib as well as in combination with checkpoint blockade is therefore being further evaluated.

Data sharing statement:

Deidentified patient data will be available upon publication after approval of proposal by the chief investigator

Author contributions:

DS, RP, TM, BB, CEC, KWH, DP, YTM, JE, DS contributed to conception or design of the work, the acquisition, analysis, and interpretation of data for the work

MHS, MP, DC, RN, HG, SF, VR, JV, SD, PA, PL, JV, AS, DB contributed to the acquisition, analysis, and interpretation of data for the work

RS, JS, SH, DP, VK, JN, SF, RH, CW, HH contributed to the analysis and interpretation of data for the work

PS, JJR, NH contributed the conception or design of the work and the analysis and interpretation of data for the work

All authors contributed to 1) drafting the work or revising it critically for important intellectual content; and 2) final approval of the version to be published; and 3) agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Figure 1: A) Mean ±SEM plasma CEBPA-51 – Q1wk regimen by dose cohort. Plasma CEBPA-51 concentration vs time profiles were collected over 7 days after the first dose and over 3 days after the second dose. Mean data is shown here for each cohort. MTL-CEBPA doses: 28 mg/m2 n=5, 47 mg/m2 n=4, 70 mg/m2 n=6, 98 mg/m2 n=3, 130 mg/m2 n=3 and 160 mg/m2 n=3. **B)** Individual patient data for B1) dose-normalised plasma CEBPA Cmax (ug/mL / mg/m2) vs dose and B2) dose normalised plasma CEBPA AUC infinity (ug.h/mL / mg/m2) vs dose after the first dose for patients treated with the Q1wk dosing regimen. (Non-HCC – red squares; HCC Viral / HCC-fibrolamellar – orange circles; HCC Non-viral (ALD and cirrhosis) - green squares; (NAFLD) green circle; HCC Unknown aetiology – blank circles). **C)** Plasma CEBPA concentration data was collected over 7 days after dosing 70 mg/m2 MTL-CEBPA twice-weekly, C1) at 0 and 24h (D1, D2, n=6); C2) at 0 and 72 (D1, D3, n=5); and three times weekly C3) at 0, 24 and 72h (D1, D2, D3, n=3). Each patient is represented by a different symbol. There was little accumulation of CEBPA after either two or three consecutive daily doses.

Figure 2 Mean changes in A) Neutrophils and B) White Cell Count following administration of MTL-CEBPA on day 1 (n=5 at 70 mg/m2)

Figure 3 illustrating radiological response in liver and lungs. Red arrow – indicating peritoneal metastasis / hepatic extension (which was irradiated on 14/7/18 due to intrahepatic bleed and severe pain). Yellow arrow – HCC. Green arrows – Lung mets which were no longer present on 5/3/18 (2 months after was started sorafenib) and 3/7/18 (2 months after sorafenib was stopped)

Supplementary Figure 1 Dose escalation flow chart. DLT – dose limiting toxicity, MTD – maximum tolerated dose

Supplementary Figure 2 qPCR of CEBPA mRNA levels at days 2, 8 and 15 following treatment

Supplementary Figure 3 Longitudinal changes in WBC mRNA expression of CEBPA, adenosine, PD-1 & CXCR4 at screening, day2, 8 and 15 of a single patient treated in the 130mg/m^2 cohort

Supplementary Figure 4 A) CT and MRI of patient with prolonged partial response with MTL-CEBPA treatment B) IFN-gamma, NFkB and IL6 trend at days 1, 8 and 15 following treatment and C) AFP change at from baseline to 4, 8 and 16 weeks on treatment

Supplementary Figure 5 showing complete radiological response of lung metastases

Tables

Table 1 Demographic and baseline characteristics

	Cohort 1 28 mg/m^2 QW	Cohort 2 47 mg/m^2 QW	Cohort 3 70 mg/m^2 QW	Cohort 4 98mg/m^2 QW	Cohort 5 130mg/m^2 QW	Cohort 6 160mg/m^2 QW	Cohort 7 70 mg/m^2 BIW (D1-3)	Cohort 8 70 mg/m^2 BIW (D1-2)	Cohort 9 70 mg/m^2 TIW	Overall
	n=5	n=4	n=6	n=3	n=3	n=3	n=5	n=6	n=3	N=38
Median age, years (range)	64 (61-78)	57 (27-74)	65 (63-80)	72 (67-74)	59 (57-61)	67 (59-70)	66 (57-69)	63 (54-77)	68 (52-77)	66 (27-80)
Gender										
Female	3	1	1	2	-	-	-	2	-	9
Male	2	3	5	1	3	3	5	4	3	29
ECOG-PS										
0	2	2	3	1	2	1	2	2	1	16
1	3	2	3	2	1	2	3	4	2	22
Tumour type, n (%)										
HCC	2	1	5	3	3	3	5	6	3	31
Fibrolamellar HCC	-	2	-	-	-	-	-	-	-	2
CRC	3	-	1	-	-	-	-	-	-	4
Ampulary	-	1	-	-	-	-	-	-	-	1
Child-Pugh										
A5	1	2	3	3	3	1	3	4	2	22
A6	-	-	1	-	-	1	1	1	1	5
B7	1	1	1	-	-	1	1	1	-	6
B8										
Extrahepatic metastasis										
Yes	4	3	4	3	2	1	2	3	1	23
No	1	1	2	-	1	2	3	3	2	15
Cause of HCC										
Hepatitis B	2	-	3	-	2	1	-	2	-	10
Alcoholic disease	-	1	2	-	-	-	2	-	1	6
Hepatitis C	-	-	-	1	-	1	1	1	-	4
NAFLD/ NASH	-	-	-	2	-	1	2	1	1	7
Haemochromatosis	-	-	-	-	-	-	-	1	1	2
Unknown	-	-	-	-	1	-	-	1	-	2
Median AFP, ng/mL (range)	85.5 (11.9-161)	5.1 (3-7)	12.0 (3-101.9)	2.2 (1.6-4737)	242.7 (78.2-407.2)	10.5 (9.6-50.4)	147.0 (2.5-6936)	249.6 (2.5-19017.64)	556.0 (1.6-1411)	20.0 (1.6-19017.64)
Prior therapy										
Surgery	4	3	2	1	1	2	1	1	1	16

Transarterial chemobolisation	2	-	3	3	2	2	1	4	1	18
RFA	1	1	-	-	-	1	-	1	-	4
IRE	1	-	-	-	-	-	-	-	-	1
Other	1	-	-	-	1	-	1	1	-	4
Radiation	2	2	-	1	-	2	-	1	1	9
None	-	1	1	-	1	-	4	2	-	9
Prior systemic therpay										
TKI	1	1	5	1	1	2	5	5	3	24
ICB	-	-	2	3	2	2	-	-	-	9
FGFRi	1	-	-	2	-	-	-	-	-	3
Other chemotherapy	4	3	1	-	1	-	-	1	-	10
None	-	-	-	-	-	-	-	-	-	-

Table 2 Most frequently (>5%) reported drug related adverse AE in each cohort presented as n (%)

	Cohort 1		Cohort 2		Cohort 3		Cohort 4		Cohort 5		Cohort 6		Cohort 7		Cohort 8		Cohort 9		Overall	
	28 mg/m^2 QW		47 mg/m^2 QW		70 mg/m^2 QW		98mg/m^2 QW		130mg/m^2 QW		160mg/m^2 QW		70mg/m^2 BIW D1&3		70mg/m^2 BIW D1&2		70mg/m^2 TIW			
	n=5		n=4		n=6		n=3		n=3		n=3		n=5		n=6		n=3		N=38	
Frequent AEs	Any grade	Gr. 3	Any grade	Gr. 3	Any grade	Gr. 3	Any grade	Gr. 3	Any grade	Gr. 3	Any grade	Gr. 3	Any grade	Gr. 3	Any grade	Gr. 3	Any grade	Gr. 3	Any grade	Gr. 3
Fatigue	2 (40.0)	-	-	-	4 (66.7)	1 (16.7)	1 (33.3)	-	-	-	-	-	1 (20.0)	-	1 (16.7)	-	-	-	9 (23.7)	1 (2.6)
Thrombocytopenia	1 (20.0)	1 (20.0)	-	-	-	-	1 (33.3)	-	-	-	-	-	1 (20.0)	-	1 (16.7)	-	1 (33.3)	1 (33.3)	5 (13.2)	2 (5.3)
Anaemia	2 (40.0)	1 (20.0)	-	-	1 (16.7)	-	1 (33.3)	1 (33.3)	-	-	-	-	1 (20.0)	-	-	-	-	-	5 (13.2)	2 (5.3)
AST increased	-	-	-	-	2 (33.3)	-	2 (66.7)	1 (33.3)	-	-	-	-	1 (20.0)	-	-	-	-	-	5 (13.2)	1 (2.6)
Blood ALP increased	1 (20.0)	-	1 (25.0)	-	1 (16.7)	-	-	-	-	-	-	-	1 (20.0)	-	-	-	-	-	4 (10.5)	-
Hypoalbuminaemia	1 (20.0)	-	1 (25.0)	-	-	-	-	-	-	-	-	-	1 (20.0)	-	1 (16.7)	-	-	-	4 (10.5)	-
ALT increased	-	-	-	-	1 (16.7)	-	1 (33.3)	-	1 (33.3)	1 (33.3)	-	-	1 (20.0)	-	-	-	-	-	4 (10.5)	1 (2.6)
Blood bilirubin increased	-	-	-	-	2 (33.3)	-	1 (33.3)	-	-	-	-	-	-	-	-	-	1 (33.3)	-	4 (10.5)	-
Pyrexia	1 (20.0)	-	1 (25.0)	-	1 (16.7)	-	-	-	-	-	-	-	-	-	-	-	-	-	3 (7.9)	-
Hypophosphataemia	2 (40.0)	1 (20.0)	-	-	-	-	1 (33.3)	1 (33.3)	-	-	-	-	-	-	-	-	-	-	3 (7.9)	2 (5.3)
Neutrophil count increased	1 (20.0)	-	1 (25.0)	-	1 (16.7)	-	-	-	-	-	-	-	-	-	-	-	-	-	3 (7.9)	-
Diarrhoea	-	-	2 (50.0)	-	1 (16.7)	-	-	-	-	-	-	-	-	-	-	-	-	-	3 (7.9)	-
Flushing	1 (20.0)	-	-	-	1 (16.7)	-	-	-	-	-	-	-	-	-	-	-	-	-	2 (5.3)	-
Ascites	-	-	-	-	1 (16.7)	-	-	-	-	-	-	-	-	-	1 (16.7)	-	-	-	2 (5.3)	-
GGT increased	-	-	-	-	-	-	1 (33.3)	1 (33.3)	-	-	-	-	1 (20.0)	1 (20.0)	-	-	-	-	2 (5.3)	2 (5.3)
Dysgeusia	-	-	-	-	1 (16.7)	-	-	-	-	-	-	-	-	-	1 (16.7)	-	-	-	2 (5.3)	-
Dizziness	-	-	-	-	1 (16.7)	-	-	-	-	-	-	-	-	-	1 (16.7)	-	-	-	2 (5.3)	-
Headache	1 (20.0)	-	-	-	-	-	-	-	-	-	-	-	-	-	1 (16.7)	-	-	-	2 (5.3)	-

Table 3 Characteristics and responses of patients receiving TKI after MTL-CEBPA. Patients below double line were those who had shown previous TKI resistance.

Dose	Age/Sex/Aatiology	Previous therapy	Metastatic disease	Therepy between MTL-CEBPA and TKI	Time to progression on MTL_CEBPA (cycles)	Primary treatment TKI?	Post TKI Best response (month)	TKI Therapy Post Study
98 mg/m^2 QW	72yrs, F, NAFLD	TACE Radiotherapy (SIRT) ICB (anti-PD1) FGFR inhibitor	Lung & Acetabulum	No	8	No	SD - ongoing for 4 months	Refgorafenib
98 mg/m^2 QW	67yrs, M, HepC	TACE ICB (anti-PDL1)	Lung	TACE	2	No	CR - ongoing for 12 months	Sorafenib
130 mg/m^2 QW	59yrs, M, HepB	TACE Surgery ICB (anti-CTLA4 + anti-PDL1)	Supraclavicular lymph node	No	2	No	PR for 2 months then PD	Levantinib
130 mg/m^2 QW	61yrs, M, HepB	Ablative therapy TACE DOXO	No	TACE	2	No	CR - ongoing for 9 months	Sorafenib
70mg/m^2 BIW (Day 1&2)	61yrs, M, HepB/C	Surgery Ablative therapy TACE DOXO	No	No	2	No	CR - ongoing for 7 months	Sorafenib
98 mg/m^2 QW	76yrs, F, NAFLD	Surgery TACE TKI (sorafenib) ICB (anti-PD1) FGFR inhibitor	Lung	No	8	Yes	SD - ongoing for 2 months	Regorafenib
70mg/m^2 BIW (Day 1&2)	73yrs, M, HepB	TKI (lenvatinib)	Para-aortic lymph node	No	2	Yes	PD - after 2 months	Sorafenib

ICB = immune-checkpoint blockade; SIRT = selective internal radiation therapy; TKI = tyrosine kinase inhibitors; TACE = transarterial chemoembolization; DOXO = doxorubicin; FGFR = fibroblast growth factor receptor

Supplementary Table 1 CEBPA-51 PK parameters – Q1wk cohorts 0-7days

Dose (mg/m^2)	n=	Tmax (h)	Cmax (µg/mL)	AUCinf (h.µg/mL)	CL ($L/h/m^2$)	Vz (L/m^2)	Half-life (h)
28	5	1.00	1.68 ± 0.98	30.7 ± 30.6	2.08 ± 2.18	98.7 ± 103.0	49.2 ± 20.4
47	4	1.00	6.33 ± 1.87	98.7 ± 106.1	0.91 ± 0.61	37.1 ± 17.6	45.2 ± 20.6
70	6	1.00	15.50 ± 1.51	219.5 ± 82.4	0.37 ± 0.18	21.3 ± 4.1	49.1 ± 20.2
98	3	1.25	20.14 ± 7.62	408.2 ± 113.8	0.26 ± 0.85	21.6 ± 7.9	59.2 ± 18.5
130	3	1.25	39.43 ± 14.24	499.1 ± 186.2	0.29 ± 0.12	20.6 ± 7.9	50.7 ± 12.0
160	3	1.00	67.45 ± 10.92	836.3 ± 51.2	0.19 ± 0.01	8.5 ± 0.7	30.8 ± 4.1

Mean non-compartmental PK parameters ± SD: Tmax – time of maximum observed concentration (median); Cmax – maximum observed concentration; AUCinf – area under the curve calculated from 0-last observed concentration and extrapolated to infinity; CL – total plasma clearance (Dose/AUCinf); Vz – apparent volume of distribution based on the terminal phase; half-life – terminal half-life calculated using at least three data points in the terminal phase.

Supplementary Table 2 RECIST 1.1 response observed overall and per cohorts in HCC patients at cut-off (12 Sep 2018) presented as n (%)

Cohort	**N**	**PR**	**SD 6m**	**SD >2m**	**SD 2m**	**PD**	**NE**
28 mg/m^2 QW	2	1 (50.0)	-	-	-	1 (50.0)	-
47 mg/m^2 QW	3	-	-	-	-	2 (66.7)	1 (33.3)
70 mg/m^2 QW	5	-	1 (20.0)	-	2 (40.0)	2 (40.0)	-
98 mg/m^2 QW	3	-	2 (66.7)	-	-	1 (33.3)	-
130 mg/m^2 QW	3	-	1 (33.3)	-	-	2 (66.7)	-
160 mg/m^2 QW	3	-	-	1 (33.3)	2 (66.7)	-	-
70mg/m^2 BIW D1&3	5	-	-	-	1 (20.0)	2 (40.0)	2 (40.0)
70mg/m^2 BIW D1&2	6	-	-	-	2 (33.3)	4 (66.7)	-
70mg/m^2 TIW	3	-	-	-	-	1 (33.3)	2 (66.7)
Overall	**33**	**1 (3.0)**	**4 (12.1)**	**1 (3.0)**	**7 (21.2)**	**15 (45.5)**	**5 (15.2)**