MTL-CEBPA, a Small Activating RNA Therapeutic Upregulating C/EBP-α, in Patients with Advanced Liver Cancer: A First-in-Human, Multicenter, Open-Label, Phase I Trial

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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 that upregulates C/EBP-α.

Patients and Methods: We conducted a phase I, open-label, dose-escalation trial of MTL-CEBPA in adults with advanced hepatocellular carcinoma (HCC) with cirrhosis, or resulting from nonalcoholic steatohepatitis 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: Thirty-eight participants have been treated across six dose levels (28–160 mg/m^2) and three dosing schedules. Thirty-four 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 three schedules evaluated. Grade 3 treatment-related adverse events occurred in nine (24%) patients. In 24 patients with HCC evaluable for efficacy, an objective tumor response was achieved in one 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 (TKIs); three 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.

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 patients with cirrhosis 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; ref. 3). In addition, lenvatinib was approved by the FDA as first-line treatment based on the REFLECT trial which showed noninferiority to sorafenib (4). Regorafenib, ramucirumab, 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

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Translational Relevance

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

inhibitors nivolumab and pembrolizumab were although granted accelerated approval by the FDA in the second-line setting, they 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 was 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/EBP-α (CCAAT/enhancer-binding protein alpha) is a leucine zipper protein that acts as a master regulator of liver homeostasis, multiple oncogenic processes (including cell-cycle control, proliferation, and angiogenesis), and the hematopoietic 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/EBP-α has been reported in several solid tumors including liver, breast, and lung (9). In addition C/EBP-α is downregulated in myeloid-derived suppressor cells from tumor-bearing mice, and C/EBP-α knockout mice display greater myeloid-derived suppressor cell tumor infiltration, vascularization, and growth (10). Upregulation of C/EBP-α in rodent models of liver cancer inhibited tumor growth (11–13). The main mechanism of action of MTL-CEBPA is therefore on myeloid cell differentiation and their effect on the tumor 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 upregulate transcription of the CEBPA gene (14). Transfection of CEBPA-51 in hepatic cell lines increased levels of C/EBP-α and inhibited cell proliferation (14, 15). Administration of MTL-CEBPA in rodent models of liver cancer increased levels of C/EBP-α and inhibited tumor growth.

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

Patients and Methods

Study design and participants

We report an international multicenter, noncomparative, 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 six patients (four colorectal liver metastases, one ampullary carcinoma metastasis, and one 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 centers and university hospitals in three countries (Singapore, Taiwan, and the United Kingdom).

Eligible patients were at least 16 years old with histologically confirmed advanced HCC with cirrhosis, or resulting from nonalcoholic steatohepatitis, with or without cirrhosis, and unsuitable for liver tumor surgery and/or refractory to radiotherapy and other therapies. Patients were required to have a Child–Pugh score of B8 or less and Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0–1. Full inclusion and exclusion criteria are described in Appendix A in Supplementary Material. 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 antihistamine administration to minimize 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 28 mg/m^2 MTL-CEBPA was considered safe in humans.

The dose-escalation phase of the study followed a standard 3+3 design (Supplementary Fig. S1) with the intention of determining the MTD. Six cohorts (cohorts 1–6) of three eligible participants were planned at the following doses: 28, 47, 70, 98, 130, and 160 mg/m^2 weekly (QW). The dose was based on body surface area 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/m^2 (BIW d1,2, BIW d1,3, and TIW d1,2,3). Steroid and antihistamine redosing was only administered before the first dose of each week.

The dose-limiting toxicities (DLTs) were determined on the basis of the incidence and severity of adverse events (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 were 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. Tumor response and progression was evaluated using the revised 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.

Secondary endpoints included incidence of toxicity as measured by AEs and serious AEs (SAEs), determination of PK and pharmacodynamic parameters, tumor 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

Table 1. Demographic and baseline characteristics.

	Cohort 1 28 mg/m² QW *n* = 5	Cohort 2 47 mg/m² QW *n* = 4	Cohort 3 70 mg/m² QW *n* = 6	Cohort 4 98 mg/m² QW *n* = 3	Cohort 5 130 mg/m² QW *n* = 3	Cohort 6 160 mg/m² QW *n* = 3	Cohort 7 70 mg/m² BIW (D1–3) *n* = 5	Cohort 870 mg/m² BIW (D1–2) *n* = 6	Cohort 9 70 mg/m² TIW *n* = 3	Overall *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
Tumor type, *n* (%)										
HCC	2	1	5	3	3	3	5	6	3	31
Fibrolamellar HCC	—	2	—	—	—	—	—	—	—	2
CRC	3	—	1	—	—	—	—	—	—	4
Ampullary	—	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
Nonalcoholic fatty liver disease/ NASH	—	—	—	2	—	1	2	1	1	7
Hemochromatosis	—	—	—	—	—	—	—	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 (1.6–4,737)	242.7 (78.2–407.2)	10.5 (9.6–50.4)	147.0 (2.5–6,936)	249.6 (2.5–19017.64)	556.0 (1.6–1,411)	20.0 (1.6–19017.64)
Prior therapy										
Surgery	4	3	2	1	1	2	1	1	1	16
TACE	2	—	3	3	2	2	1	4	1	18
RFA	1	1	—	—	—	1	—	1	—	4
IRE	1	—	—	—	—	—	—	—	—	1
Other	1	—	—	—	1	—	1	1	—	4
Radiotherapy	2	2	—	1	—	2	—	1	1	9
None	—	1	1	—	1	—	4	2	—	9

(Continued on the following page)

Table 1. Demographic and baseline characteristics. (Cont'd)

	Cohort 1 28 mg/m^2 QW $n = 5$	Cohort 2 47 mg/m^2 QW $n = 4$	Cohort 3 70 mg/m^2 QW $n = 6$	Cohort 4 98 mg/m^2 QW $n = 3$	Cohort 5 130 mg/m^2 QW $n = 3$	Cohort 6 160 mg/m^2 QW $n = 3$	Cohort 7 70 mg/m^2 BIW (D1–3) $n = 5$	Cohort 870 mg/m^2 BIW (D1–2) $n = 6$	Cohort 9 70 mg/m^2 TIW $n = 3$	Overall $N = 38$
Prior systemic therapy										
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	—	—	—	—	—	—	—	—	—	—

Abbreviations: ICB, immune-checkpoint blockade; NASH, nonalcoholic steatohepatitis.

concentration of CEBPA-51 encapsulated in intact MTL-CEBPA nanoparticles. A fluorescently labeled peptide nucleic acid 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 high-performance liquid chromatography 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 72 hours after administration of the second dose. After more frequent dosing with 70 mg/m^2 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 (ie, between the first and the second doses), at trough (prior to the next dose), 24 hours after the last dose, and at 168 hours after the first dose (ie, prior to the next cycle).

Pharmacodynamics

Ten milliliters 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 white blood cells (WBCs) from whole blood, whereas all remaining blood components 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 analyzed 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, and CD274 (PD-1) for select samples of individual patients. Samples were collected at pretreatment (before start of infusion/day 1) and then 24 hours after 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 factors such as C3b and Bb as well as a cytokine panel (IL2, IL4, IL6, IL10, IL17a, TNFa, and IFNg) 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 characterize safety analyses. Kaplan–Meier methodology was used to determine mean and 95% confidence intervals (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 noncompartmental 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 28 mg/m^2 ($n = 5$), 47 mg/m^2 ($n = 4$), 70 mg/m^2 ($n = 6$), 98 mg/m^2 ($n = 3$), 130 mg/m^2 ($n = 3$), and 160 mg/m^2 ($n = 3$) and 14 patients in the dose-frequency escalation at 70 mg/m^2 on weekly days 1 and 2 ($n = 6$), days 1 and 3 ($n = 5$), and days 1, 2, and 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 cutoff date (29 disease progression, four study drug toxicity, one unrelated adverse event, and one 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 enrollment was 1 (range, 1–5). Of the overall patient cohort, the mean index platelet count was 189.1 $\times$ 10^9/L ($\pm$84), mean index bilirubin 17.7 μmol/L ($\pm$12.5), and mean index international normalized ratio was 1.056 ($\pm$0.28).

Dose escalation and safety

Thirty-four patients were evaluable for safety endpoints at 28 days. An MTD was not reached. Grade 3 treatment-related AEs occurred in nine (24%) patients. Treatment-related AEs (all grades) that occurred in more than 10% of patients were fatigue (23.7%), thrombocytopenia (13.2%), anemia (13.2%), elevated AST (13.2%), elevated ALP (10.5%), hypoalbuminemia (10.5%), increased ALT (10.5%), and increased bilirubin (10.5%) as shown in **Table 2**. The changes in liver function tests were generally transient such that overall there were no significant changes in liver function tests at the end of the first and second cycles of treatment compared with baseline. Treatment-related SAEs were reported in four (11%) patients. Two of these patients are described below under treatment withdrawal (acute coronary syndrome and hyperbilirubinemia). Of the two patients who were not withdrawn, one experienced hemorrhage from a stoma and the other from an upper respiratory tract infection. Three (7.9%) patients died while on study (two from disease progression and one related to upper gastrointestinal 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 was 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 premorbid atherosclerotic disease and self-limiting back pain following drug infusion). One patient was withdrawn from the study due to a drug-related toxicity (hyperbilirubinemia 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 Gamma-glutamyl transpeptidase following two units of alcohol consumption and was withdrawn on day 8 of the third cycle.

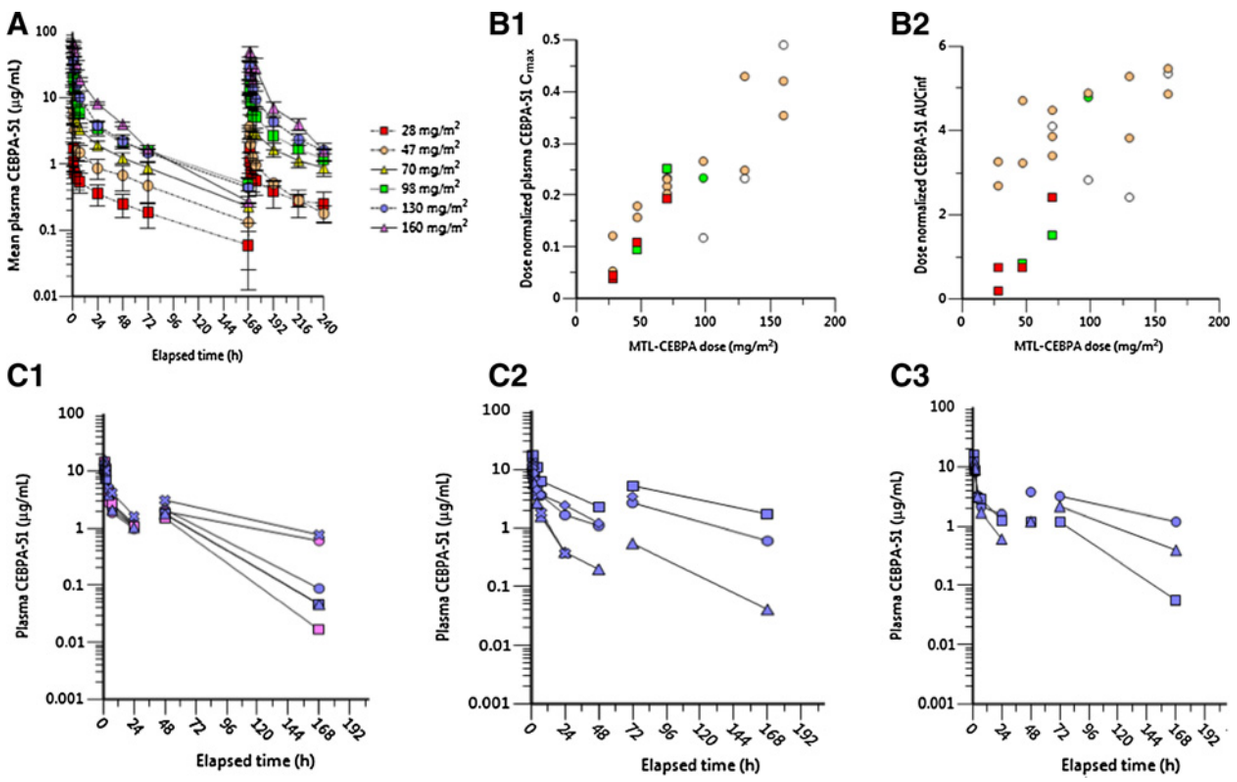

Figure 1.

A, Mean $\pm$ SEM plasma CEBPA-51—Q1wk regimen by dose cohort. Plasma CEBPA-51 concentration versus time profiles were collected over 7 days after the first dose and over 3 days after the second dose. Mean data are shown here for each cohort. MTL-CEBPA doses: 28 mg/m^2 ($n = 5$), 47 mg/m^2 ($n = 4$), 70 mg/m^2 ($n = 6$), 98 mg/m^2 ($n = 3$), 130 mg/m^2 ($n = 3$), and 160 mg/m^2 ($n = 3$). **B,** Individual patient data for (B1) dose-normalized plasma CEBPA C_{max} (μg/mL/mg/m^2) versus dose and (B2) dose-normalized plasma CEBPA AUC infinity (μg h/mL/mg/m^2) versus 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 etiology, blank circles]. **C,** Plasma CEBPA concentration data were collected over 7 days after dosing 70 mg/m^2 MTL-CEBPA twice weekly, (C1) at 0 and 24 hours (D1, D2, $n = 6$); (C2) at 0 and 72 hours (D1, D3, $n = 5$); and three times weekly (C3) at 0, 24, and 72 hours (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.

Pharmacokinetics

Mean plasma CEBPA-51 concentration versus time profiles for each Q1wk cohort are shown in **Fig. 1A**. Overall, there was an increase in exposure with increasing dose, and the plasma CEBPA-51 concentration versus time profiles were similar for the first and the second doses—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 supraproportional increase in exposure across the dose range of 28 to 160 mg/m^2 (**Fig. 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 6 hours 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 (**Fig. 1C**). Plasma CEBPA concentration at 24 hours 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/m^2.

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

The analysis of complement and cytokine assays as stipulated by Medicines & Healthcare Products Regulatory Agency 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).

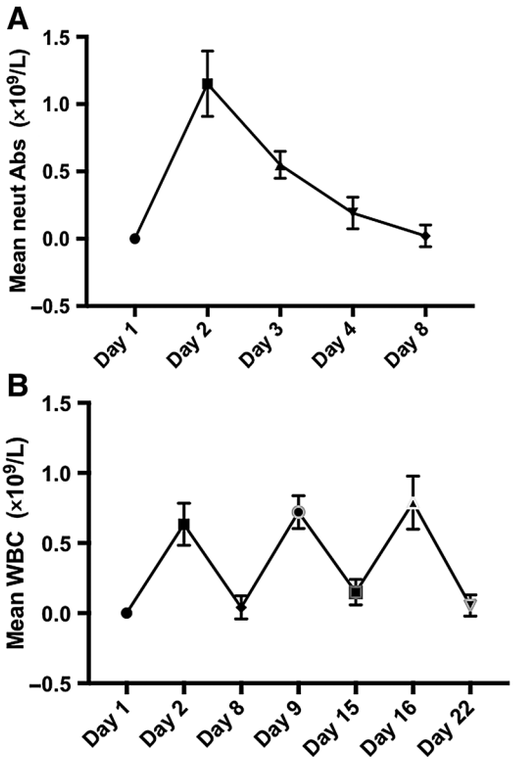

Figure 2.
Mean changes in (**A**) neutrophils and (**B**) WBC following administration of MTL-CEBPA on day 1 (n = 5 at 70 mg/m^2).

Pharmacodynamics

CEBPA mRNA levels were measured from WBCs of patients treated with MTL-CEBPA 24 hours after treatment (posttreatment) by quantitative real-time PCR and presented as relative expression to baseline at days 2, 8, and 15 following treatment (see Supplementary Fig. S2). 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 and a 1.4-fold increase at days 8 and 15. Changes in white cell count and neutrophils following drug administration are demonstrated in **Fig. 2**. There were incremental decreases in expression of WBC adenosine, PD-1, and CXCR4 mRNA following drug administration to day 15 (see Supplementary Fig. S3).

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 28 mg/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 radiofrequency ablation, transarterial chemoembolization (TACE), surgery, sorafenib, enzalutamide/placebo in a randomized clinical trial, 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 Fig. S4). 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 dose levels. In addition, 12 patients at different dose levels achieved stable disease as the best RECIST response at 2 months with four 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; SE, 1.33) when excluding the patients who did not have HCC as primary pathology.

Follow-up

After discontinuation of MTL-CEBPA, seven patients were treated with TKI as illustrated in **Table 3**. Of these, one patient who was previously treated with ablative therapy, TACE, and anti–CTLA-4 with anti-PDL1 was challenged with lenvatinib after MTL-CEBPA therapy and had a partial response but progressed at 6 months after 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 and 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, he experienced a complete radiological response in both liver and lungs 4 months after treatment was started. This response is sustained at 12-month follow-up, and the longitudinal cross-sectional imaging is illustrated in **Fig. 3**. The third patient is a 61-year-old male with hepatitis B and C previously treated

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

	Cohort 1 28 mg/m² QW		Cohort 2 47 mg/m² QW		Cohort 3 70 mg/m² QW		Cohort 4 98 mg/m² QW		Cohort 5 130 mg/m² QW		Cohort 6 160 mg/m² QW		Cohort 7 70 mg/m² BIW D1&3		Cohort 8 70 mg/m² BIW D1&2		Cohort 9 70 mg/m² TIW		Overall	
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**
	n = 5		*n* = 4		*n* = 6		*n* = 3		*n* = 3		*n* = 3		*n* = 5		*n* = 6		*n* = 3		*N* = 38	
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)
Anemia	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)	—
Hypoalbuminemia	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)	—
Diarrhea	—	—	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/ Etiology	Previous therapy	Metastatic disease	Therapy 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	72 yrs, F, NAFLD	TACE radiotherapy (SIRT) ICB (anti-PD-1) FGFR inhibitor	Lung and acetabulum	No	8	No	SD—ongoing for 4 months	Regorafenib
98 mg/m^2 QW	67 yrs, M, HepC	TACE ICB (anti-PDL1)	Lung	TACE	2	No	CR—ongoing for 12 months	Sorafenib
130 mg/m^2 QW	59 yrs, M, HepB	TACE surgery ICB (anti-CTLA-4 + anti-PDL1)	Supraclavicular lymph node	No	2	No	PR for 2 months then PD	Lenvatinib
130 mg/m^2 QW	61 yrs, M, HepB	Ablative therapy TACE DOXO	No	TACE	2	No	CR—ongoing for 9 months	Sorafenib
70 mg/m^2 BIW (Day 1&2)	61 yrs, M, HepB/C	Surgery ablative therapy TACE DOXO	No	No	2	No	CR—ongoing for 7 months	Sorafenib
98 mg/m^2 QW	76 yrs, F, NAFLD	Surgery TACE TKI (sorafenib) ICB (anti–PD-1) FGFR inhibitor	Lung	No	8	Yes	SD—ongoing for 2 months	Regorafenib
70 mg/m^2 BIW (Day 1&2)	73 yrs, M, HepB	TKI (lenvatinib)	Para-aortic lymph node	No	2	Yes	PD—after 2 months	Sorafenib

Abbreviations: DOXO, doxorubicin; ICB, immune-checkpoint blockade; SIRT, selective internal radiation therapy.

with ablative therapy, TACE, and doxorubicin who progressed after two cycles of MTL-CEBPA (during which time he developed lung metastases) and was treated with sorafenib. He has shown a complete radiological response to both lung and liver lesions 1 month after TKI treatment which is sustained on follow-up for 7 months. CT images of the lung lesions are shown in Supplementary Fig. S5. 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-month follow-up; however, regorafenib was then discontinued due to toxicity.

Discussion

This first-in-human and first-in-class multicenter phase I dose and dose-escalation study of the RNA oligonucleotide MTL-CEBPA has shown the drug to be well tolerated with no MTD reached. Based on a combination of safety, PK, and pharmacodynamics, the recommended dose of MTL-CEPBA for further evaluation is 130 mg/m^2 QW. The toxicity profile was favorable and comparable with the other drugs used in this patient population including sorafenib (3), regorafenib (5), and nivolumab (16). The nonlinear PK behavior of MTL-CEBPA is suggestive of a saturable capacity-limited tissue/cellular uptake process, dominant in the first 6 hours 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 antitumor activity with a mean progression-free survival of 4.6 months in pretreated patients, despite a relatively modest overall response rate 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 tumor. KRAS mutations are known to be associated with a protumor inflammatory microenvironment through activation of NF-κB and IL22 (17) as well as IL6 (18) signaling, 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 five patients with lung metastases (19). In the original trial that led to the approval of sorafenib, out of 299 patients randomized to the drug, there were no complete responses and only two 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. In addition, 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 subpopulations of WBC results in a tumor microenvironment which is

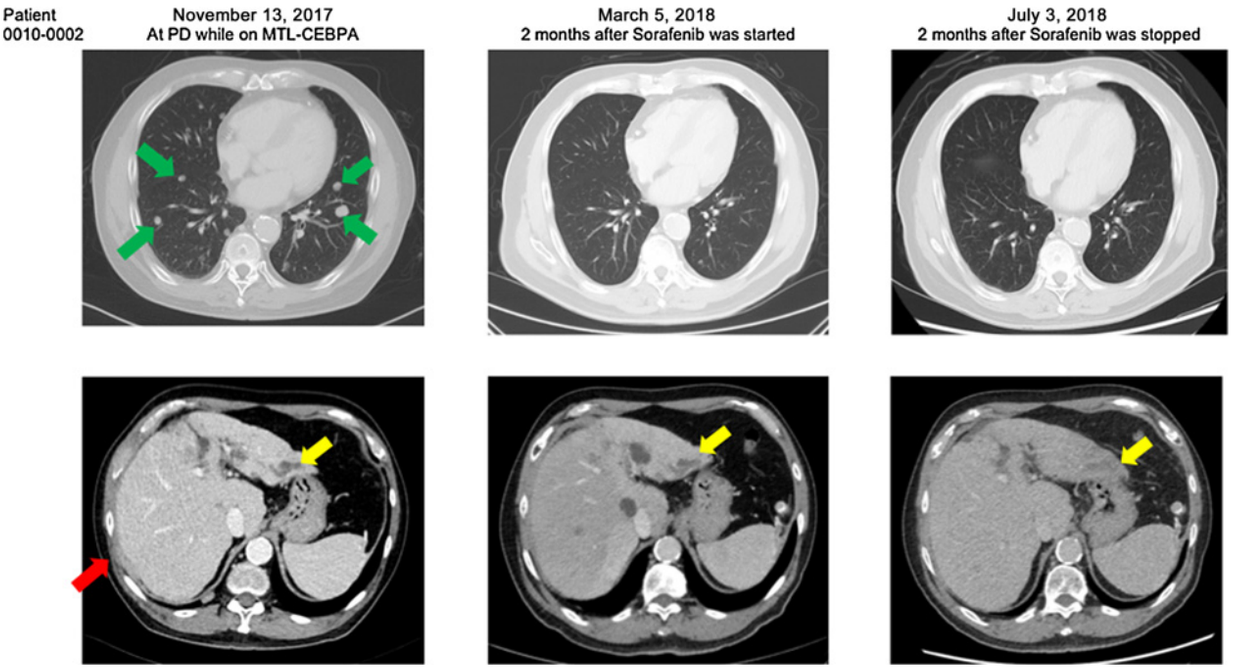

Figure 3.

Radiological response in liver and lungs. Red arrow, peritoneal metastasis/hepatic extension (which was irradiated on July 14, 2018, due to intrahepatic bleed and severe pain). Yellow arrow, HCC. Green arrows, Lung mets which were no longer present on March 5, 2018 (2 months after was started sorafenib) and July 3, 2018 (2 months after sorafenib was stopped).

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 tumors, 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 tumor dissemination (21), highlighting the importance and plasticity of these cells in cancer progression.

In an HCC preclinical mouse model, Zhou and colleagues have shown that TANs recruit macrophages and regulatory T cells to HCCs to promote their growth, progression, and resistance to sorafenib (22). Chang and colleagues (23) observed that tumor-infiltrating $Ly6G^+$ myeloid-derived suppressor cells (MDSC) and other immune suppressors were increased in orthotopic liver tumors using a syngeneic mouse liver cancer cell line. They found that tumor-infiltrating $Ly6G^+$ MDSCs of sorafenib-treated tumors significantly induced IL10 and TGFβ expressing $CD4^+$ T cells and downregulated the cytotoxic activity of $CD8^+$ T cells. The combination of anti-Ly6G antibody or anti-IL6 antibody with sorafenib significantly reduced the cell proportion of $Ly6G^+$ MDSCs in orthotopic liver tumors, enhanced T-cell proliferation, and improved the therapeutic effect of sorafenib. They concluded that modulating the tumor microenvironment through targeting tumor-infiltrating $Ly6G^+$ MDSCs represents a strategy to improve the oncological efficacy of sorafenib (23).

The C/EBP-α transcription factor is known to regulate multiple cellular pathways relevant to HCC. Deregulation of C/EBP-α expression has been reported in a variety of human cancers, and in HCC, C/EBP-α 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 upregulation by saRNA inhibits tumor growth in multiple tumor models (9, 11, 15). It is well described that C/EBP-α regulates hematopoiesis by inducing myeloid differentiation. It has been observed that myeloid lineage–specific deletion of C/EBP-α results in significantly enhanced MDSC proliferation and expansion, as well as an increase of myeloid progenitors and a decrease of mature cells. Deletion of C/EBP-α in MDSCs enhanced the pro-angiogenic, immune suppressive, and protumorigenic behavior of these cells by upregulating the production of inducible nitric oxide synthase and arginase, as well as MMP-9 and VEGF (10). In this study, we found a consistent and reversible increase in white cell count and neutrophils in keeping with the hypothesis that peripheral blood mononuclear cell 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 toward immunosuppressive HCC microenvironment during Sorafenib treatment and that it is also associated with antivascular and antimetastatic effects and HCC progression delay (25).

We hypothesize that pretreatment of the HCC tumor microenvironment with MTL-CEBPA renders it more susceptible to the effect of TKIs and based on the proposed mechanism and preclinical 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.

Disclosure of Potential Conflicts of Interest

D. Sarker is an employee/paid consultant for Eisai, Novartis, Ipsen, and Surface Oncology, reports receiving speakers bureau honoraria from Eisai, MSD, and Bayer, and other remuneration from MiNA Therapeutics. T. Meyer is an employee/paid consultant for AstraZeneca, Eisai, Roche, BTG, and Ipsen. M. Sodergren reports receiving commercial research grants from MiNA Therapeutics. B. Basu is an advisory board member/unpaid consultant for Eisai and reports receiving other remuneration from Bayer PlC. D.H. Palmer is an employee/paid consultant for Bayer, Eisai, Bristol-Myers Squibb, AstraZeneca, Sirtex, Roche, and Nucana, and reports receiving commercial research grants from Bristol-Myers Squibb, AstraZeneca, Sirtex, BTG, and Nucana. K.W. Huang holds ownership interest in MiNA Therapeutics. Y.T. Ma is an employee/paid consultant for Bayer and Eisai. T.R.J. Evans reports receiving commercial research grants from Beigene, Bayer, AstraZeneca, Bristol-Myers Squibb, MiNA Therapeutics, Roche, Eisai, MSD, and Medivir, speakers bureau honoraria from Bristol-Myers Squibb, Eisai, MSD, and Medivir, and is an advisory board member/unpaid consultant for Roche, Eisai, MSD, Medivir, and Bristol-Myers Squibb. R. Sharma reports receiving other commercial research support from Incyte Pharmaceuticals and Boston Scientific, and speakers bureau honoraria from Roche and Bayer. D.J. Pinato is an employee/paid consultant for MiNA Therapeutics, Roche, AstraZeneca, Eisai, reports receiving commercial research grants from Bristol-Myers Squibb and MSD, and speakers bureau honoraria from Roche and Bayer. J.P. Nicholls, J. Voutila, R. Habib, and D.C. Blakey are employees/paid consultants for and hold ownership interest (including patents) in MiNA Therapeutics Ltd. D. Collin, R. Nutbrown, H. Glenny, S. Fairbairn, and H.E. Huber are employees/paid consultants for MiNA Therapeutics Ltd. V. Reebye reports receiving other remuneration from MiNA Therapeutics Ltd. P. Lloyd is an employee/paid consultant for KinDyn Consulting Ltd. S. Felstead is an employee/paid consultant for Deljay Consulting Limited. P. Saetrom reports receiving commercial research grants from, holds ownership interest (including patents) in, and is an advisory board member/unpaid consultant for MiNA Therapeutics Ltd. N. Habib is an employee/paid consultant for and reports receiving commercial research grants from MiNA Therapeutics Ltd. No potential conflicts of interest were disclosed by the other authors.

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