Targeting myeloid-derived suppressor cells and T cells: combination treatment with MTL-CEBPA and PD-1 antibody in a mouse syngeneic CT26 model

#3089

Mikael H Sodergren[1,3], Choon Ping Tan[2,3], Vikash Reebye[1], Robert Habib[2], David Blakey[1] and Nagy Habib[2]

[1]Department of Surgery, Imperial College London, UK; [2]MiNA Therapeutics, London, UK; [3]joint first authors

Background

- CCAAT/Enhancer-Binding Protein alpha (CEBPA) is a transcription factor governing myeloid development. CEBPA is downregulated in several solid tumours and CEBPA knockout mice display greater myeloid-derived suppressor cell tumour infiltration, vascularisation and tumour growth[1].
- MTL-CEBPA is the clinical candidate comprising of CEBPA-51, a small RNA duplex designed to activate endogenous CEBPA expression, encapsulated in a NOV340 liposome[2]. MTL-CEBPA treatment reverses rodent models of cirrhosis, fibrosis, hepatosteatosis and significantly reduces tumour burden in cirrhotic hepatocellular carcinoma[3].
- MTL-CEBPA is the first small activating RNA (saRNA) therapy and the first drug targeting CEBPA to enter clinical trials. Clinically, MTL-CEBPA is observed to alter the ratio of myeloid populations.
- We hypothesise that targeting myeloid-derived suppressor cells (MDSCs) with MTL-CEBPA and T cells with PD-1 antibody may have complementary effects on the tumour immune microenvironment and thereby enhance the therapeutic efficacy of each individual therapy.

Here we demonstrate the outcome of combining MTL-CEBPA with anti-PD-1 treatment in a mouse syngeneic CT26 model. Mice were implanted with CT26 tumour cells and were treated with MTL-CEBPA, anti-PD-1 or a combination of both therapies over 3 weeks.

Methods

Female Balb/c mice (6 weeks old) were transplanted subcutaneously with $5x10^5$ CT26.WT cells/mouse with the order of cell implant randomized by box. One day after implantation, mice were allocated to one of the following experimental groups:

Groups - 10 animals per group:

1. Control group
2. MTL-CEBPA - 5mg/kg, i.v, d1/d3 schedule, 7 doses
3. anti-PD1 - 10mg/kg, i.p., d1/d4 schedule, 7 doses
4. anti-PD1+ MTL-CEBPA – refer to group 2 and 3 for dosage and treatment schedule

Tumour volumes were monitored for 23 days and RNA extracted from tumours at termination were analysed by nanostring Mouse 360 IO codeset (LBL-10545-01) and mouse myeloid innate Immunity codeset (LBL-10398-02).

Results

1. Tumour volume

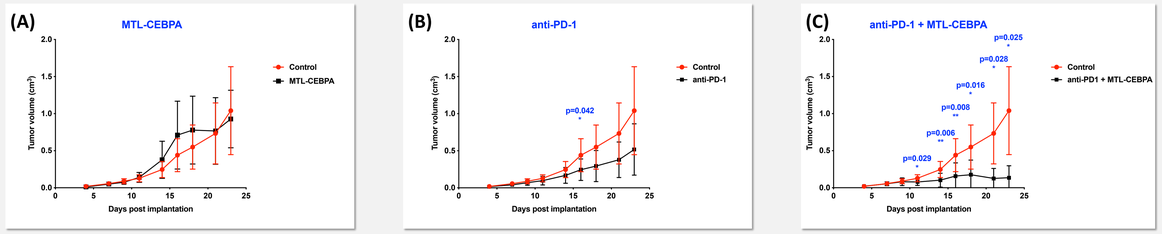

Figure 1. Combination of anti-PD-1 and MTL-CEBPA synergistically reduce CT26 tumor volume

Changes in tumour volume from day 4 to the end of study at day 23. Mean tumour volume of (A) MTL-CEBPA, (B) anti-PD-1 and (C) anti-PD-1 + MTL-CEBPA treated animals were compared against the control group. (red line). Statistical significance is calculated by unpaired t test with Welch's correction. * p< 0.05 and ** p < 0.01.

2. Gene expression by treatment groups

Genes	anti-PD-1	MTL-CEBPA	anti-PD-1 + MTL-CEBPA
Cd4	1.81	1.53	7.63 (*)
Cd8a	1.29	1.22	3.68 (*)
Cd8b1	1.51 (*)	1.02	4.62 (*)
Cd3e	1.22	1.11	4.48 (*)
Gzma	1.38	1.14	2.39 (*)
Gzmb	1.76	1.51 (*)	3.86
Ifng	2.20	1.24	4.71 (**)
Vegfa	1.18	1.04	0.94
Mki67	1.00	0.84 (**)	0.62 (**)

Table 1. Tumours from anti-PD-1 + MTL-CEBPA treated mice display enhanced anti-tumour gene expression profile

RNA extracted from tumours were subjected to nanostring analysis. Expression levels of genes from each group were compared against a control group.

Individual genes that show synergistic effect of anti-PD-1 and MTL-CEBPA are tabulated. Values are calculated as fold versus vehicle control. Statistical significance is calculated by unpaired t test with Welch's correction. * p < 0.05 and ** p < 0.01

3. Correlating gene expression with tumour progression

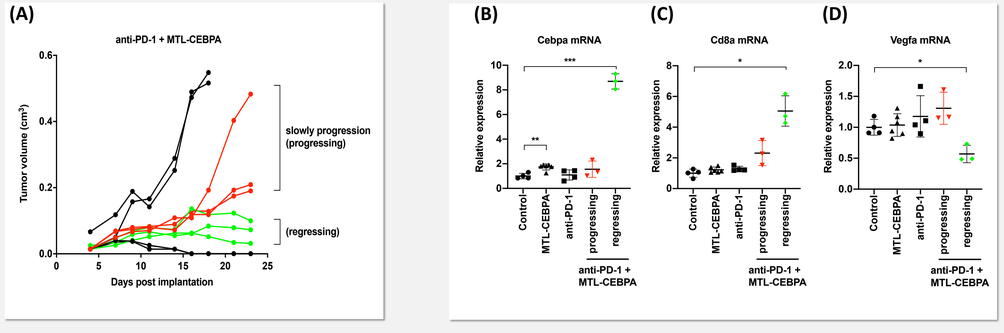

Figure 2. Expression of genes that correlate with favourable disease outcome in anti-PD-1 + MTL-CEBPA treated group

(A) Further classification of tumour samples from the anti-PD-1 + MTL-CEBPA group for nanostring analysis, selected regressing tumour subgroups are indicated with the green lines and slowly progressing tumour subgroups are indicated with the red lines.

Relative expression of (B) Cebpa, (C) Cd8a and (D) Vegfa mRNA from progressing and regressing tumour subgroup in (A) were compared to all other groups. Statistical significance is calculated by unpaired t test with Welch's correction. * p< 0.05 and ** p < 0.01.

4. Tumour infiltrating lymphocytes

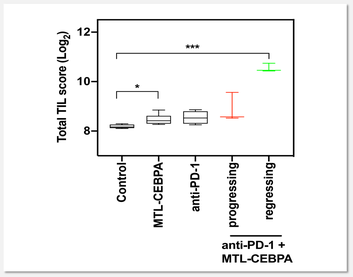

Figure 3 – Tumour regressing subgroup of anti-PD-1 + MTL-CEBPA treated mice have increased tumour infiltrating lymphocytes

Tumour infiltrating lymphocyte score for all treatment groups reported by mouse I/O 360 nanostring codeset. Statistical significance is calculated by unpaired t test with Welch's correction. * p< 0.05 and *** p < 0.001.

Discussion

The results here indicate that anti-PD-1 combined with MTL-CEBPA significantly inhibits tumour growth when compared to single agent treatment. Anti-tumour gene transcriptional profiles were more prominent in the anti-PD-1 + MTL-CEBPA combination group and correlated more strongly with regressing tumours. Increase in tumour infiltrating lymphocytes was also observed when mice were treated with anti-PD-1 + MTL-CEBPA.

These results suggest that MTL-CEBPA improved the efficacy of anti-PD-1 in a syngeneic CT26 model, indicating a promising therapeutic option for therapies relying on anti-PD-1.

References

1. Mackert, J. R. et al. Dual negative roles of C/EBPα in the expansion and pro-tumor functions of MDSCs. Sci Rep 7, 14048 (2017)
2. Voutila, J. et al. Development and Mechanism of Small Activating RNA Targeting CEBPA, a Novel Therapeutic in Clinical Trials for Liver Cancer. Mol Ther 25, 2705 (2017)
3. Reebye, V. et al. Gene activation of CEBPA using saRNA: preclinical studies of the first in human saRNA drug candidate for liver cancer. Oncogene 375, 767 (2018).

Conflict of interest

CPT, VR, RH DB and NH are employees of MiNA Therapeutics.
MHS Receives a research grant from MiNA Therapeutics.

Contact

For partnership enquiries, please contact bd@minatx.com