#B029

CM24, a Novel Anti-CEACAM1 mAb, Suppresses Neutrophil Extracellular Trap (NET)-Induced Migration and Metastasis of Cancer Cells

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BACKGROUND

- Neutrophil Extracellular Traps (NETs) are web-like DNA structures decorated with histones, proteases, and cytotoxic proteins such as myeloperoxidase (MPO) released by activated neutrophils
- NETs are involved in cancer immunoediting, progression, metastatic spread and play a key role in the tumor microenvironment
- Targeting NETs is a valid strategy and may serve as a novel treatment modality in cancer therapeutics
- CEACAM1 is involved in tumor immune evasion and metastasis and was recently proposed to be a part of the NET structure
- In this study, we present for the first time the NET-related activity of CM24, a novel anti-CEACAM1 mAb. We demonstrate that CM24 binds to the NET structure, and significantly suppresses the NET-induced migration of various cancer cells. This novel mechanism supports the inhibition of cancer metastasis by CM24 in mouse models

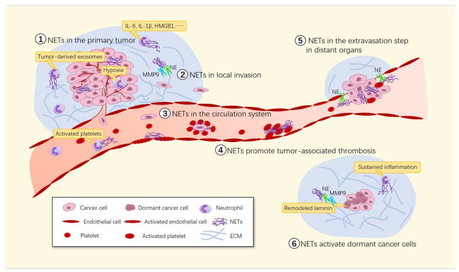

Figure 1. Involvement of NETs in tumor metastasis and immune evasion (adapted from 'Chen, Q et al. Cancers 2021, 13, 2832')

RESULTS

CEACAM1 is Highly Expressed on Tumor Infiltrating CD11b+ Myeloid Cells

Downregulation by CM24 in animal models

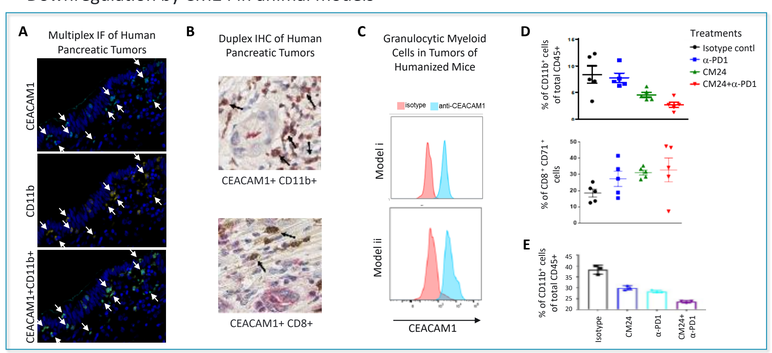

Figure 2: CEACAM1 expression profile in tumor-infiltrating immune cells (A-C) and the *in-vivo* effect of CM24 (D-E) (A) Multiplex IF staining of human pancreatic tumor microarray showed enhanced expression of CEACAM1 on the myeloid immune subset; DAPI (blue), CEACAM1 (light blue), CD11b (yellow). (B) Duplex staining for CEACAM1 (red, MRG1) and TIL markers (brown) for myeloid cells (CD11b) and cytotoxic T cells (CD8) in human pancreatic cancer samples. (C) FACS analyses of human melanoma (i) and NSCLC (ii) tumors of humanized mouse models, using anti-CEACAM1 (MRG1), demonstrates high expression of CEACAM1 on granulocytic myeloid cells; Adapted from Barbara Joyce Shaikh (Merck) 'Human Immune System (HIS) mouse models for translational research. (D) In a Hu-CD34+ NSG mouse model of human pancreatic tumors, FACS analysis demonstrated a decrease in CD11b+ population and CD8+ cell activation in the tumors, following treatment of the mice with CM24. The combination with anti-PD1 enhanced the effects. (E) The same effect was demonstrated in human melanoma mouse model.

Enhanced Expression of CEACAM1 on both Tumor Cells and Tumor-Infiltrating Immune Cells

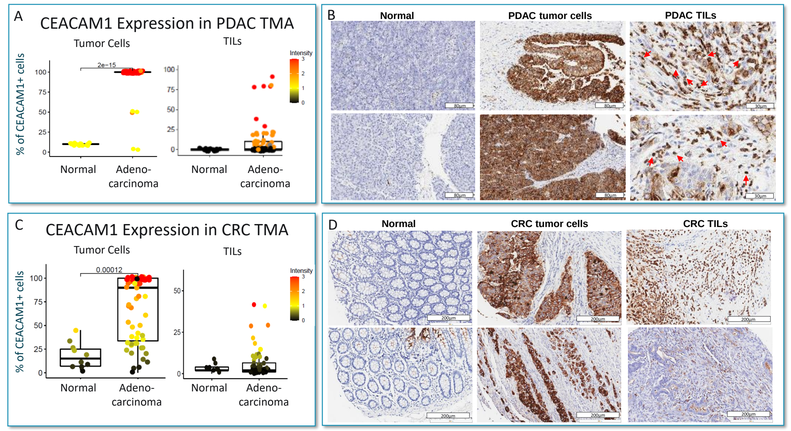

Figure 3. CEACAM1 immunostaining in tumor microarrays (TMA) of pancreatic cancer (A,B) and colorectal cancer (C,D) showed enhanced expression of CEACAM1 on both the tumor cells and tumor infiltrating leukocytes (TILs) as compared to normal tissues. Red arrows indicate CEACAM1+ Tumor-associated myeloid cells, including neutrophils (TANs). % CEACAM1 positive cells and CEACAM1 intensity analysis included TMA of 38 cases/76 cores of PDAC and 10 cases/20 cores of normal pancreatic tissues (A), as well as TMA of 67 cases of CRC and 10 cases of normal colorectal tissues (C).

Direct Binding of CM24 to the NET Structure

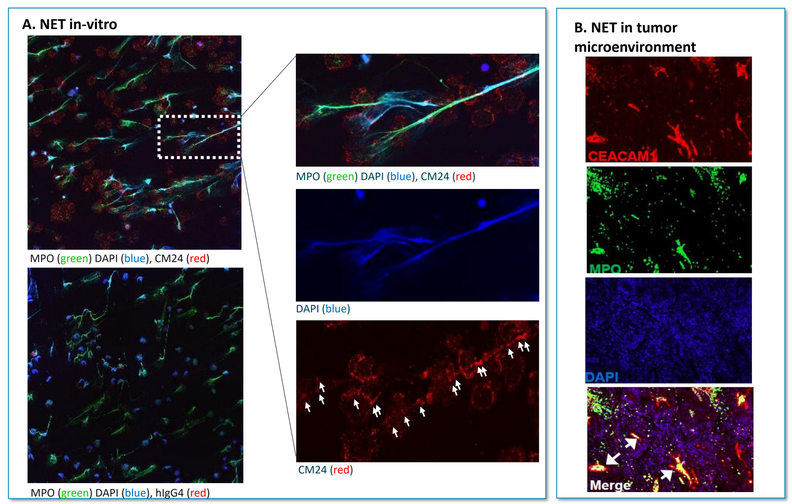

Figure 4. Co-localization of CEACAM1, extracellular DNA and MPO (myeloperoxidase, NET marker) in-vitro (A) and in tumors (B) . Fresh Neutrophils were stimulated by PMA to induce NETosis, fixed and stained as indicated above, and imaged using confocal microscopy (A). Multiplex IF staining of tumors derived from a humanized mouse model demonstrates a similar pattern (B).

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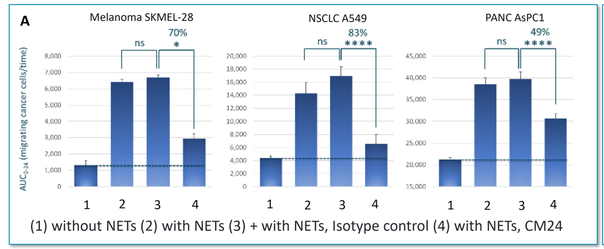
CM24 Inhibits NET-Induced Migration of CEACAM1 Expressing Cancer Cells



	CEACAM1+ cells (%)	CEACAM1 MFI
SKMEL-28	84.8	48,115
A549	73.1	566
AsPC1	99.9	11,688

Figure 5. In-vitro NET-promoting cancer cell migration is suppressed by CM24. (A) NET-induced migration was defined by measuring fluorescent cancer cell migration following NET addition [(2) vs (1)]. Human cancer cells were treated with CM24, isotype control, or left untreated in a serum-free medium with or without NETs. Serum was used as the chemotactic stimulus for cancer cell migration in a transwell Boyden chamber. Fluorescence of the migrating cells was measured every 2hr for 24hr and the mean AUC ± SEM is presented. Statistical significance was shown by Two-way ANOVA with multiple comparisons **** p-value < 0.0001, * p-value < 0.05; (B) CEACAM1 Expression was verified in the cancer cells used for migration. % CEACAM1-positive cells and the intensity of CEACAM1 expression was tested using flow cytometry with MRG1 anti-CEACAM1 Ab.

CM24 Suppressed Metastasis in a Xenograft Lung Lesion Model

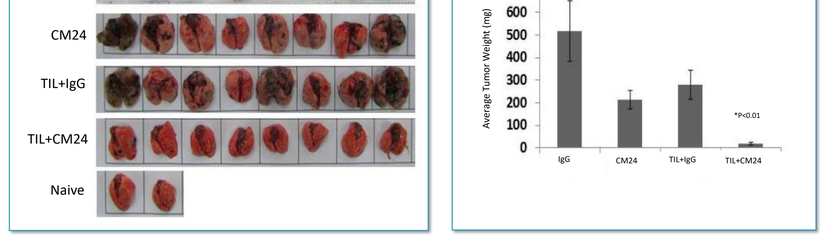


Figure 6. CM24 demonstrated anti-metastatic activity in human melanoma xenograft lung lesion model. SCID-NOD mice were engrafted with human melanoma MEL 526 cells (IV, 5M cells/mouse) and were treated as indicated above for 44 days. Antibodies were injected (IV, 250µg/mouse) twice a week, and TILs were administered (IV, 10M cells/mouse) at days 2,12,22,32. Mice were sacrificed on day 49, and the lungs were removed, photographed (A) and weighed (B).

CONCLUSIONS

- By blocking CEACAM1, CM24 inhibits NET-mediated cancer cell migration in-vitro and metastasis in-vivo
- This novel MOA provides a rationale for the anti-cancer activity of CM24 and may contribute to improving patient outcomes.
- Inhibiting NETs may provide a novel therapeutic approach as an adjuvant treatment with CM24 to prevent metastasis.
- Clinical studies to evaluate CM24, anti-CEACAM1 mAb, and efficacy are being conducted in PDAC patients.

The study is sponsored by Purple Biotech Ltd; no conflicts of interest to declare