Effective treatment of colorectal peritoneal metastases by exploiting a molecular subtype specific vulnerability

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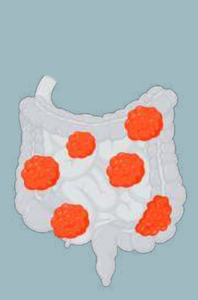
Background & Aim

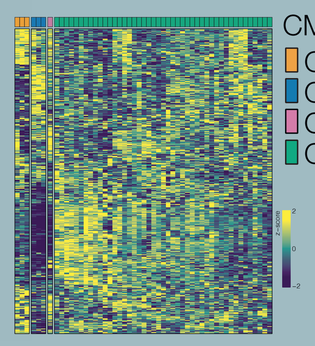
In colorectal cancer (CRC), peritoneal metastases (PMs) associate with severe morbidity and dismal prognosis. Given the incidence of this disease and the lack of adequate treatments currently available, PMs pose a large unmet clinical need. Although PMs can be accompanied by more widespread metastatic disease, it often occurs as the only sign of dissemination. This implies that the route of metastatic spread to the peritoneum differs from that to distant organs. PMs are thought to result from cancer cells that spill into the abdominal cavity, and are able to attach to the peritoneal lining and form tumor deposits. This cascade places specific demands on the cancer cells.

- Improve molecular understanding of CRC PM
- Identify therapeutic vulnerabilities
- Test new, effective treatment strategies

Methods

Molecular profiling of patient samples





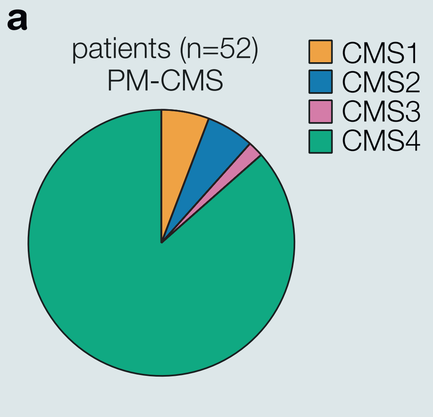
- 52 patients with CRC PM
- RNAseq of peritoneal lesions
- CMS subtyping

Identification of effective treatment strategies

Drug screen database (GDSC) → **IC50 CMS2 vs. CMS4 cell lines** → **In vitro & in vivo validation**

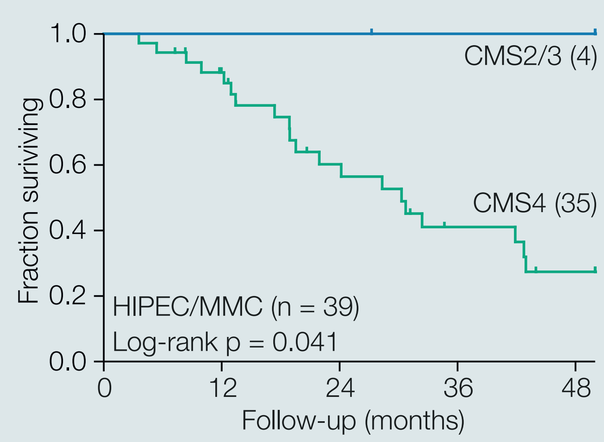
Results

1 Peritoneal metastases represent CMS4 colorectal cancer



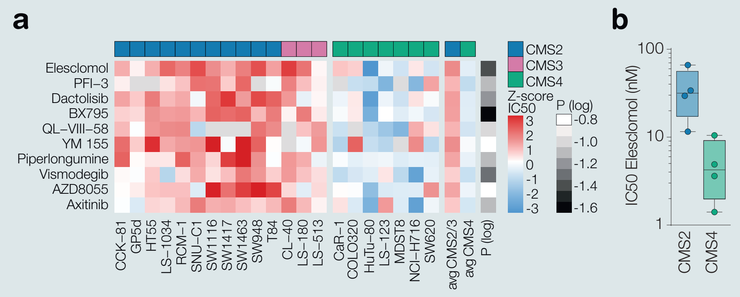
a. Distribution of CMS subtypes amongst peritoneal metastasis tissue samples.
b. Percentage of patients in the AMC-AJCCII-90 dataset with primary CRC that developed PM, displayed per CMS subtype.

2 Current HIPEC treatment is of limited efficacy in CMS4 PM



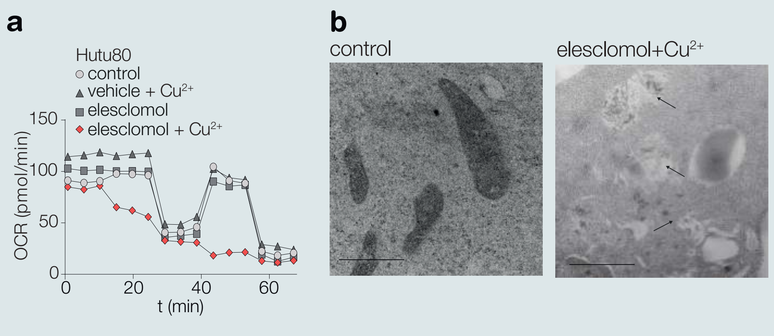
Prognostic value of CMS2/3 (blue) and CMS4 (green) for overall survival in the HIPEC/MMC treated patients, analyzed by Kaplan-Meier survival analysis and log-rank testing.

3 Identification of copper ionophore elesclomol as CMS4 specific vulnerability



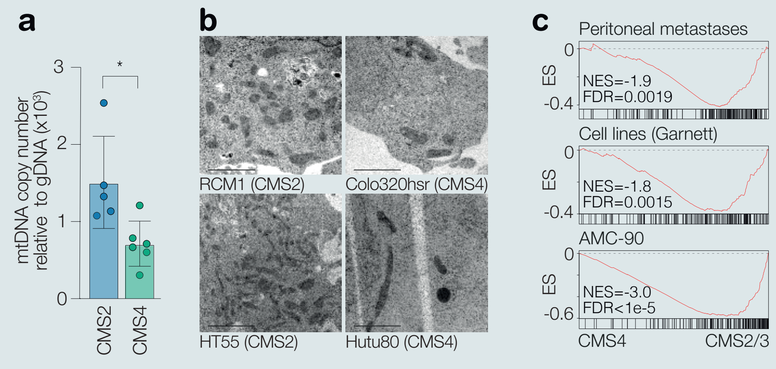
a. Heatmap showing the ΔZ-score in drug sensitivity (IC50) in colorectal cell lines and between CMS2&3 and CMS4. **b.** IC50 values of CMS2 and CMS4 cell lines treated with Elesclomol for 72 hours.

4 Elesclomol is a copper ionophore that targets mitochondria



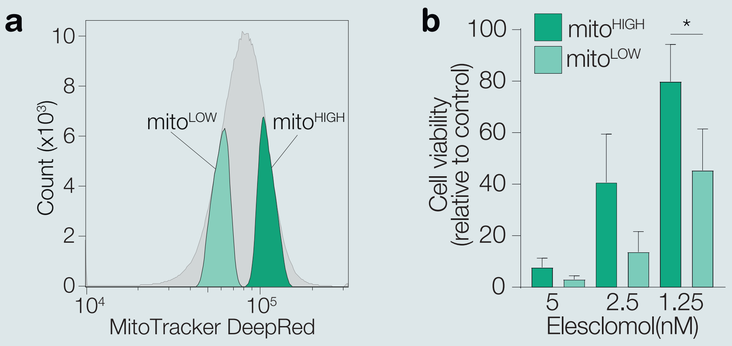
a. Seahorse flux analysis of Hutu-80 cells. Elesclomol +/- Cu^{2+} was added at 5μM at t=12 min by injection in the analyzer **b.** Transmission electron microscopy images of Hutu-80 cells after 2h exposure to control or 5μM Elesclomol:Cu^{2+}. Scale bars 2 μm.

5 Mesenchymal cancers have less mitochondria



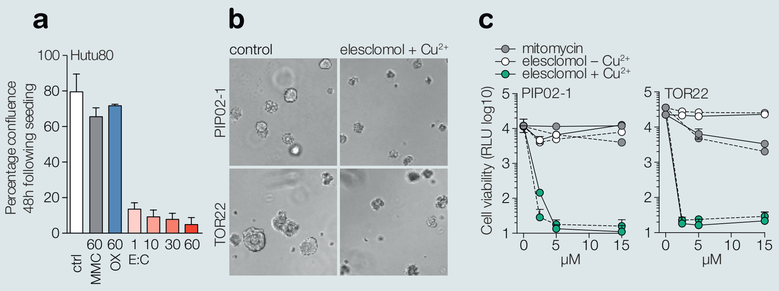
a. Mitochondrial abundance, quantified by mtDNA copy number in 11 cell lines.
b. EM images of CMS2 vs. CMS4 cell lines (scale bars: 2 μm) **c.** Geneset mRNA enrichment analysis depicting Oxidative Phosphorylation signature, comparing CMS4 vs. CMS2/3 in PM, cell lines and primary CRC patient dataset AMC90.

6 Low mitochondrial content confers vulnerability to elesclomol



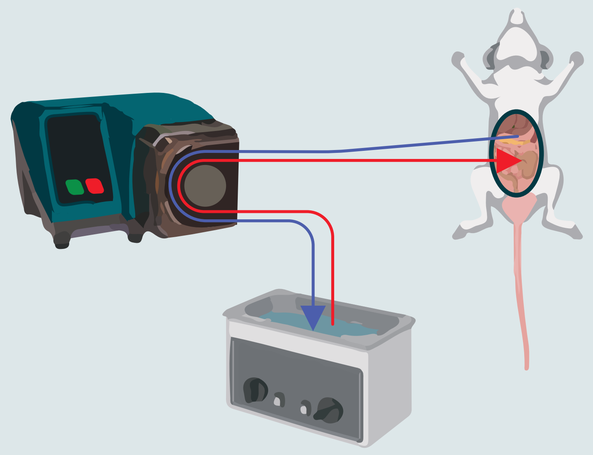
a. Histogram depicting the sorting strategy for figure. **b.** Bargraph depicting the IC50 values of Elesclomol in sorted populations ($mito^{LOW}$ and $mito^{HIGH}$) in CMS4 cell line Hutu-80, stained with MitoTracker DeepRed.

7 Short exposure of elesclomol is effective in CMS4 cell lines and PM organoids



a. Confluency after 1, 10, 30 and 60 minutes of exposure to Elesclomol-Cu^{2+} (2.5μM), 60 minutes Mitomycin C (2.5 μM) or 60 minutes Oxaliplatin (400 μM) **b + c.** PM-derived organoids were treated for 60 minutes with 5μM Elesclomol-Cu^{2+}.

8 Ongoing: in vivo HIPEC rat model



Schematic representation of the hyperthermic intraperitoneal chemotherapy (HIPEC) treatment model in small animals.

Conclusions

- Peritoneal metastases represent the CMS4 subtype
- Elesclomol targets a mitochondrial vulnerability in this subtype
- Elesclomol is effective after short exposure time
- Elesclomol is a promising candidate for the local treatment of PM

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