Nanometronomic treatment of breast cancer with Doxorubicin loaded H-Ferritin prevents drug resistance and circumvents cardiotoxicity
Cancer chemotherapy

Treatment of approximately 50% of human cancers includes the use of chemotherapy.
MTD vs. LDM
drug administration

MTD = maximum tolerated dose
LDM = low-dose metronomic

I. Kareva et al. Metronomic chemotherapy: An attractive alternative to maximum tolerated dose therapy that can activate anti-tumor immunity and minimize therapeutic resistance. *Cancer Lett.* 2015, **358**, 100-106
Anti-angiogenic mechanism of LDM

Metronomic chemotherapy: Possible new directions?

Adapted from:
N. André et al. Has the time come for metronomics in low-income and middle-income countries? *Lancet Oncol.* 2013, 14, e239-e248

“NANOMETRONOMIC” (LDNM) CHEMOTHERAPY
H-Ferritin nanocages (HFn)

- Easily produced as a recombinant protein in *E. coli*
- Polymer of 24 subunits of Heavy (H) or Light (L) ferritin chain which self-assembles in a cave sphere structure of 12 nm
- **Thermal** (≤70 °C for 15 min) and **chemical stability** (Denaturants such as urea or guanidinium chloride)
- Low immunogenicity and high stability in biological fluids
- **Controlled disassembly** (pH-dependent), which makes HFn easily loaded with drugs
- Recognizes with high specificity (95%) and high sensitivity (98%) the transferrin receptor 1 (TfR1), which is overexpressed by cancer cells

*Truffi M, Fiandra L, Sorrentino L, Monieri M, Corsi F, Mazzucchelli S. Pharmacological Research. 2016, accepted*
HFn promotes DOX nuclear translocation

HFn-DOX mediates self-triggered nuclear delivery of DOX increasing:
- Drug cellular uptake
- Nuclear accumulation
- Efficacy in blocking proliferation and in inducing cell death and DNA damage

HFn-DOX is a good candidate for LDNM chemotherapy?
HFn uptake in 4T1-L Breast Cancer cells

Murine 4T1-L cell line as breast cancer model:

- high level of proliferation, migration and invasiveness
- DOX-inducible expression of MDR-1 (or P-glycoprotein)
- stable luciferase expression

Dose-dependent recognition of tumor cells

Internalization:

- HFn was partly compartmentalized in early endosomes and partly free in the cytosol
- Absence of interaction with lysosomes, Golgi apparatus and recycling endosomes. HFn did not follow any lysosomal degradation, elimination or recycling
HFn activity in 4T1-L BC cells

- Free DOX reduced cell proliferation for 24 h only, consistent with onset of chemoresistance
- Proliferation was arrested for at least 72 h after treatment with HFn-DOX

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HFn in vitro:
- Improves chemotherapeutic efficacy
- Enhances tumor cell selectivity
- Circumvents MDR mechanisms

Good candidate for LDNM chemotherapy

15.2-fold higher concentration of DOX inside 4T1-L nucleus within 3 h (HFn-DOX vs free DOX)
AlexaFluor660-labeled HFn (5 μg kg⁻¹) i.v. injected by tail vein and imaged by live fluorescence

**Tumor targeting**

- Epifluorescence over time:
  - 1 h: Low fluorescence
  - 2 h: Increased fluorescence
  - 24 h: Highest fluorescence
  - 48 h: Decreasing fluorescence

- Averaged tumor Epif intensity (psec/cm²/μW/cm²) 10⁻⁷

**Biodistribution**

- Renal excretion:
  - 1 h: Minimal fluorescence
  - 2 h: Increased fluorescence
  - 24 h: Highest fluorescence
  - 48 h: Decreasing fluorescence

**Renal excretion**

- Urine mean fluorescence intensity (a.u.)
  - 1 h: High intensity
  - 2 h: Medium intensity
  - 24 h: High intensity
  - 48 h: Decreasing intensity
LDNM treatment of 4T1-L tumor bearing mice with HFn-DOX

4T1-L cells implanted at day 0

Our metronomic setting: drug administration = 1.24 mg DOX kg\(^{-1}\) at day 5, 9, 13 and 17

In vivo

HFn-DOX in metronomic setting significantly slow tumor progression increasing apoptosis in tumor tissue
LDNM inhibits neo-angiogenesis and prevents drug resistance

**Tumor angiogenesis**

Induction of MDR-1 expression

In vivo
LDNM overcomes DOX cardiotoxicity and systemic dysfunction

Cardiotoxicity: morphological evaluations

Cardiomyocyte area (pixels)

Transmission Electron Microscopy of Heart tissue

Cardiotoxicity: functional evaluations

Membrane potential (a.u.)

Hepatic and renal functionalities

HFn-DOX ~ placebo
Conclusions

IN SUMMARY...

Developed highly aggressive metastatic BC model based on murine 4T1-L cells to monitor tumor progression and spread

- DOX monotherapy **does not STOP** tumor progression
- LDNM chemotherapy ⇒ reappraised **key role of targeted action** on cancer cells?
  ⇒ metronomic administration associated with cell nucleus targeting could **circumvent DOX resistance** and **enhance cancer cell killing**
    - Mazzucchelli et al., manuscript in preparation
  ⇒ LDNM chemotherapy has the potential to combine the advantages of both MTD and LDM

PERSPECTIVES...

- elucidate the individual contributions of targeted therapy, immune system activation, and neo-angiogenesis inhibition in the strong enhancement of antitumor efficacy of HFn-DOX
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