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

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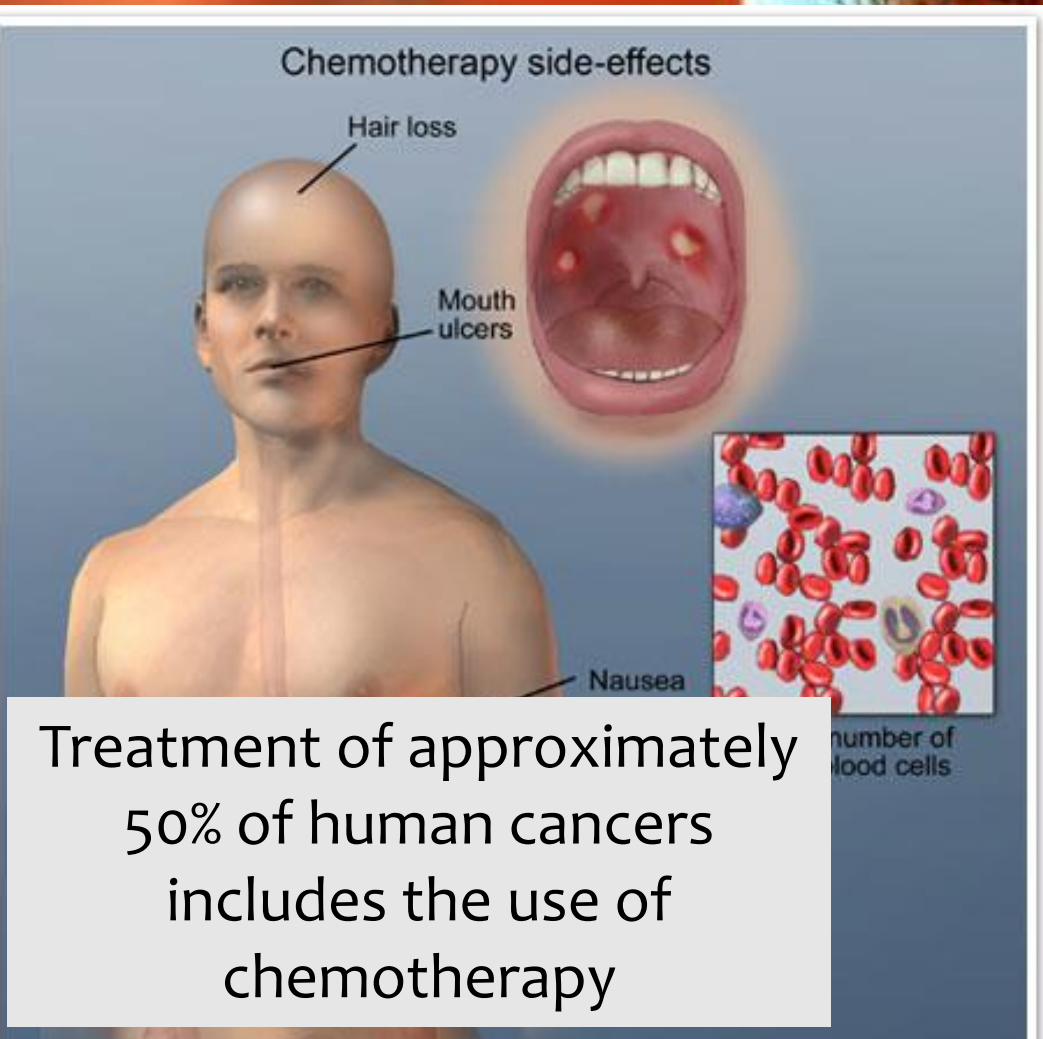
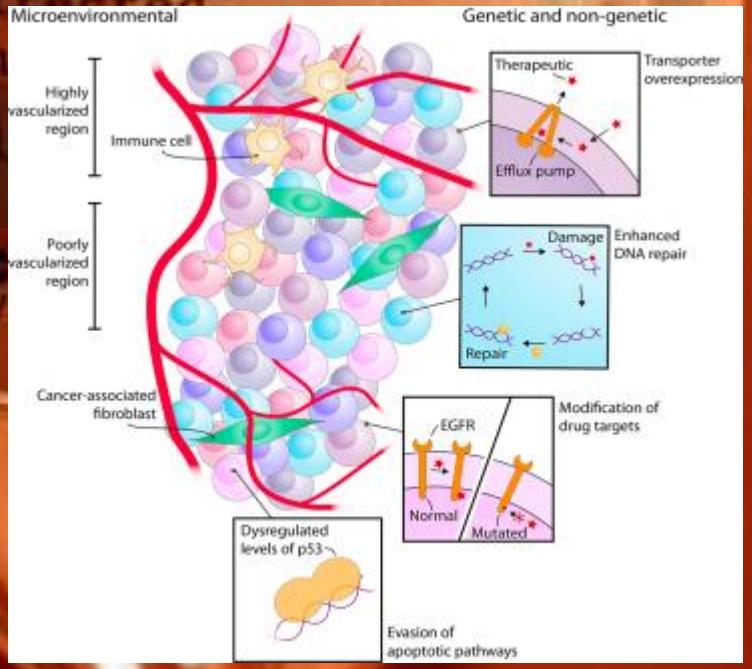
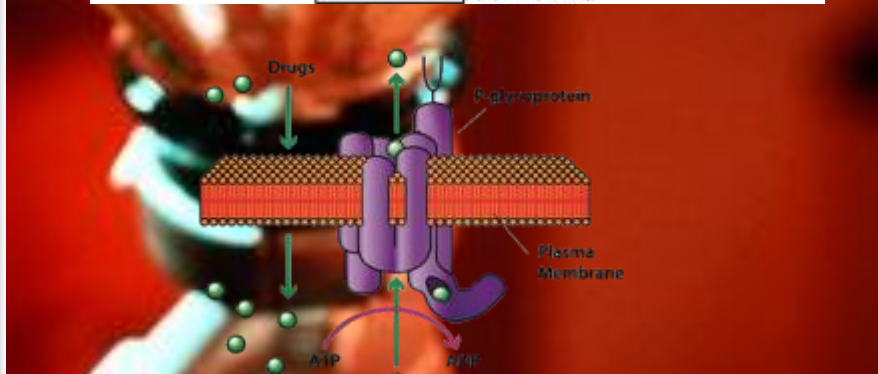
Cancer chemotherapy



Treatment of approximately 50% of human cancers includes the use of chemotherapy

MULTIDRUG RESISTANCE

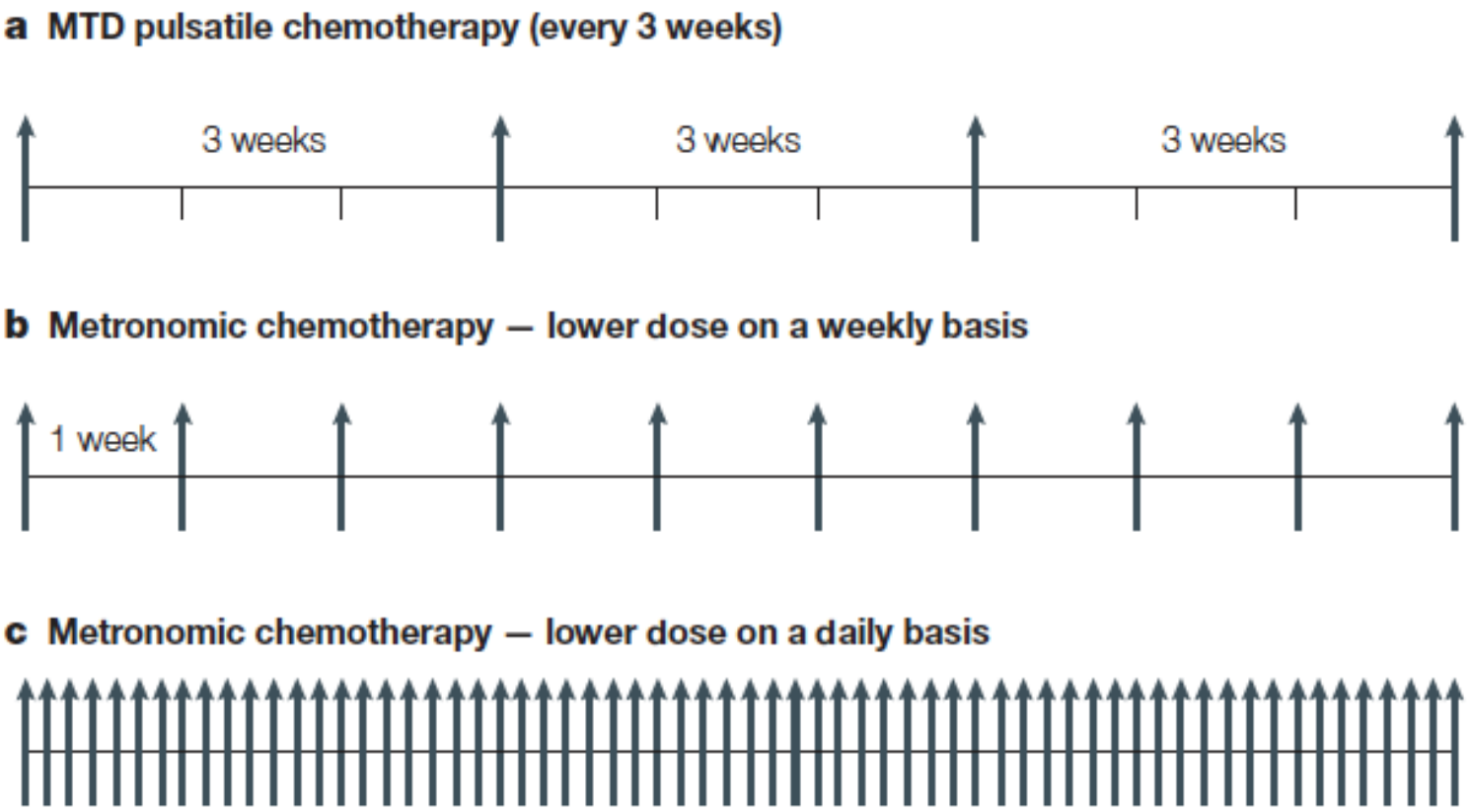




MTD *vs.* LDM drug administration

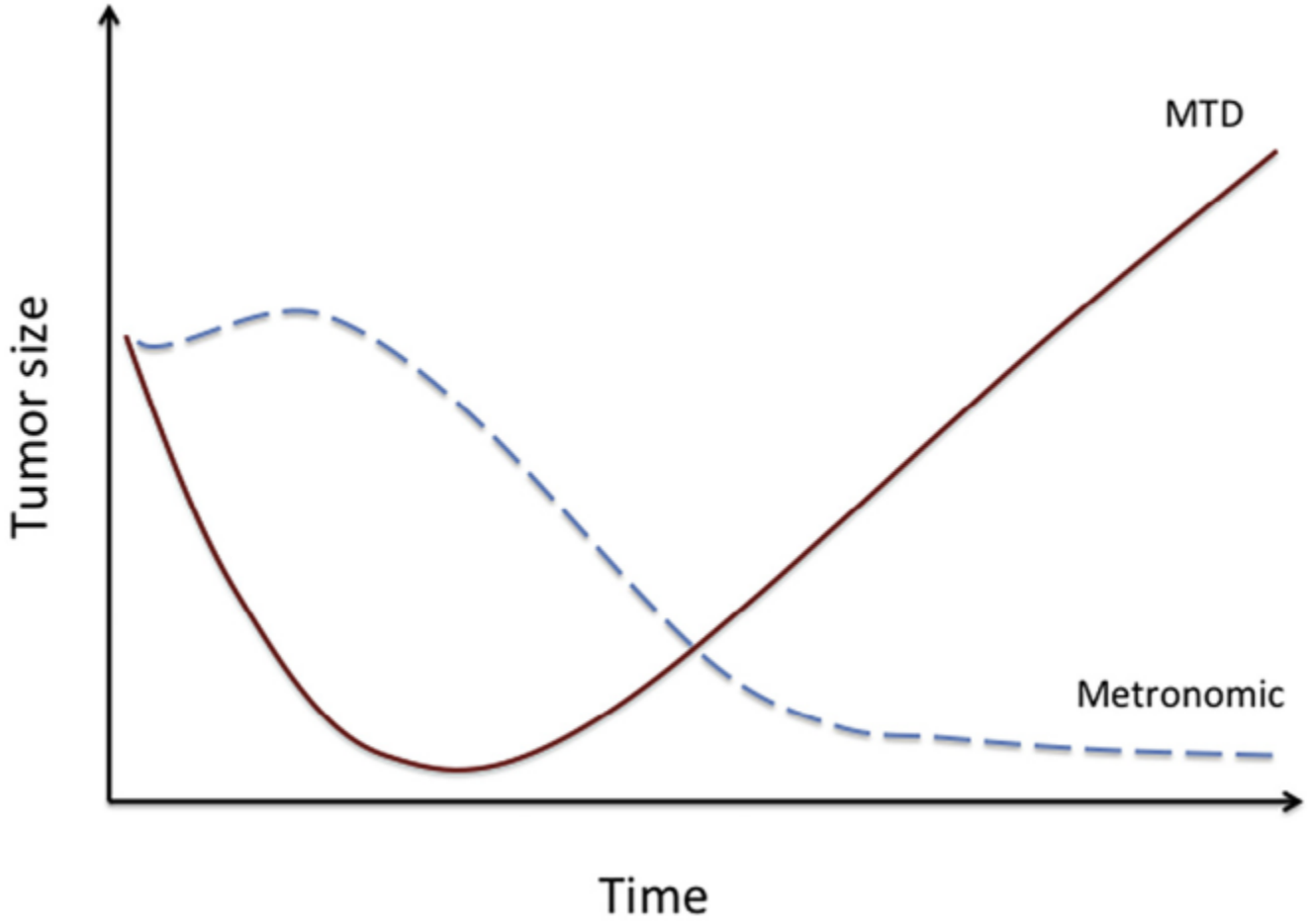
MTD = maximum tolerated dose

LDM = low-dose metronomic



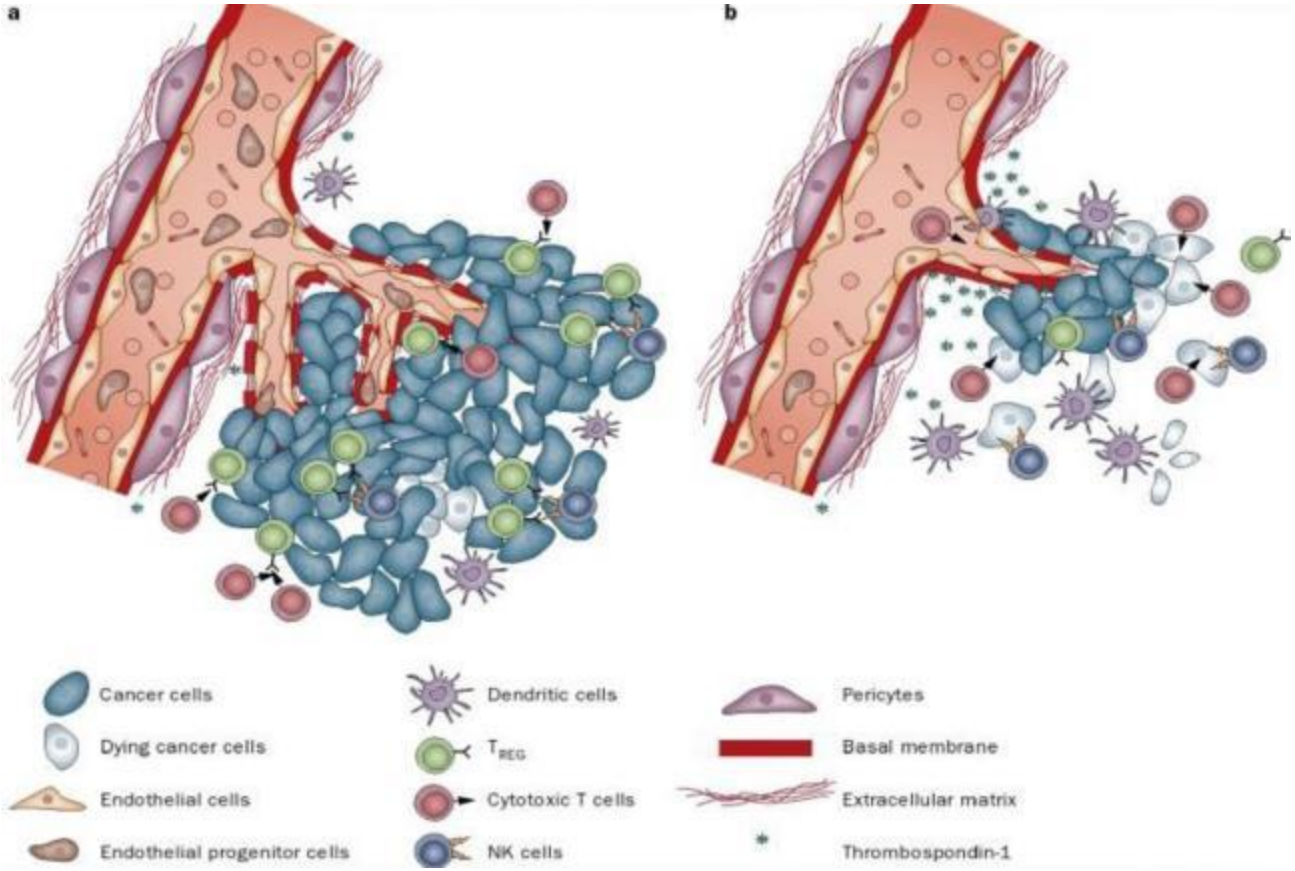
R. S. Kerbel et al. The anti-angiogenic basis of metronomic chemotherapy. *Nat. Rev. Cancer* 2004, 4, 423-436

MTD *vs.* LDM drug administration



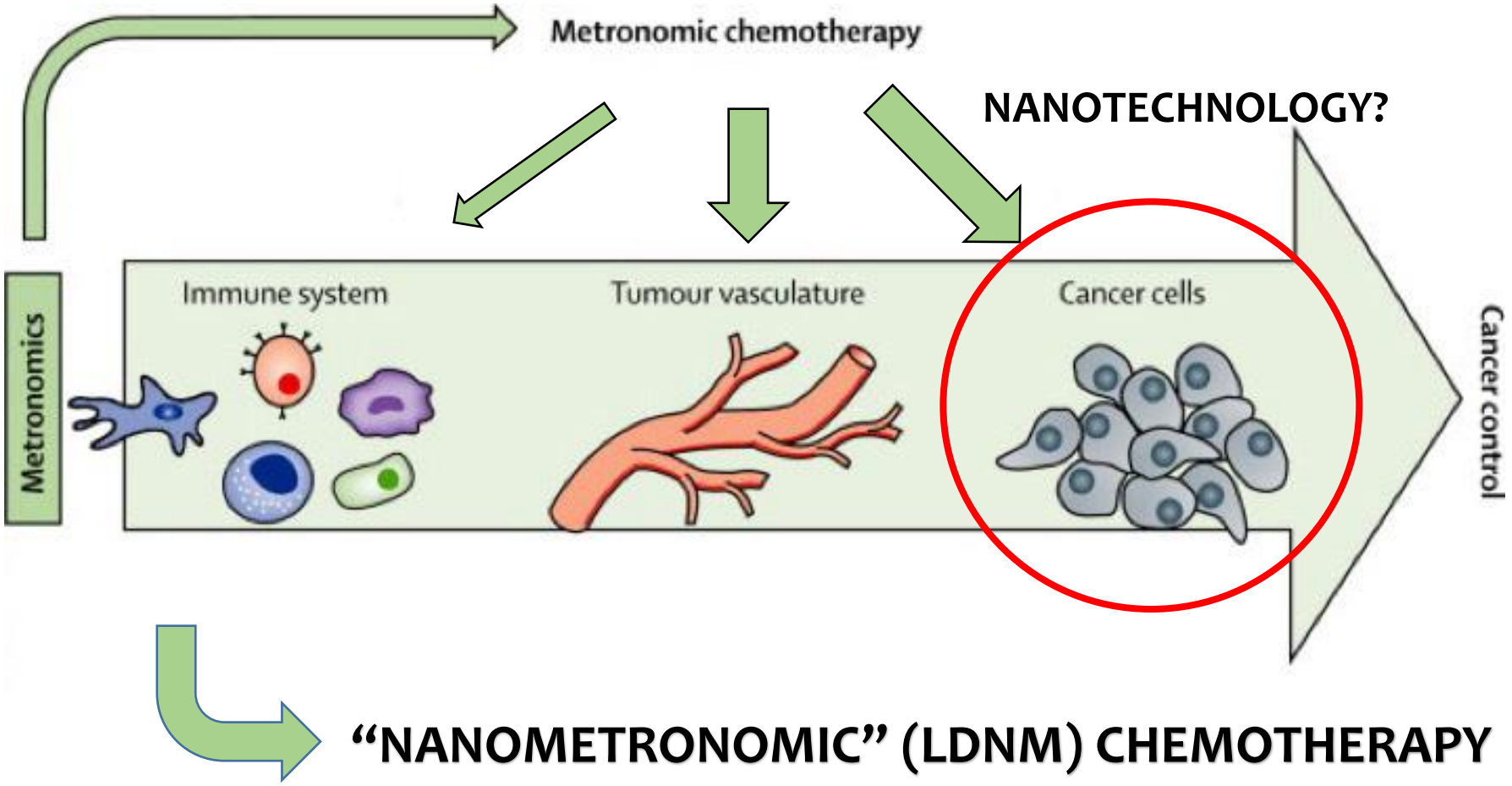
I. Kareva et al. Metronomic chemotherapy: An attractive alternative to maximum tolerated dose therapy that can activate anti-tumor immunity and minimize therapeutic resistance. *Cancer Lett*. 2015, *358*, 100-106

Anti-angiogenic mechanism of LDM



E. Pasquier et al. Metronomic chemotherapy: new rationale for new directions. *Nat Rev Clin Oncol.* 2010, *7*, 455-465

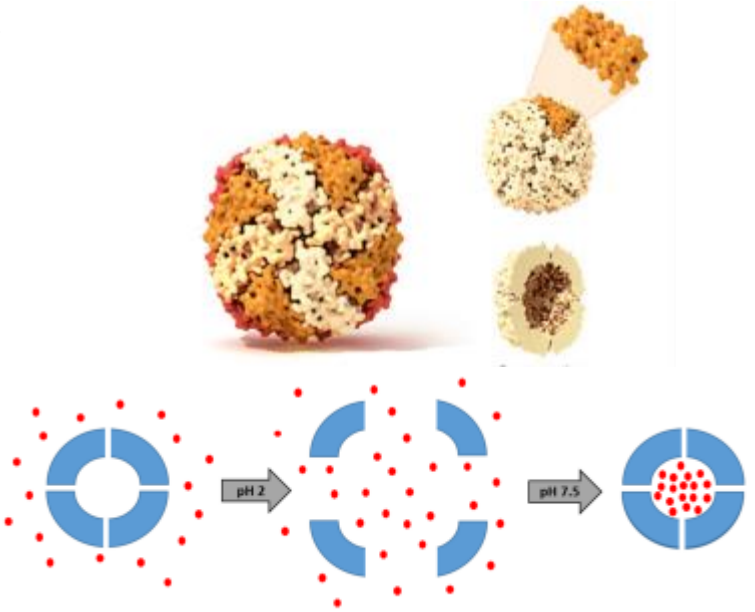
Metronomic chemotherapy: Possible new directions?

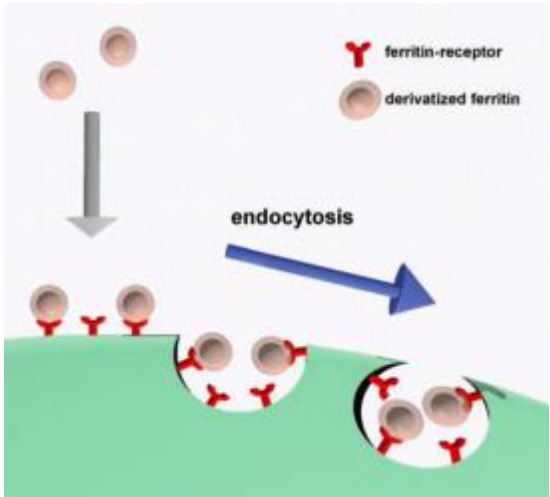


Adapted from:
N. André et al. Has the time come for metronomics in low-income and middle-income countries? *Lancet Oncol.* 2013, 14, e239-e248

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**





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HFn promotes DOX nuclear translocation

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Protein nanocages for self-triggered nuclear delivery of DNA-targeted chemotherapeutics in Cancer Cells

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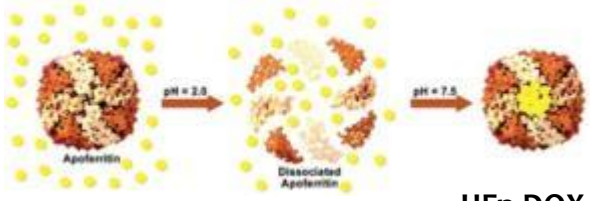


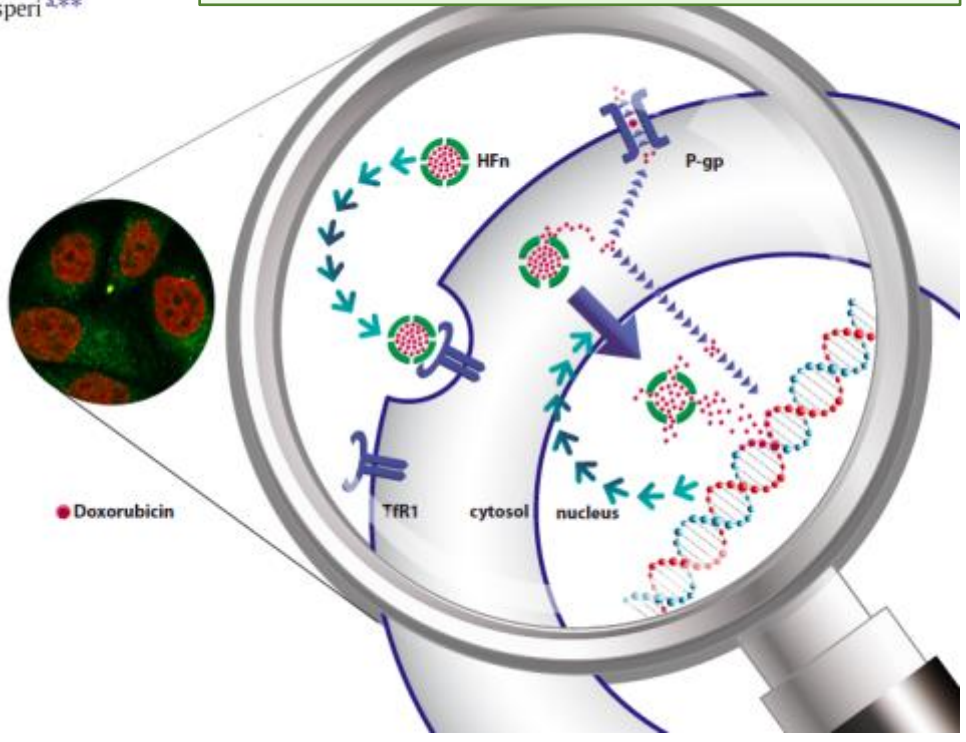
HFn-DOX

● ⟶ Doxorubicin (~29 molecules/HFn shell)

HFn-DOX is a good candidate for LDNM chemotherapy?

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

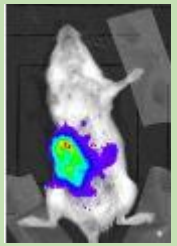


HFn uptake in 4T1-L Breast Cancer cells

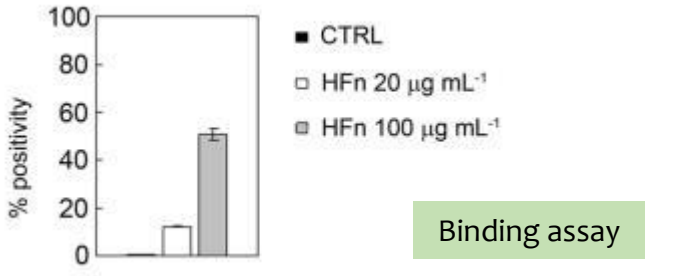
In vitro

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

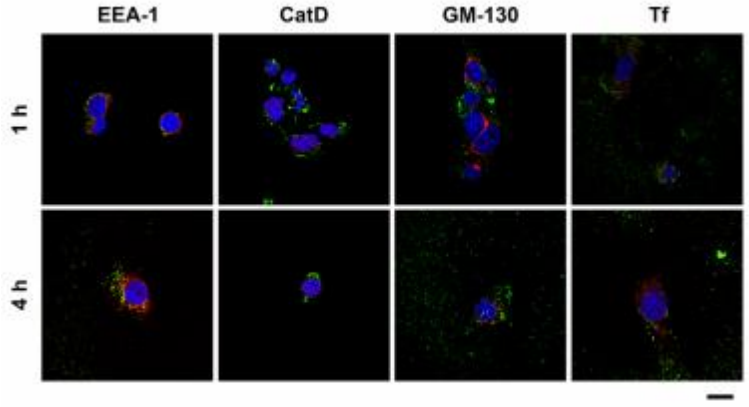
- high level of proliferation, migration and invasiveness
- DOX-inducible expression of MDR-1 (or P-glycoprotein)
- stable luciferase expression

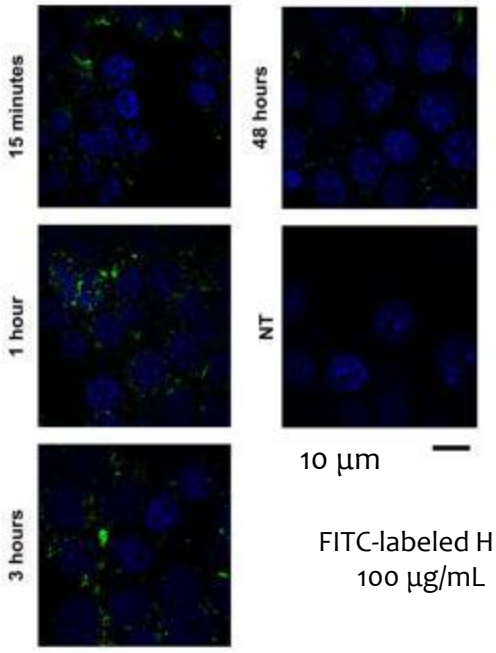


Dose-dependent recognition of tumor cells



Binding assay





FITC-labeled HFn
100 μg/mL

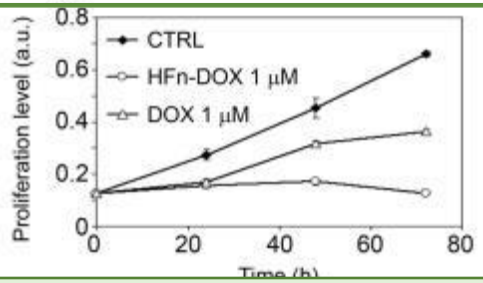
Internalization:

- 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

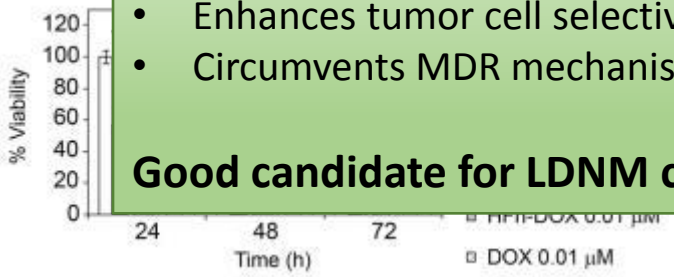
HFn activity in 4T1-L BC cells

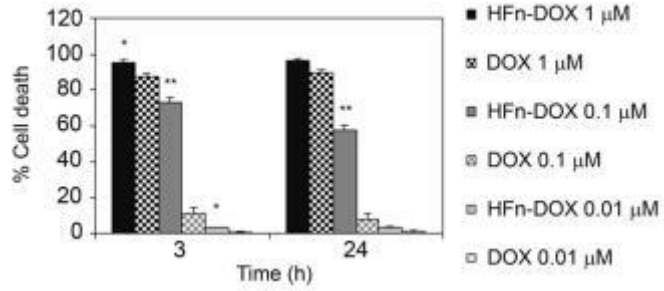
In vitro

- 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



HFn-DO
increase





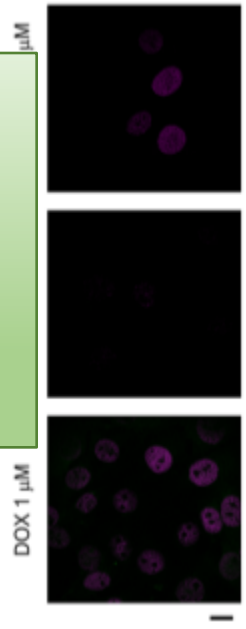
Annexin V

HFn in vitro:

- Improves chemotherapeutic efficacy
- Enhances tumor cell selectivity
- Circumvents MDR mechanisms

Good candidate for LDNM chemotherapy

HFn promotes DOX nuclear translocation



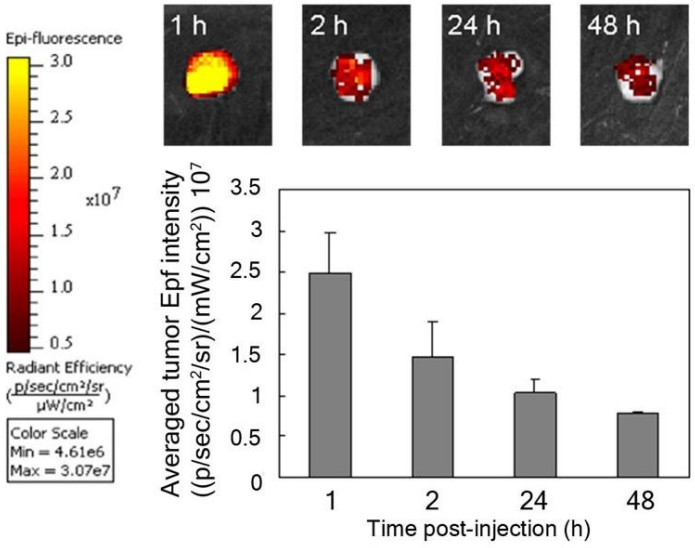
15.2-fold higher concentration of DOX inside 4T1-L nucleus within 3 h (HFn-DOX *vs* free DOX)

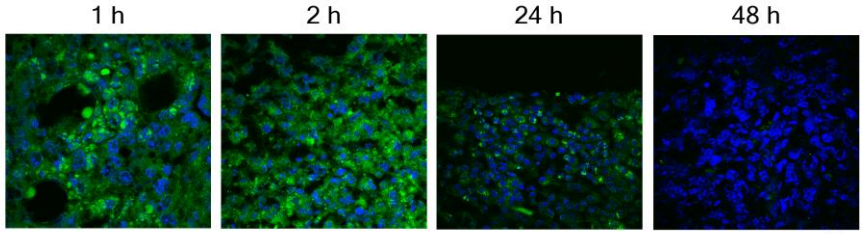
HFn tumor targeting and biodistribution

In vivo

AlexaFluor660-labeled HFn (5 μg kg^{-1}) i.v. injected by tail vein and imaged by live fluorescence

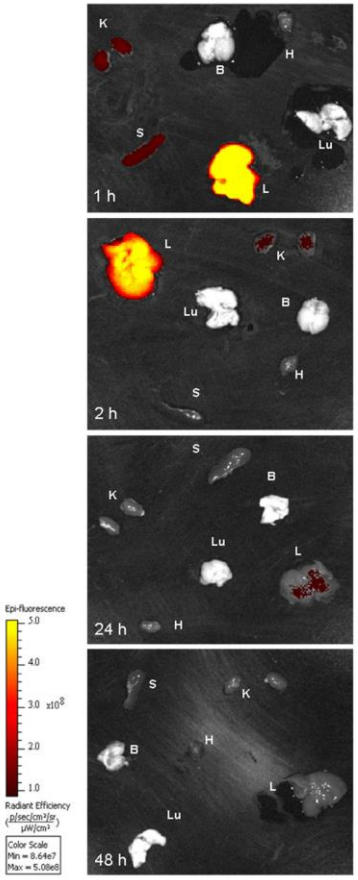
Tumor targeting



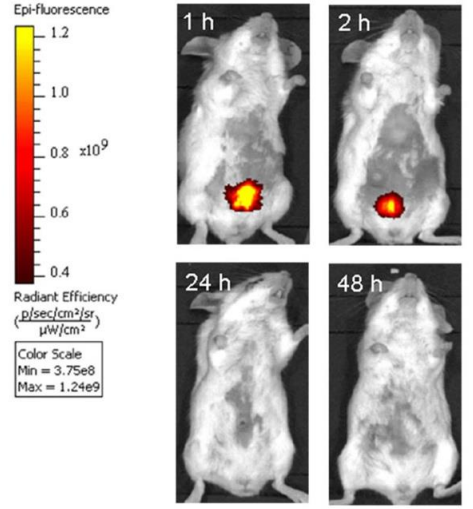


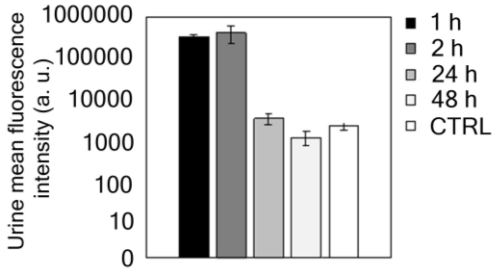
Tumor cryosections nuclei = blue (DAPI)

Biodistribution



Renal excretion



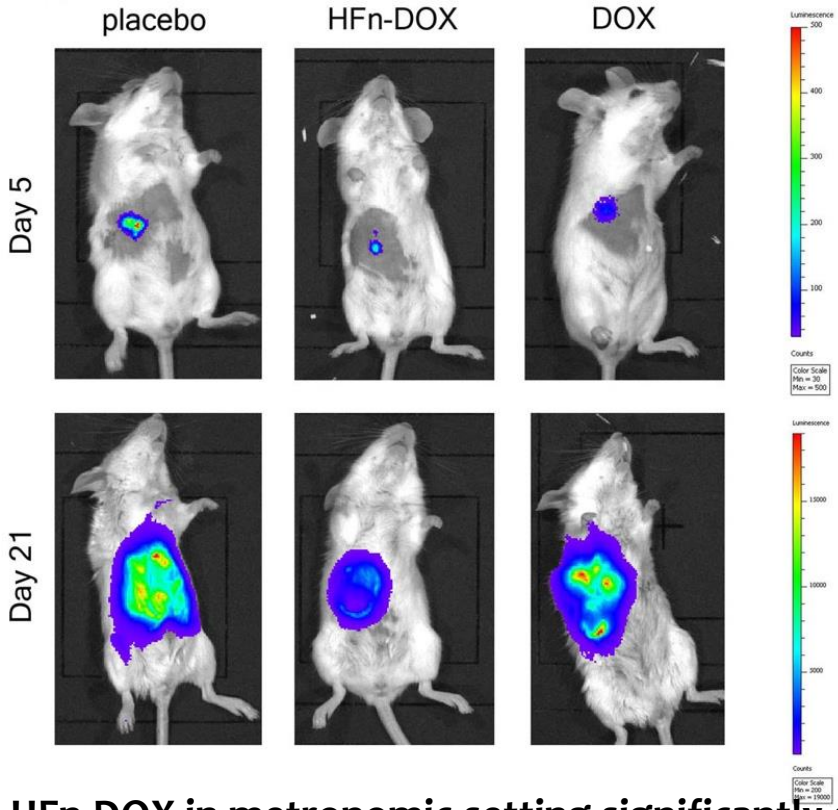


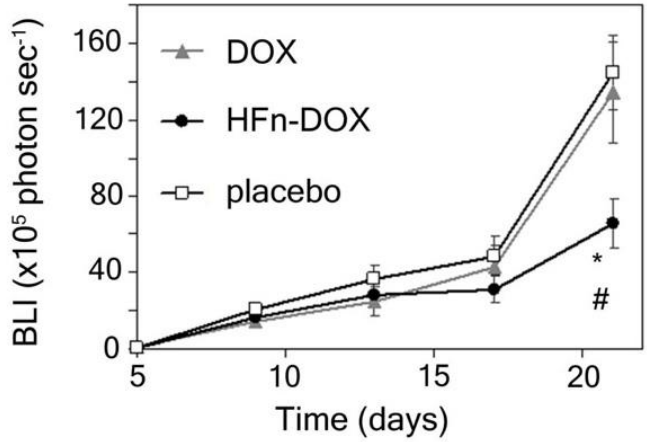
LDNM treatment of 4T1-L tumor bearing mice with HFn-DOX

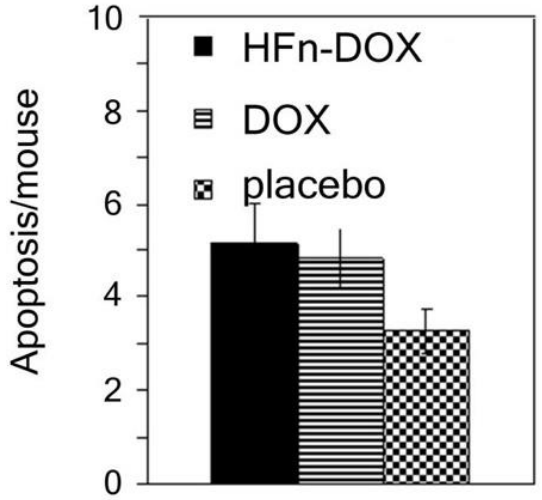
In vivo

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







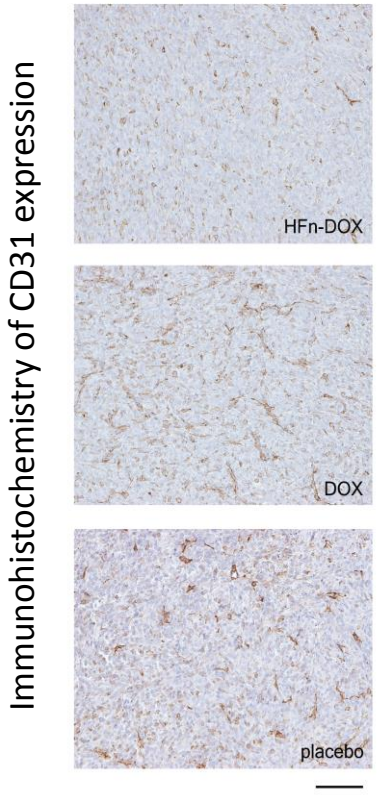
Immunohistochemistry

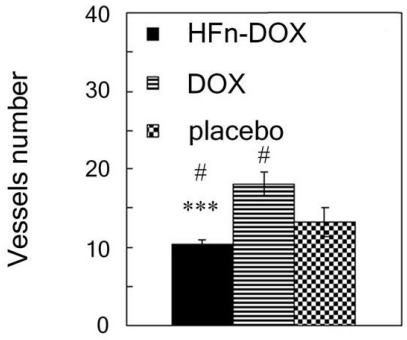
HFn-DOX in metronomic setting significantly slow tumor progression increasing apoptosis in tumor tissue

LDNM inhibits neo-angiogenesis and prevents drug resistance

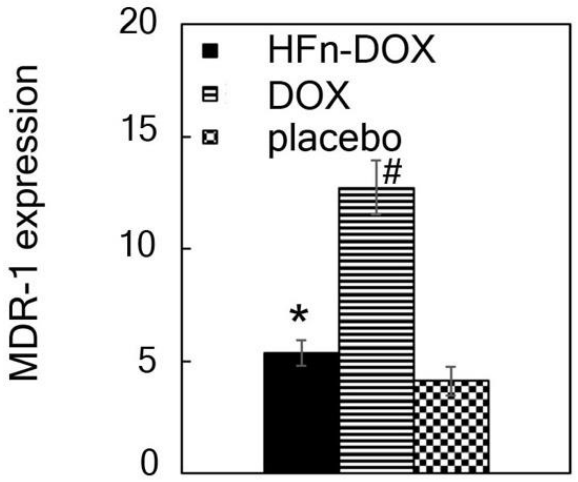
In vivo

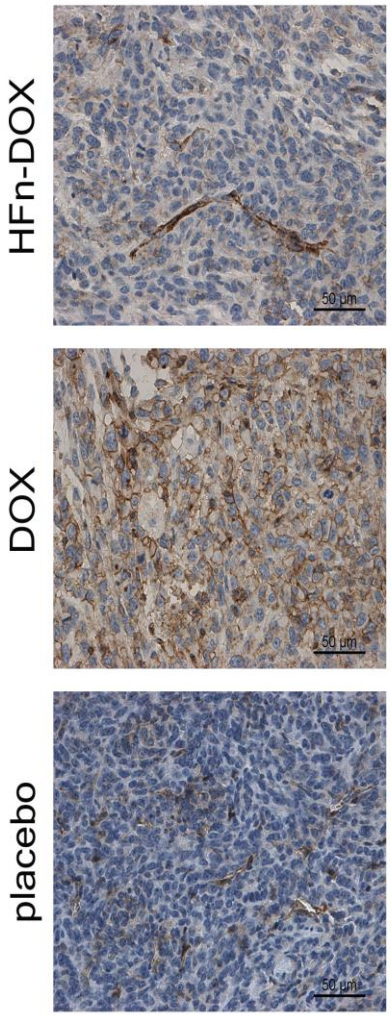
Tumor angiogenesis





Induction of MDR-1 expression



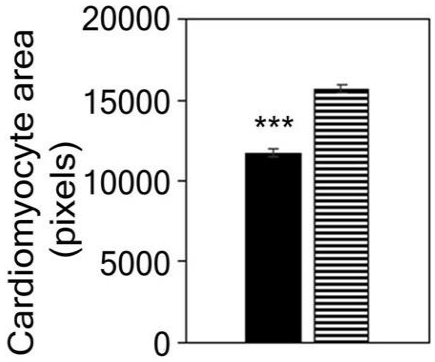


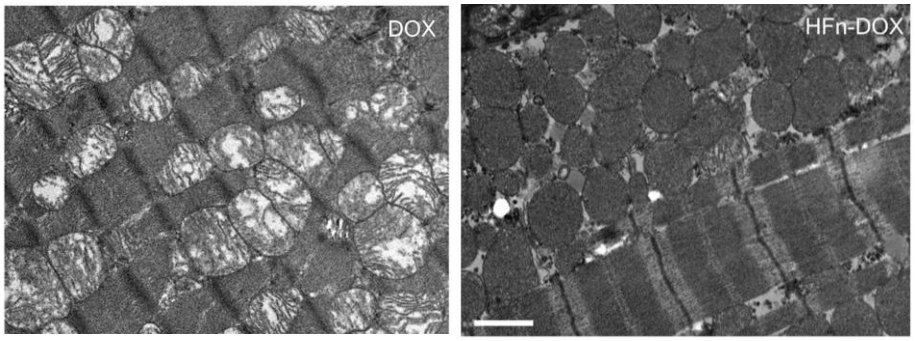
LDNM overcomes DOX cardiotoxicity and systemic dysfunction

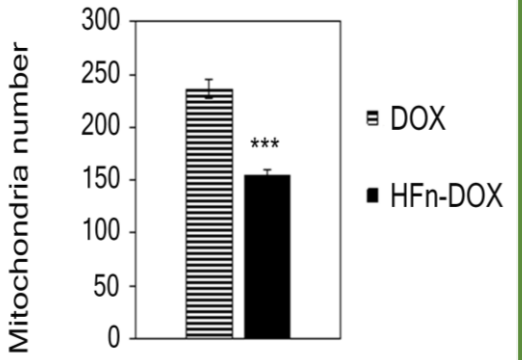
In vivo

Cardiotoxicity:

morphological evaluations



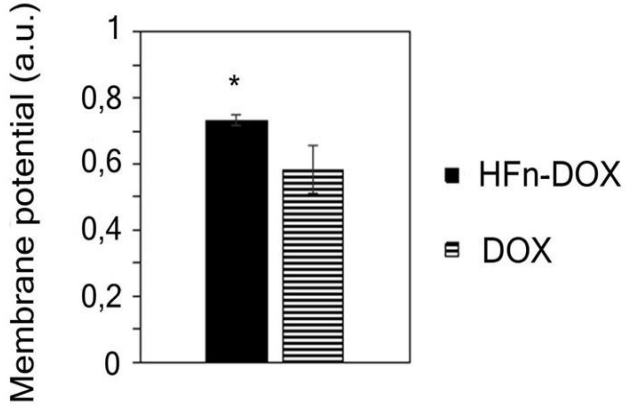




Transmission Electron Microscopy of Heart tissue

Cardiotoxicity:

functional evaluations



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

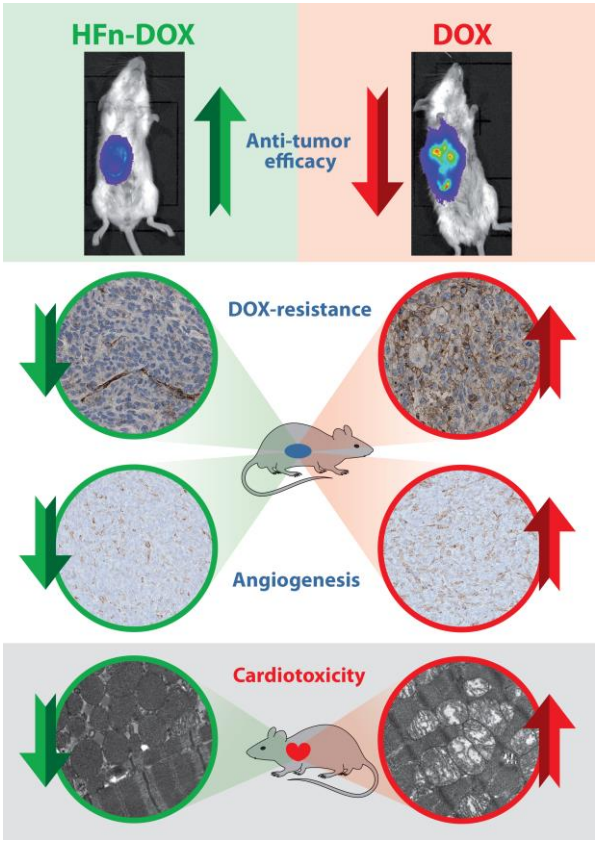
☞ DOX monotherapy **does not STOP** tumor progression

☞ 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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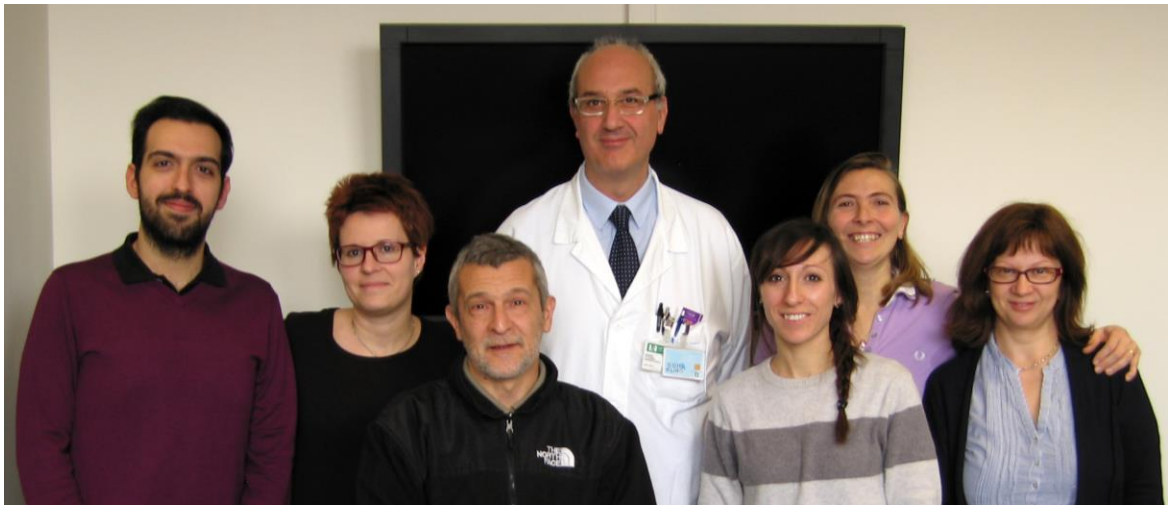

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