Targeted Paclitaxel Delivery Via Metalloproteinase-Sensitive Liposomes To Prevent Peripheral Neurotoxicity: A Proof Of Concept

Laura Molteni[1], Cinzia Bragato[2], Andrea Persico[2], Gabriella Nicolini[1], Francesca Re[3], Paride Mantecca[2], Marcelo Henrique Kravicz[3]

[1] Experimental Neurology Unit, School of Medicine and Surgery, University of Milano-Bicocca, Monza, Italy
[2] Dept. of Earth and Environmental Sciences, University of Milano-Bicocca, Milano, Italy
[3] NanoMedicine Center, School of Medicine and Surgery, University of Milano-Bicocca, Monza, Italy

Introduction
Paclitaxel (PTX) is one of the most effective chemotherapeutic drugs for treating breast cancer. However, its use can lead to peripheral neurotoxicity. Delivery strategies are needed to overcome these side effects. For this purpose, our project has exploited the concept of triggered drug release to characterize liposomes loaded with PTX and functionalized with a lipopeptide sensitive to metalloproteinases. The idea was to design a formulation of PTX that is inactive until it comes into contact with metalloproteinases, which are known to be increased in tumor microenvironments.

Methods
Liposomes containing PTX (Lip-PTX), liposomes containing PTX and functionalized with a metalloproteinase sensitive lipopeptide (MSLP-Lip-PTX), and unloaded liposomes were prepared by film formation and hydration followed by extrusion in a thermobarrel.
The efficacy of liposomes in reducing viability of breast cancer cells was evaluated by MTT assay. The absence of neurotoxic peripheral effects of liposomes was evaluated in sensory neurons primary cultures.
Transgenic zebrafish embryos (Tg(isl2b:GFP)^{zb7}) were injected with liposomes, and morphological, behavioral and molecular analysis were performed to evaluate liposomes neurotoxic effects.

Results
In vitro. Lip-PTX and MSLP-Lip-PTX showed an anti-tumor effect against MCF-7 cells, and exerted a neuroprotective effect in sensory neurons primary cultures, inducing a minor shortening of neurites compared to PTX.
In vivo. Zebrafish embryos injected with Lip-PTX and MSLP-Lip-PTX showed a reduced mortality rate, less epithelial damages and an increase of response to a mechanical stimulus compared to those injected with PTX.

Conclusions
Our findings reveal that Lip-PTX and MSLP-Lip-PTX effectively mitigate, both *in vitro* and *in vivo*, the neurotoxic effects commonly associated with Paclitaxel. This study highlights the potential of the liposome system as a safe and promising therapeutic strategy that warrants further investigation. These results lay the groundwork for assessing the behavior of liposomes in a zebrafish xenograft model established using human breast cancer cells.