Stromal depletion and control of metastases with a polymeric conjugate of docetaxel

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ABSTRACT SUMMARY
Cellax is a polymer conjugate of docetaxel (DTX) which self-assembles into well-defined 120 nm nanoparticles in saline. This delivery system extends blood circulation, increases tumor uptake, and interacts preferentially with stromal cells. In a 4T1 orthotopic breast cancer model in mice, 85% of Cellax particles were taken up by cancer-associated fibroblasts (CAF), a major component of stroma. Further, Cellax treatments reduced CAF by 82%, while native DTX and Nab-paclitaxel exerted no anti-stromal activity. Concomitant effects were ~70-fold increased perfusion, ~3-fold decreased interstitial fluid pressure (IFP), and a 7-fold reduction in lung metastases. Taken together, the data suggests that stromal depletion enhances control of tumor growth and metastases, and that stromal control can be an important component of therapy.

INTRODUCTION
Nanoparticle delivery of drugs can enhance blood circulation and tumor uptake, and reduce toxicity by limiting non-selective uptake. Increasingly, investigators are addressing particle fate in the tumor: drug must be released or interact with the target cells in order to have an effect.

We designed a drug delivery system around a carboxymethylcellulose scaffold, in which a high hydrophobic DTX content (37 wt%) and a PEG component (5 wt%) drive self-assembly into a defined nanoparticle (120 nm), that extends blood circulation, enhances tumor uptake, and is readily internalized by cells (1-4). The Cellax therapeutic demonstrates significant enhancements to efficacy and safety, relative to approved taxane therapies (1-4). Here we report on the unique therapeutic impact of Cellax in the tumor microenvironment compared to native DTX and Nab-paclitaxel (Abraxane®).

EXPERIMENTAL METHODS
Cellax polymer was synthesized as previously reported (1): carboxymethylcellulose was converted to a free acid and acetylated, followed by EDC coupling of DTX and PEG, to yield a polymer with 37.1 +/- 1.5 wt% DTX and 4.7 +/- 0.8 wt% PEG (Fig 1). Cellax particles were prepared by precipitation of acetonitrile solutions of polymer into 0.9% saline, followed by dialysis, concentration, and sterile filtration. Dose was determined by UV measurement.

Figure 1: Depiction of the Cellax polymer
Animal studies: The experimental protocols in this study were approved by the Animal Care Committee of the University Health Network (UHN, Toronto, ON, Canada). DTX was formulated in a Tween80/ethanol/saline (20:13:67) solution. Nab-paclitaxel (Abraxane) was sourced from the UHN pharmacy.
Orthotopic 4T1 models: 4T1 cells (1x10⁶) were inoculated to the mammary fat pad of female Balb/c mice (n=10 per group). Intratumoral distribution study: when 4T1 tumors reached 4-5 mm in diameter, mice were treated with Cellax (170 mg DTX/kg) and were sacrificed 6, 16, 24, 72 and 168 h after treatment. Fixed tumor sections were stained for α-SMA+ CAF and with H&E, and images were analyzed by Definiens Tissue Studio software for total tumor area, non-viable component, and stromal coverage. In a related study, mice were treated with Cellax containing fluorescent DiI, were sacrificed 24h later, and the fixed tumors were stained for α-SMA+ CAF. Tumor sections were imaged and co-localization of CAF with Cellax-DiI was calculated in Definiens software. Efficacy model: when tumors reached 5-7 mm, mice were treated with the MTD of Cellax (170 mg DTX/kg), native DTX (40 mg DTX/kg), Nab-paclitaxel (50 mg PTX/kg) or saline via tail vein injection. Six days post therapy, mice were injected with FITC-lectin, tumor interstitial fluid pressure (IFP) was measured, and the primary tumors were then resected and fixed for histology. Tumor sections were stained for CD31 (blood vessels) and for α-SMA (CAF). On day 13, mice were treated with a second round of therapy, and on day 20 the entire cohort was sacrificed, and
lung tissues were harvested for histology (H&E staining, quantification of tumor burden with Definiens).

RESULTS AND DISCUSSION

Cellax nanoparticles were 118 +/- 3 nm, and dose was adjusted based on UV measurements. Full characterization of Cellax polymer and particles, including serum drug release profiles, is described in references 1-4.

High stroma content is a biomarker for poor prognosis in people with cancer, and is associated with metastasis (5). For example, women with breast cancer expressing high tumor α-SMA+ stroma (primary >50% stroma) exhibit a higher rate of relapse at 5 years than women whose tumors have low stroma (<=50%) (6). Stroma has been considered as an important therapeutic target. However, developing agents effective against this target has not been successful.

Cellax particles were labelled with fluorescent Dil, and from analysis of histology sections, it was found that 85% of Cellax particles were internalized by α-SMA+ CAF. Subsequent to treatment with Cellax, CAF in the 4T1 tumors declined by 50% in the first 16 h, and declined continuously thereafter to almost undetectable after 1 week. On the other hand, significant death of the tumor cells only occurred after complete stromal depletion. The data suggest that stroma is the primary target of Cellax.

In the efficacy model, Cellax treatment induced a significant (p<0.05) 82% reduction in α-SMA+ CAF coverage in the 4T1 tumors, compared to non-significant effects in tumors treated with Nab-paclitaxel or DTX (Fig 2 A-B). No observable differences in blood vessel density were recorded. Tumors treated with Cellax were significantly (p<0.05) more perfused than other groups, exhibiting a 68% increase. DTX and Nab-paclitaxel treated tumors exhibited no increase in perfusion relative to control. In addition to enhanced perfusion, IFP was reduced by 2.6-fold in Cellax treated tumors, whereas no significant changes were observed in DTX or nab-paclitaxel mice relative to control.

Cellax treated mice exhibited a significant (p<0.05) reduction in the presentation of metastases in the lung tissues: 100% of control, 90% of DTX, and 86% of Nab-paclitaxel treated mice presented with lung nodules, compared to 40% of Cellax treated mice. Definien image analysis of the lung sections (Fig 2 C-D) indicated that lung tumor burden was reduced by 6.7 fold compared to non-significant decreases in the other taxane groups.

CONCLUSION

Cellax nanoparticle delivery of DTX has a significant impact on tumor stroma, an effect associated with increased tumor perfusion, decreased IFP, and reduced metastases compared to native DTX and Nab-paclitaxel.

REFERENCES


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