

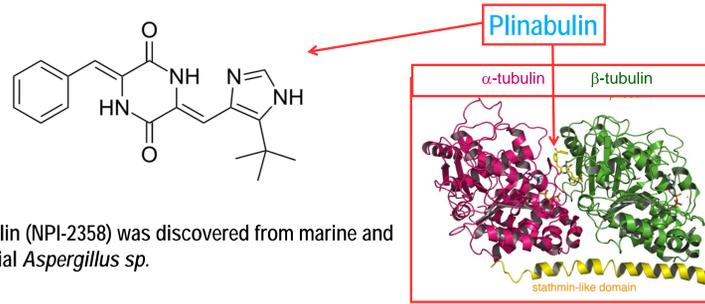
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## Introduction

- Chemotherapy-induced neutropenia (CIN) is a major side effect of cancer chemotherapy associated with life-threatening infections, hospitalization, chemotherapy dose reduction and/or delay
- CIN is currently treated with recombinant human G-CSF but it has significant limitations such as bone pain and inconvenient administration regimen for patients receiving chemotherapy



- Plinabulin (NPI-2358) was discovered from marine and terrestrial *Aspergillus sp.*

- Plinabulin is a microtubule de-polymerizing agent (MDA) which binds to the  $\beta$  tubulin of the tubulin monomer near the colchicine binding site and prevents cell cycle progression of tumor cells
- Plinabulin is currently in Phase 3 clinical trial for NSCLC in patients undergoing docetaxel chemotherapy
- Plinabulin modulates tumor microenvironment by activating DCs and priming anti-tumor T cells
- It inhibits angiogenesis, disrupts existing tumor vasculature and induces cancer cell apoptosis
- Plinabulin also reduces CIN in cancer patients by mechanisms which remain unaddressed and is the focus of this study

## Tubulin network in neutrophil under arrest

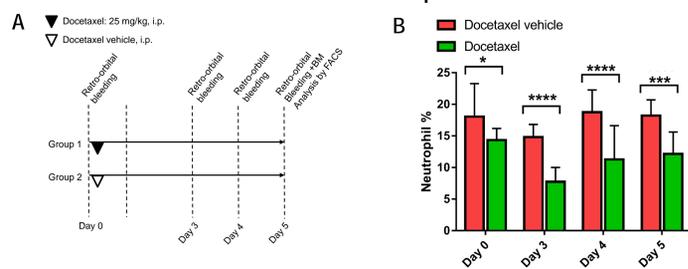
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Fig. 1. TIRF imaging with 100x oil objective of human neutrophils isolated from blood of healthy donors, labeled with 3uM Sir-Tubulin dye at the 2x10<sup>6</sup>/ml cell concentration at 37° C for 60 min before imaging. The cells were incubated with mAb24-AF488 and KIM127-DL550, 5ug/ml each, at RT for 3 min prior to image acquisition. They were perfused in the microfluidic chamber coated with 2ug/ml rhP-selectin-Fc, 10ug/ml rhICAM-1-Fc, and 10ug/ml rhIL-8 at RT for 2 h, blocked with 1% casein for 30 min at a wall shear stress of 6 dyn/cm<sup>2</sup>.

## Results

### Docetaxel causes neutropenia in mice



### Plinabulin effect on severe neutropenia in mice

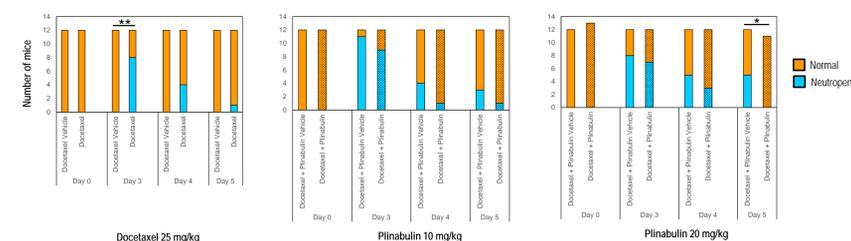


Fig. 4. Docetaxel 25 mg/kg causes severe (grade 4) neutropenia in most mice defined as below mean - 2SD (outside 95% confidence interval of normal). Plinabulin 10 or 20 mg/kg significantly reduces severe neutropenia caused by docetaxel on day 4 and almost eliminates severe neutropenia on day 5.

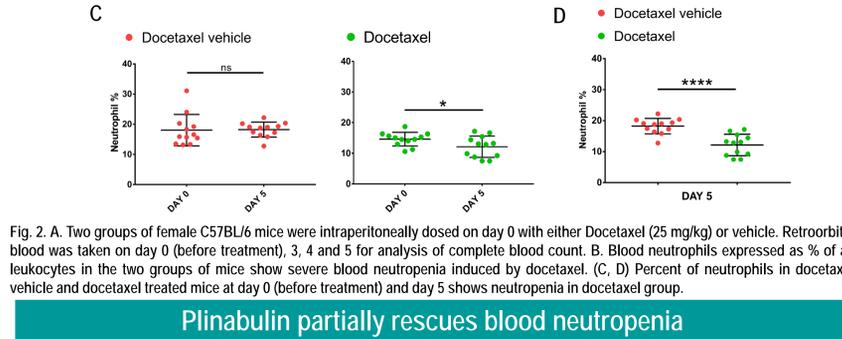


Fig. 2. A. Two groups of female C57BL/6 mice were intraperitoneally dosed on day 0 with either Docetaxel (25 mg/kg) or vehicle. Retroorbital blood was taken on day 0 (before treatment), 3, 4 and 5 for analysis of complete blood count. B. Blood neutrophils expressed as % of all leukocytes in the two groups of mice show severe blood neutropenia induced by docetaxel. (C, D) Percent of neutrophils in docetaxel vehicle and docetaxel treated mice at day 0 (before treatment) and day 5 shows neutropenia in docetaxel group.

### Plinabulin partially rescues blood neutropenia

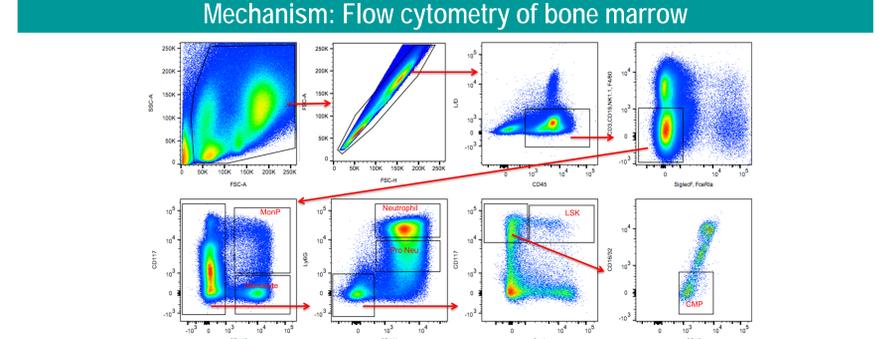
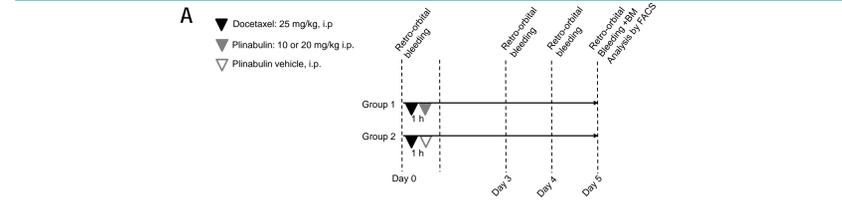


Fig. 5. Gating strategy for bone marrow neutrophils and other cell subsets.

### Docetaxel causes neutropenia by blocks at LSK and promyelocyte levels

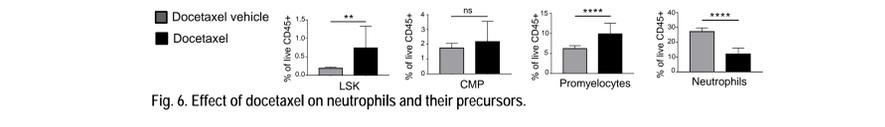


Fig. 6. Effect of docetaxel on neutrophils and their precursors.

### Plinabulin partially restores bone marrow neutrophils

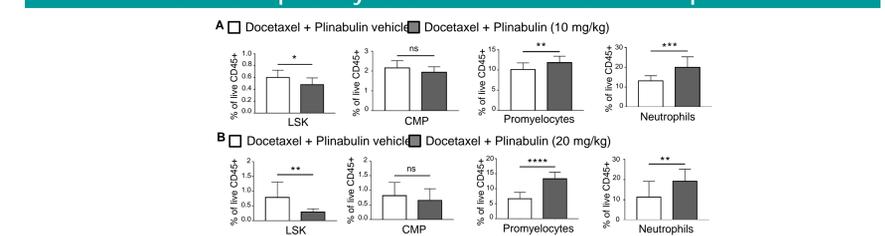


Fig. 7. Plinabulin restores bone marrow neutropenia and improves low promyelocytes in bone marrow of docetaxel-treated mice.

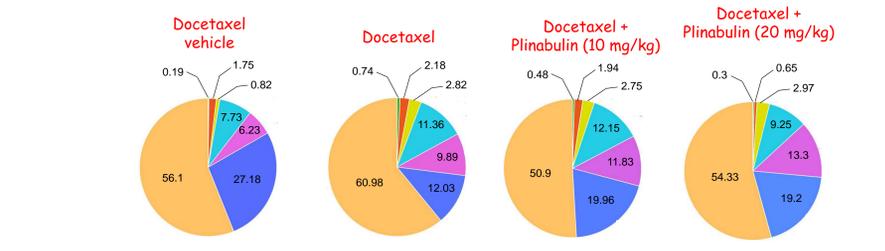


Fig. 8. Docetaxel increases monocyte precursors and monocytes. (B, C) Plinabulin has no effect on monocyte lineage.

### Plinabulin has no effect on monocyte lineage

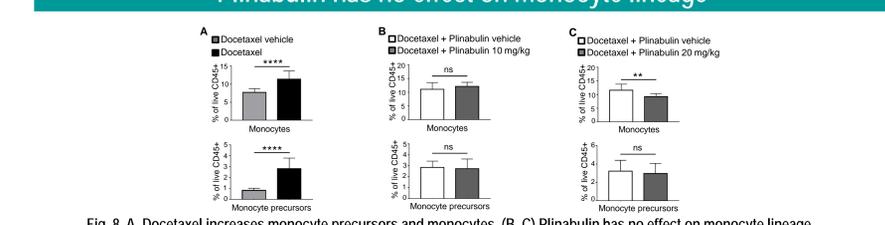


Fig. 8. A. Docetaxel increases monocyte precursors and monocytes. (B, C) Plinabulin has no effect on monocyte lineage.

### Conclusions

- Plinabulin effectively blunts docetaxel-induced neutropenia in mice
- One mechanism could be by relieving the docetaxel-induced accumulation LSK cells in the bone marrow
- Plinabulin specifically affects neutrophil production and has little effect on the monocyte lineage
- Convenient administration regimen over G-CSF shows promise for treatment of CIN