# Plinabulin boosts antitumor efficacy and improves tolerability of topoisomerase inhibitor-based antibody-drug conjugates without or with immune checkpoint inhibitor

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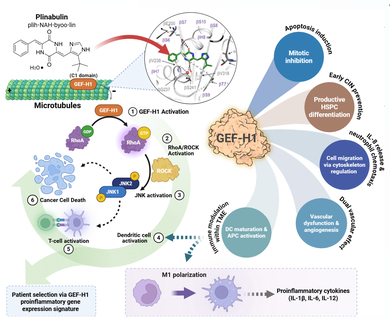
## Introduction

Antibody drug conjugates (ADC) with topoisomerase inhibitors (TOP1-ADC) have transformed treatments for cancers expressing specific tumor antigens. The FDA-approved TOP1-ADC includes TROP2- or HER2-directed datopotamab deruxtecan (Dato-DXd) and trastuzumab deruxtecan (T-DXd), respectively. To boost ADC efficacy and overcome drug resistance, combination strategies involving immune checkpoint inhibitors (ICI) are being investigated in preclinical-to-clinical translational studies.[1-2]

## Plinabulin

### Background

Plinabulin is a distinct GEF-H1 agonist with differentiated binding to tubulin that exerts anticancer activity primarily by activating GEF-H1 mediated immune pathways in dendritic cell (DC) maturation, M1 polarization and T cell activation.[3-5] In Dublin-3 phase 3 study, plinabulin and docetaxel doublet outperformed docetaxel with significant OS/PFS/ORR benefits and doubling of 2- and 3-year survival rates, and reduced docetaxel induced neutropenia.[6] In post-ICI settings given after radiation, plinabulin potentiates PD-1 inhibitors with significantly higher GEF-H1 immune activation signature in responders of mixed cancer types.[7-8]




*Illustrated by Daniel Wu via Biorender.com

**Mechanism-of-Action:**

Plinabulin binds tubulin with a fast off-rate,[4] leading to the release of **GEF-H1, a microtubule C1 domain-associated and highly spatio-temporally regulated RhoA activator**.[9-12]

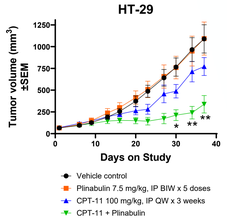
**As a GEF-H1 agonist, plinabulin has the following anti-cancer mechanism:**

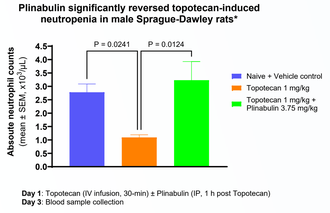
a) Activates the RhoA/ROCK signaling that leads to **DC maturation/M1 polarization and T-cell activation**, which has been validated in preclinical and clinical studies.[7-8,13]

b) Promotes **HSPC proliferation** biasing towards the GMP lineage during GEF-H1-dependent productive hematopoiesis, contributing to its **CIN prevention benefit.**[14-15]

c) Due to GEF-H1's role in vasculature,[12] plinabulin **modulates angiogenesis**.

- Preclinically, plinabulin enhances antitumor activity of irinotecan in human colon cancer xenografts and reduces topotecan induced neutropenia in rats.




**Day 1**: Topotecan (IV infusion, 30-min) ± Plinabulin (IP, 1 h post Topotecan)
**Day 3**: Blood sample collection

## Methods

Here we investigated plinabulin synergy with TOP1-ADC *in-vitro* (Crown Biosciences, Taicang, China) and without or with PD-1/L1 inhibitor pembrolizumab or atezolizumab *in-vivo* (Pharmaron, Beijing, China).

For *in-vitro* combination, HER2+ KPL-4 breast cancer cells were treated with plinabulin (1.46 - 46.8 nM), T-DXd (0.105 - 3.37 μM) or both.

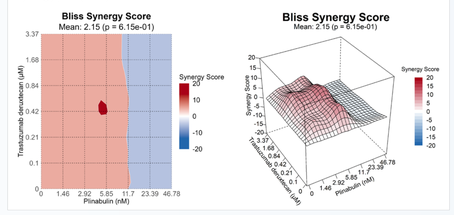
For *in-vivo* combinations, MC38 mouse colon adenocarcinoma cells overexpressing hTROP2 (hTROP2-MC38) or hHER2 (hHER2-MC38), were inoculated in B-hPD-1 or B-hPD-1/hPD-L1/hHER2 mice respectively, and antitumor activities of plinabulin (7.5 mg/kg) plus Dato-DXd (5 mg/kg) ± pembrolizumab (0.3 mg/kg) or T-DXd (3 mg/kg) ± atezolizumab (8 mg/kg) were evaluated.

## Results

### *In-vitro* synergy with T-DXd

- Plinabulin demonstrated synergistic activity with T-DXd with median CI <1 at 1:1, 2:1 and 4:1 ratios (T-DXd:plinabulin).

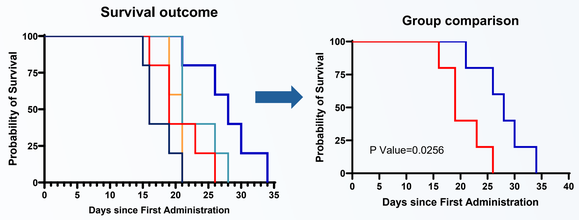
| Cell Line | DrugA | DrugB | Bliss_Average | Bliss_Median |
|---|---|---|---|---|
| KPL-4 | Plinabulin | Trastuzumab deruxtecan | 2.15 | 1.46 |




The **Bliss independence model** is expected to hold true for **non-interacting drugs that elicit their responses independently**, e.g., by targeting separate pathways.

### Prolonged survival when combined with T-DXd and atezolizumab *in-vivo*

- The triple combination of T-DXd + Plinabulin + Atezolizumab most significantly prolonged the survival of hHER2+ MC38 tumor-bearing mice.

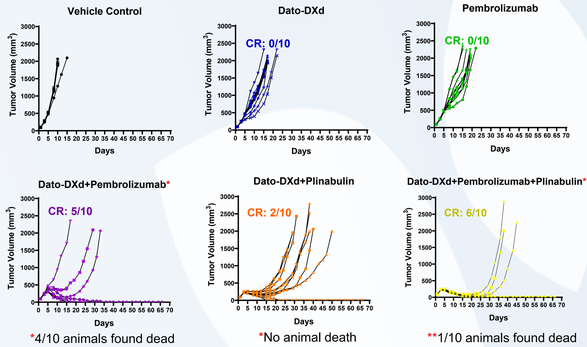



## Results Continued

### Synergizes with Dato-DXd ± pembrolizumab

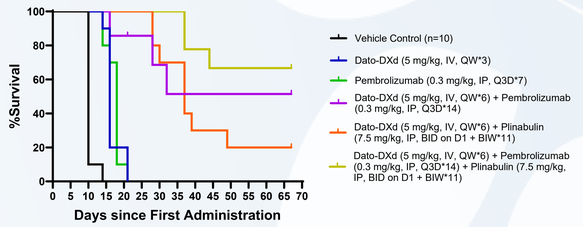
- The dosing regimen consisted of Dato-Dxd (5 mg/kg, IV QW), plinabulin (7.5 mg/kg, IP BIDx1/BIW) and pembrolizumab (0.3 mg/kg, IP Q3D).
- Dato-DXd antitumor activity was significantly enhanced by plinabulin (doublet) and further boosted by pembrolizumab (triplet) with complete response rates of 0%, 20% and 60% respectively.
- The triple and double combo significantly prolonged the animal survival and **was better tolerated than Dato-DXd plus pembrolizumab.**

**hTROP2+ MC38 tumor model**



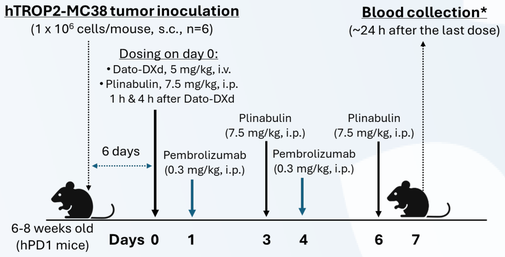

*4/10 animals found dead
*No animal death
**1/10 animals found dead

**Survival outcome***




*Non-tumor-related deaths due to suspected toxicity were exluded.

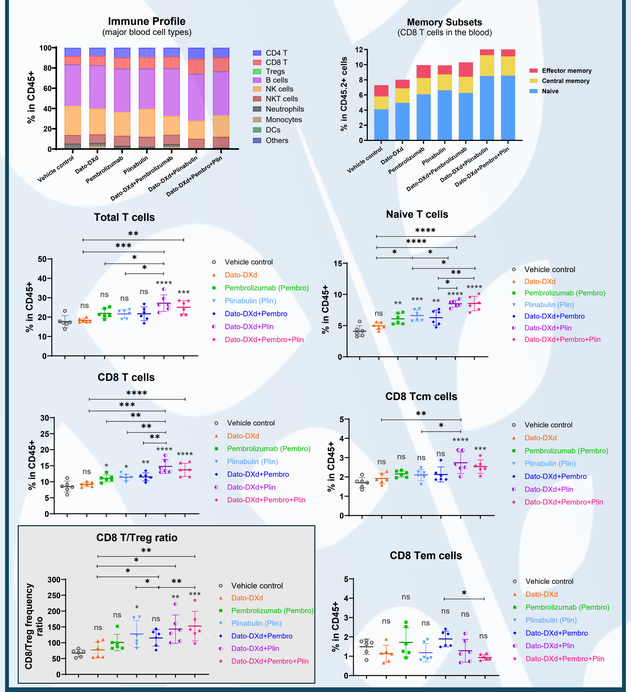
### Mechanistic study treatment schedule




*Whole blood samples were collected on Day 7 and subjected to flow cytometric immunophenotyping, following the workflow and gating strategies outlined in Miyauchi S et al., Protocol to study the immune profile of syngeneic mouse tumor models. STAR Protoc. 2024 Sep 20;5(3):103139. Statistical analyses were performed comparing all treatment groups vs. vehicle control using one-way ANOVA or comparing across groups with Tukey's multiple comparison posttest. *P* ≤0.05*, 0.01**, 0.001***, 0.0001****.

## Results Continued

**Plinabulin plus Dato-DXd ± pembrolizumab increases total and central memory CD8 T cells and CD8/Treg ratios in blood**




## Conclusions

Plinabulin has shown to improve the anti-cancer efficacy of TOP1-ADCs with or without a PD-1/PD-L1 inhibitor in relevant preclinical models. By sequential administration of plinabulin after Dato-DXd (± pembrolizumab), we observed significantly higher complete response rates and extended animal survival. Importantly, plinabulin addition significantly increased CD8+/Treg ratio in the blood, underscoring plinabulin's immune activating profile by strengthening the cancer-immunity cycle.[16] Clinical investigations assessing plinabulin safety and efficacy combined with 'neutropenia-limiting' TOP1-ADCs[2] alone or plus ICI are in the planning phase.

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