Application of a dual human cell system to measure potential off-target induced peripheral neuropathy (PN) for Antibody Drug Conjugate (ADC) candidates and other developing chemotherapeutics.

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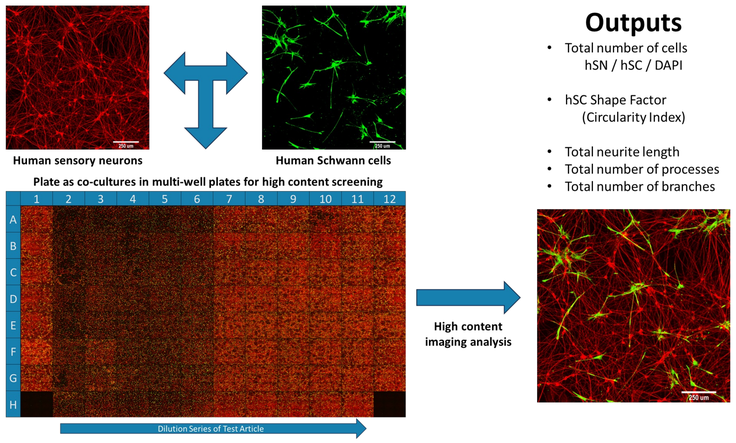
Overview

Antibody-drug conjugates (ADCs) are intended for targeted delivery of highly potent payloads to cancer cells and may cause peripheral neuropathy (PN) by a variety of mechanisms including bystander effect.

In this study, we have characterized the co-culture of human iPSC-derived sensory neurons (hSNs) and primary human Schwann cells (hSCs) using four well-characterized chemotherapeutics (oxaliplatin, vincristine, paclitaxel and bortezomib) and monomethyl auristatin E (MMAE), which is commonly used as the toxic payload in ADC drugs.

These experiments show the potential of high content screening for investigating mechanisms of PN and assessing new therapeutics.

Methods



The co-culture system of hSNs and hSCs was plated into a 96-well format for high throughput screening capabilities and dosed with various testing articles (TAs) in a range of concentrations for a predetermined length of time (including media controls).

The system was optimized for cell health, distribution, attachment and outgrowth before introduction of testing articles. **Figure 1** shows consistent cell distribution, and the average number of cells within the 3x3 frame matrix close to the theoretical number (for perfectly even distribution).

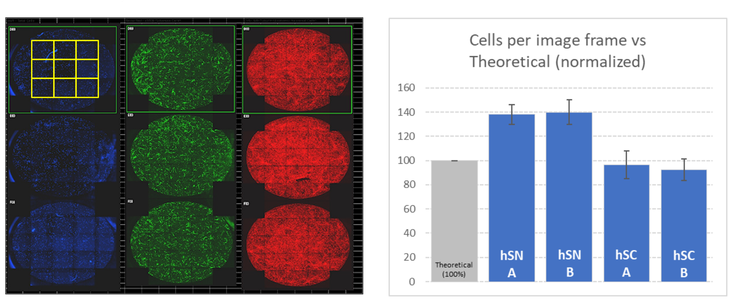


Fig.1 Distribution of cells and normalized cell numbers (adhesion) within two separate sets of media control wells (designated A and B).

Results: ICC Imaging

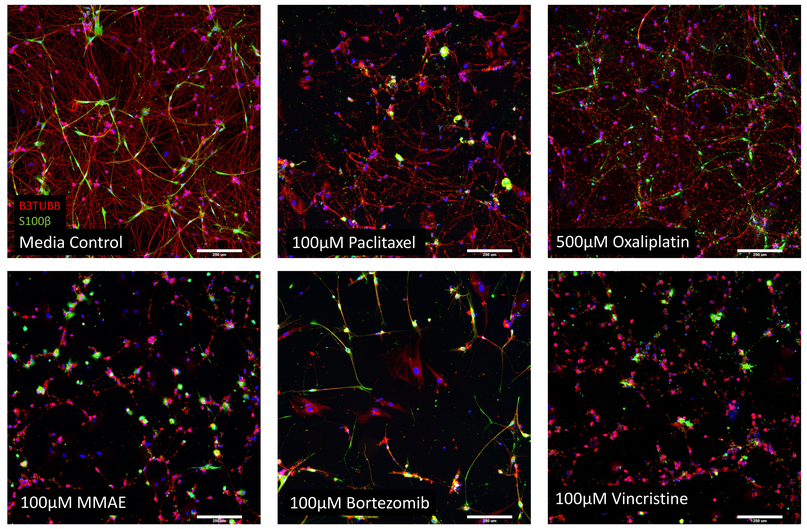


Fig.2 Images showing co-cultures exposed to the highest dosing concentration of each test article (Paclitaxel, Oxaliplatin, MMAE, Bortezomib and Vincristine).

Results: Metrics Characterization

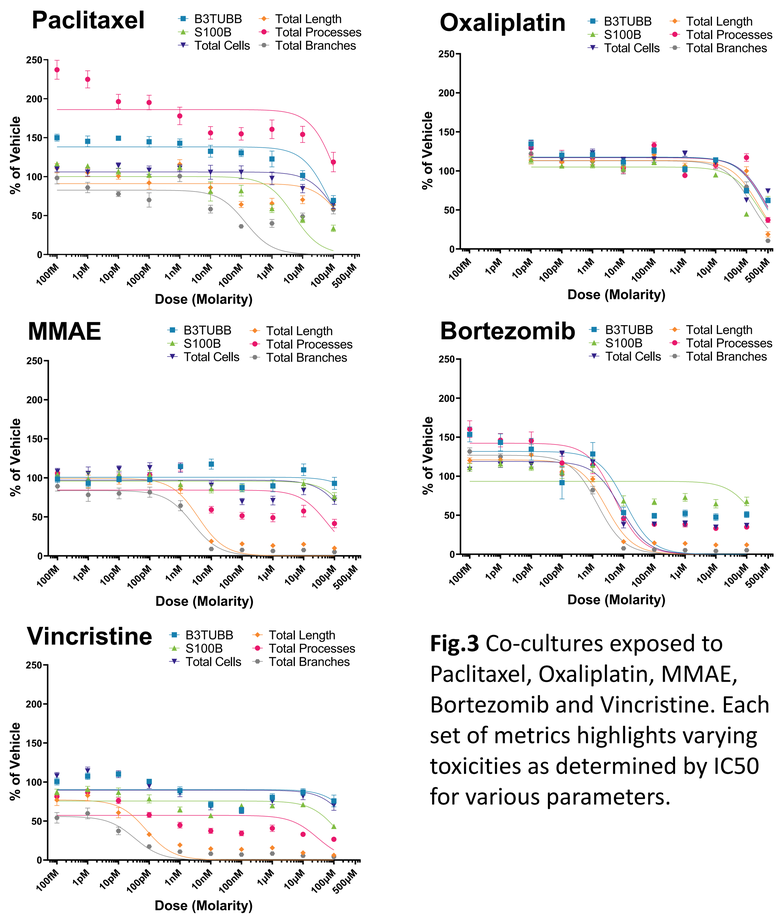


Fig.3 Co-cultures exposed to Paclitaxel, Oxaliplatin, MMAE, Bortezomib and Vincristine. Each set of metrics highlights varying toxicities as determined by IC50 for various parameters.

Table 1. IC50 comparison for co-cultures treated with known PN-causing chemotherapeutics.

	Paclitaxel	Oxaliplatin	MMAE	Bortezomib	Vincristine
Neuron Count	76.9 µM	366 µM	> 100 µM	11.3 nM	> 100 µM
Schwann Cell Count	5.15 µM	249 µM	> 100 µM	> 100 µM	> 100 µM
Total Cells	> 100 µM	427 µM	> 100 µM	8.07 nM	> 100 µM
Total Neurite Length	> 100 µM	244 µM	3.73 nM	2.69 nM	80.5 pM
Total Processes	> 100 µM	398 µM	58.1 µM	5.19 nM	29.3 µM
Total Branches	137 nM	151 µM	2.44 nM	1.44 nM	33.8 pM

Results: hSC circularity index

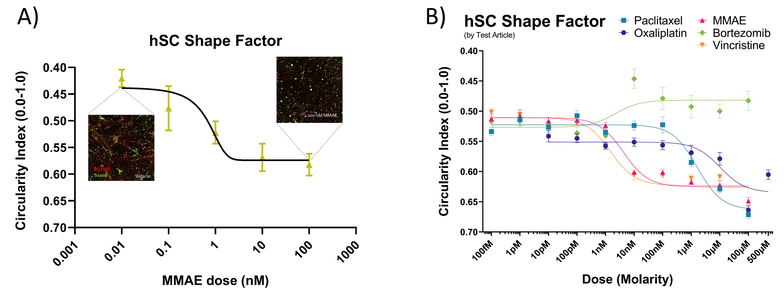


Fig.4 A) A representative graph showing a dose dependent response in Schwann cell shape after test article exposure (0 = completely linear; 1 = completely circular). B) Dose response curves for Schwann cells in co-culture after exposure with known PN-causing chemotherapeutics.

Table 2. EC50 comparison of morphological changes in the human Schwann cell population when co-cultures were treated with known PN-causing chemotherapeutics.

	Paclitaxel	Oxaliplatin	MMAE	Bortezomib	Vincristine
hSC Circularity Index	1.62 µM	10.3 µM	4.26 nM	2.18 nM	1.49 nM

Summary & Conclusions

In summary, this fully human co-culture cell system of hSNs and hSCs provides a fast, cost-effective pathway to human-relevant data for the evaluation of potential ADC and other chemotherapy-induced PN effects.

The variety of metrics allows for detailed insights into mechanistic and cell-specific compound effects with additional value in evaluating multiple ADC candidates for off-target toxicity as compared to one another and/or to MMAE toxicity when narrowing the candidate field during development.

Current efforts are focused on further characterizing the system with an opportunity for continued commercial work with those in the field of ADC and other therapeutic drug development; most notably, where off-target peripheral neuropathy is of interest.