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PRINCIPAL INVESTIGATOR: In Hong Yang

CONTRACTING ORGANIZATION:

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14. ABSTRACT

Chemotherapy-induced peripheral neuropathy (CIPN) is the most common dose-limiting neurotoxicity from anti-cancer drug therapy [1,2]. Platinum drugs, taxanes, proteasome inhibitors, vinca alkaloids, epothilones, and immunomodulators are the standard of anti-cancer therapies for the six most cancers. An estimated 2010 incidence of 994, 680 cases for these six cancers is reported. Therapeutic clinical trials indicate from 25 to 100% of CIPN with symptoms lasting months to years. Several hundred thousand patients per year are experiencing CIPN. The number of cancer survivors living with CIPN is unknown. Even when CIPN does not involve dose-limiting side effects, its onset may severely affect the patient's quality of life and can cause dosage reductions, delay in treatment, and even treatment discontinuation [3]. The pathologic changes in most cases of CIPN revolve around the distal to proximal axonal degeneration, rather than cell body death, which has been referred to as "dying-back neuropathy." [4] Currently, there are no effective therapies aimed at halting the progression of, or reversing distal axonal degeneration through, the usage of anti-breast cancer drugs [5]. All of the available therapies are aimed at symptomatic control of neuropathic pain however they do not protect against the underlying issue of distal axonal degeneration

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Introduction:
Chemotherapy-induced peripheral neuropathy (CIPN) is the most common dose-limiting neurotoxicity from anti-cancer drug therapy [1,2]. Platinum drugs, taxanes, proteasome inhibitors, vinca alkaloids, epothilones, and immunomodulators are the standard of anti-cancer therapies for the six most cancers. An estimated 2010 incidence of 994, 680 cases for these six cancers is reported. Therapeutic clinical trials indicate from 25 to 100% of CIPN with symptoms lasting months to years. Several hundred thousand patients per year are experiencing CIPN. The number of cancer survivors living with CIPN is unknown. Even when CIPN does not involve dose-limiting side effects, its onset may severely affect the patient's quality of life and can cause dosage reductions, delay in treatment, and even treatment discontinuation [3]. The pathologic changes in most cases of CIPN revolve around the distal to proximal axonal degeneration, rather than cell body death, which has been referred to as "dying-back neuropathy." [4] Currently, there are no effective therapies aimed at halting the progression of, or reversing distal axonal degeneration through, the usage of anti-breast cancer drugs [5]. All of the available therapies are aimed at symptomatic control of neuropathic pain however they do not protect against the underlying issue of distal axonal degeneration.

Keywords: Chemotherapy, Neuropathy, CIPN, Taxol

Accomplishments:

High Throughput Compartmentalized Culture Platform (HTCCP)

We have built a high throughput microfluidic platform which compartmentalizes soma and axon terminal regions to screen the protective drugs for the degenerative role of Taxol. 2 compartments (soma and axon terminal) molded in PDMS were connected by microchannels. The microgrooves were 3 µm high and 6 to 10 µm wide, to guide the axons from the cell body compartment to the axonal compartment, as well as to prevent cell bodies from passing between channels. HTCCP have microchannels in between and a barrier can be formed through which axons and dendrites are able to extend, but not their cell bodies. The hydrostatic pressure in between the compartments maintains the fluidic isolation of the cell bodies and their extensions, where specific biochemicals and drugs can be selectively delivered either on cell body or axon part. DRGs are cultured in one compartment and is allowed to grow its neurite through the micro channels and to the other fluidically isolated compartment. Drugs and taxol can be added either compartments and axonal degeneration after 24 hours is evaluated.

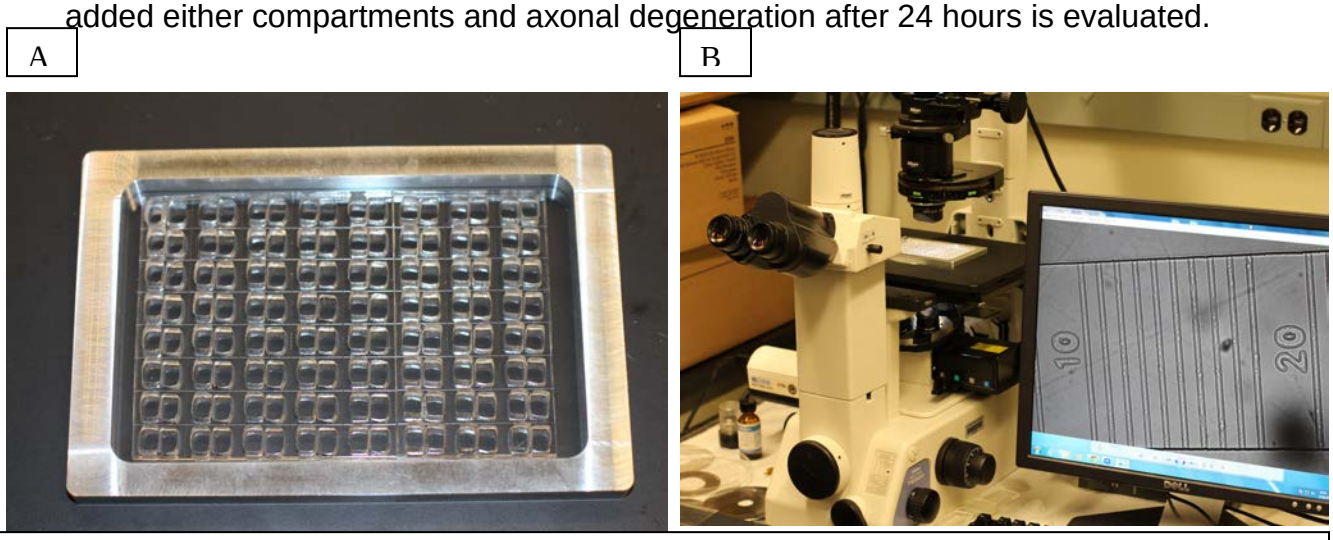


Figure 1. High throughput compartmentalized culture platform (HTCCP). **A.** Image of HCCP, 64 compartmentalized units per HTCCP. **B.** Image of microchannels of HTCCP

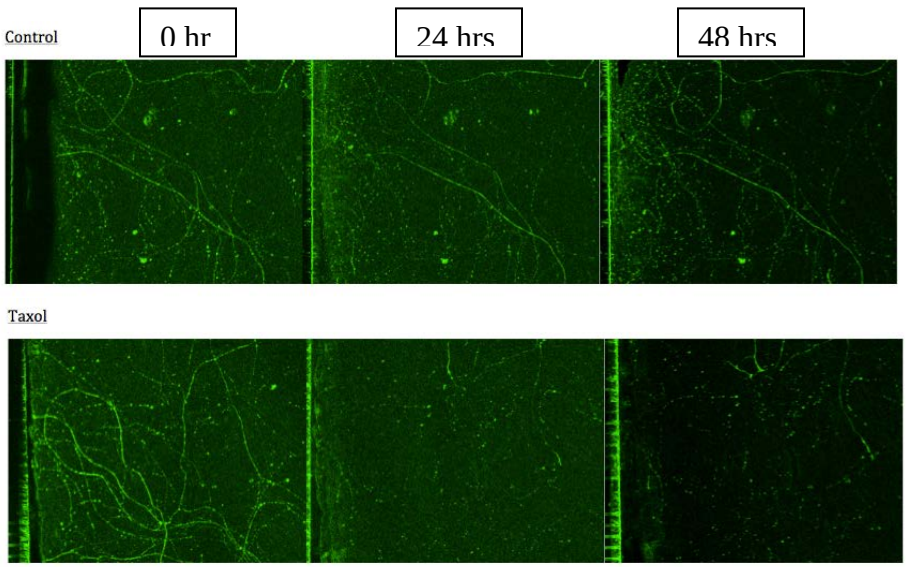
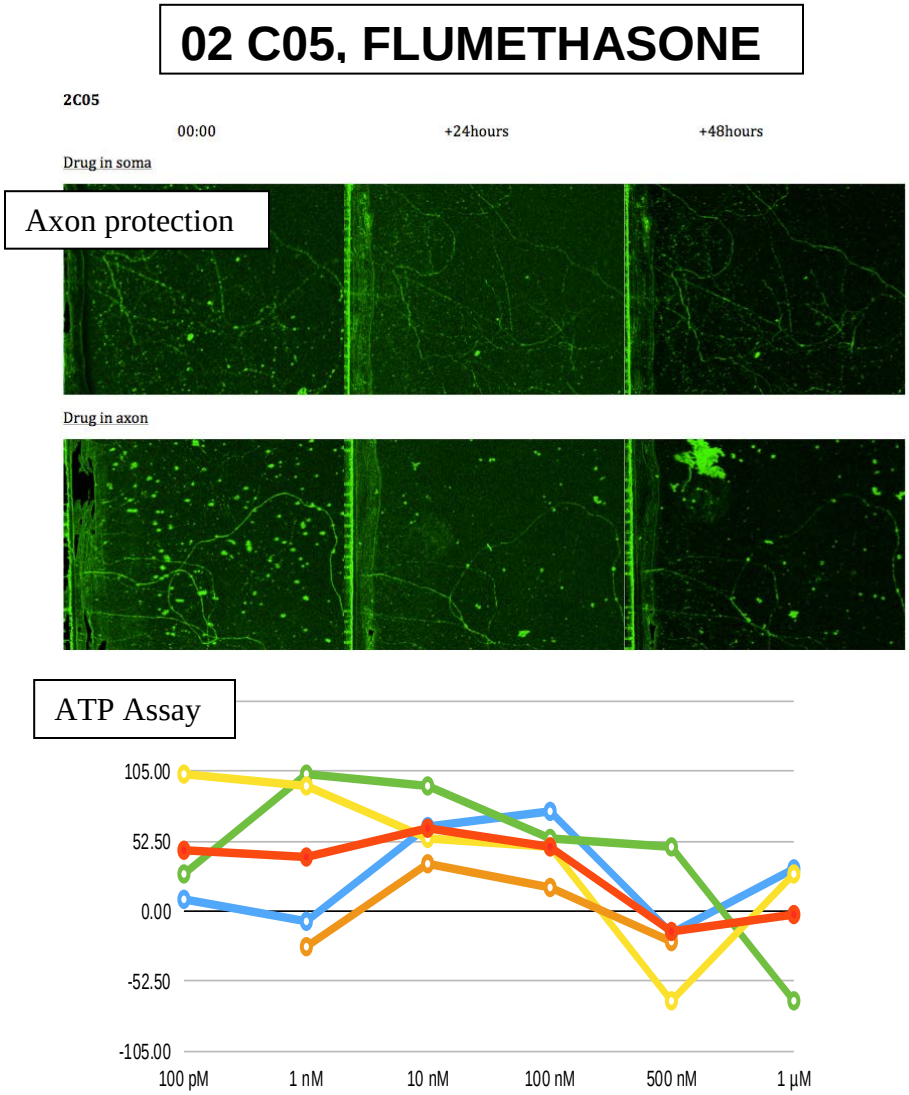
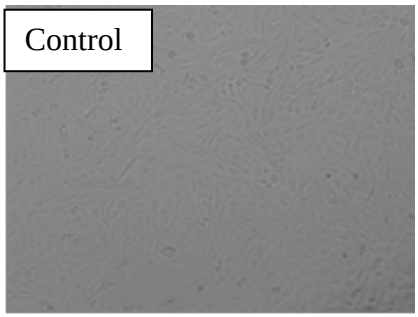


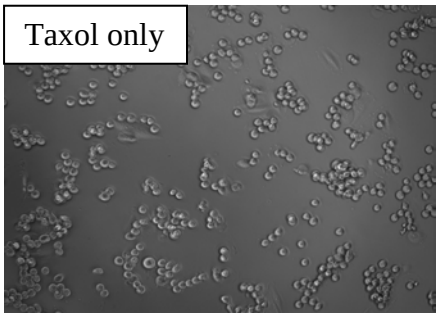
Figure 2. In vitro model of Taxol (Paclitaxel, 50nM) induced axonal degeneration in HTCCP. Image of axons with/without Paclitaxel with 0 hr, 24 hrs, and 48 hrs.

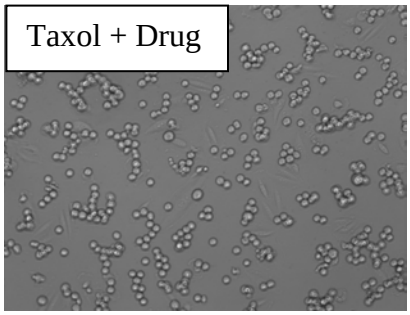
Neuroprotective drugs against Taxol:
An ATP light assay kit is used to check the cell viability in order to asses the toxicity/ protective effect of each drug. ATP is a molecule found in and around living cells, and as such it gives a direct measure of biological concentration and health. ATP is quantified by measuring the light produced through its reaction with the naturally-occurring firefly enzyme Luciferase using a Luminometer. The amount of light produced is directly proportional to the amount of biological energy present in the sample. 5 drugs were identified using HTCCP and ATP assay. We have identified 5 neuroprotective drugs against Taxol.

Evaluation of neuroprotective drugs in anti-cancer ability of Taxol:
To evaluate the impact of neuroprotective drugs on Taxol's ability to kill breast cancer cells, conditions for culturing cancer cells and measuring the ATP levels were optimized for the 96-well plate format. Briefly, 1,500 cells=well in media SUM159 in DMEM=F-12 with 5% FBS, 500ll of 10mg=ml insulin and 25ll of 10mg=ml hydrocortisone) were plated in 96-well plates for 24 hours. Constant concentrations of Taxol with or without neuroprotective drugs were added to the wells for another 24 hours.

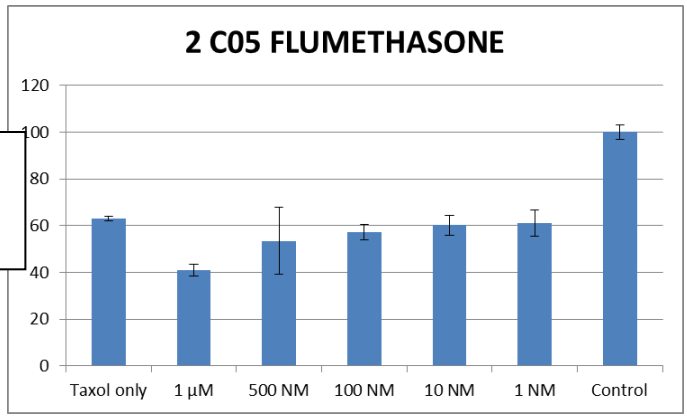






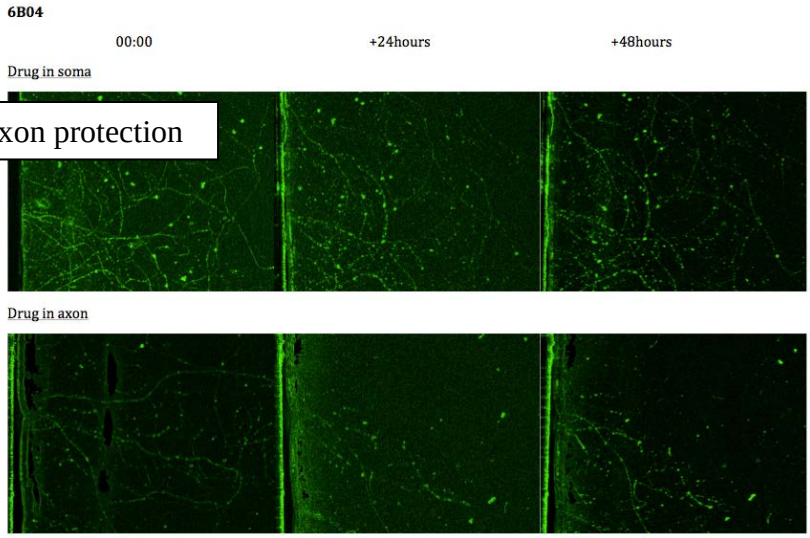


ATP assay of Cancer cells with drug + Taxol



6B04, Fluocinolone acetonide

Axon protection



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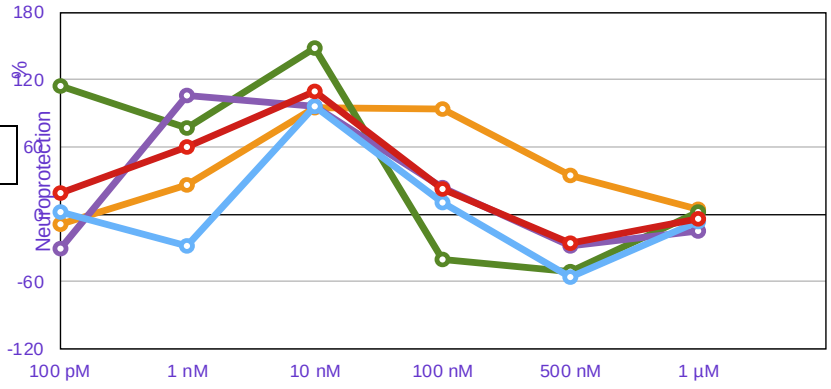
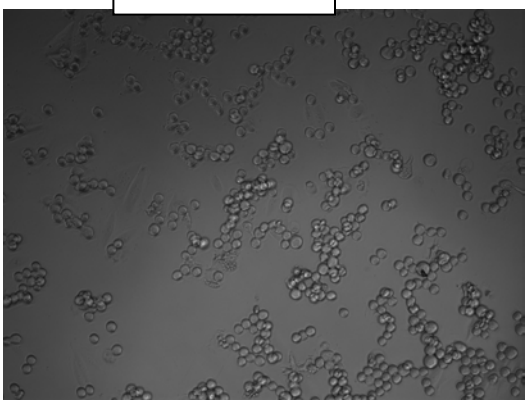


Taxol + Drug



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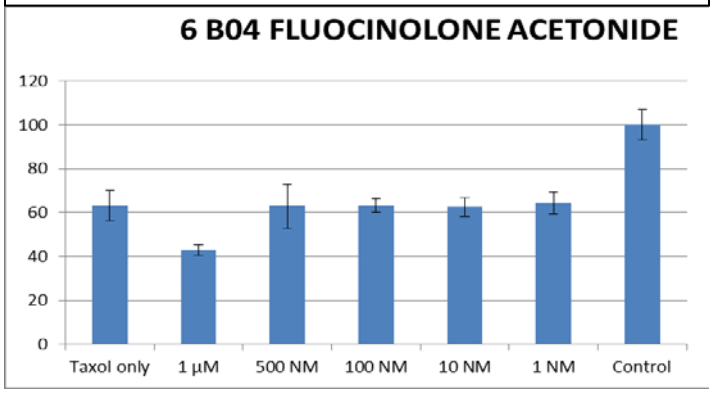
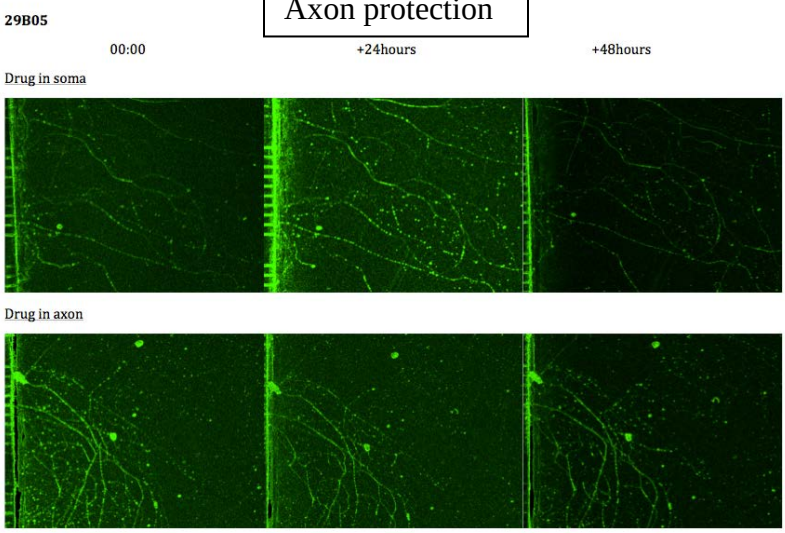
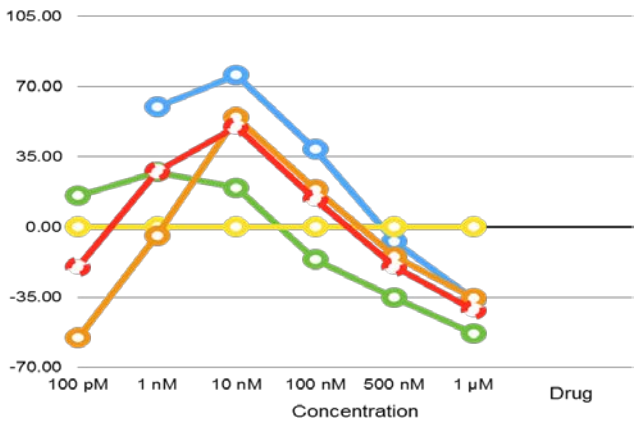


29 B05 CHOLEST-5-EN-3-ONE

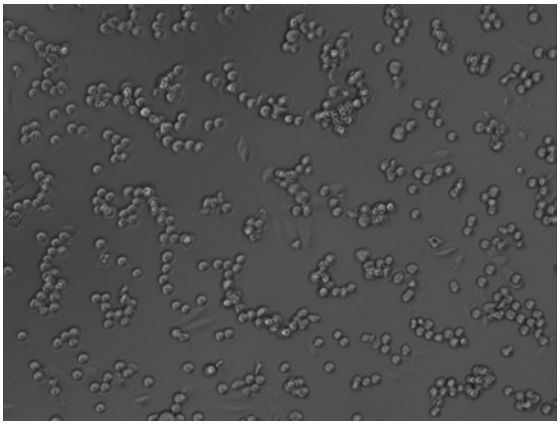
Axon protection



ATP Assay



Taxol + Drug



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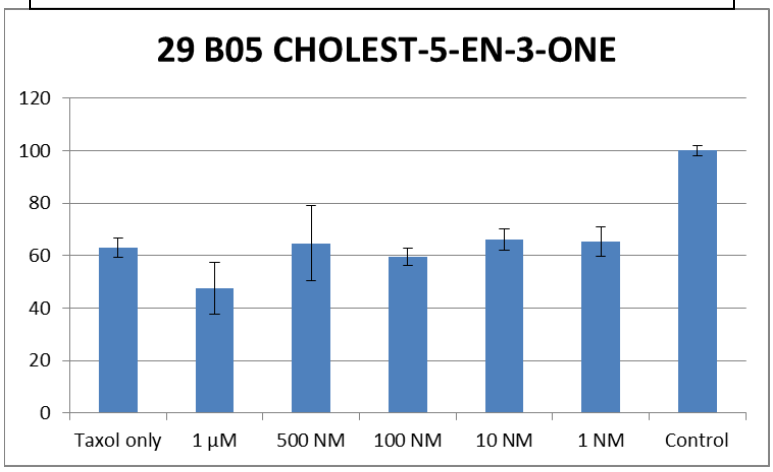
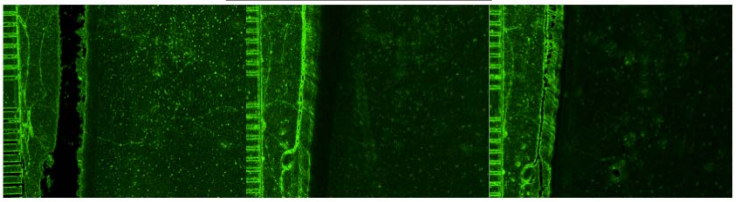


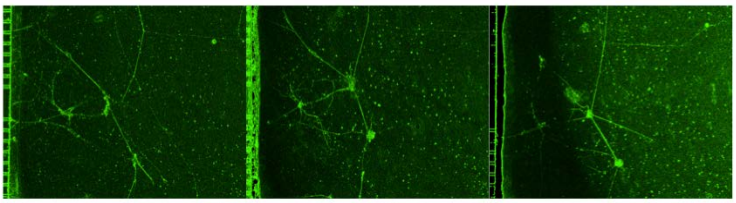
23 E05, XANTHOXYLIN

Axon protection

Taxol and Drug in axon



Taxol in axon, Drug in soma



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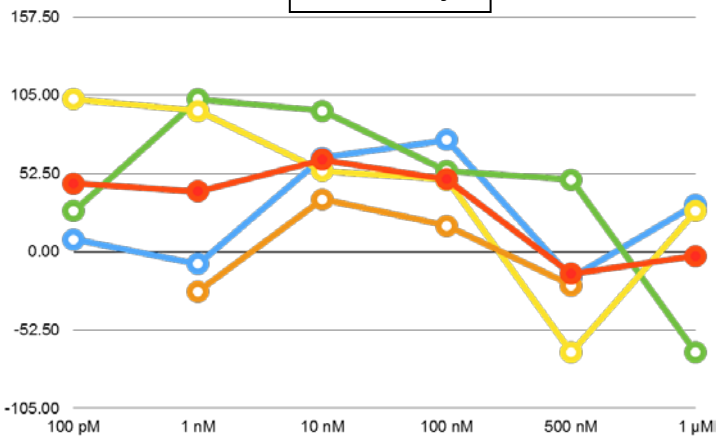
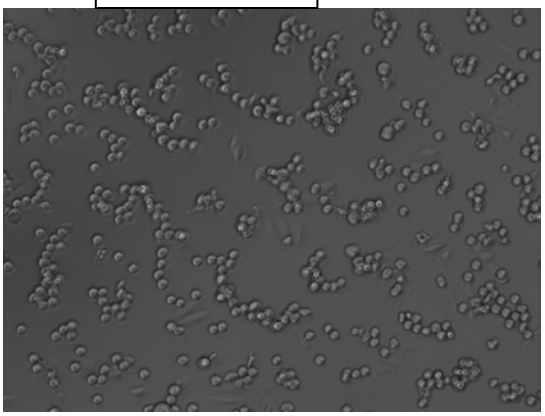


Taxol + Drug



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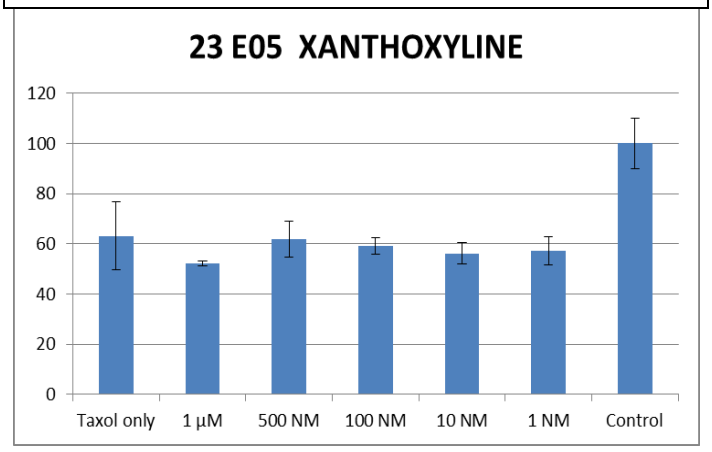
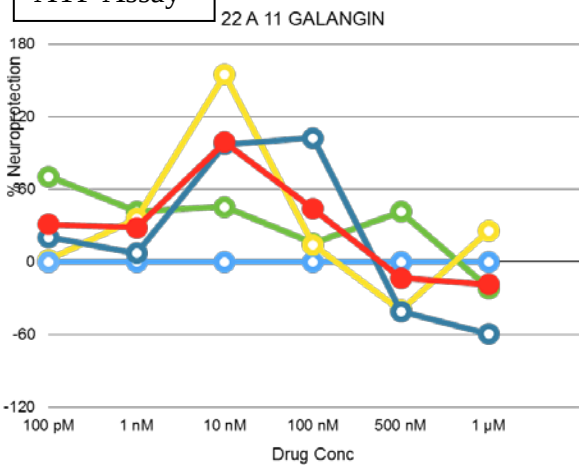
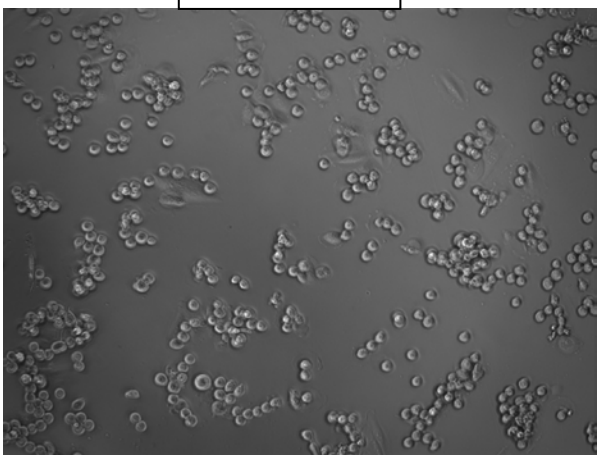


22 A11 GALANGIN

ATP Assay



Taxol + Drug



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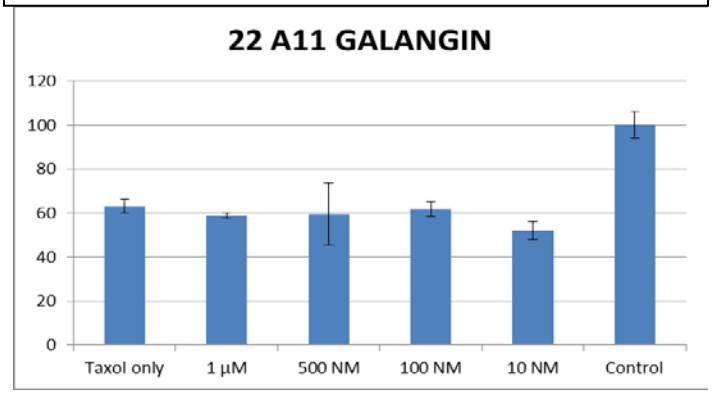

We have identified more than 50 neuroprotective drugs from the initial screening. In order to identify truly neuroprotective drugs, repetitive experiments have been done to get reproducible results. In addition, toxicity studies of some of identified drugs haven't been well studied in vivo. In order to get the toxicity data of identified drugs, multiple animal experiments with different dosages of drugs have been done to identify the optimal dosage of drugs. In conclusion, we have identified 5 neuroprotective drugs (GALANGIN, XANTHOXYLIN, CHOLEST-5-EN-3-ONE, FLUOCINLONE ACETONIDE, FLUMETHASONE) against Taxol. The identified drugs did not induce the proliferation of breast cancer cells which can be readily applicable to CIPN. This is very important in the therapeutic development in CIPN. Currently, in vivo experiments are being carried out with identified neuroprotective drugs with 5 different dosages. We will identify the in vivo neuroprotective effects of identified drugs soon.

Impact:
From this research, we have developed a novel High Throughput Compartmentalized Culture Platform (HTCCP) and identified 5 neuroprotective drugs against CIPN. The identified 5 drugs have great potentials to prevent CIPN. The identified drugs are filed patents though Johns Hopkins Tech Transfer. Further analysis and modification of identified drugs in CIPN can lead to the commercialization stages.

Changes/Problems:
XANTHOXYLIN and CHOLEST-5-EN-3-ONE have low water solubility. To improve the water solubility of drugs, chemical structures should be changed. Specific concentrations of drugs in tissues and organ are not well established. In order to identify potential toxicities of drugs in vivo, we are characterizing in vivo concentrations of drugs.

Products:
Patent: cholest-5-en-3-one, flumethasone, galangin, xanthoxyline, fluocinolone acetonide
Patent application date: 4/9/2014.
Patent application type: Provisional application
Application number: C13018-22

Participants & Other Collaborating Organizations:

Name:	In Hong Yang
Project Role:	PI
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	30
Contribution to Project:	Dr. Yang has performed work in the area of the development of HTSS and in vitro and vivo drug screening for CIPN
Funding Support:	

Name:	Ahmet Hoke
Project Role:	Co-PI
Researcher Identifier (e.g. ORCID ID):	

Nearest person month worked:	3
Contribution to Project:	Dr. Hoke has performed work in the area of vivo validation of drug in CIPN
Funding Support:	

Name:	Nitish Thakor
Project Role:	Co-PI
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	3
Contribution to Project:	Dr. Thakor has performed work in the area of development of microfluidic system.
Funding Support:	

Name:	Shuo Wang
Project Role:	Technician
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	12
Contribution to Project:	Shuo Wang has performed work in the area of drug screening in vitro.
Funding Support:	

Special Reporting Requirements: Nothing to report.

Appendices

1. Pike CT, Birnbaum HG, Muehlenbein CE, Pohl GM, Natale RB (2012). Healthcare costs and workloss burden of patients with chemotherapy-associated peripheral neuropathy in breast, ovarian, head and neck, and nonsmall cell lung cancer. Chemother Res Pract 2012:913848.

2. Marmiroli P, Nicolini G, Miloso M, Scuteri A, Cavaletti G (2012). The fundamental role of morphology in experimental neurotoxicology: the example of chemotherapy-induced peripheral neurotoxicity. Ital J Anat Embryol 117:75-97.

3. Postma TJ, Heimans JJ (2000). Grading of chemotherapy-induced peripheral neuropathy. Ann Oncol 11:509-513.

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5. Hershman DL, Weimer LH, Wang A, Kranwinkel G, Brafman L, Fuentes D, Awad D, Crew KD(2011). Association between patient reported outcomes and quantitative sensory tests for measuring long-term neurotoxicity in breast cancer survivors treated with adjuvant paclitaxel chemotherapy. Breast Cancer Res Treat 125:767-774.