Investigating the Mechanism of Action Behind Novel Anti-Cancer Drugs

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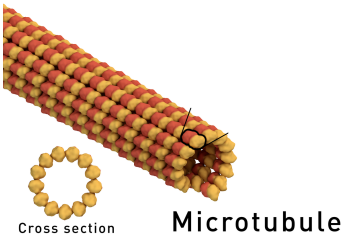
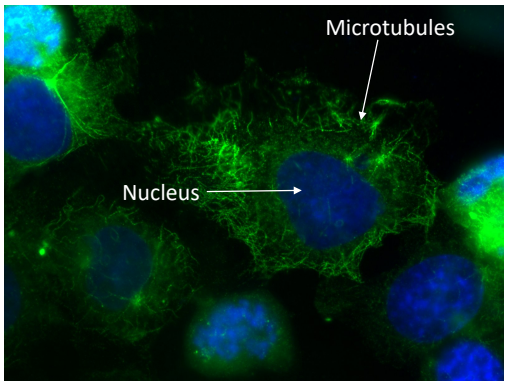
Contributions to medicine – why does this research matter?

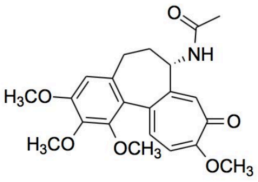
- Minimize side effects and toxicity in cancer patients receiving chemotherapy
- Advance current knowledge in the area of microtubule-targeted drugs



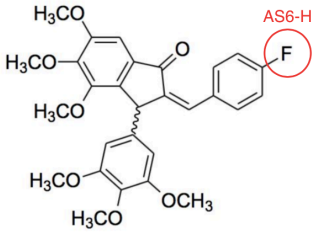


Research goals

- **Long-term:** determine a mechanism of action for Ind-V and AS6-H (microtubule-targeting drugs)



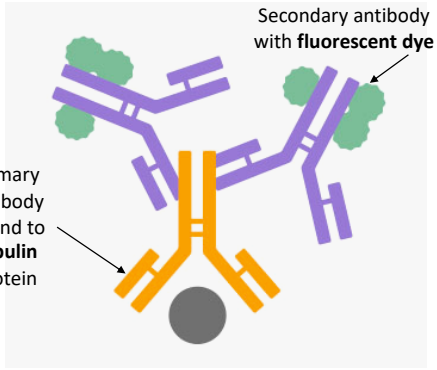
Colchicine



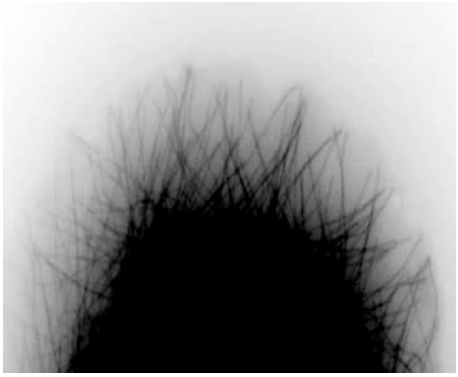
Ind-V

- **Short-term:** develop new assays to measure drug activity
  1. Immunofluorescence assay – qualitative info on microtubule structure
  2. Viacount assay – measure cell proliferation on a large scale
  3. Light scattering assay – measure amount of *in vitro* polymerized tubulin
  4. *In vivo* microinjection assay

In vivo methods: immunofluorescence (1) and microinjection for future experiments

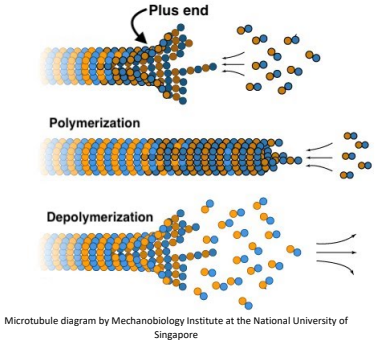


Immunofluorescence schematic by Thermo-Fisher Scientific

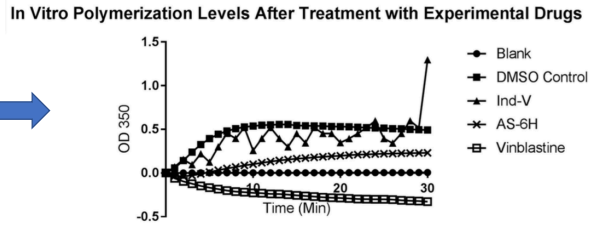


Rhodamine-tubulin injected MCF7 breast cancer cell recorded by Kathy Kamath

In vitro methods: light scattering assay (3)

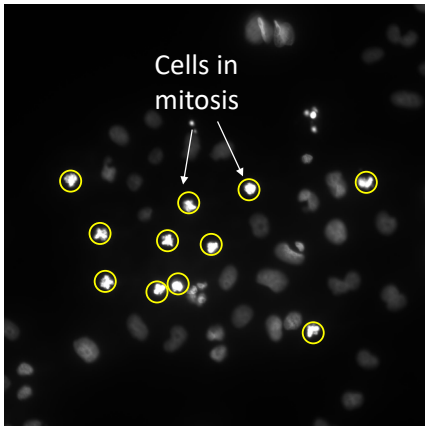


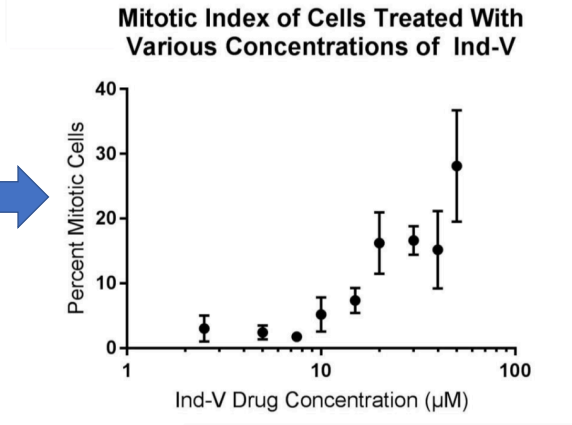
Microtubule diagram by Mechanobiology Institute at the National University of Singapore



- *In vitro* = "test-tube experiment"
- Measures the amount of protein that polymerizes in the presence of different drug concentrations
- Less polymerized protein suggests that the drug inhibits microtubule polymerization, freezing the cells in mitosis

1. Immunofluorescence data shows the proportion of cells arrested in mitosis

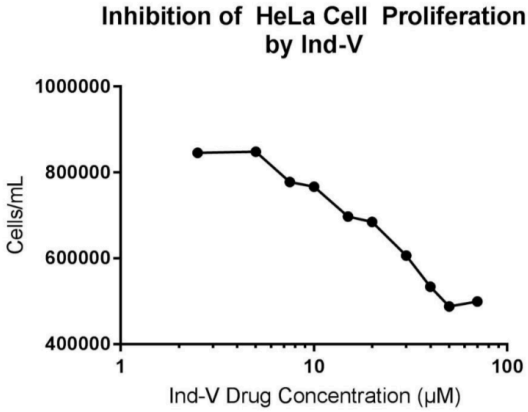




Mitotic index = $\frac{\#\ Mitotic\ cells}{Total\ \#\ of\ cells}$

2. Viacount assay data shows that higher drug concentrations lead to an increase in cell death

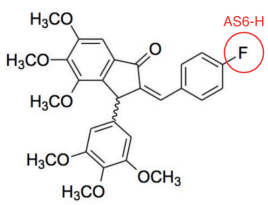
Date - 28-JUL-2018		
Instrument Serial Number - GTI-020435		
Software Name - ViaCount		
Version Number - 2.5.2		
Sample No.	**Sample ID**	**Number of Events**
1	F11	1000
2	F12	1000
Cell Concentration Information(cells/mL)		
Viable	Dead	
137010.09	166781.69	
155819.8	170163.03	
% of Total Information		
Viable	Dead	
45.1	54.9	
47.8	52.2	



Summer 2018 in review

Summer goals:

1. Immunofluorescence assay
2. Viacount assay
3. Light scattering assay



Ind-V

Results:

1. Mitotic index from **immunofluorescence experiments** increases with drug concentration, suggesting that Ind-V induces cell cycle arrest in a concentration-dependent manner
2. Data from **Viacount assays** support the idea that the effects of Ind-V are concentration-dependent
3. Past data from **light scattering assays** shows that AS6-H decreases the amount of polymerized protein formed

Future goals and acknowledgements

- **In the future:**
  - Experiments:
    - Microinjection experiment with Ind-V and AS6-H
    - Additional flow cytometry experiments
  - Long-term goals:
    - Determine a mechanism of action for Ind-V & AS6-H
    - Eventually introduce the drug to human clinical trials

- **Special thanks to:**
  - CSEP and the Gorman Scholars Program
  - Dr. Thrower and the Wilson Lab
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