Nature as a Remarkable Chemist
The Discovery and Development of Taxol

Taxus brevifolia Western Yew

December 2019 Joint BSA/NCAB Meeting

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NATIONAL INSTITUTES OF HEALTH
BETHESDA, MARYLAND 20014

NATIONAL CANCER INSTITUTE

April 21, 1977

Dr. Susan Horwitz
Assistant Professor
Department of Pharmacology
Albert Einstein College of
Medicine of Yeshiva University
1300 Morris Park Avenue
Bronx, New York 10461

Dear Susan:

At a recent Decision Network meeting, NSC-125973 (Taxol) was approved for further study. We have some information about it (folder enclosed) and believe that it may be a protein synthesis inhibitor. Would you please study this compound in your systems.

The compound is quite insoluble in aqueous vehicle, but DMA and DMSO can be used effectively.

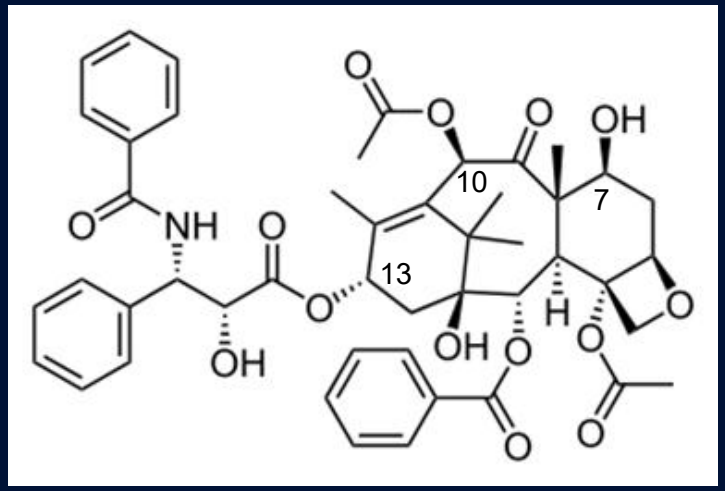
Sincerely,

David

David Abraham, Ph. D.
Investigational Drug Branch
Cancer Therapy Evaluation Program
Division of Cancer Treatment
National Cancer Institute

Taxus brevifolia

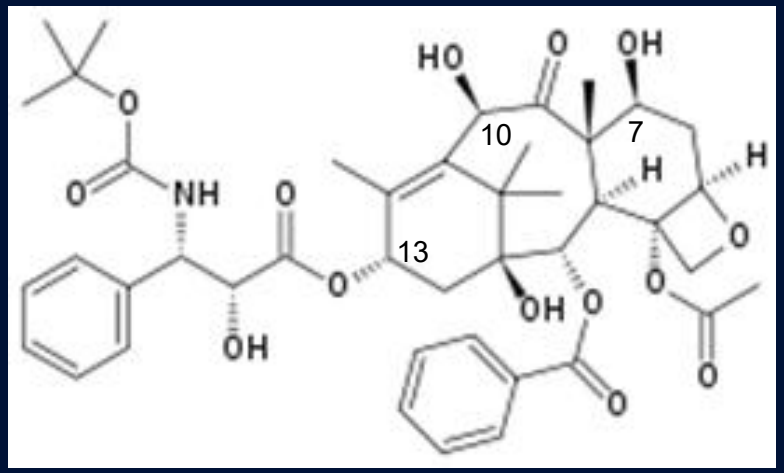
Paclitaxel (Taxol®)



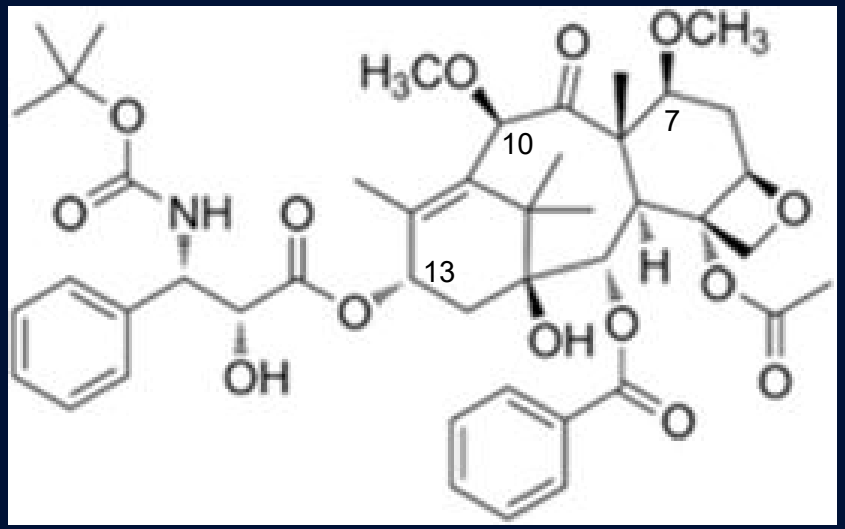
Abraxane®

Protein-bound particles for injectable suspension (albumin-bound)

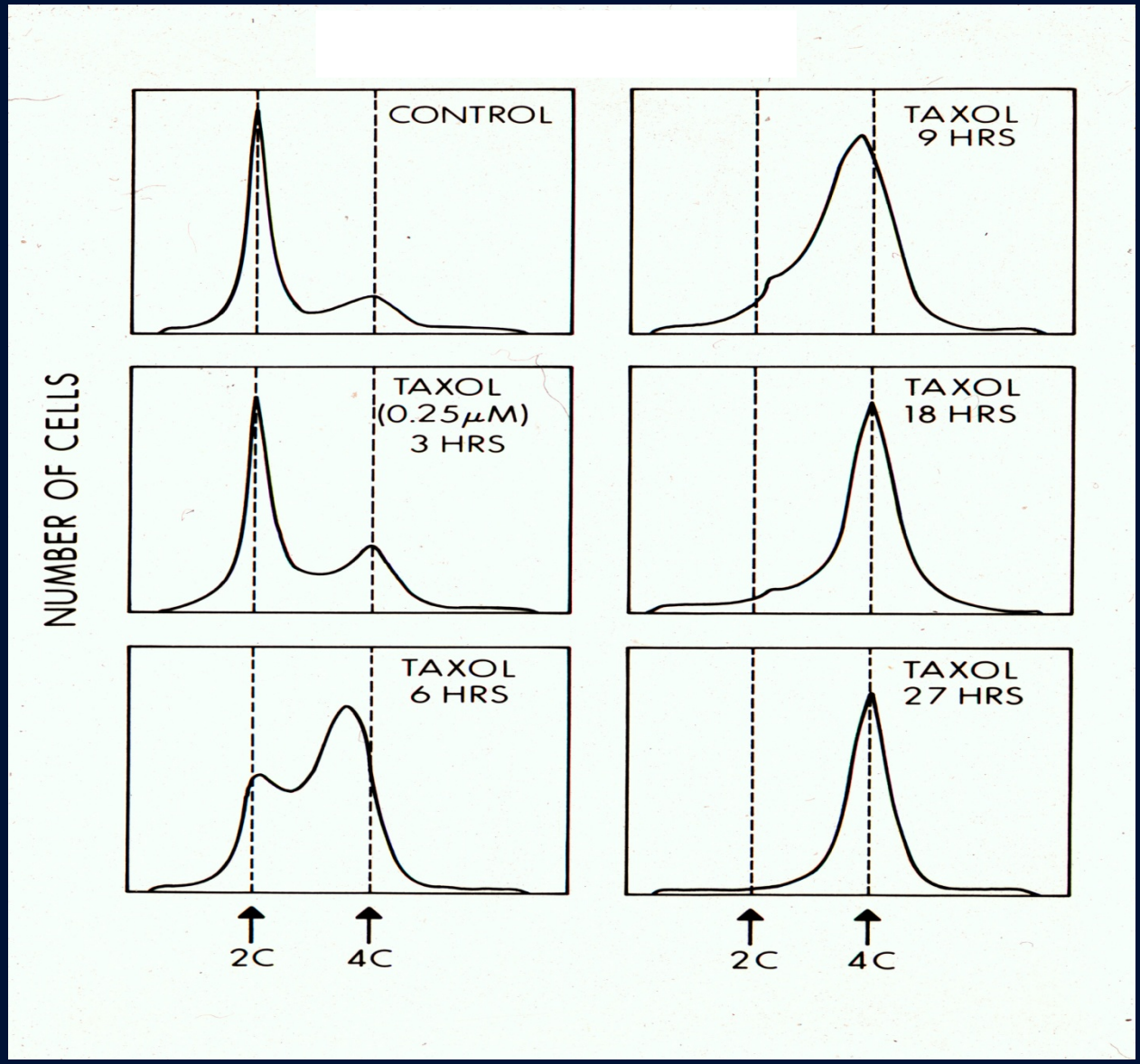
Docetaxel (Taxotere®)



Cabazitaxel (Jevtana®)

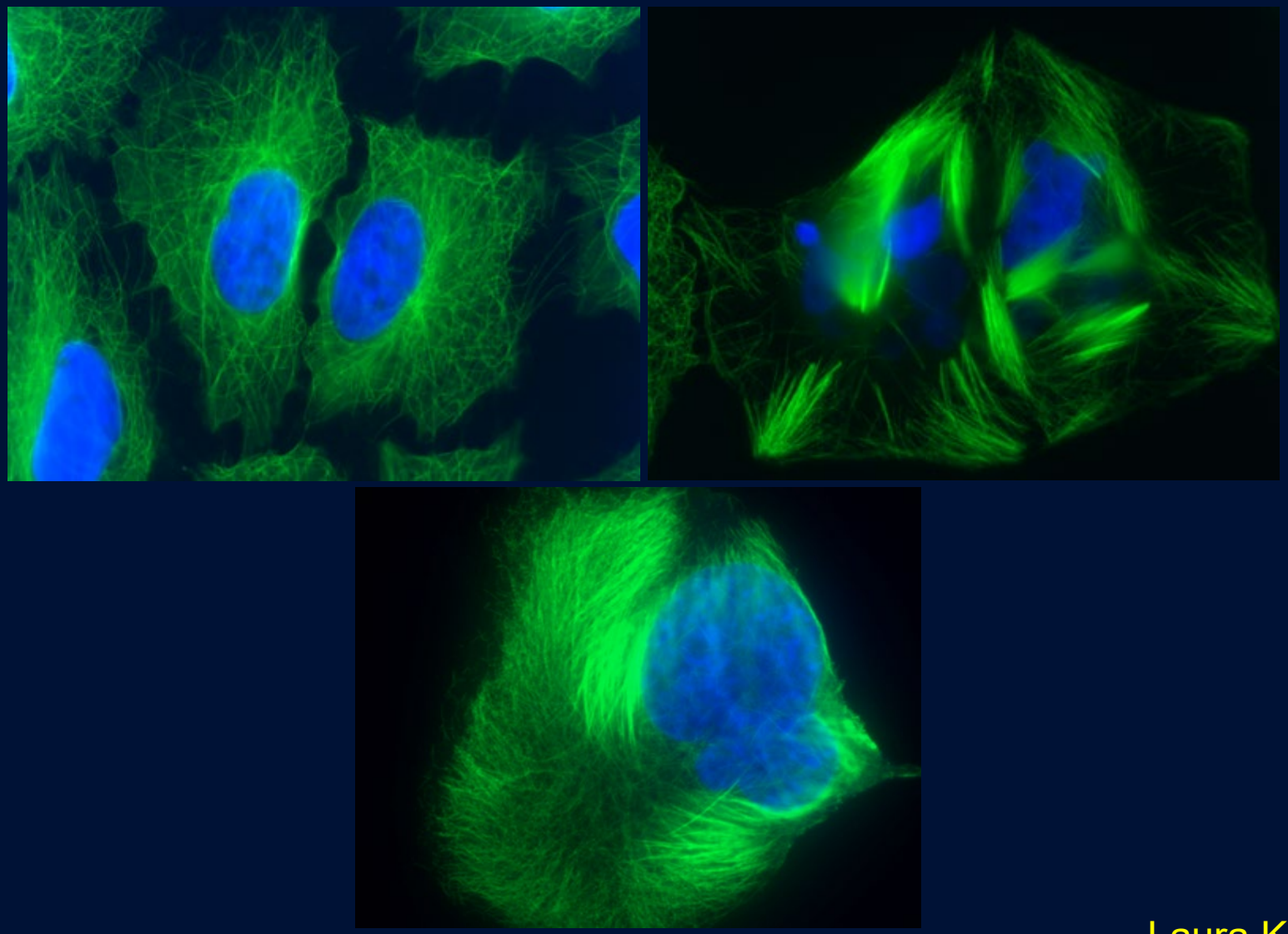


Taxol Blocks HeLa Cells in Mitosis



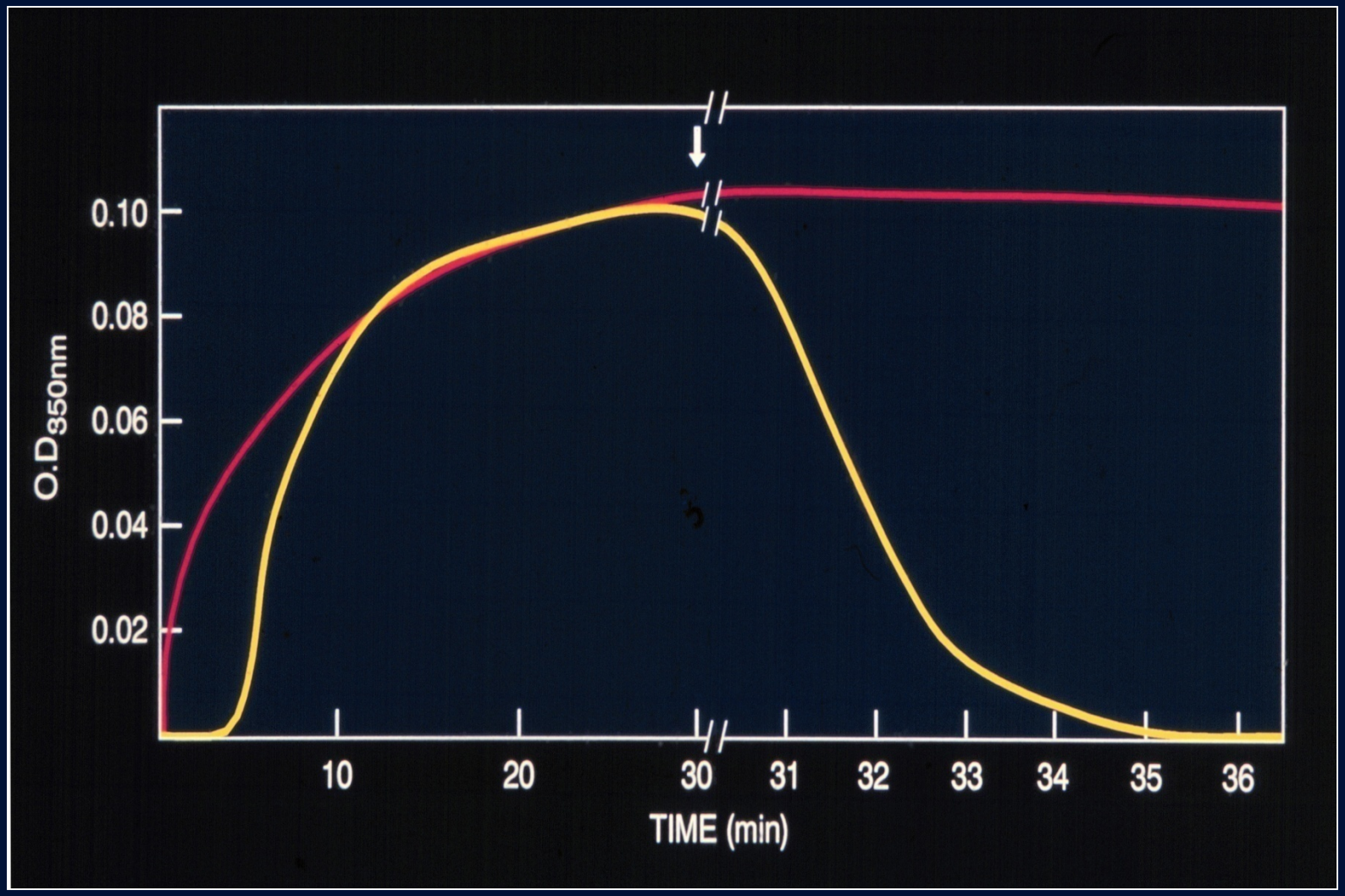
Schiff & Horwitz, 1980, PNAS.

Taxol induces microtubule bundling



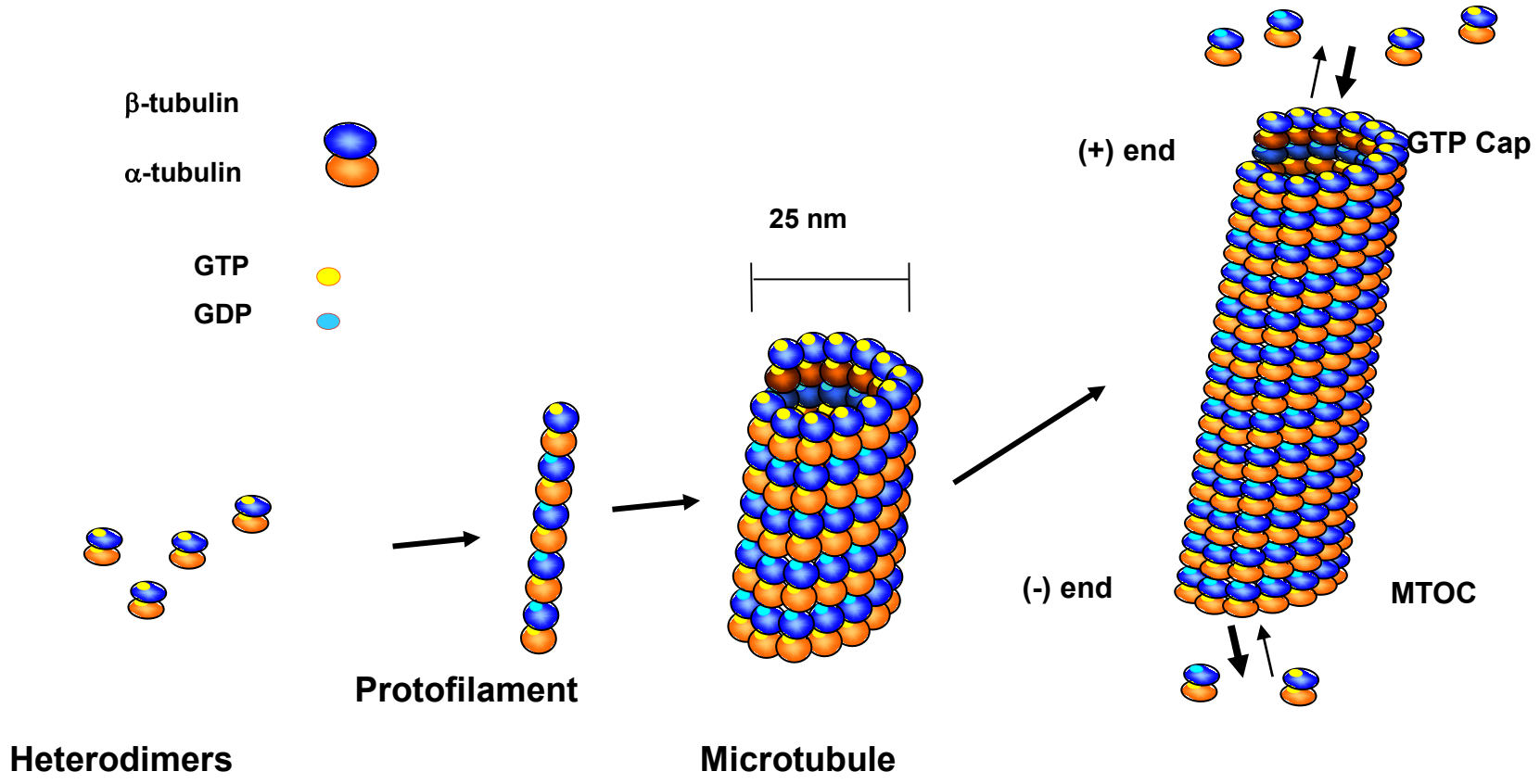
Laura Klein

Taxol Enhances *In vitro* Tubulin Polymerization and Microtubule Stabilization



Schiff et. al., 1979, Nature

Microtubule Structure and Dynamics



- Microtubules (MTs) are highly dynamic and switch stochastically from shrinking (catastrophe) to growing (rescue) both in *vivo* and in *vitro*—DYNAMIC INSTABILITY

- Numerous isotypes (7 alpha, and 8 beta) differ mainly at their acidic, negatively charged termini that interact with various intrinsic proteins (MAPs, kinesins, stathmin, etc.)

ALBERT EINSTEIN COLLEGE OF MEDICINE
OF YESHIVA UNIVERSITY

1300 MORRIS PARK AVENUE. BRONX, N.Y. 10461. CABLE EINCOLLMED, N.Y.

DEPARTMENT OF MOLECULAR PHARMACOLOGY PHONE: (212) 430-2000

August 9, 1978

Dr. John Douros
Drug Development Branch
National Cancer Institute
National Institutes of Health
Bethesda, Maryland 20014

Dear John:

As I mentioned to you in Hawaii, my laboratory has become very interested in the mechanism of action of taxol. We have been working intensely with this drug during the past year and find that it is extremely cytotoxic to cells growing in culture. Although we have not completely defined its site of action, we know that it is quite different from any other drug that we have previously studied and we plan to pursue its activity. In order to do this, we need radio-actively labeled taxol. Monroe Wall and M.C. Wani isolated taxol and would certainly be the most knowledgeable concerning the preparation of labeled drug. I would, of course, include them in any publications that might develop from material they prepared. I would appreciate it if you could bring this problem to their attention. We would also like to test the two major products isolated from taxol after mild base-catalyzed methanolysis, $C_{17}H_{17}NO_4$ and $C_{29}H_{36}O_{10}$, as described in JACS 93:9, 1971.

I enjoyed talking with you in Hawaii. Thank you very much for your help.

Sincerely,

Susan

Susan B. Horwitz, Ph.D.
Associate Professor

SBH:mr

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
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BETHESDA, MARYLAND 20014

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NATIONAL CANCER INSTITUTE

August 22, 1978

Dr. Monroe E. Wall
Research Triangle Institute
P. O. Box 12194
Research Triangle Park, NC 27709

Dear Monroe:

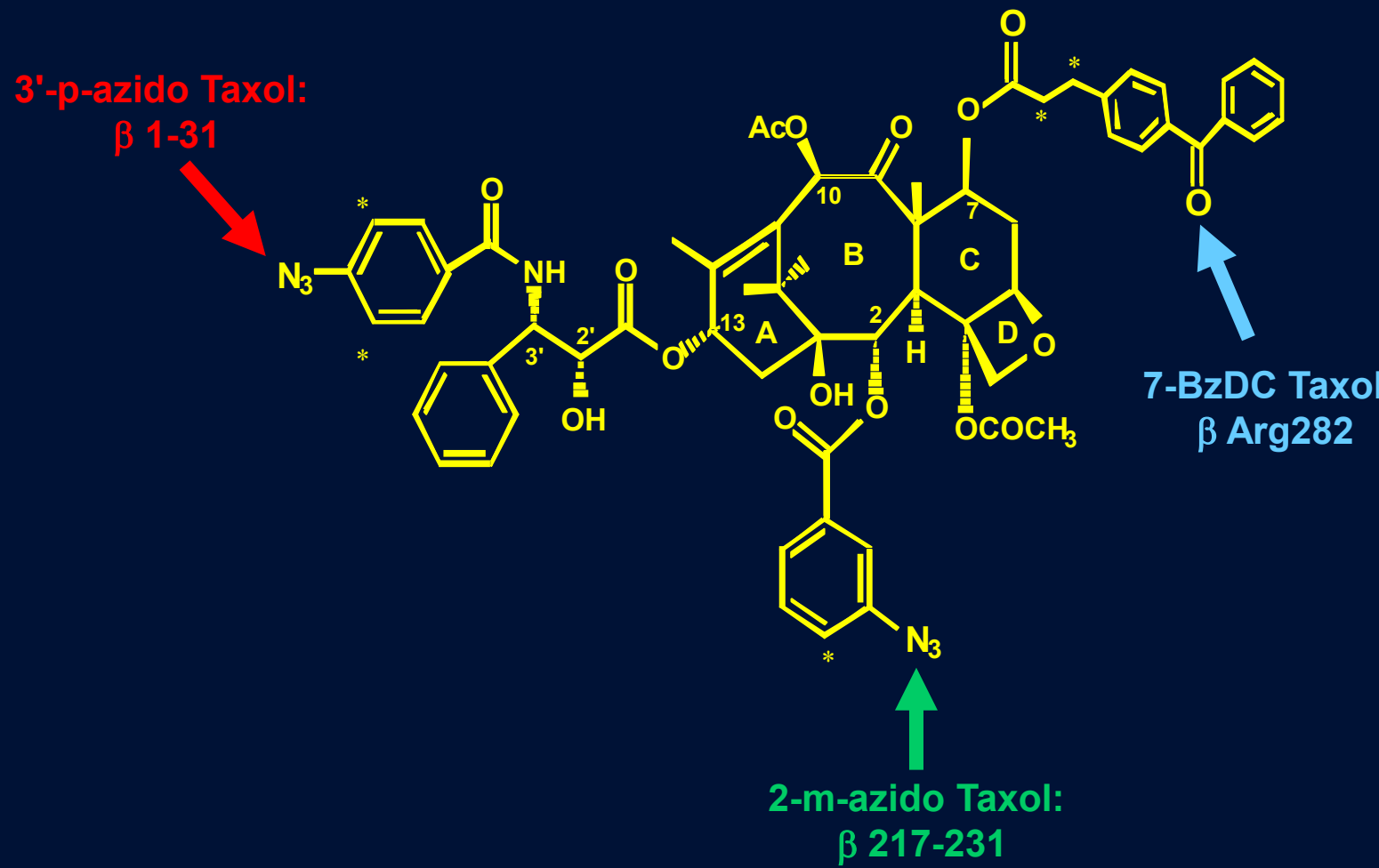
Can you help this poor girl (enclosed letter). Please send me a quote on these radiolabeled materials and I will buy them from you.

Sincerely,

John D. Douros, Ph.D.
Chief, Natural Products Branch
Developmental Therapeutics Program
Division of Cancer Treatment, NCI

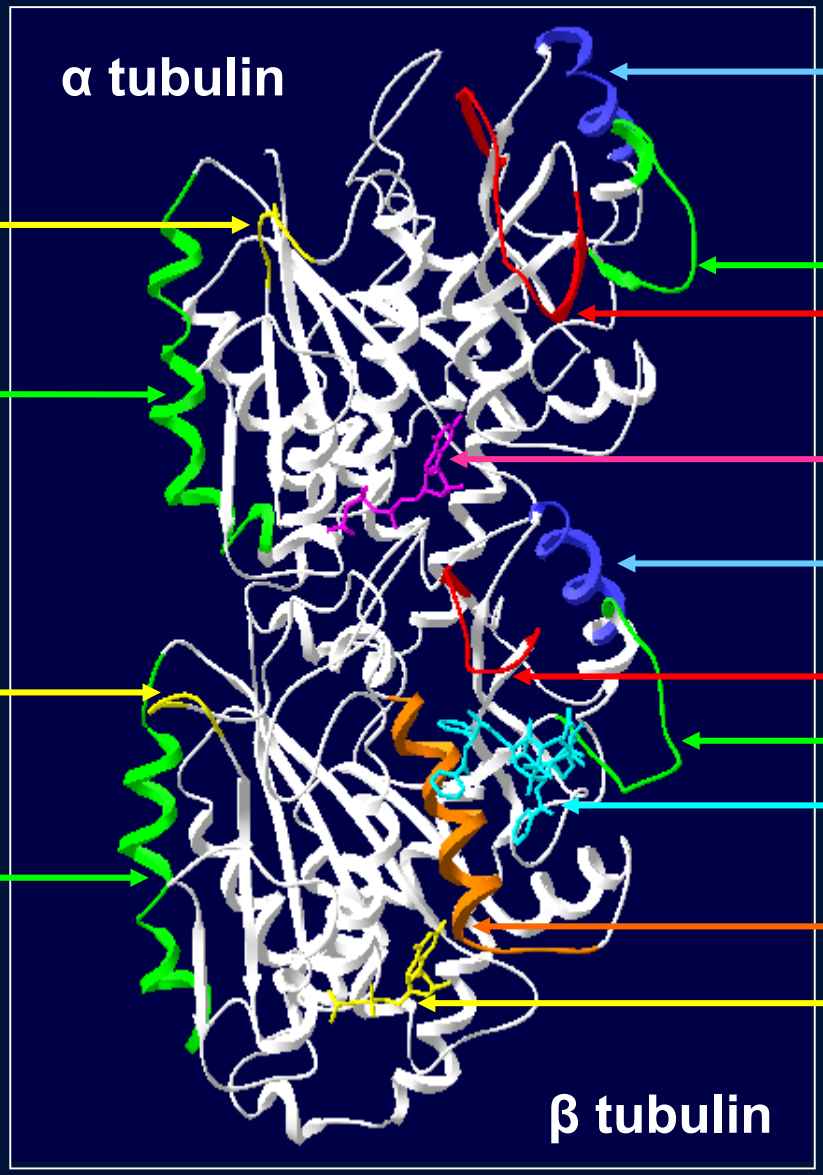
enclosure

Photoaffinity Labeling Results



Rao, et. al., 1994, 1995, 1999, J. Biol. Chem.

Taxol in the three-dimensional model of αβ-tubulin dimer



H10 Helix
H1- S2 Loop
M loop
S9-S10 Loop
H3 Helix
GTP
H10 Helix
H1- S2 Loop
S9-S10 Loop
M loop
Taxol
H3 Helix
H7 Helix
GDP

Nogales et. al. 1998

Taxol Chronology I

1958	Collaboration NCI/USDA
1962	NCI/USDA random plant collections Extract of bark from *Taxus brevifolia*, cytotoxic to KB cells
1971	Taxol isolated and structure published cytotoxic to L-1210, P-388, P-1543 leukemias, WM-256 carcinosarcoma
1975	Excellent activity against murine B16 melonoma
1977	Selected for clinical development by NCI
1978	Good activity against some human xenografts, MX-1 mammary tumor
1979	Unique mechanism of action described

Taxol Chronology II

1980	Preclinical toxicology Aqueous insolubility (Cremophor); Neutropenia
1982	Approved by NCI for IND application
1983	Clinical Trials Hypersensitivity reactions; Scarcity of taxol
1988	Clinical activity Advanced drug-refractory ovarian carcinoma Metastatic breast carcinoma Evaluation in a variety of malignancies Combination chemotherapy / radiation / G-CSF
1991	January 23rd. Cooperative Research & Development Agreement (CRADA) NCI and BMS
1992	July 22nd. NDA by BMS for ovarian cancer
1992	December 29th. FDA approval, refractory ovarian cancer
1994	FDA approval, breast carcinoma
1999	FDA approval, non-small cell lung carcinoma
2013	FDA approval, Abraxane® in combination with Gemcitabine for metastatic adenocarcinoma of the pancreas

Names for hi-jacking

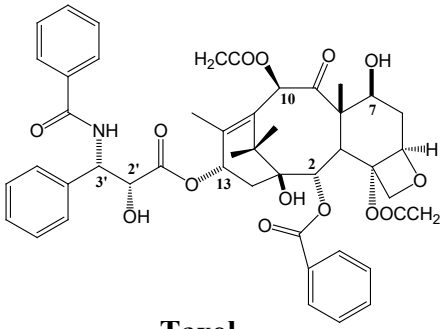
"Taxol is a trademark now, but Bristol-Myers Squibb should return it to the research community".

Nature, Feb 2nd, 1995

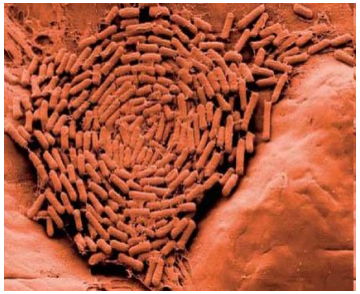
Trademark	Generic
Taxol®	Paclitaxel
Taxotere®	Docetaxel

Microtubule Stabilizing Agents (MSAs)

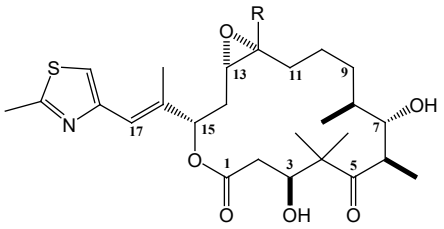
Pacific Yew



Taxol



Sorangium Cellulosum

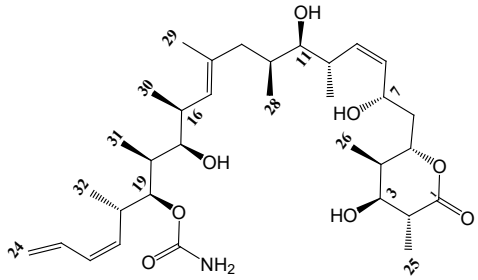


Epothilone A (R=H)
Epothilone B (R=Me)

↓ O → N

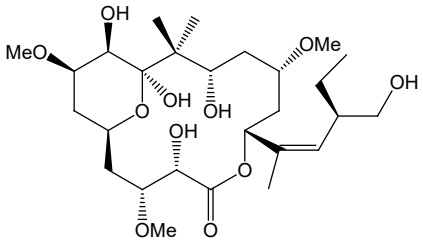
Ixabepilone (IXEMPRA ™)

Discodermia dissoluta

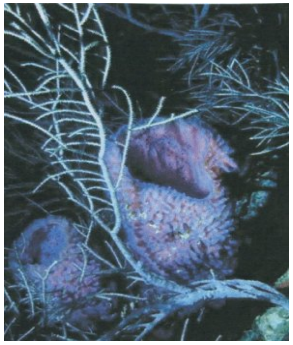


(+)-Discodermolide

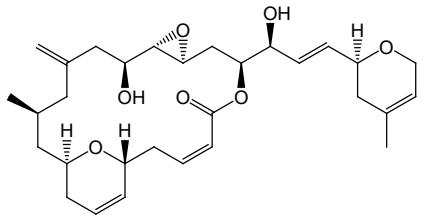
Mycale hentscheli



(+)-Peloruside A



Fasciospongia rimosa



(-)-Laulimalide

β-Tubulin Isotypes

Tubulin Class	Gene Name	Protein Name	Chromosome Location	Organ Expression	Cellular Expression
Class I	TUBB	βI	6q21.33	Constitutive	All Cells
Class II	TUBB2A	βIIa	6p25.2	Brain, Nerves, Muscles	Some Neurons
Class II	TUBB2B	βIIb	6p25.2	Brain, Muscles, Tonsils	Some Neurons
Class III	TUBB3	βIII	16q24.3	Brain, Testis, Colon	Neurons Sertoli Cells Epithelial Cells
Class IV	TUBB4A	βIVa	19p13.3	Brain	Neurons Glia
Class IV	TUBB4B	βIVb	9q34.3	Most Organs	Testis Ciliated Cells
Class V	TUBB6	βV	18p11.21	Tissue Specific	Muscle Endothelial Secretory Cells
Class VI	TUBB1	βVI	20q13.32	Blood, Marrow, Spleen	Erythroid Cells Platelets

Distribution of βV-Tubulin in Normal Tissues by Immunohistochemistry

Tissue		Intensity
Skin	Sebaceous glands	+++
	Squamous epithelium	+ (variable)
	Basal cells	+
Muscle	Smooth muscle	+++
	Skeletal muscle	++
	Cardiac Muscle	++
Blood vessel	Endothelial cells	+++
	smooth muscle	++
GI tract[a]	Epithelium	–
Liver	Hepatocytes	–
	Bile ducts	++
Pancreas	Ducts	+
	Islets	++
	Acini	–
Kidney	Renal tubules	– to ++ (variable)
	glomeruli	++
Testis	Seminiferous tubules	–
	Mature germ cells	–
	Immature germ cells	++
	Sertoli cells	–

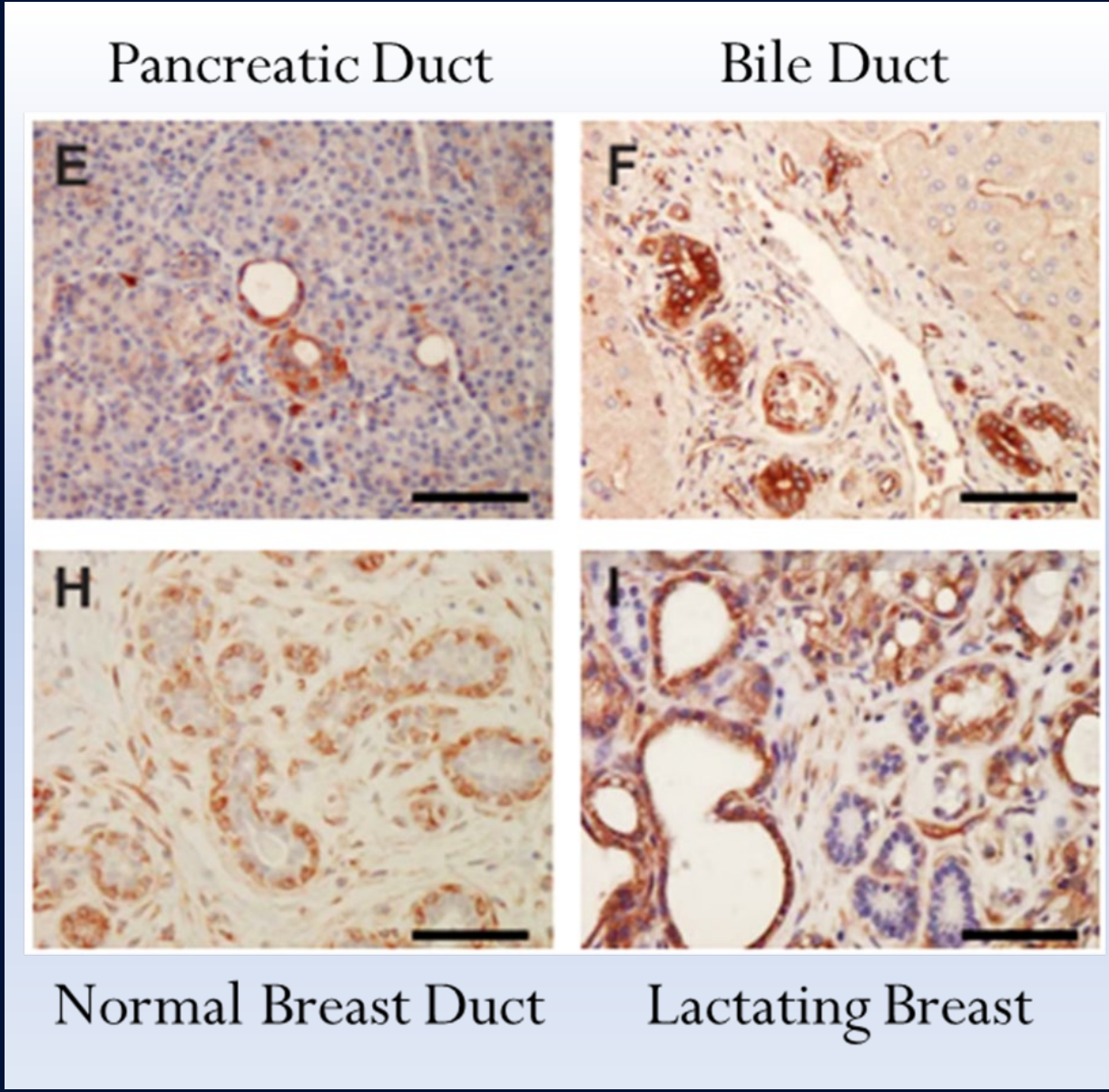
Tissue		Intensity
Prostate	Glandular cells	++ (variable)
	Basal cells	–
Mammary gland	Luminal cells	–
	Lactating secretory cells	++
	Myoepithelial cells	+++
Ovary	Surface epithelium	–
	Stromal cells	+
Fallopian tube	Epithelium	–
Lung	Pneumocytes	–
	Respiratory epithelium	–
Thyroid	Follicular cells	– to ++ (variable)
Adrenal gland	Cortex and medulla	–
Salivary Gland	Acini	+ (variable)
	Ducts	++
Nerve	Neuronal cells	–

- Tissues with secretory function showing βV-tubulin expression

[a] Stomach, Small Intestine, Appendix, Colon, Gall Bladder

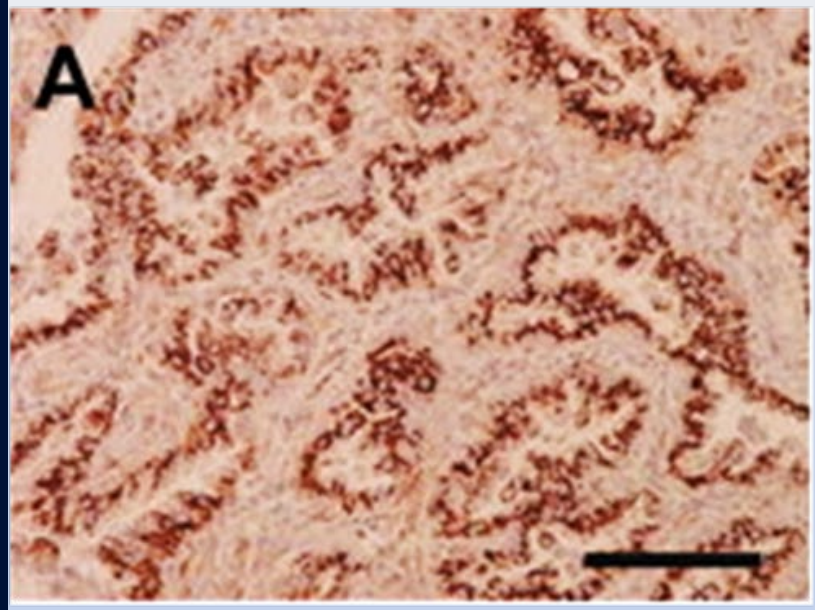
Chao et al (2012) *Cytoskeleton*, 69:566–576

βV-tubulin in Secretion

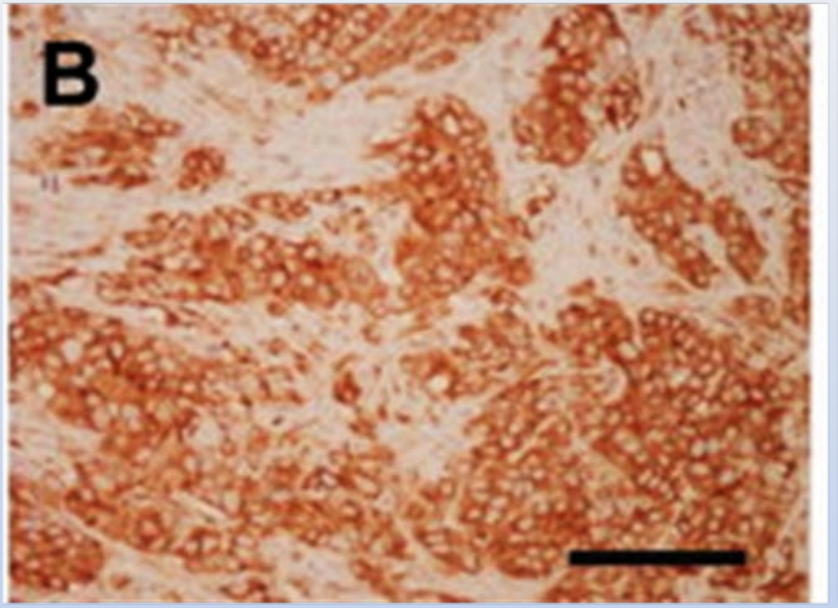


βV-tubulin in Malignancies

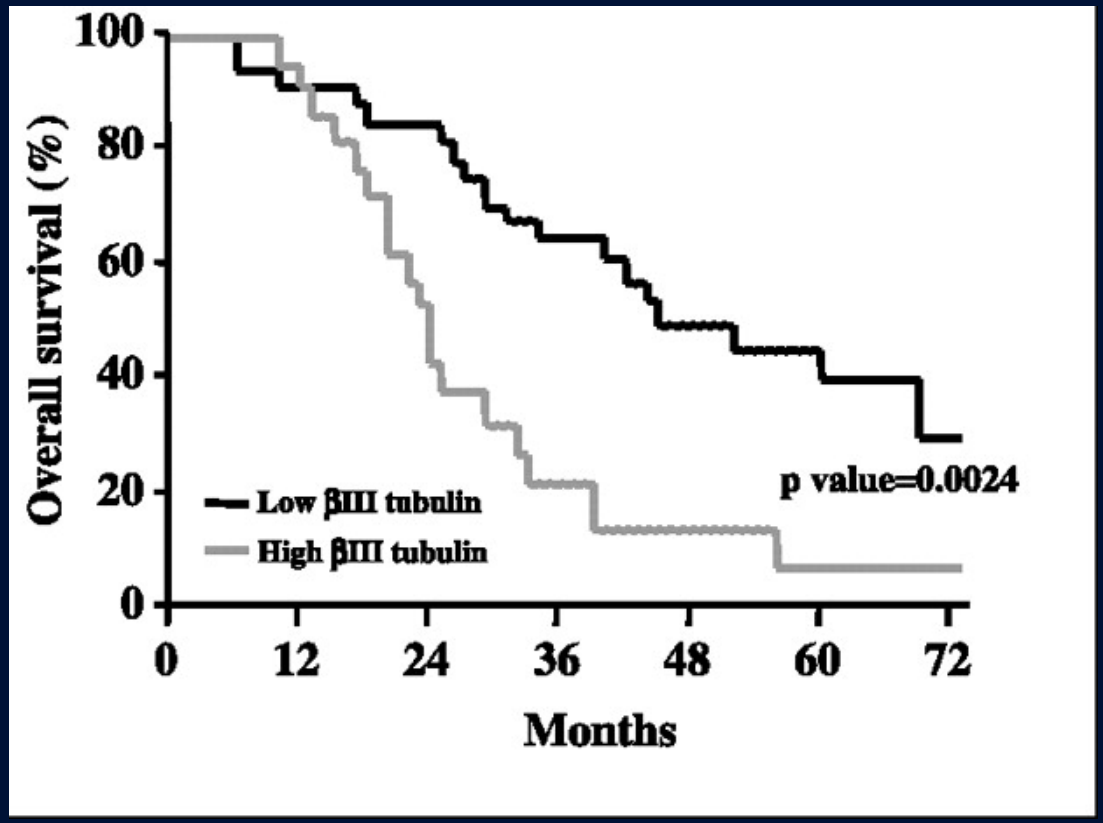
Lung Adenocarcinoma



Invasive Breast Cancer

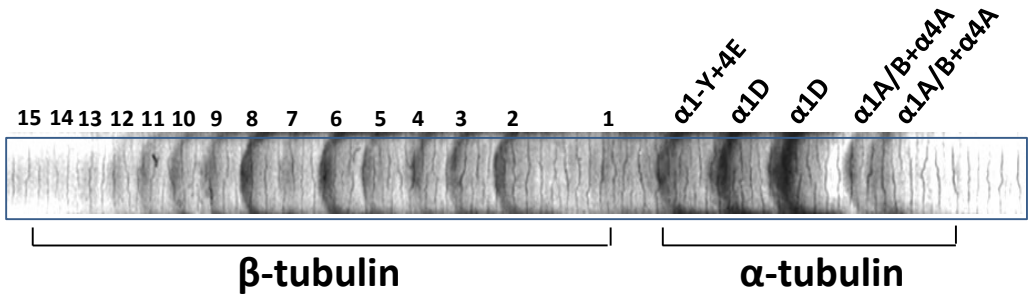


Overall survival curve according to low versus high βIII-Tubulin expression in advanced ovarian cancer patients. βIII is a Marker of Poor Outcome in Advanced Ovarian Cancer

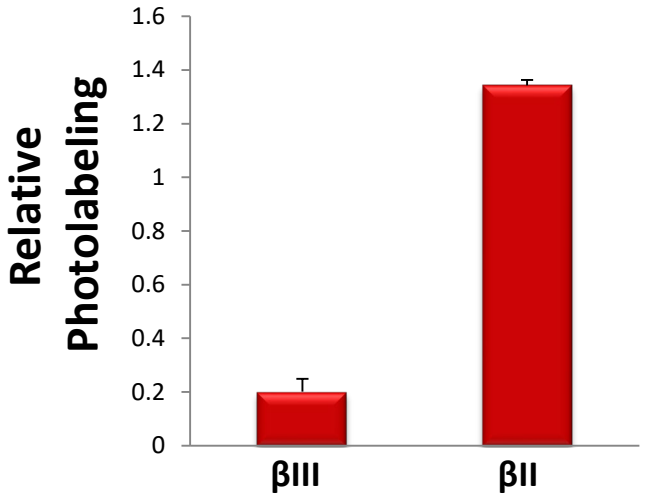


Ferrandina, G. et al. *Clin Cancer Res,* 2006

Photoaffinity Labeling of Tubulin Isotypes with [^{3}H]2-(m-azidobenzoyl)taxol

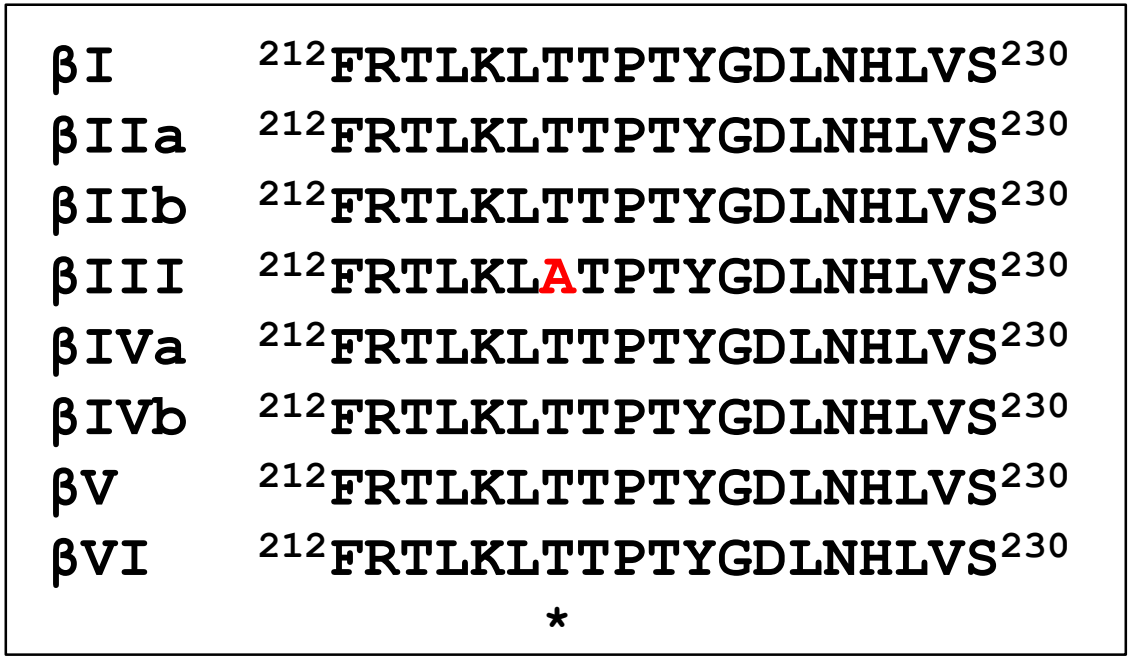


Band #	Tubulin Isotype	% Total β-tubulin	% Total Photolabeling
1	βIII	7.3 ± 0.3	1.5 ± 0.3
2	βIVb	9.7 ± 0.7	2.8 ± 0.5
3	βIVb	8.7 ± 0.4	3.4 ± 0.7
4	βI + βII + βIVa	7.8 ± 0.5	6.9 ± 0.4
5	βIVa	8.1 ± 0.4	7.5 ± 0.7
6	βII + βIVb	10.7 ± 0.3	7.1 ± 0.7
7	polyglutamylated βII (+3E)	7.0 ± 0.3	9.2 ± 0.8
8	polyglutamylated βII (+4E)	10.8 ± 0.3	9.1 ± 1.6
9	polyglutamylated βII (+4E)	8.4 ± 0.3	10.9 ± 1.1
10	polyglutamylated βII (+4E)	7.9 ± 0.2	11.1 ± 0.8
11	polyglutamylated βII (+4E)	6.7 ± 0.4	8.0 ± 0.2
12	polyglutamylated βII (+5E)	4.2 ± 0.3	7.4 ± 0.9
13	polyglutamylated βII (+6E)	1.9 ± 0.9	7.3 ± 1.7
14	polyglutamylated βII (+nE)	0.9 ± 0.4	4.3 ± 0.8
15	polyglutamylated βII (+nE)	--	3.7 ± 0.8

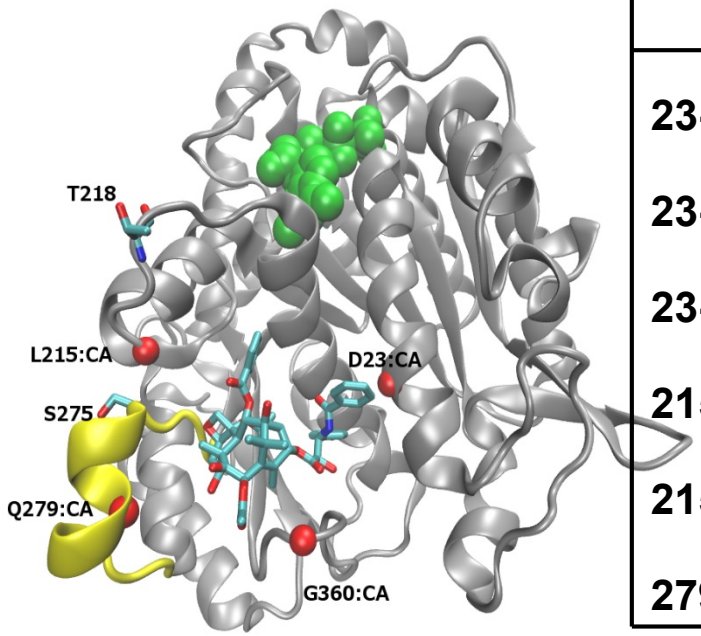


Chia-Ping H Yang

Sequence Alignment of the Leucine Cluster of Human β-Tubulin Isotypes



Distances Across Binding Pocket in Monomers of βI-Tubulin and T218A Variant



Distance definition	Distance in Taxol complex (Å)	βI-Tubulin Av distance (Å)	*	T218A Variant Av distance (Å)	*
23-215	18.39	20.1	97.3	18.9	63.5
23-279	23.34	26.5	99.2	23.1	51.5
23-360	11.34	12.4	73.3	12.0	67.2
215-279	9.8	11.3	95.2	12.8	85.3
215-360	16.43	17.6	80.3	17.5	78.7
279-360	16.06	18.5	90.7	14.7	36.3

Eng-Hui Yap
Andras Fiser

* % of snapshots with distance ≥ distance in Taxol complex
Molecular dynamic simulations – frequency of Taxol accommodating conformations decreased in the variant.

Conclusions

Tumors from different origins express distinct tubulin isotypes.

Taxol binds differentially to distinct β-tubulin isotypes.

Tubulin isotypes have a role in the response of tumors to Taxol and other antitumor drugs. Therefore, knowing the isotype content of tumors may affect treatment in a positive way.

Tubulin isotypes may have effects in cells unrelated to their role as components of the microtubule cytoskeleton.

Acknowledgements

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Pascal Verdier- Pinard

Ruth Angeletti
Andras Fiser
Gloria Huang
Hayley McDaid
Hui Xiao
Chia Ping H. Yang
Yihong Wang