ACADEMIC DRIVEN DRUG DEVELOPMENT: FROM MOLECULES TO PROOF-OF-CONCEPT CLINICAL TRIALS

Marian Hajduch, MD, PhD

Institute of Molecular and Translational Medicine,
Faculty of Medicine and Dentistry,
Palacky University in Olomouc
Czech Republic

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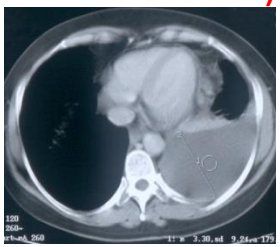
Outline

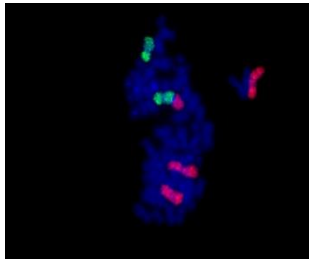
- Successful case studies in drug development – current drugs on the market
- Philosophy of academic drug development
- Case studies in drug research and development
- What we have learned?

Major determinants of therapeutic outcome

ANATOMIC STAGE OF DISEASE (DIAGNOSTICS - IMAGING)

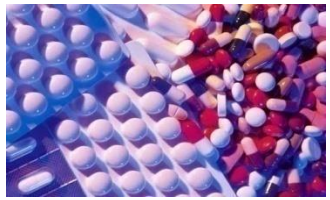


BIOLOGY (BIOMARKERS & MOLECULAR TARGETS)



THERAPEUTIC OUTCOME
PERSONALIZED MEDICNE

THERAPIES (SMALL MOLECULES, BIOLOGICS, ATMPs)



Academic drug development?

- Partially de-risking novel drug discovery targets; through the parallel running of milestone driven drug discovery projects alongside academically driven development of the underpinning target biology.

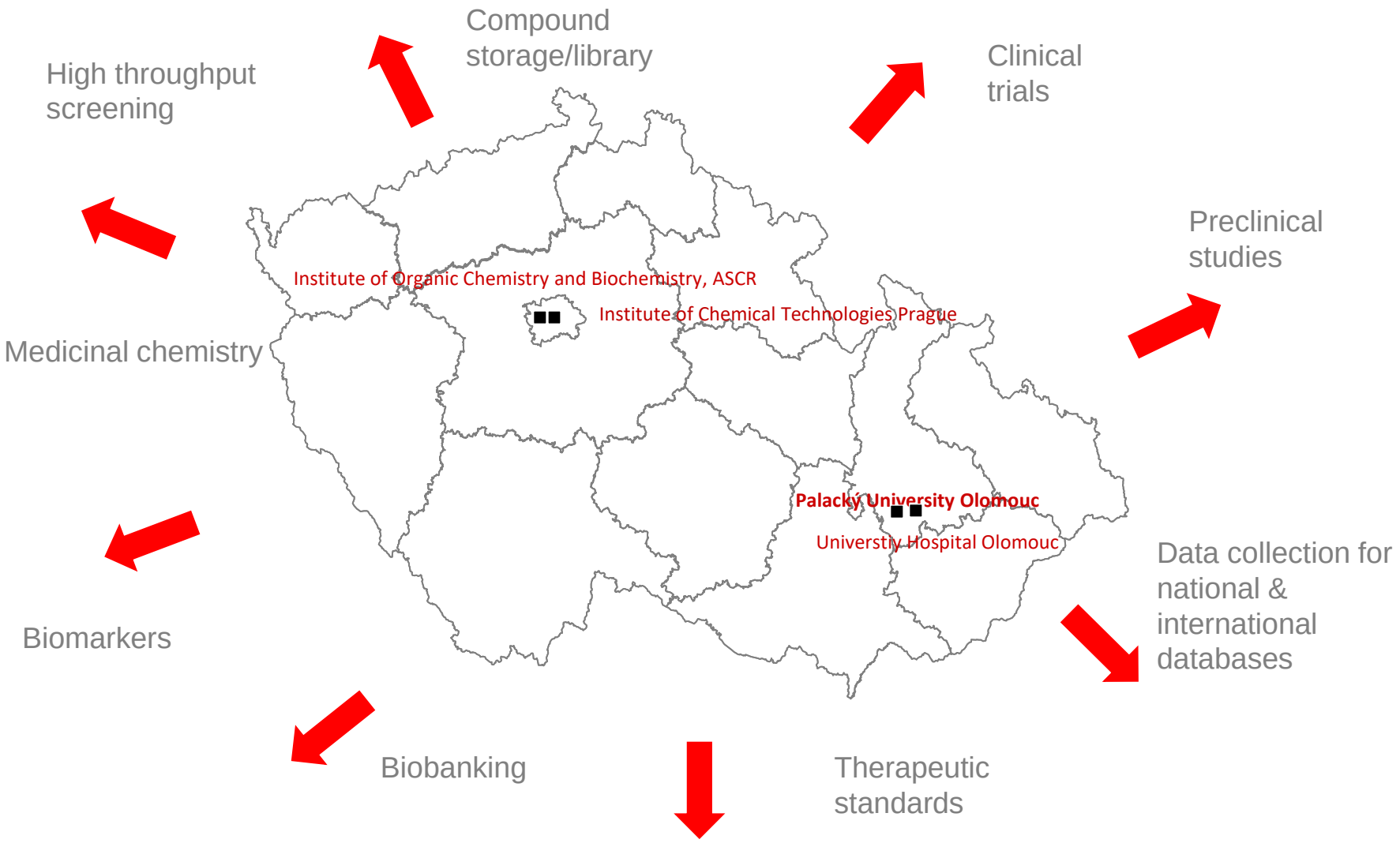
- Neglected and orphan diseases; through the development of therapies for diseases whose patient base demographics or numbers will not generate a sufficient commercial return to offset the cost of drug development.

- New paradigms for drug discovery; universities are an excellent environment for the development of new paradigms for drug discovery, „undrugabble“ targets and „drug unlike“ molecules.

- Access to new and/or more predicitve models, techniques requieing the human materials and/or patients (PDXs, iPSCs, etc.).

- Training young scientists in the practice of drug discovery and educating basic scientists in the requirements for the translation of fundamental research into drug discovery.

Infrastructural project for chemical biology and translational medicine (BIOMEDREG) – concentrating, evaluating and developing the national chemical knowledge



Betulinic acid derivatives
Chemistry: Dr. Sarek, Faculty of Sciences, UP

Anticancer

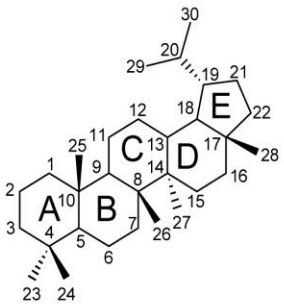
Antimicrobial

Antiinflammatory (Nrf2 activators)

Aniviral (HIV – maturation inhibitors)

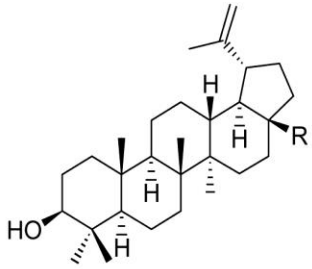
Hedgehog inhibitors

Formal skeleton



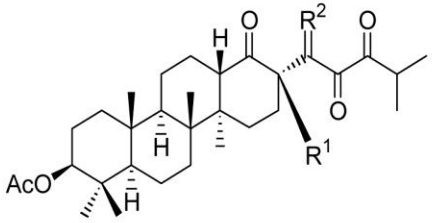
Lupane skeleton

Lead structures for derivatization



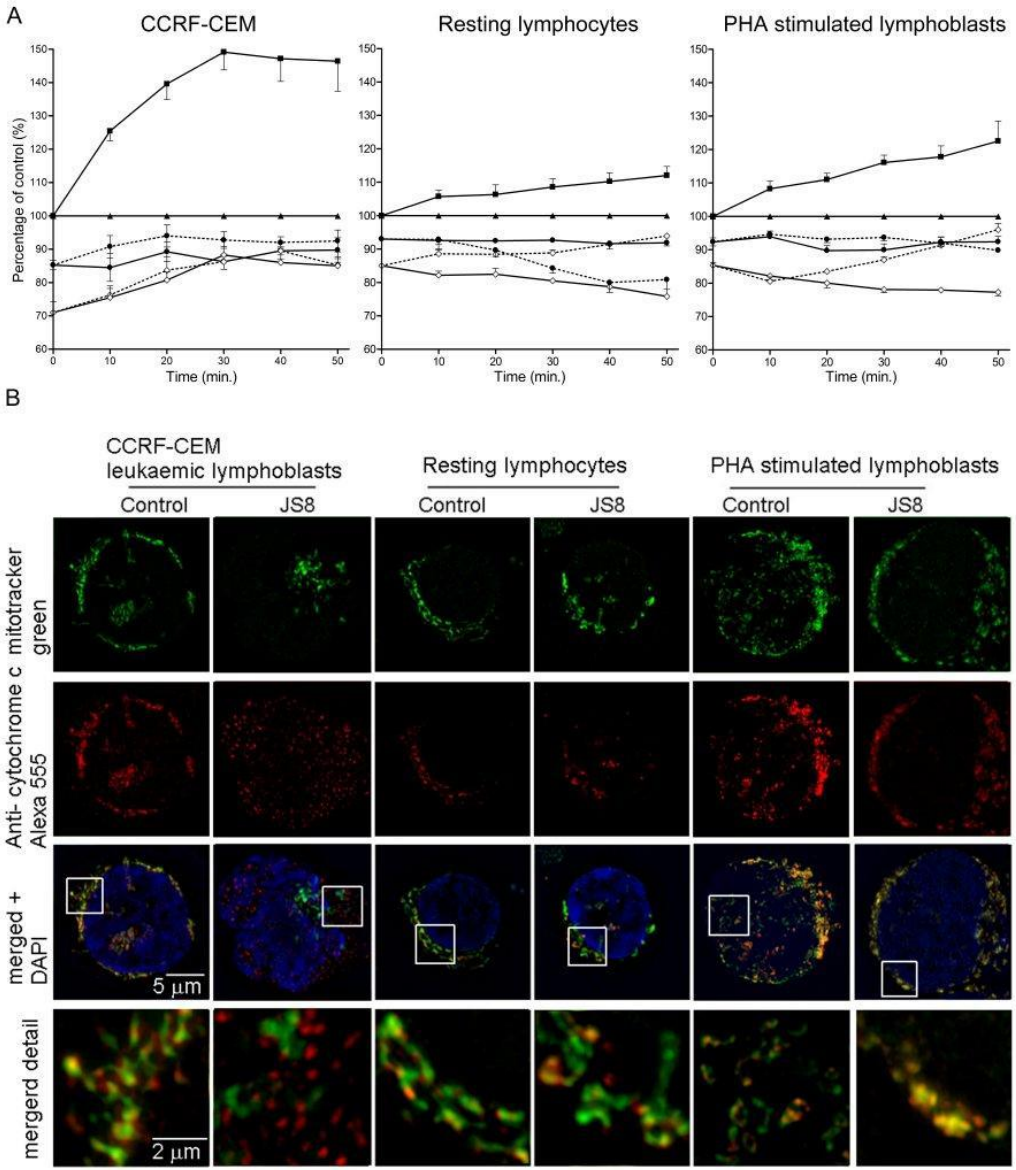
Betulin, R = CH_2OH
Betulinic acid, R = COOH

Example of new structure(s) with pro-apoptotic activity

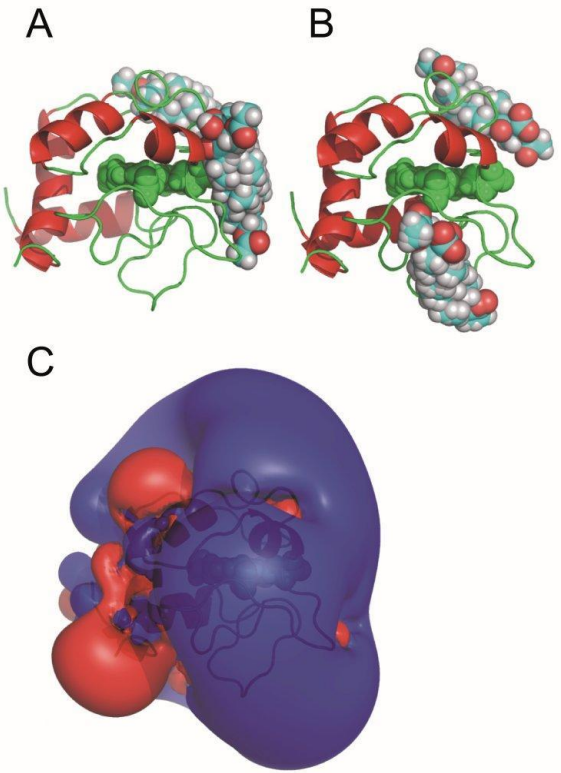


Highly oxygenated 18,19-secolupane type; R^1 is CH_2OAc or CO_2R^3, R^2 is O or H,H and R^3 is ester group

Anticancer activity – mechanism of action



Betulinic acid derivative JS8 – selective binding to cytochrome c in tumor cells and consequent induction of apoptosis



Compounds antiviral activity: HIV

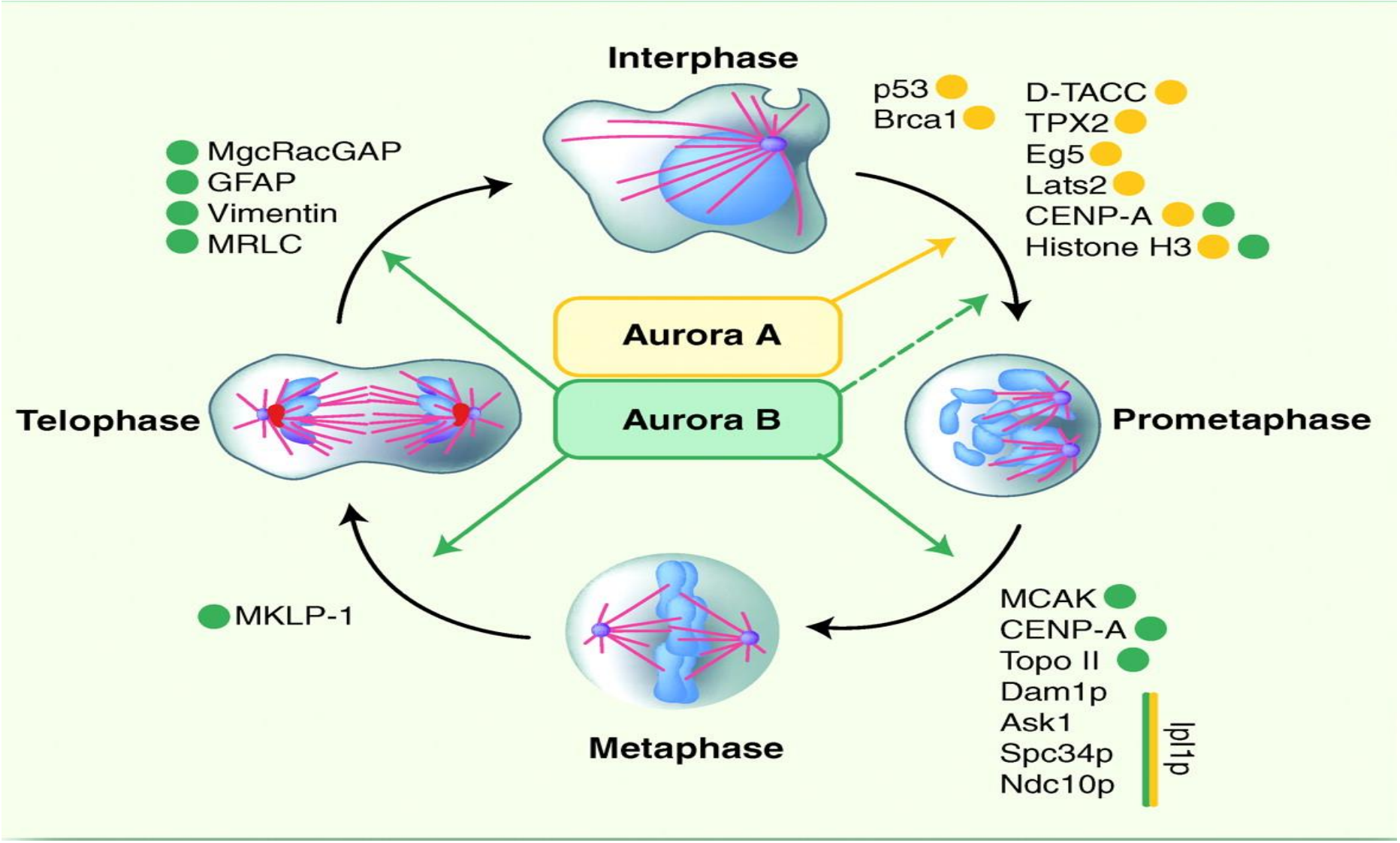
Maturation inhibitors – block the last cleavage site of the HIV gag protein
4th class of anti-HIV drugs active against all clinical drug resistant isolates

WHAT WE HAVE LEARNED?

- Market driven decision are important.
- Merging companies may result in different priorities.

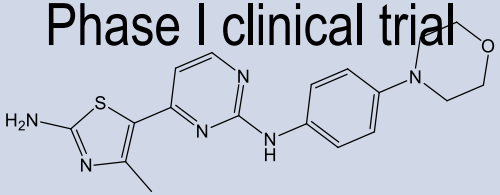
150			**2**	**9**	**5500**	**2750**
80118			**7 - 35**	**60-315**	**15,000**	**3,350**
151			**36**	**920**	**15,000**	**416**
PA-457			**2.7**	**16.7**	**23,000**	**8,500**

Aurora A and/or Aurora B targets



Gautschi Clin Cancer Res 14:1639, 2008

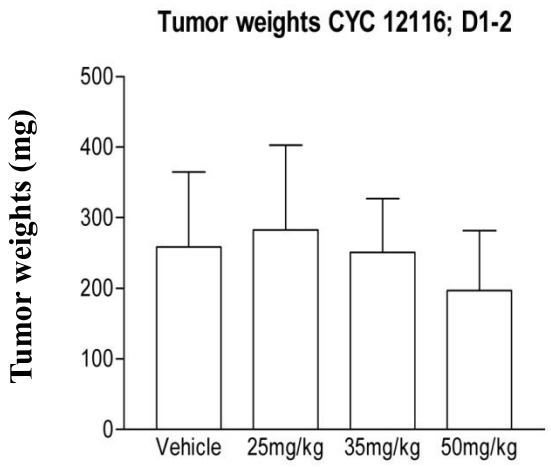
CYC12116, a Novel Pan Aurora kinase inhibitor

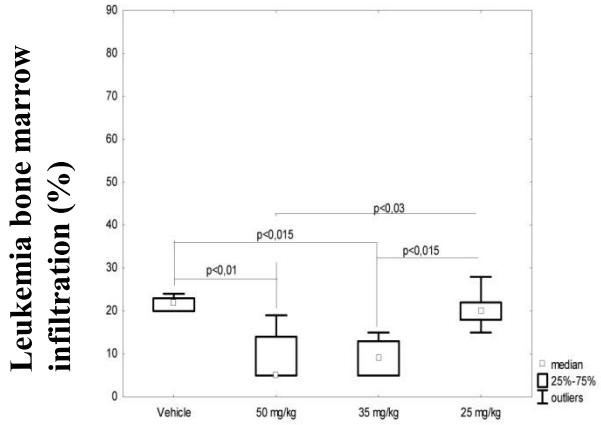
Compound	IC50	Comments
CYC116 (Cyclacel) Phase I clinical trial 	*ATP competitive assay* Aurora A - 44 nM B - 19 nM C - 65 nM *Cellular* 34-1370 nM	Antitumor activity in *in vivo* animal models. 50mg/kg dose significantly reduced tumor weights.
ZM447439 (A.Zeneca) Reference compound	*ATP competitive assay* Aurora A - 110 nM B - 130 nM	Using ZM447439, Aurora B roles in mitosis discovered.

- Potential Tumor resistance mechanisms towards CYC116 was not identified
- Helps in predicting clinical response and to design alternative strategies to combat resistance

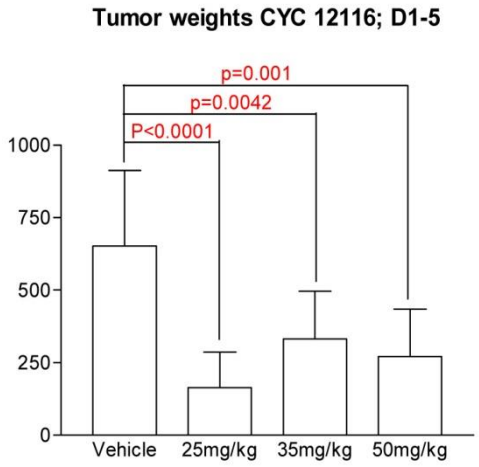
The effect of CYC12116 on tumor weights and leukemia bone marrow infiltration

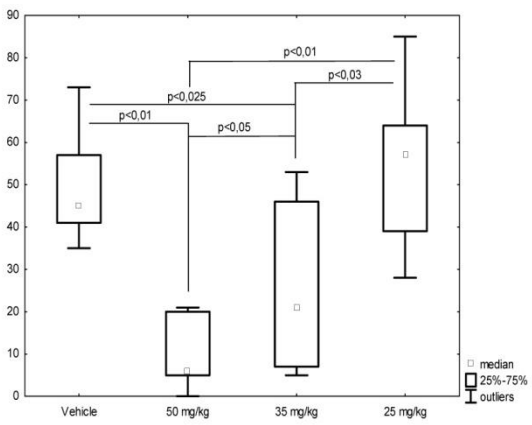
Day 2



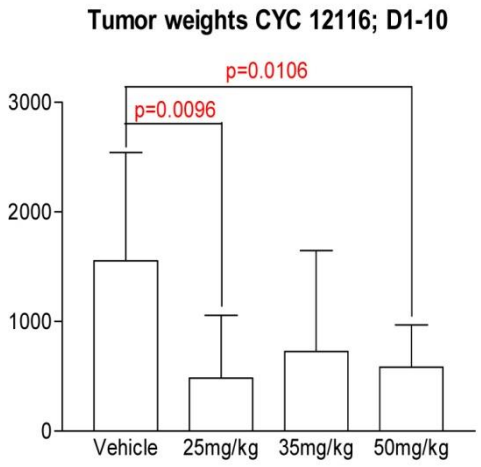


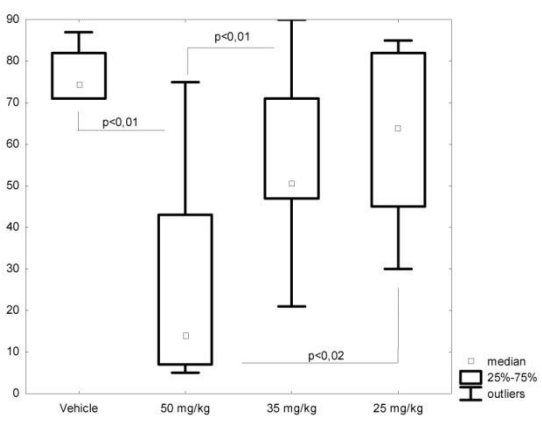
Day 5



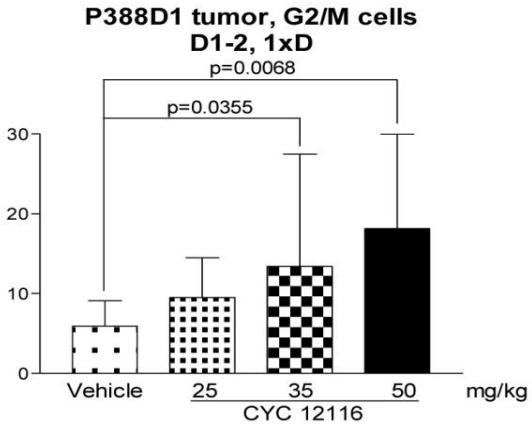
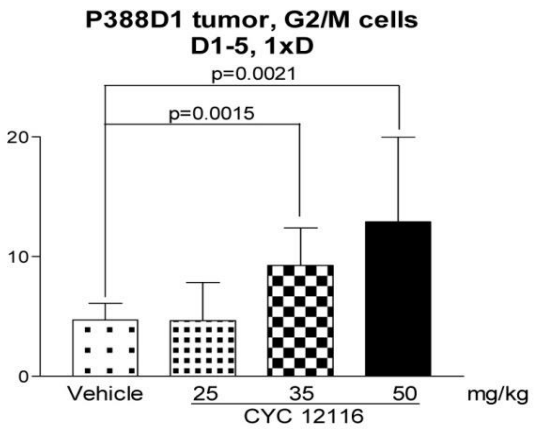
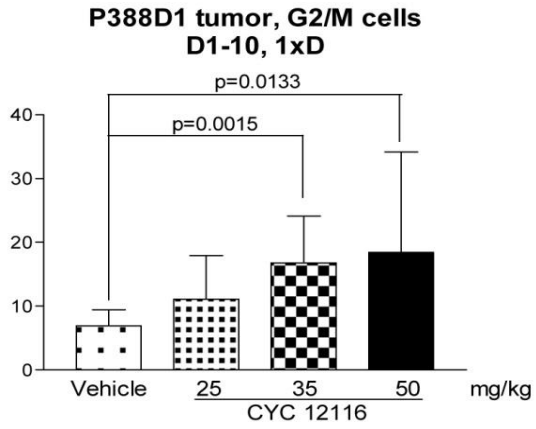
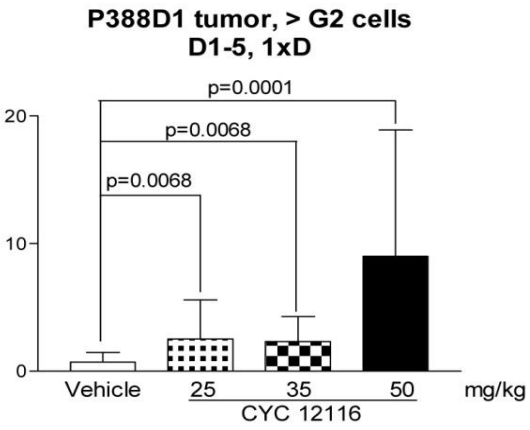
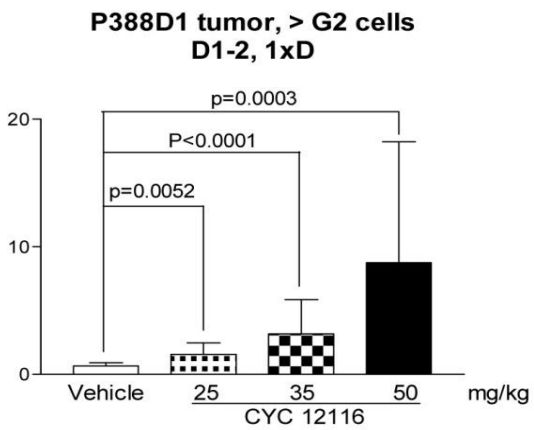
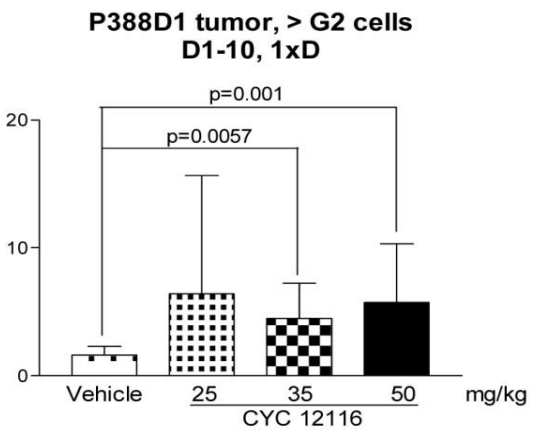


Day 10



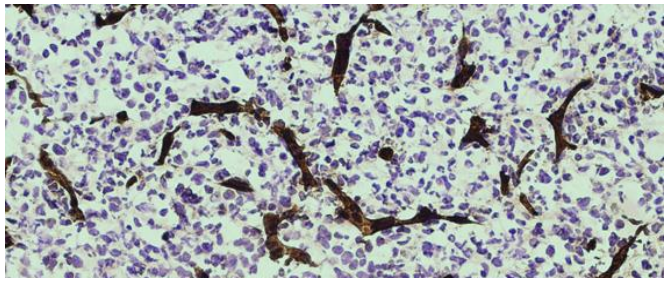


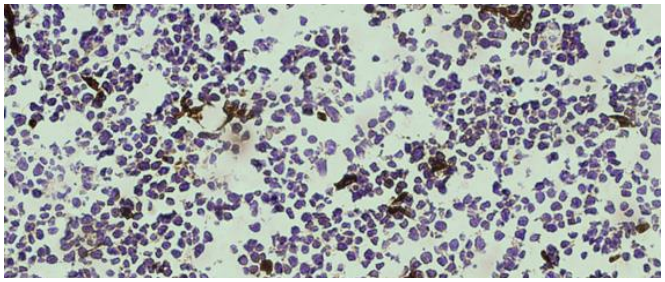
The effect of CYC12116 on tumor cell cycle and ploidy

	Day 2	Day 5	Day 10
G2/M cells			
Polyploid >G2 cells			

Suppression of angiogenesis and histone H3 phosphorylation in CYC12116 treated P388D1 tumors (50 mg/kg p.o., 1xD, D1-5)

Tumor microvessel density (anti-CD31 staining)

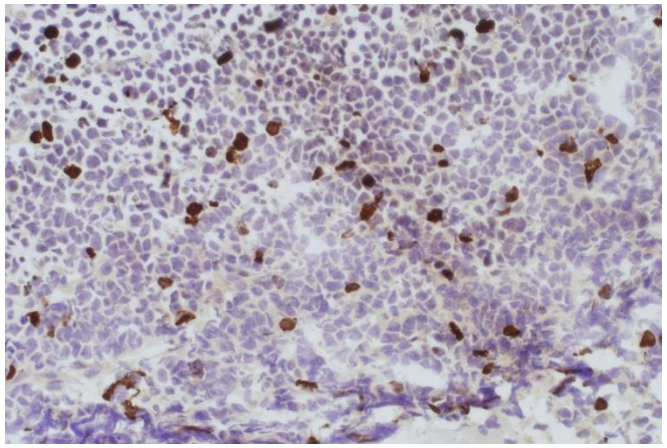


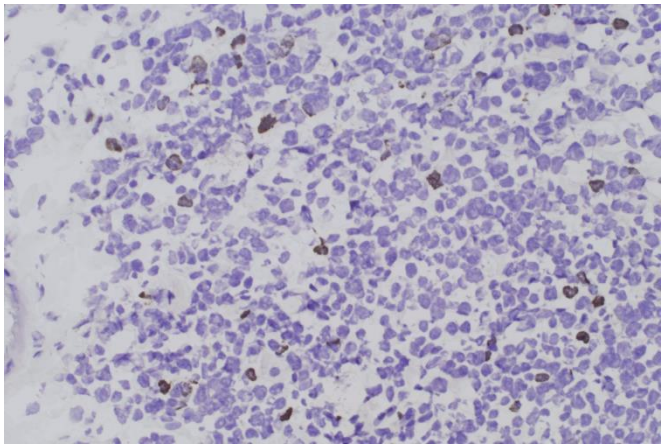


WHAT WE HAVE LEARNED?

- Collaborative research with academia may have added value.

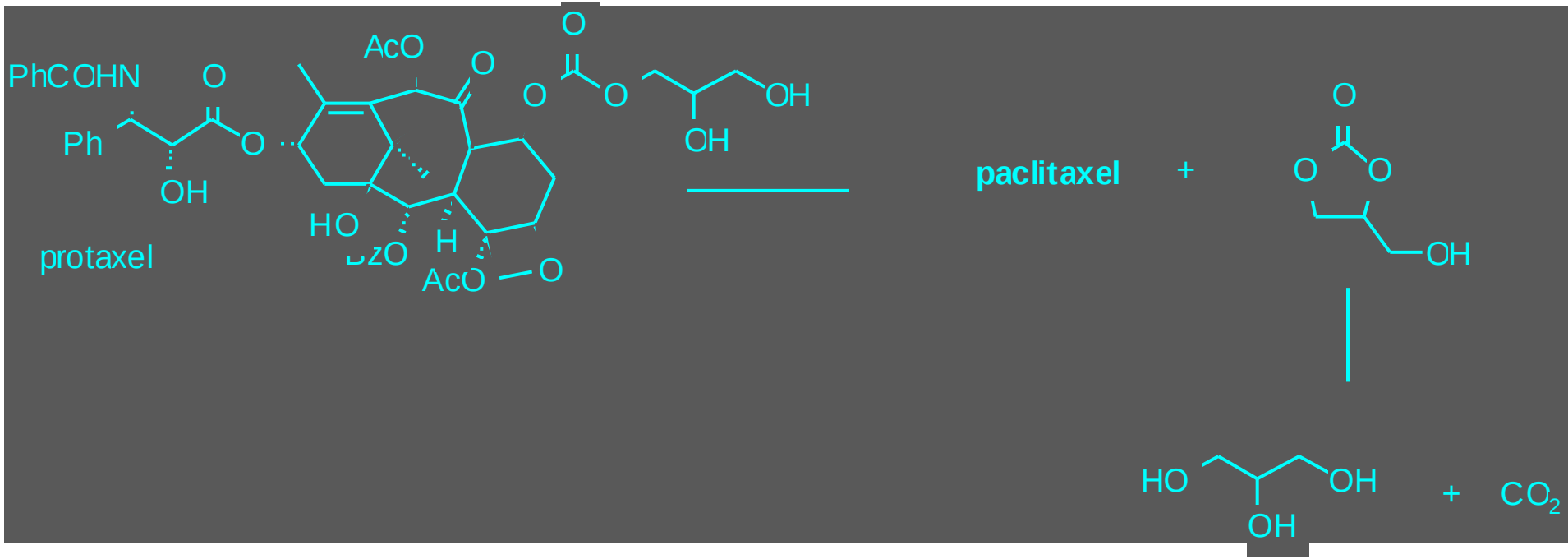
Histon H3 phosphorylation

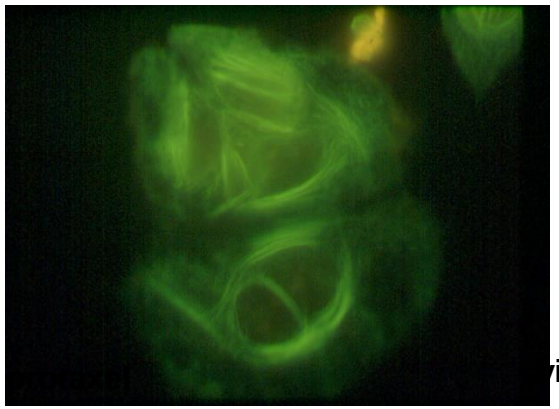


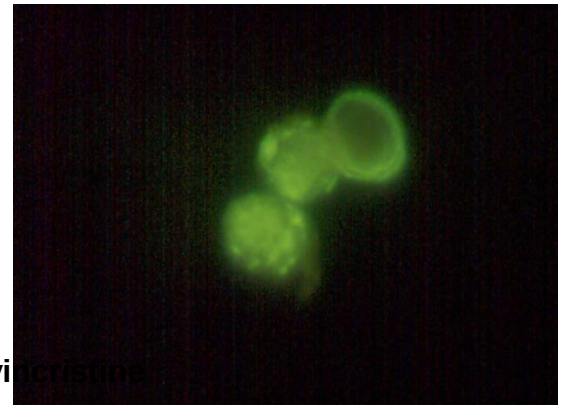


vehicle **CYC12116**

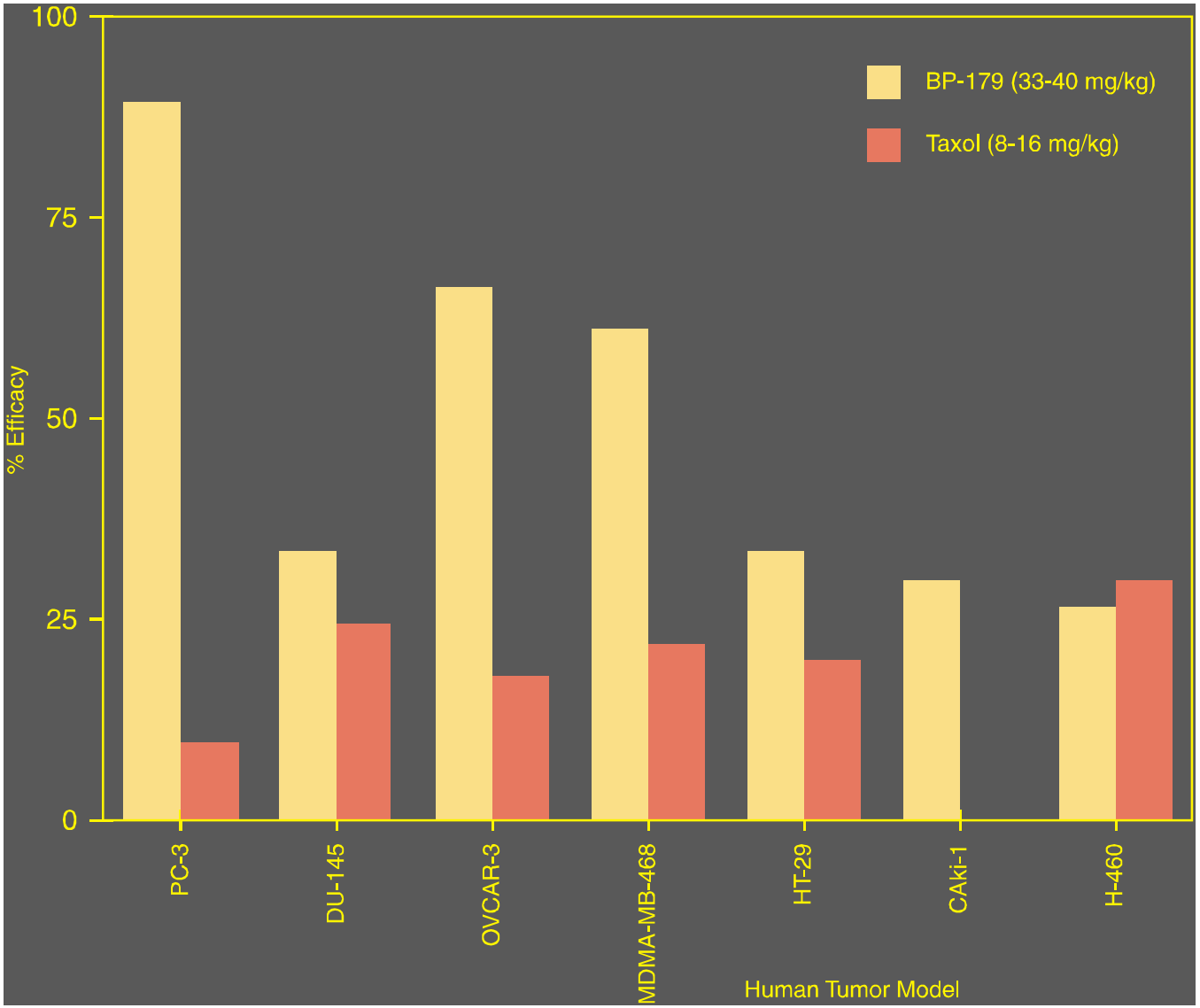
Protaxel – paclitaxel pro-drug – mechanism Chemistry: Biophysica Fnd.



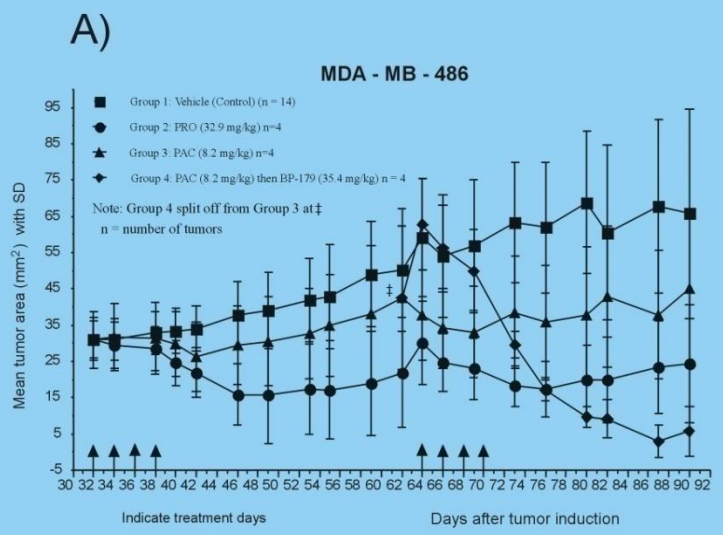


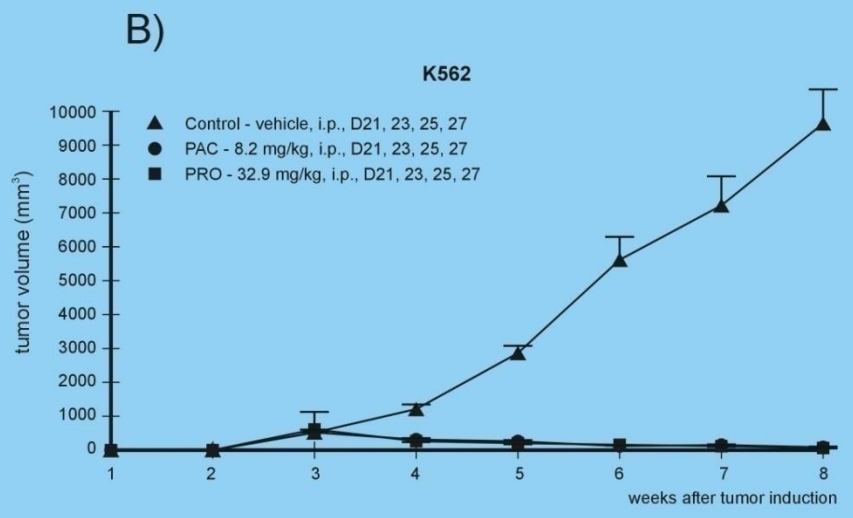


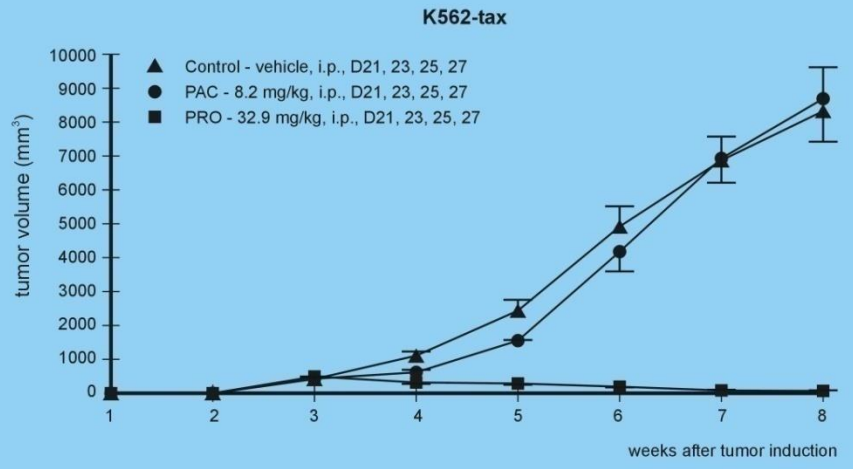
IN VIVO COMPARISON OF BP-179 AND TAXOL'S % EFFICACIES AGAINST SEVERAL HUMAN TUMOR MODELS



Protaxel works in paclitaxel resistant tumors

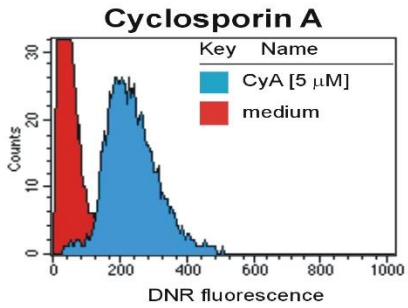


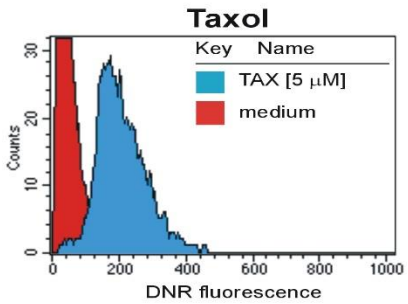


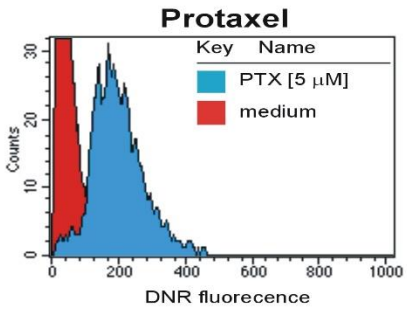


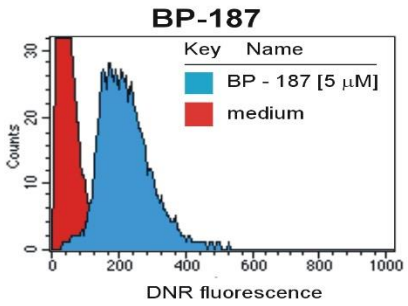
A) Nude mice were xenotransplanted with the breast carcinoma cells, MDA-MB-486, and then treated with PAC or PRO. While PRO induced an effective antitumor effect, animals treated with PAC relapsed. However, re-treatment of PAC-failed mice with PRO resulted in a rapid shrinkage of the tumor.
B) An athymic mice model of the human K562 myeloid leukemia and its PAC-resistant counterpart, K562-tax. Transplanted animals were treated with or without PAC/PRO. Nonetheless only the PRO treatment induced an effective response in the K562-tax PAC-resistant line.

INHIBITION OF DAUNORUBICIN EFFLUX IN TAXOL RESISTANT K562-tax CELL LINE









Protaxel phase clinical I trial

- Study design: single center opened study

- Chemotherapy resistant and paclitaxel refractory/relapsed ovarian cancers (orphan drug status)

- Antiandrogen resistant prostate cancer patients

- Intrapatient escalation in modified Fibonacci schema

- Primary endpoints: toxicity, tolerability, maximum tolerated dose, dose limiting toxicity

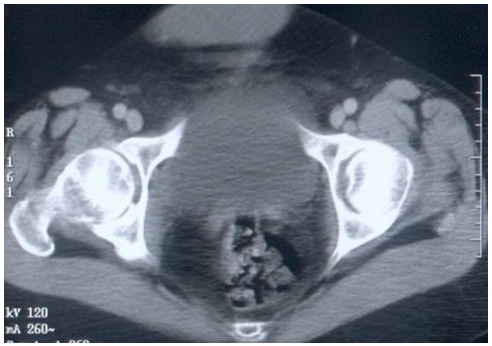
- Secondary endpoints: efficacy

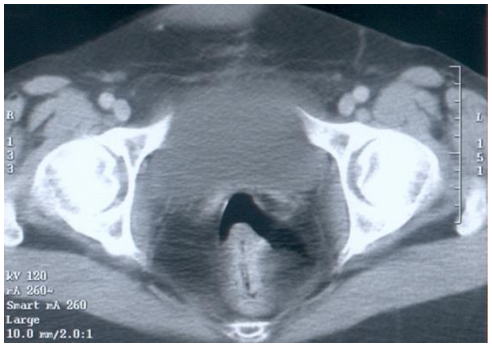
Clinical efficacy of Protaxel therapy in ovarian carcinoma patient

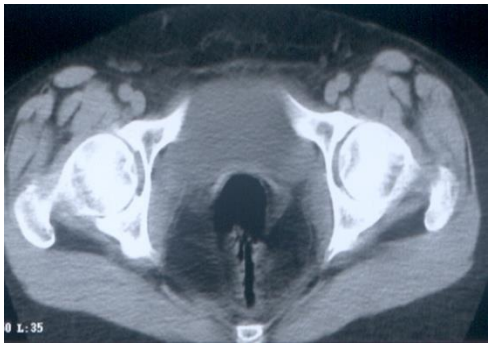
before treatment

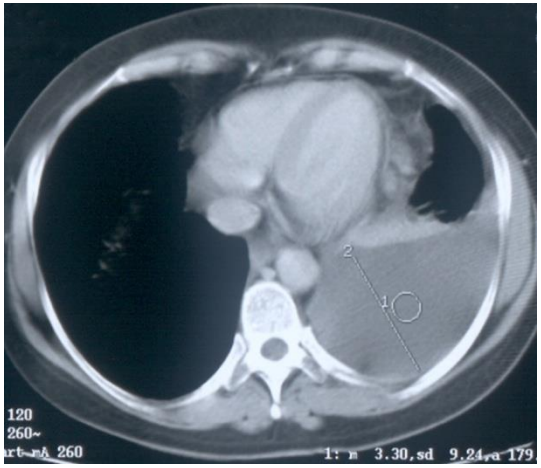
after 3 cycles

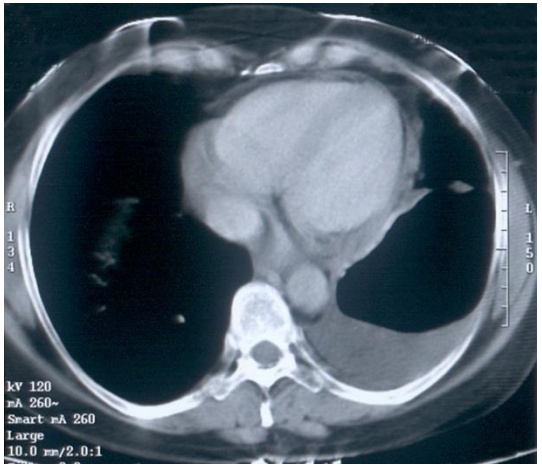
after 5 cycles

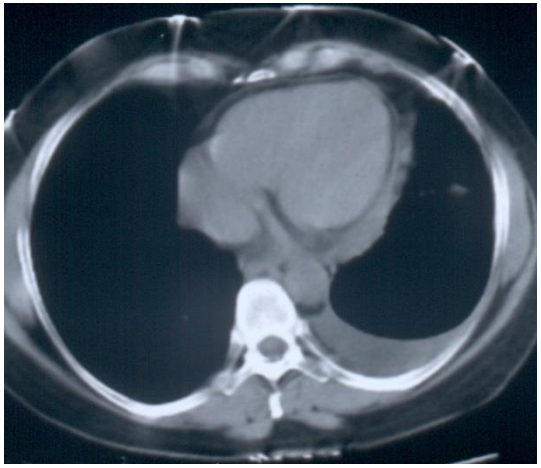








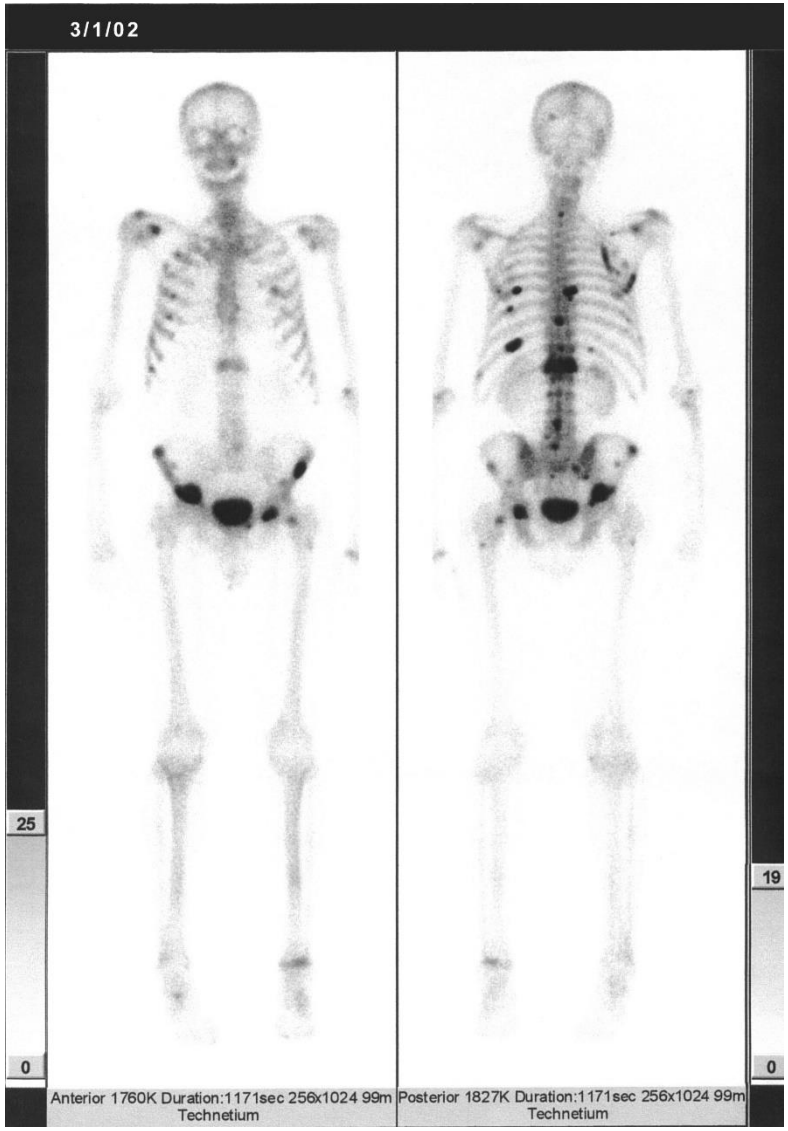




CA-125: 1400 640 106 ng/ml

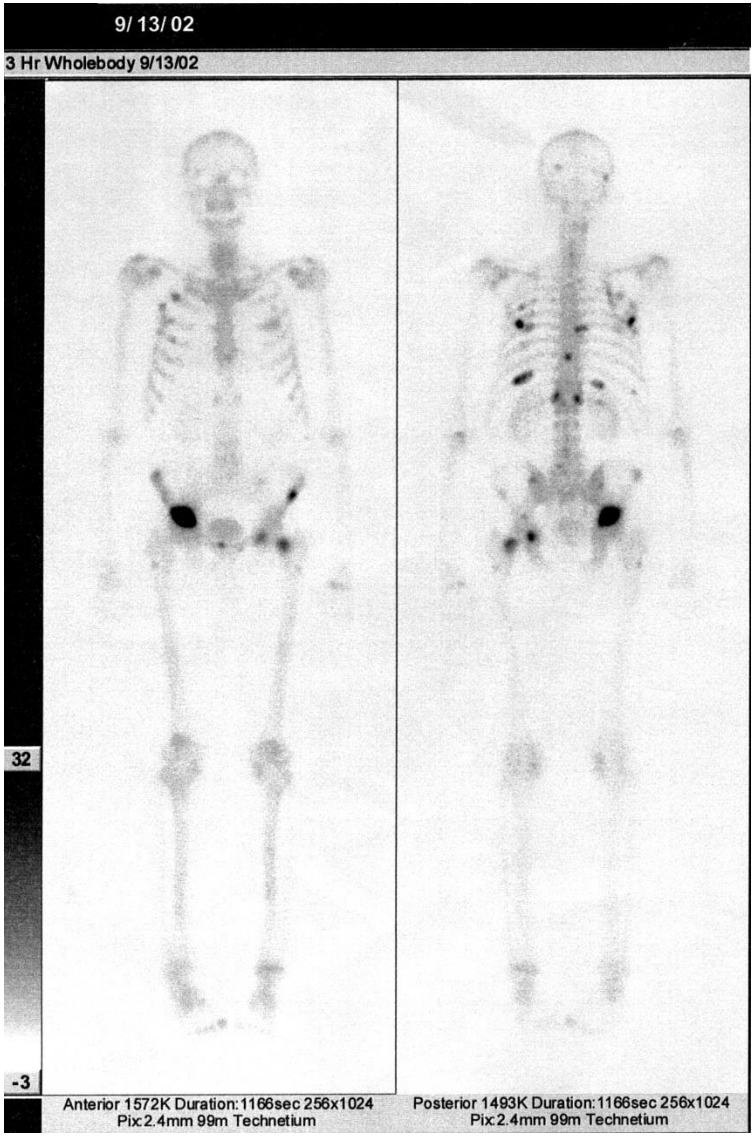
^{99m}Tc scintigraphy: bone metastases in Protaxel treated prostate cancer patient

before treatment



PSA = 64.3 ng/ml

after 5th cycle of chemotherapy



PSA = 9.1 ng/ml

Drug Discovery Process Perspective

ACADEMIC/INDUSTRIAL DILEMA

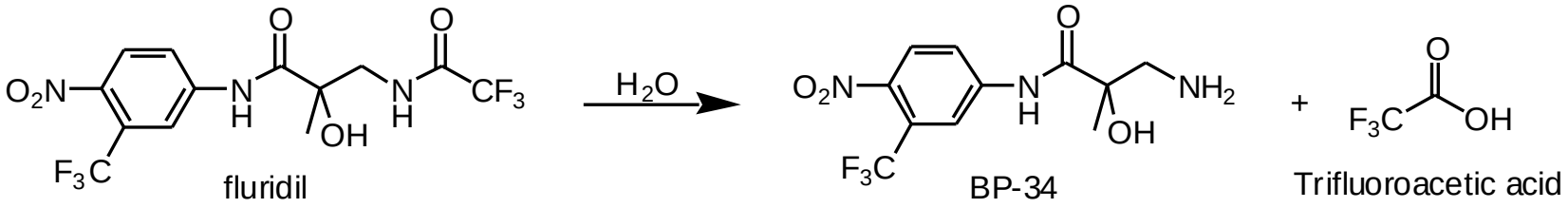
Publish or perish *versus* Publish and perish.

WHAT WE HAVE LEARNED?

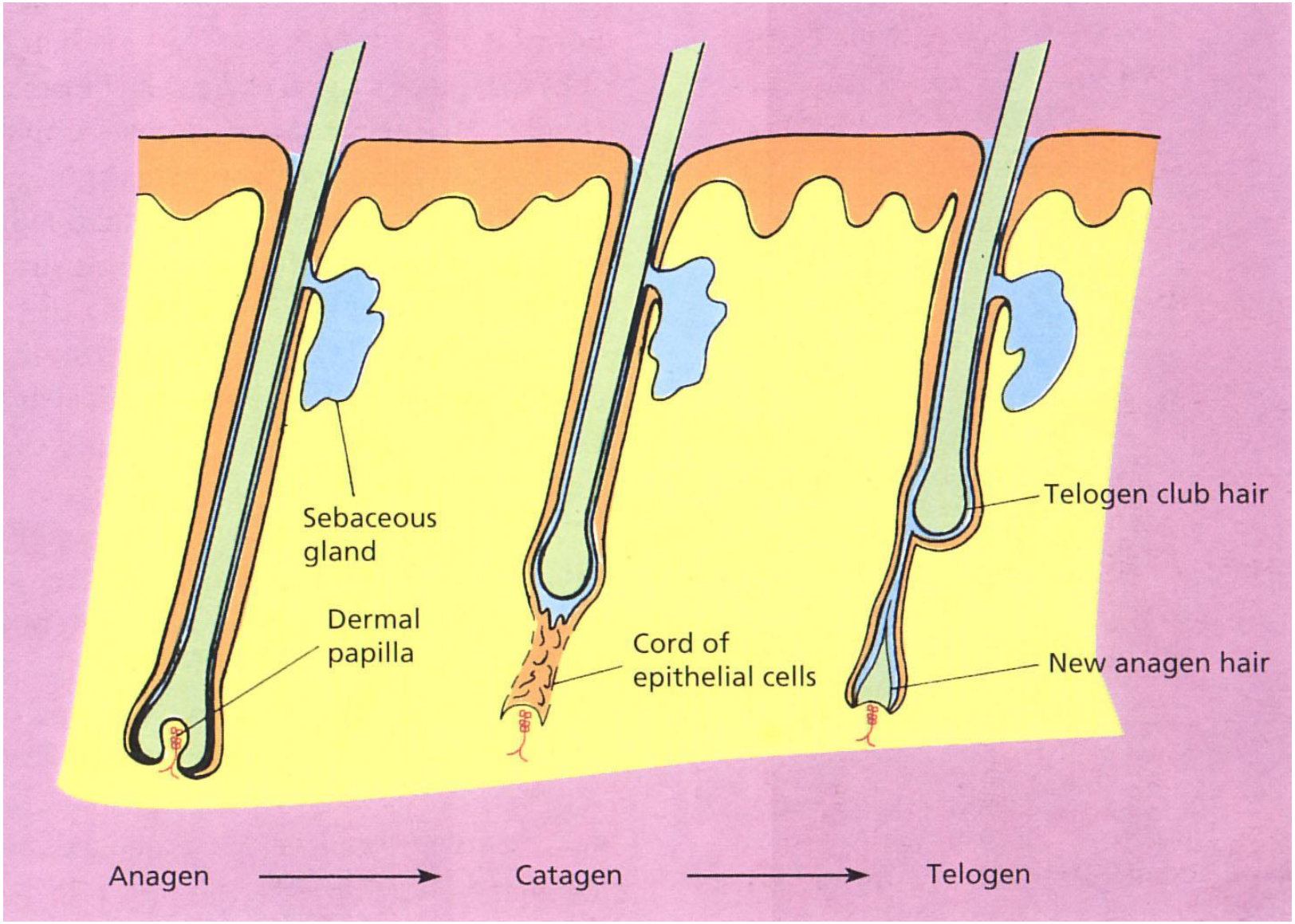
- Time is money.

Fluridil – topical antiandrogen

- Originally developed for hormonal therapy of prostate cancer
- Blockage of androgen receptor (AR) in hair follicle
- hydrophobic property
- hydrolytic instability-quick disintegration of fragments, systemic tolerance and rapid excretion

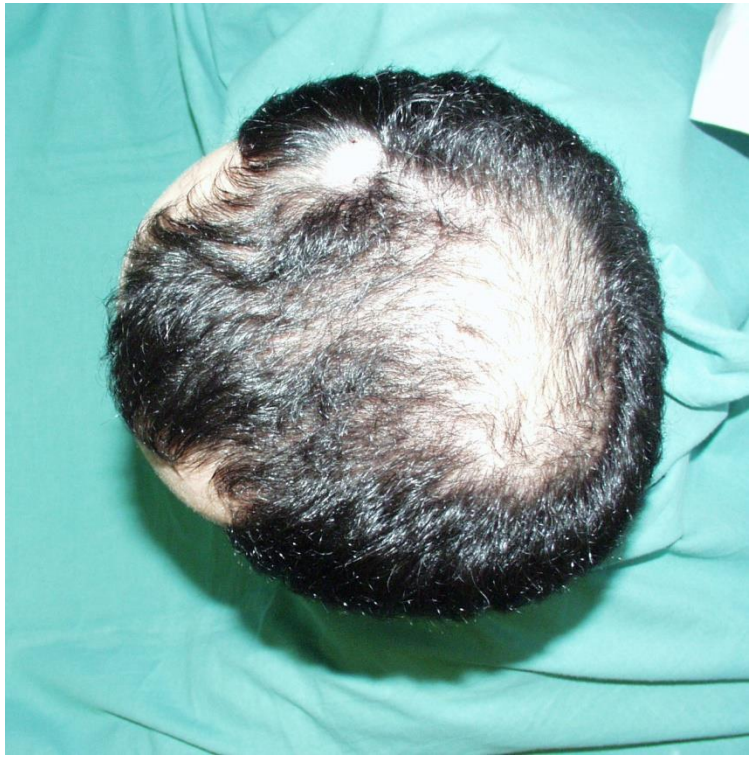


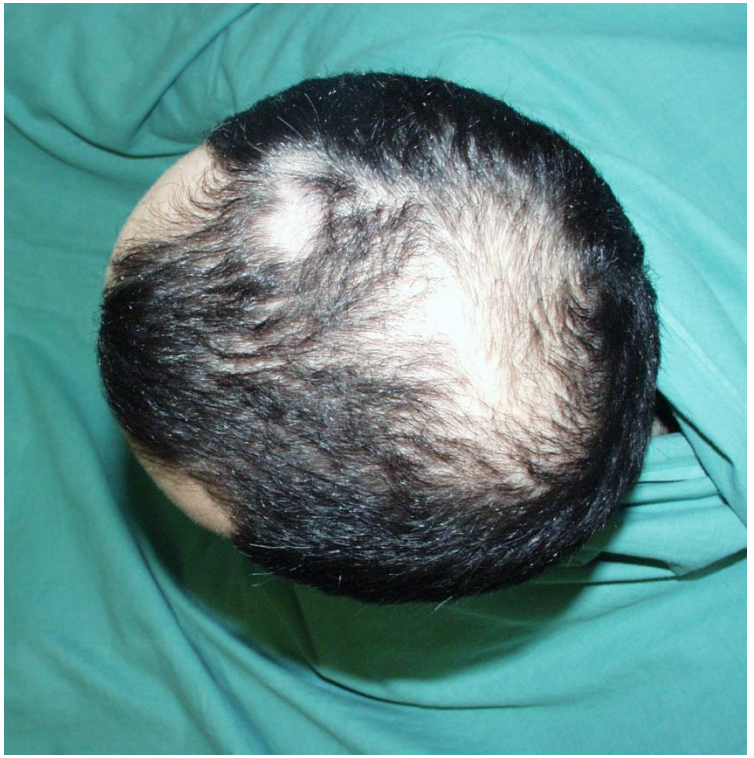
Hair cycle


Sebaceous gland
Dermal papilla
Cord of epithelial cells
Telogen club hair
New anagen hair
Anagen
Catagen
Telogen

Survey of pictures of thatches

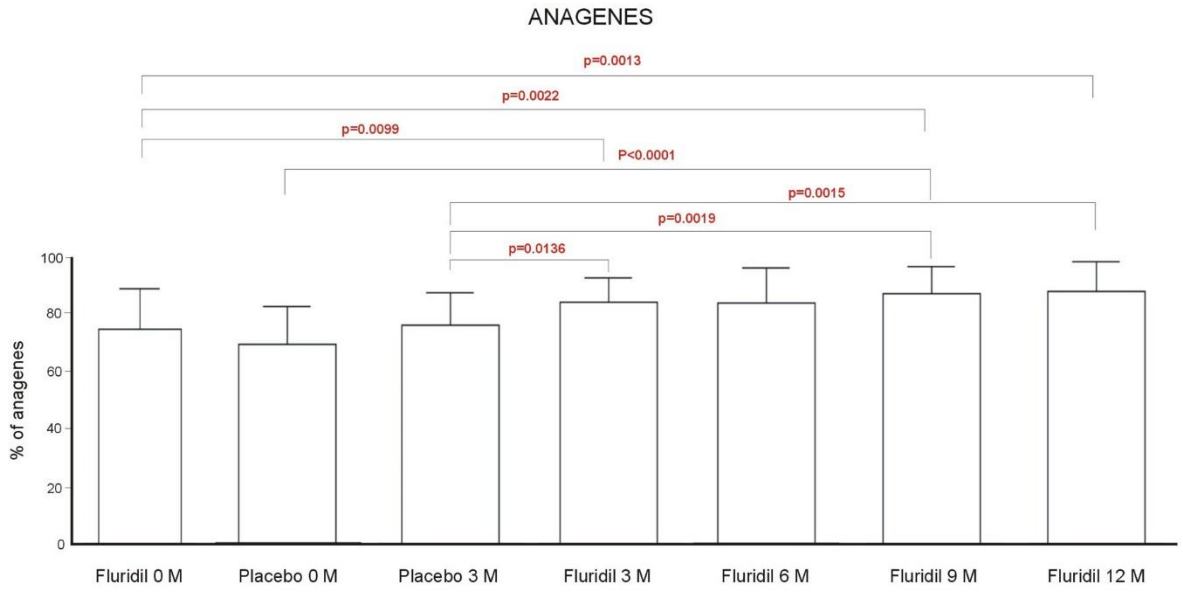
0 **12 months**

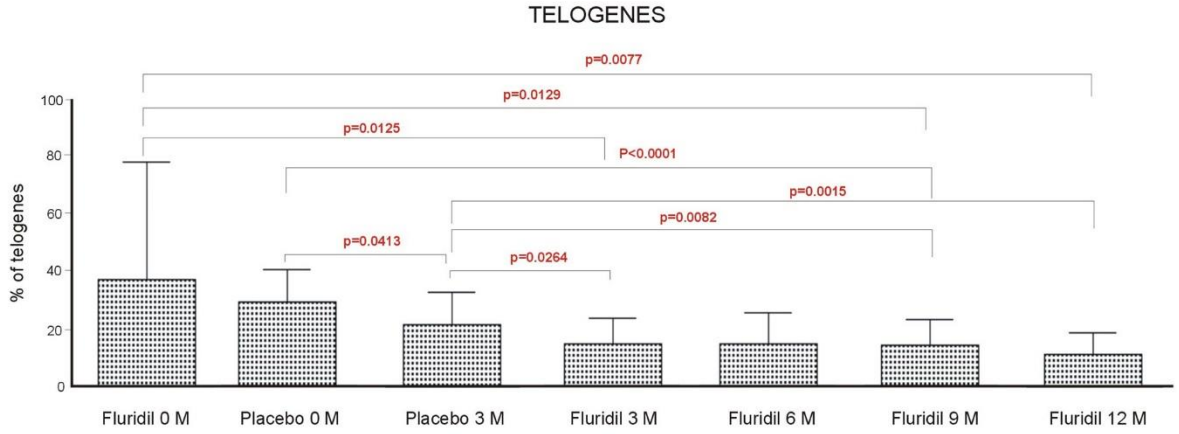




Sovak M, Seligson AL, Kucerova R, Bienova M, Hajduch M, Bucek M. Fluridil, a rationally designed topical agent for androgenetic alopecia: first clinical experience. Dermatol Surg. 2002 Aug;28(8):678-85.

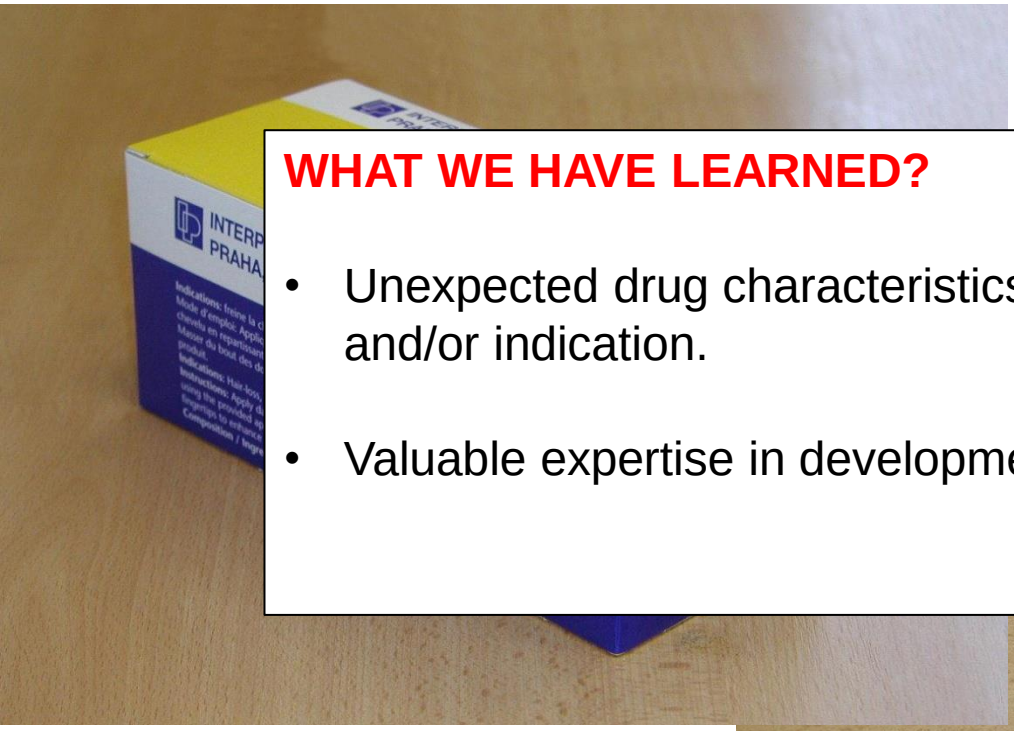
Evaluation phototrichograms





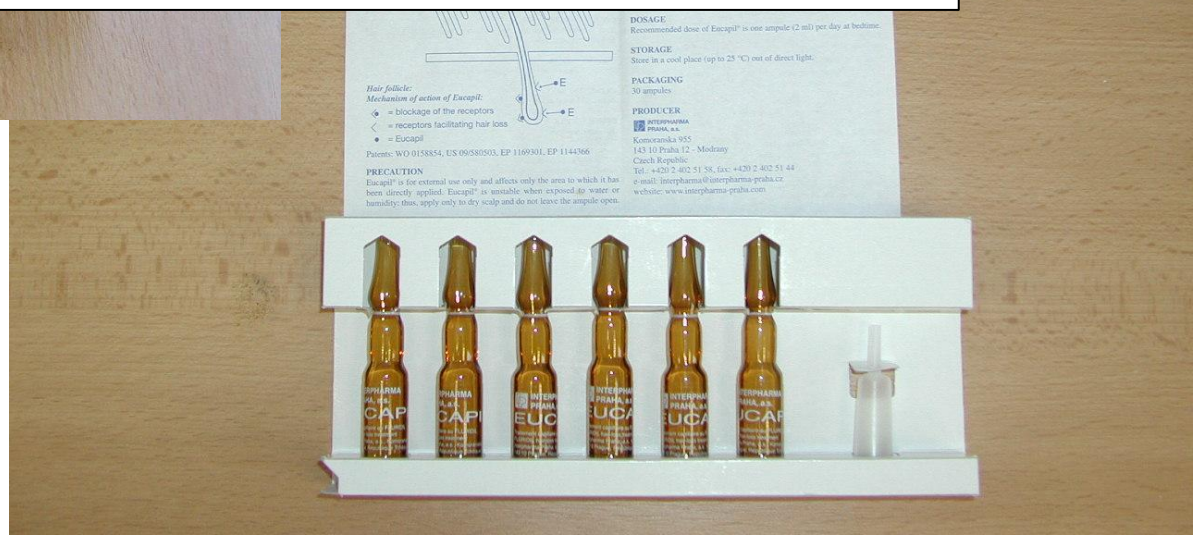
Sovak M, Seligson AL, Kucerova R, Bienova M, Hajduch M, Bucek M. Fluridil, a rationally designed topical agent for androgenetic alopecia: first clinical experience. Dermatol Surg. 2002 Aug;28(8):678-85.

Fluridil - Eucapil



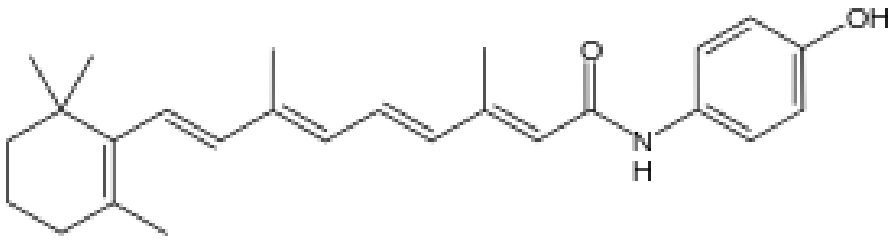
WHAT WE HAVE LEARNED?

- Unexpected drug characteristics may result in new product and/or indication.
- Valuable expertise in development of cosmetic products.

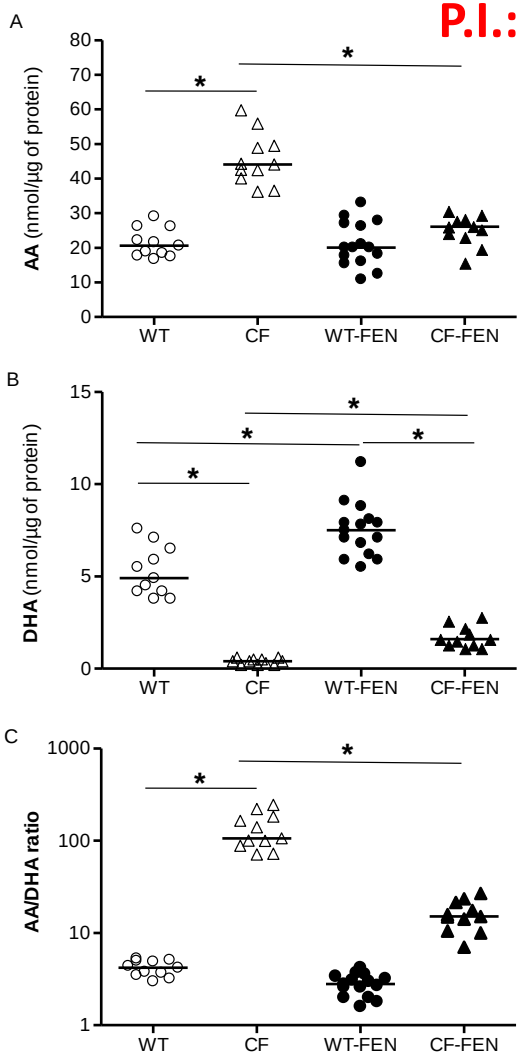


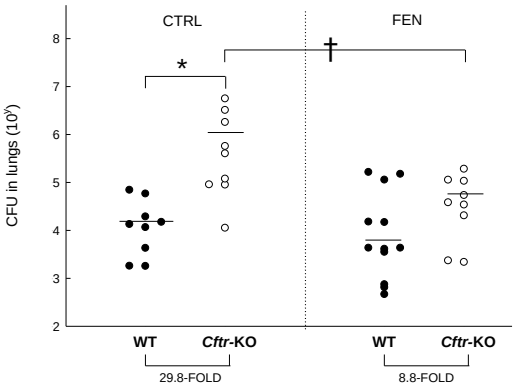
Fenretinide drug reporposing

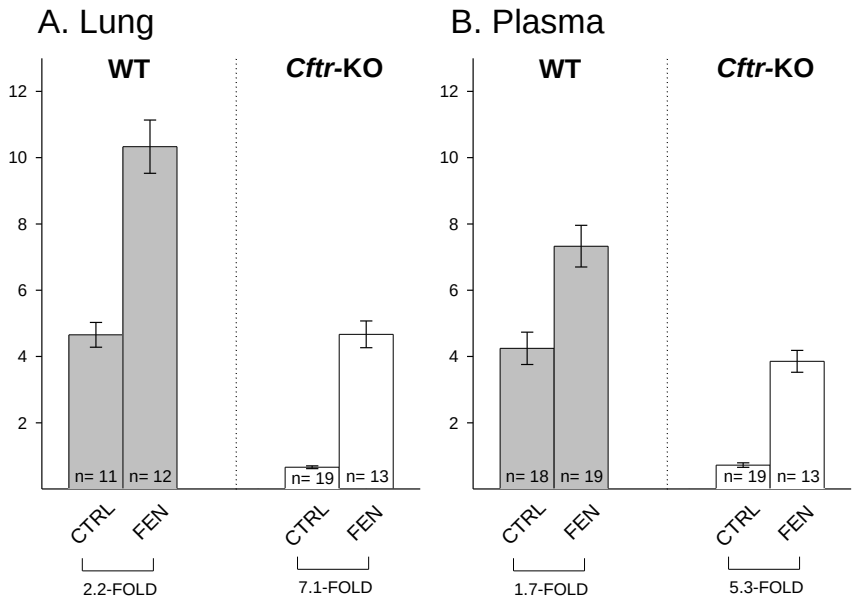
- Synthetic retinoid: 4-hydroxy(phenyl)retinamide
- Anticancer activity: neuroblastoma (ROS, ceramide metabolism, AA/DHA ratio, apoptosis)
- Chemopreventive trials
- Antiinflammatory activity in RA, psoriasis, CF…



Drug repurposing/Rare diseases projects: Fenretinide in Cystic Fibrosis
P.I.: Prof. Radzioch, McGill University



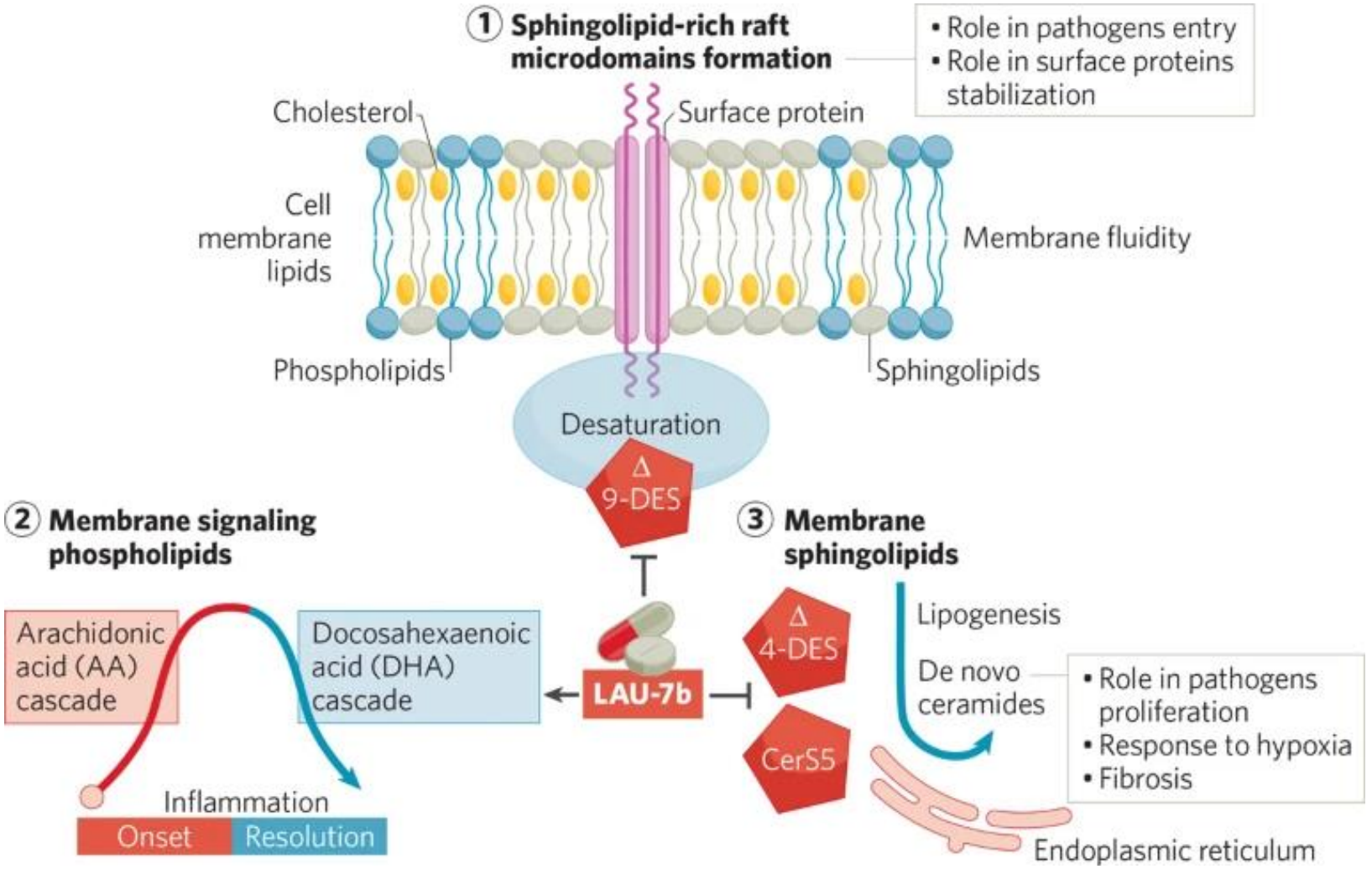




Gibault et al. AJRCMB 2009, Wojewodka et al. AJRCMB2009, AJRCMB 2014

Fenretinide for colonized CF patients – FDA approval for orphan drug status

Fenretinide mechanism of action



Laurent Pharma diagram

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Fenretinide phase I trial

Table 1 Demographic data of patients and healthy controls and response of patients to fenretinide in the third cycle of phase Ib clinical trial

Patient number	Sex	Age (years)	BMI (kg/m^2)	Genotypes		Fen (μM)	C24:0 (pmol/nmol of phosphate) at the baseline	C24:0 (pmol/nmol of phosphate) in C3 after fenretinide administration	C16:0(pmol/nmol of phosphate) at the baseline	C16:0 (pmol/nmol of phosphate) in C3 after fenretinide administration
				Traditional nomenclature	HGVS nomenclature					
High responders (C24:0 ↑, C16:0 ↓)										
CF3	M	29	18.9	F508del/F508del	p.[Phe508del]; [Phe508del]	2.278	3.9 3.96 ± 0.5	4.2 5.1 ± 0.6	1.2 1.2 ± 0.22	1.1 0.92 ± 0.21
CF5	M	22	23.5	F508del/F508del	p.[Phe508del]; [Phe508del]	4.594	4.5	5.2 (C3 vs. baseline;	0.9	0.6 (C3 vs. baseline;
CF7	M	18	19.0	F508del/621+1G>T	c.[1521_1523delCTT]; [489+1G>T]	3.326	3.9	5.9 $p = 0.03$)	1.1	0.8 $p = 0.03$)
CF10	M	25	19.4	F508del/F508del	p.[Phe508del]; [Phe508del]	1.978	4.3	5.2	1.3	1.1
CF14	M	33	19.0	F508del/F508del	p.[Phe508del]; [Phe508del]	N/A[b]	3.2	4.9	1.5	1.0
Partial responders										
CF1	M	36	24.4	F508del/F508del	p.[Phe508del]; [Phe508del]	1.008	3.5 4.06 ± 0.8	3.5 4.8 ± 0.84	1.7 1.0 ± 0.43	1.2 1.0 ± 0.43
CF6	M	18	21.0	F508del/G85E	p.[Phe508del]; [Gly85Glu]	1.702	4.2	5.7 (C3 vs. baseline;	0.8	1.1 (C3 vs. baseline;
CF9	M	36	24.9	F508del/3849+10KbC>T	c.[1521_1523delCTT];[3717+12191C>T]	3.981	4.0	4.9 $p = 0.13$)	0.6	1.1 $p = 0.41$)
CF12	M	57	16.5	F508del/R352G	p.[Phe508del]; [Arg352Gly]	2.966	3.3	5.3	0.8	1.0
CF15	F	52	19.7	F508del/F508del	p.[Phe508del]; [Phe508del]	N/A[b]	5.3	4.5	1.1	0.9
Placebos										
CF4	F	21	24.2	F508del/F508del	p.[Phe508del]; [Phe508del]	0	3.8 4.2 ± 0.5	3.3 4.9 ± 1.1	0.9 0.8 ± 0.1	1.1 0.8 ± 0.2
CF8	M	29	26.6	F508del/S549N	p.[Phe508del]; [Ser549Asn]	0	4.8	6.0 (C3 vs. baseline;	0.8	0.7 (C3 vs. baseline;
CF11	F	31	23.6	F508del/Unknown	p.[Phe508del]; [?]	0	4.5	5.0 $p = 0.18$)	0.7	0.7 $p = 0.50$)
CF13	M	29	21.2	F508del/711+1G>T	c.[1521_1523delCTT]; [579+1G>T]	0	3.8	5.6	0.9	0.8
HC ($n = 15$)	M/F[a]	41.6 ± 12.9	24.7 ± 3.1	Wild-type/Wild-type	p.[=];[=]	0	5.72 ± 0.4	N/A	0.44 ± 0.20	N/A

BMI body mass index, *HGVS* Human Genome Variation Society, *Fen* fenretinide, *N/A* not applicable, *HC* healthy controls, *ns* $p>0.05$, *$p \leq 0.05$

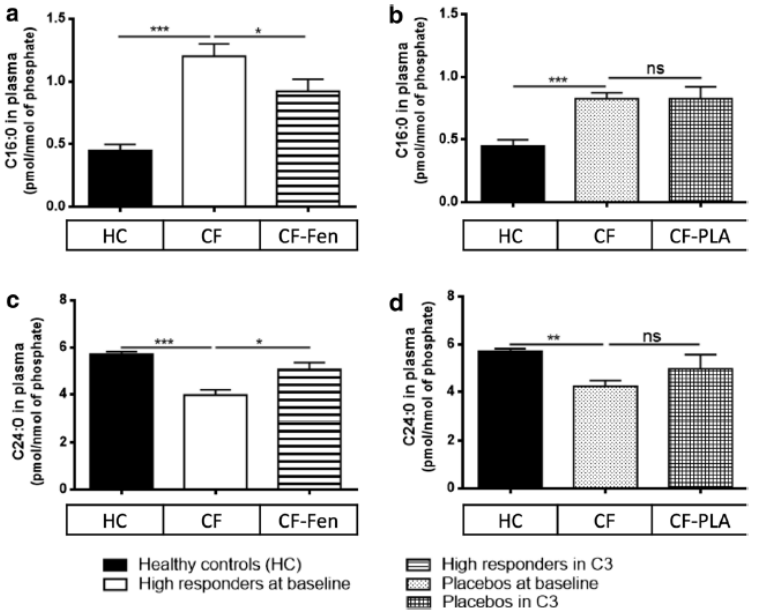
[a] Male sex, 46%

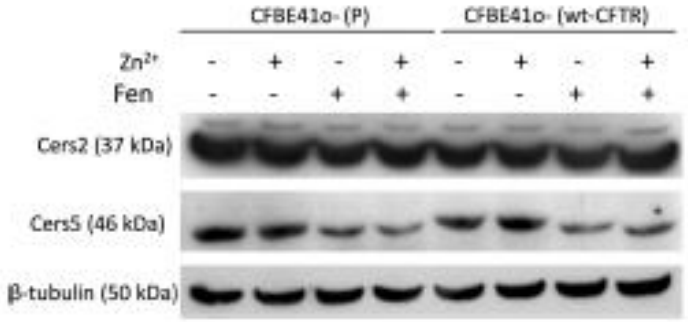
[b] According to the approved ethical protocol for clinical trial phase Ib, pharmacokinetic analysis was approved for 12 patients, including placebos

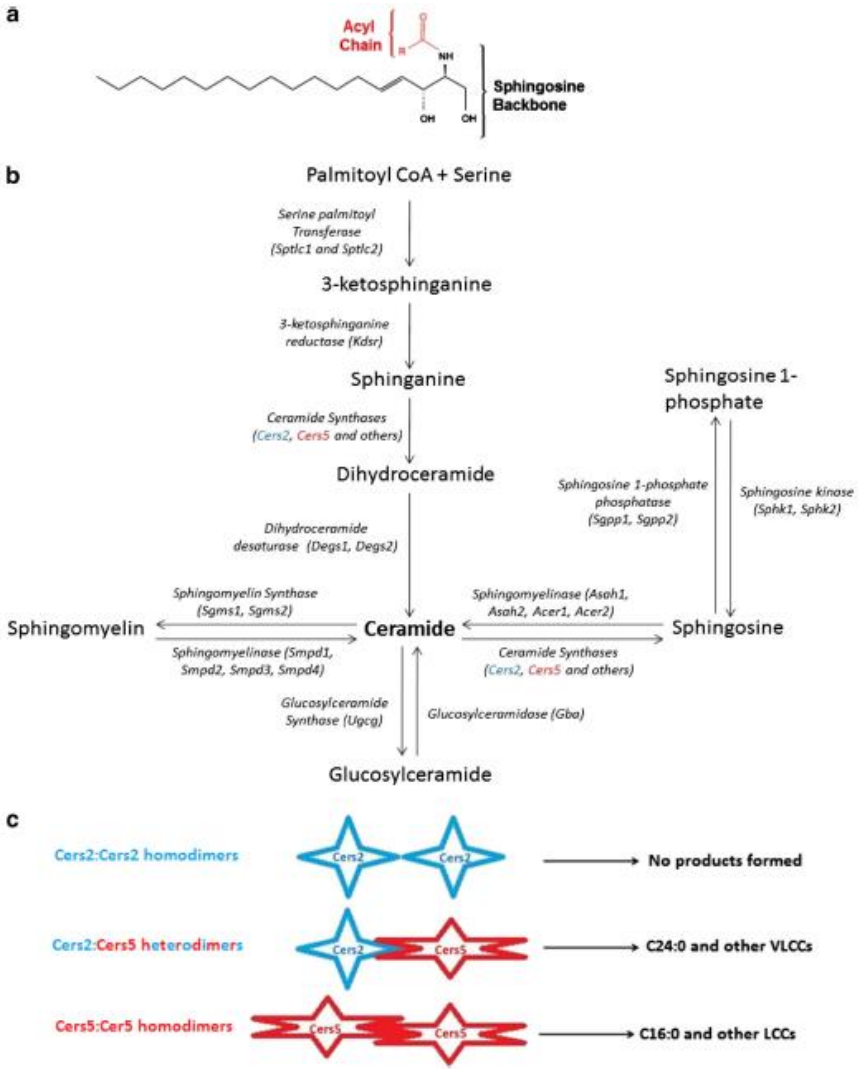
Garic D. http://www.laurentpharma.com/ et al. J Mol.Med. 2017

http://www.laurentpharma.com/

Fenretinide phase I trial







General Conclusions phase II trial

Study of LAU-7b in the Treatment of Cystic Fibrosis in Adults (APPLAUD). Phase II NC265288

- Patients with CFTR correctors therapy benefit with Lau7b treatment, increase FEV1, decrease primary inflammatory markers.
- The response to FEN is better in younger CF patients, in similar fashion in the trials of breast cancer.
- LAU-7b first inflammation-controlling drug to demonstrate clinical benefit in adults with CF, complementary to administration of CFTR modulators.
- Study achieves clinically meaningful reduction in lung function loss at 6 months, measured as absolute change in ppFEV1, reaching statistical significance in the Per Protocol population.

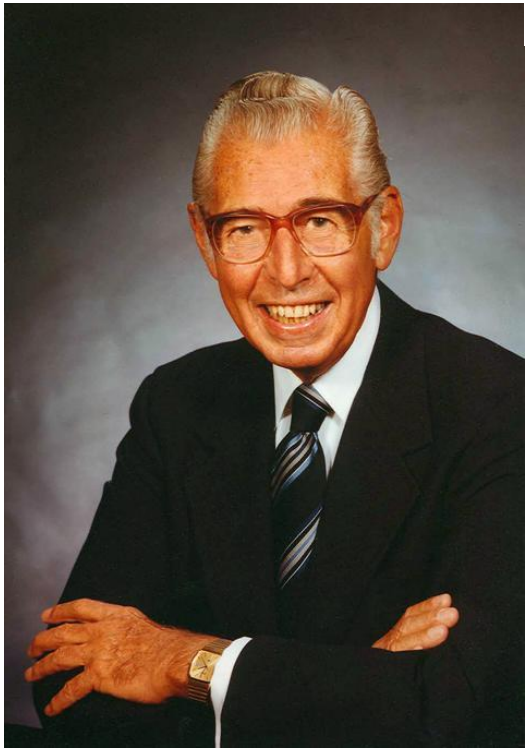
Study of LAU-7b for the Treatment of COVID-19 Disease in Adults (RESOLUTION) NCT04417257

- Patient goal 508 individuals.
- Dou[illegible] days
- Prim
  - P[illegible]%
    - Decreased lung inflammation observed only in Lau 7b treated patients.
  - Underway
    - Analysis of post Covid symptoms.
- Patent of use application WO/2021/189153

WHAT WE HAVE LEARNED?

- Differences in US – EMA – regulations.
- New reformulation strategy improved pharmacology of the drug resulting in new IP.
- Triple drug repurposing for COVID-19.

Has disulfiram indeed anticancer effect?



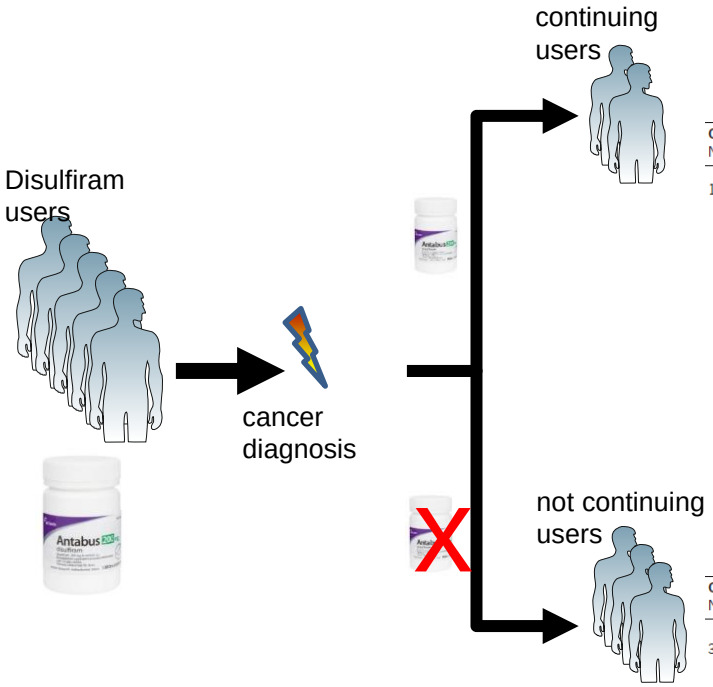
SPONTANEOUS REGRESSION OF BREAST CANCER

Edward F. Lewison, M.D.

Johns Hopkins Hospital
Baltimore, Maryland

E.T. A 35 year old female was operated upon for breast cancer in 1956. She remained well for three years until 1959. At that time severe back pain developed as the result of metastases to the spine, ribs and pelvis with collapse of T-5. She was treated by oophorectomy, radiation therapy and hormone therapy and her condition improved for the next two years. However, in 1961 she became a severe alcoholic and it was necessary to discontinue all hormone therapy and antabuse (Disulfiram) was started. Over the next 10 years--from 1961 to 1971--complete resolution of all bone lesions in the spine, skull, pelvis and ribs gradually occurred and the patient remained clinically free of cancer with no further hormone therapy, chemotherapy, or radiation therapy. Frequent psychiatric care was required and she remained on and off Antabuse therapy for her continued drinking problem. She died in 1971 when she accidentally fell from a third floor window. The coroner's report showed a high blood alcohol level and residual nests of metastatic carcinoma in the bone marrow.

Epidemiological data for Danish Cancer Registry

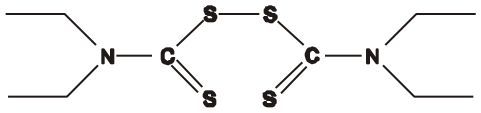


Overall				Localized stage				Non-localized stage			
Number*	HR	95% CI	*P* value	Number*	HR	95% CI	*P* value	Number*	HR	95% CI	*P* value
1,177	0.66	0.58–0.76	0.000	602	0.69	0.64–0.74	0.000	355	0.71	0.59–0.87	0.001
3,038	1.00			1,429	1.00			1,054	1.00		

Skrott Z, et al.. Alcohol-abuse drug disulfiram targets cancer via p97 segregase adaptor NPL4. Nature. 2017 Dec 14;552(7684):194-199. doi: 10.1038/nature25016.

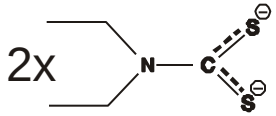
Repurposing of disulfiram: Targeting cancer via *p97* segregase adaptor NPL4

DSF, Disulfiram (tetraethylthiuram disulfide)



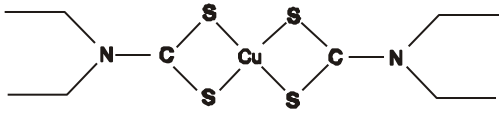
in the stomach and/or the blood

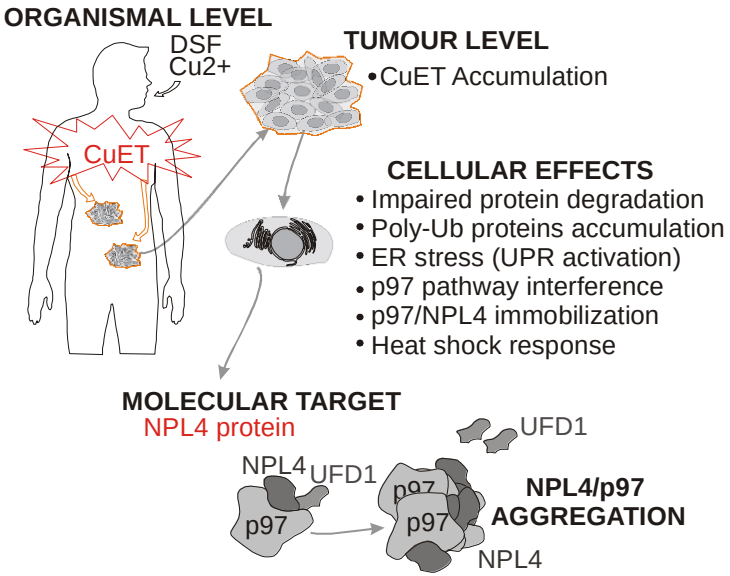
DTC, (diethyldithiocarbamate)



in the presence of Cu2+

CuET, bis(diethyldithiocarbamate)-copper





Alcohol-abuse drug disulfiram targets cancer via p97 segregase adaptor NPL4, *Nature,* 2017, 552, 194

Disulfiram's anti-cancer activity reflects targeting NPL4, not inhibition of aldehyde dehydrogenase, *Oncogene,* 2019, 38, 6711

Targeting the NPL4 Adaptor of p97/VCP Segregase by Disulfiram as an Emerging Cancer Vulnerability Evokes Replication Stress and DNA Damage while Silencing the ATR Pathway, *Cells,* 2020, 9, pii: E469

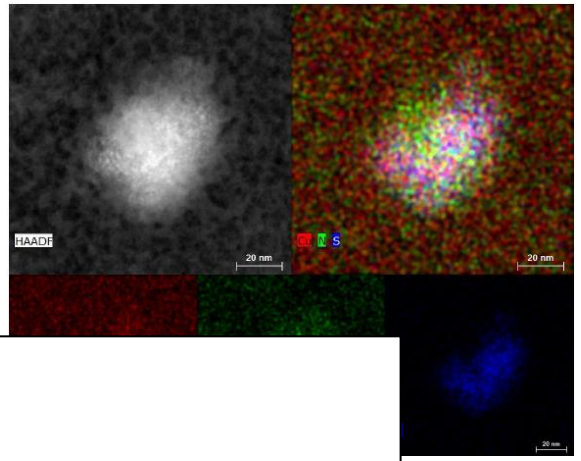
CuET in albumin nanoparticles

water

solubility <0.5

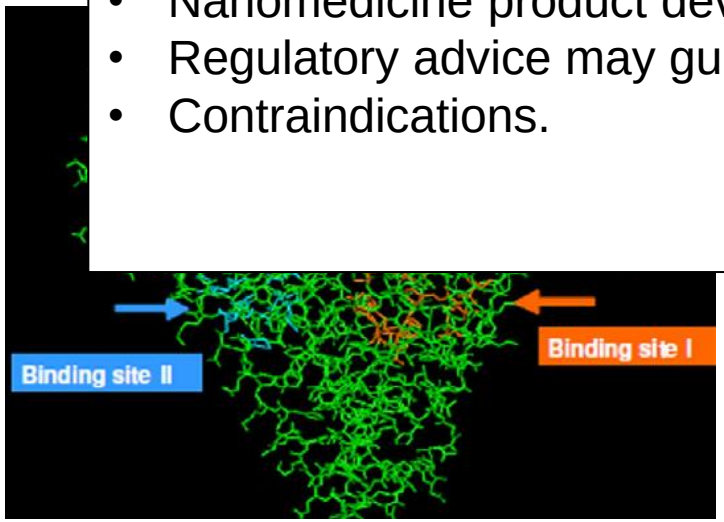
$2(DTC^{-}) + Cu^{2+} \rightarrow$ CuET

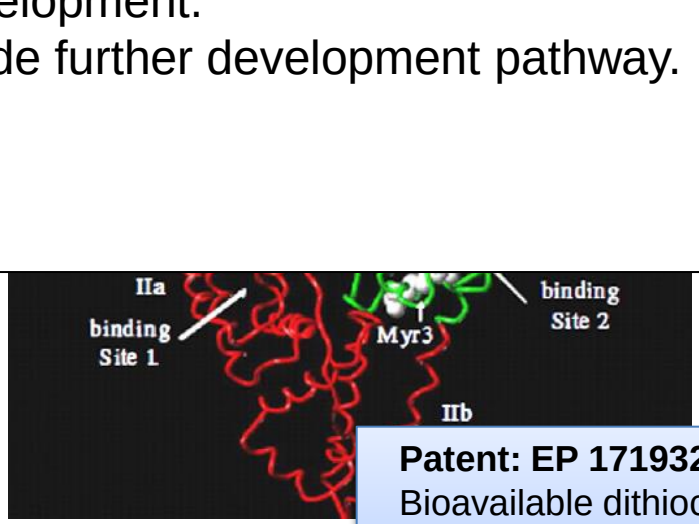
albumin in water



WHAT WE HAVE LEARNED?

- Good science is important.
- Nanomedicine product development.
- Regulatory advice may guide further development pathway.
- Contraindications.





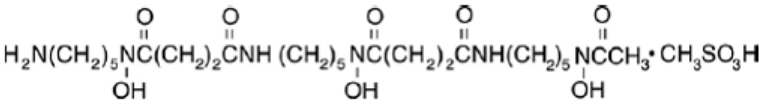
Patent: EP 17193240.3
Bioavailable dithiocarbamate-metal complex nanoparticles, method of preparation and use thereof

Radiolabelled siderophores for detection of microbial infections

- **Many bacterial**, **fungal** and viral **pathogens** can cause nosocomial **infections**, which can be **life-threatening**, especially in **immunocompromised hosts**
- **Early** and **accurate diagnosis** is often **essential** for succesfull treatment
- Currently used **methods lack specificity** and/or **sensitivity**
- **Siderophores** are low molecular mass **iron-chelating molecules** serving as iron transporters for nearly all **bacteria**, **fungi** and some plants
- Many microorganisms employ a **specific** and highly efficient **iron transporter mechanism** based on iron binding **siderophores**, that are **upregulated** in the pathogen especially **during infection**
- **Siderophores labelled** with a suitable **radionuclide** could be useful tool for the **detection of infections** caused by pathogenic microorganisms using **imaging methods of nuclear medicine**
- **Suitable radionuclide** useful for **labelling of siderophores** seems to be for example **gallium-68**
- **^{68}Ga** is a **positron emitter** with complexing properties comparable to those of Fe(III) and readily available from a **^{68}Ge/^{68}Ga-generator**

Desferrioxamine-B (Desferal, DFO-B) repurposing

+



^{68}Ge/^{68}Ga-generator
(with marketing authorization)

- **FDA approved** drug for long-term metal chelation therapy for decades
- testing of **^{68}Ga-DFO-B** for **bacterial infection imaging**

^{68}Ga-DFO-B: PET/CT imaging in infected animals

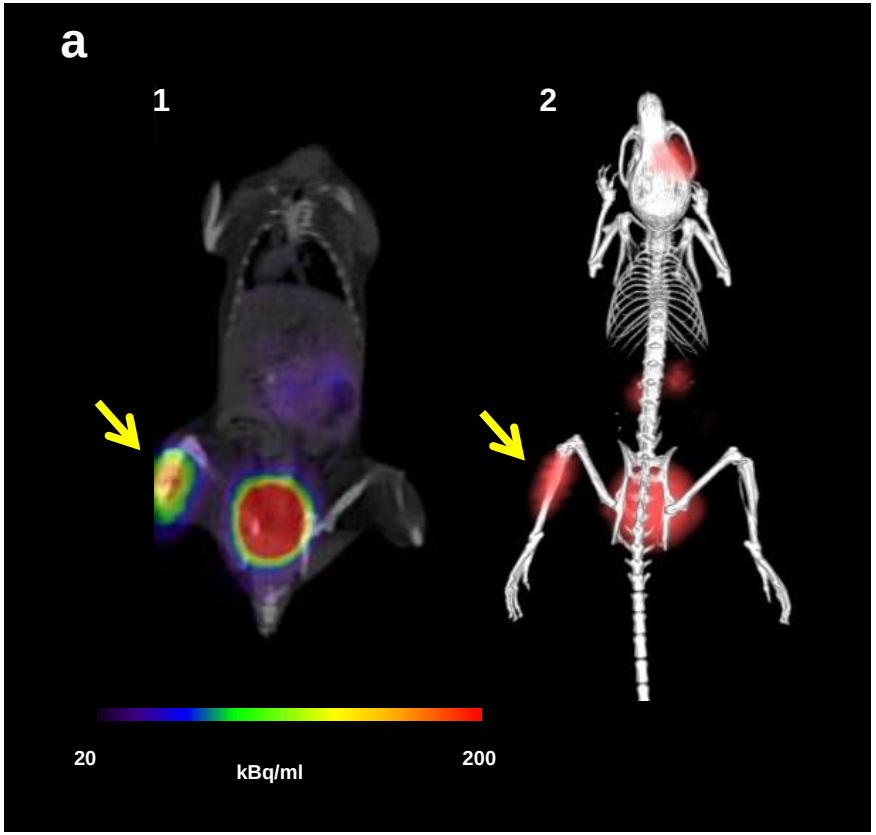
European Journal of Nuclear Medicine and Molecular Imaging (2021) 48:372–382
https://doi.org/10.1007/s00259-020-04948-y

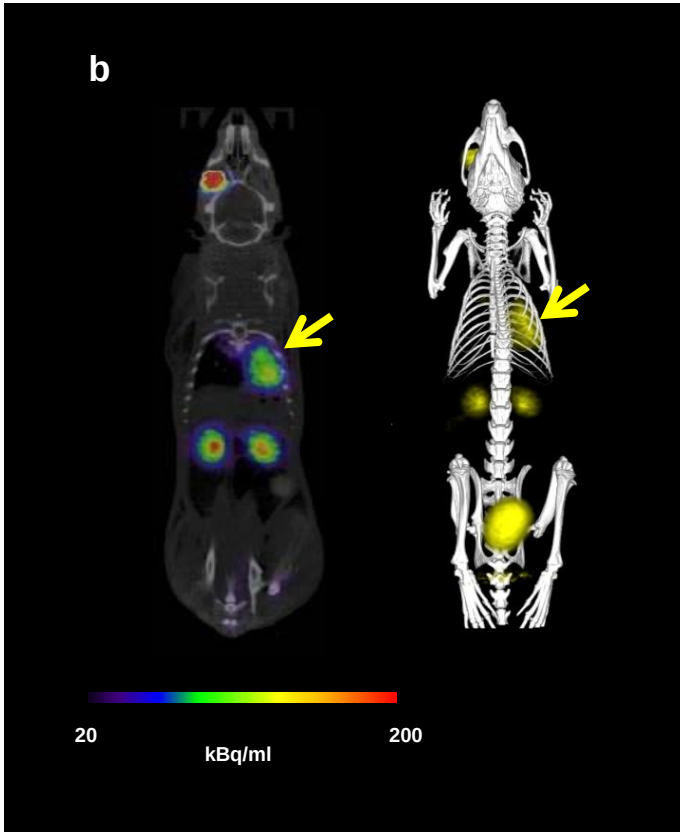
ORIGINAL ARTICLE

^{68}Ga-labelled desferrioxamine-B for bacterial infection imaging

Milos Petrik[1] · Eva Umlaufova[1] · Vladislav Raclavsky[2] · Andrea Palyzova[3] · Vladimir Havlicek[3,4] · Joachim Pfister[5] · Christian Mair[5] · Zbynek Novy[1] · Miroslav Popper[1] · Marian Hajduch[1] · Clemens Decristoforo[5]

Received: 21 May 2020 / Accepted: 29 June 2020 / Published online: 30 July 2020





^{68}Ga-DFO-B: clinical trial

- **prospective phase I/IIA study (EudraCT 2020-002868-31)**

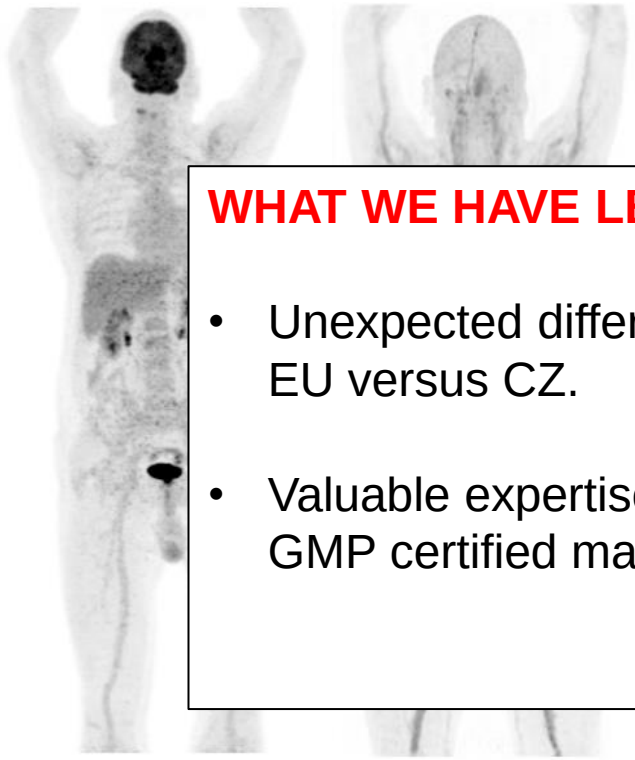
MEDIZINISCHE UNIVERSITÄT INNSBRUCK + Palacký University Olomouc

- primary objectives:
  - **safety and tolerability** of ^{68}Ga-DFO-B in human subjects
  - preliminary **diagnostic sensitivity** of ^{68}Ga-DFO-B
- secondary objectives:
  - **radiation dosimetry** of ^{68}Ga-DFO-B in human subjects
  - characterization of **pharmacokinetics** of ^{68}Ga-DFO-B in human subjects
- total number of participants: **15** (four with radiation dosimetry)

regulations in AT are more simple and straightforward

no need of „full GMP“ requirements in AT

^{68}Ga-DFO-B: first patients (biodistribution and dosimetry)



Target Organ	Total [mSv/MBq]	ICRP-103 ED [mSv/MBq]	Dose actual study [mSv]
Adrenals	1,57E-02	1,45E-04	3,12
Total Body	1,00E-02	0,00E+00	1,99
Effective Dose		9,18E-03	1,82

- **no adverse events**
- **< 2 mSv**
- **ion**
- **g**
- **nination**
- **ability**
- **olites in**
- **low protein binding**

WHAT WE HAVE LEARNED?

- Unexpected difference in regulation of radiopharmaceutics in EU versus CZ.
- Valuable expertise in development of radiopharmaceutics, GMP certified manufacturing site under preparation.

^{18}F-FDG **^{68}Ga-DFO-B**

60 min

ED = 1.82 mSv

Czech contribution to nuclear medicine

Prof. Josef Pacák
and
Prof. Miloslav Černý

Synthesis of 2-Deoxy-2-fluoro-D-glucose

By Josef Pacák,* Zdeněk Točík, and Miloslav Černý

(*Department of Organic Chemistry, Charles University, Prague–Albertov, Czechoslovakia*)

J. Chem. Soc. D, 1969, 77-77

1990's: ^{18}F-FDG approved by FDA for PET

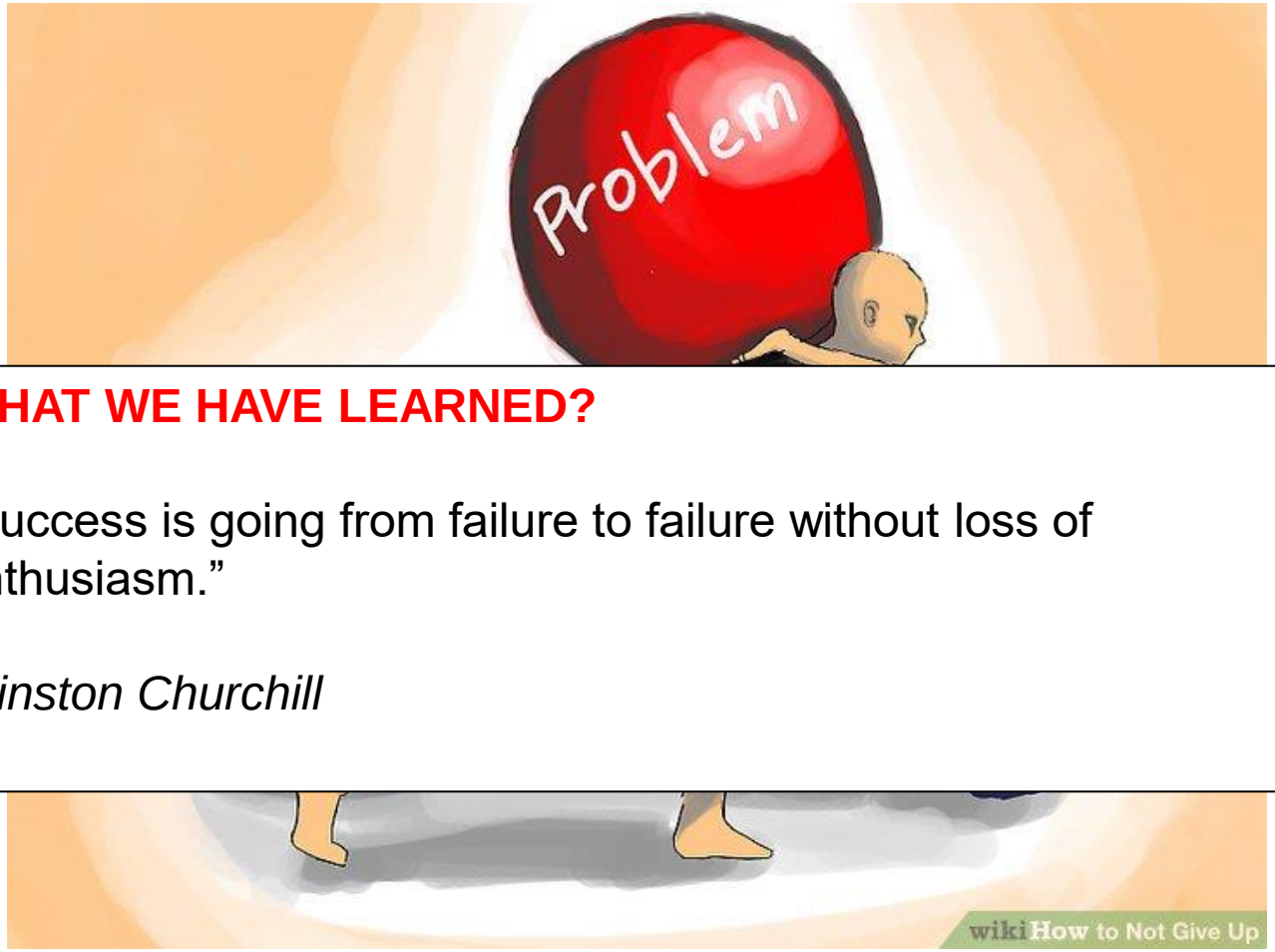
Dr. Martina Benešová

^{177}Lu-PSMA-617 therapy (β^-)

2022: FDA approved Pluvicto (^{177}Lu-PSMA 617)

Academic drug development? YES, but what is important?

- Good science to rise interest.
- Clear and logical plan to develop a product.
- Strong team of dedicated and experienced scientists.
- Positive attitude.
- Do not get disappointed - rejection/failure rates in drug development funding/licensing are much higher than in paper submissions or grant applications.



WHAT WE HAVE LEARNED?

"Success is going from failure to failure without loss of enthusiasm."

Winston Churchill

Thank you for attention!

IMTM

marian.hajduch@upol.cz; www.imtm.cz ; www.eatris.eu