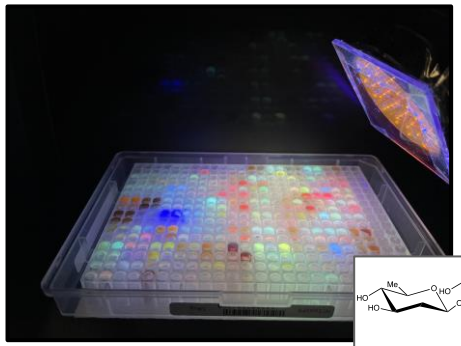
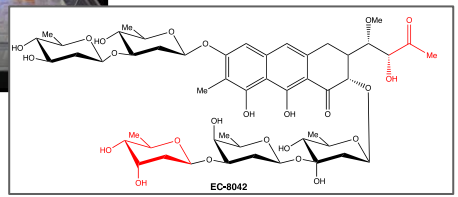
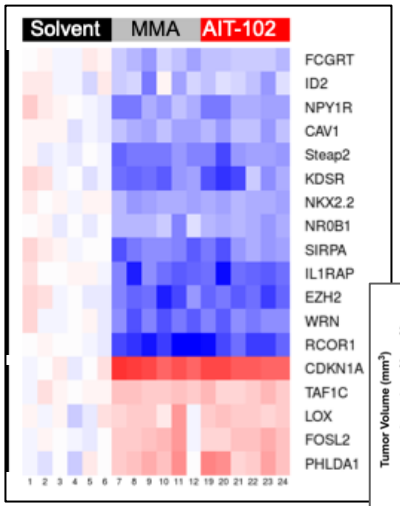
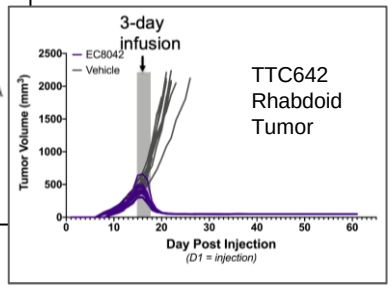
The Discovery and Development of AIT-102 as a Targeted Therapy for Ewing Sarcoma and Rhabdoid Tumor









Patrick J. Grohar

Russell G. Adderley Professor of Pediatric Oncology, Professor of Pediatrics, Department of Pediatrics, University of Michigan

and

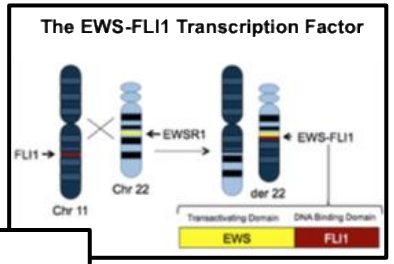
Barry R. O'Keefe

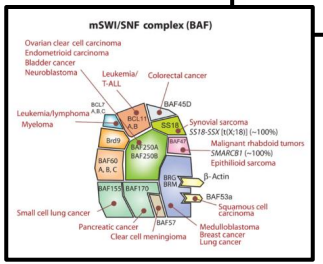
Natural Products Branch, Developmental Therapeutics Program, Division of Cancer Treatment and Diagnosis, National Cancer Institute

Frederick National Laboratory Advisory Committee, October 23, 2024

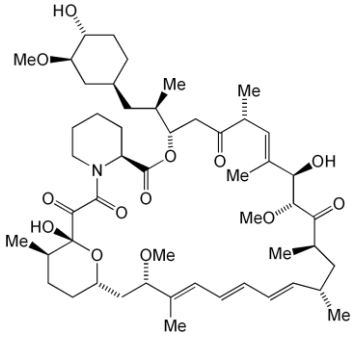
Presentation Outline

- NCI Natural Products Branch (NPB)
  - Natural products in drug discovery
  - NPB resources available to researchers
- EWS-FLI1 Reporter High Throughput Screen of NPB libraries identified natural product active against Ewing Sarcoma
  - Discovery of mithramycin as an active agent
  - Identification of a better performing analog of mithramycin
- Further pre-clinical development of AIT-102
  - Mechanism of action in Ewing sarcoma and rhabdoid tumor
  - Mechanism guides the schedule of administration

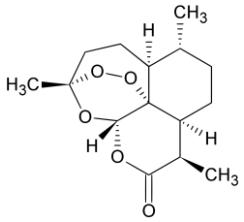




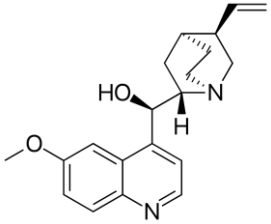
Natural Products: A History of Pharmaceutical Utility



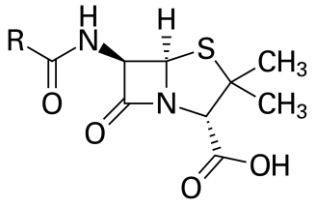
Rapamycin



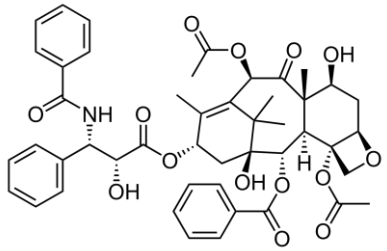
Artemisinin



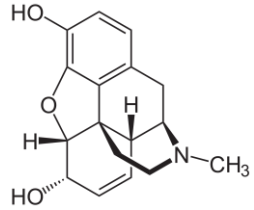
Quinine



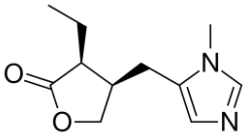
Penicillin



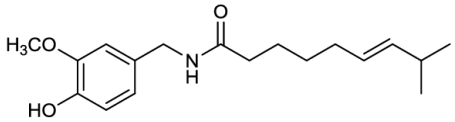
Paclitaxel



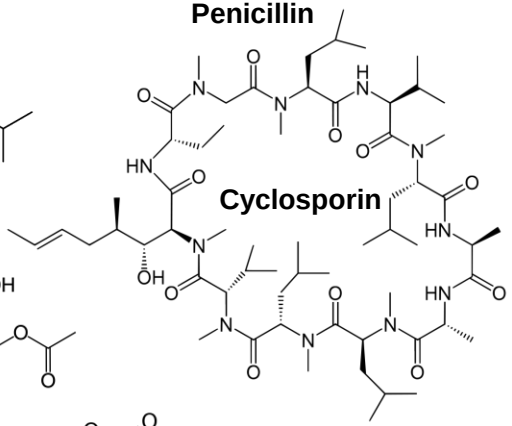
Morphine



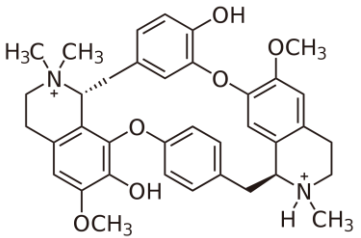
Pilocarpine



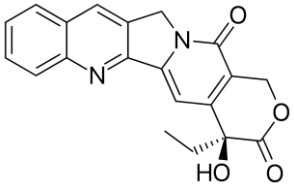
Capsaicin



Cyclosporin

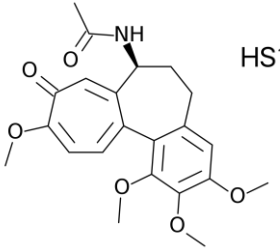


Tubocurarine

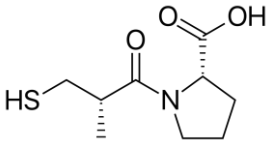


Camptothecin

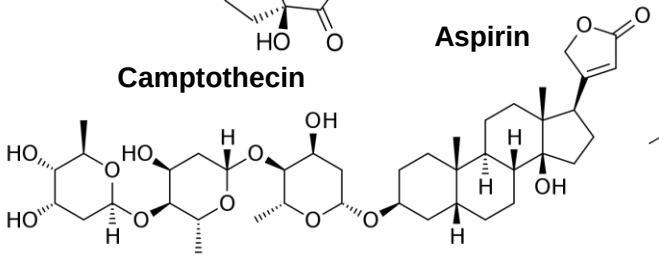
Aspirin



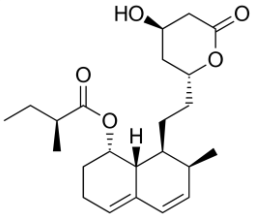
Colchicine



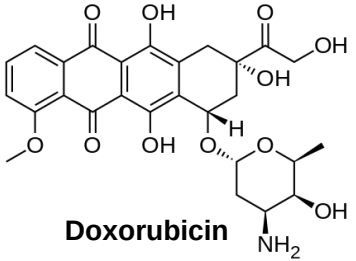
Captopril



Digitoxin



Mevastatin



Doxorubicin

Natural Products: A History of Pharmaceutical Utility





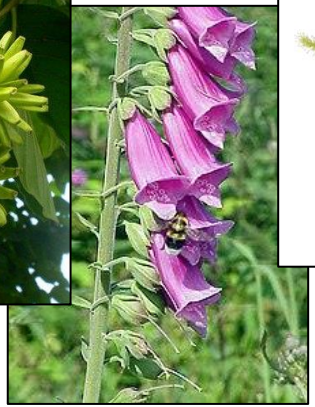


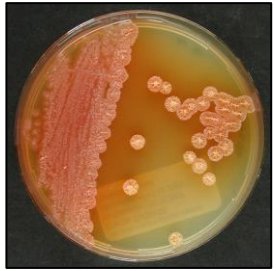




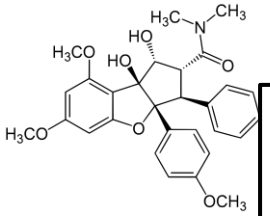




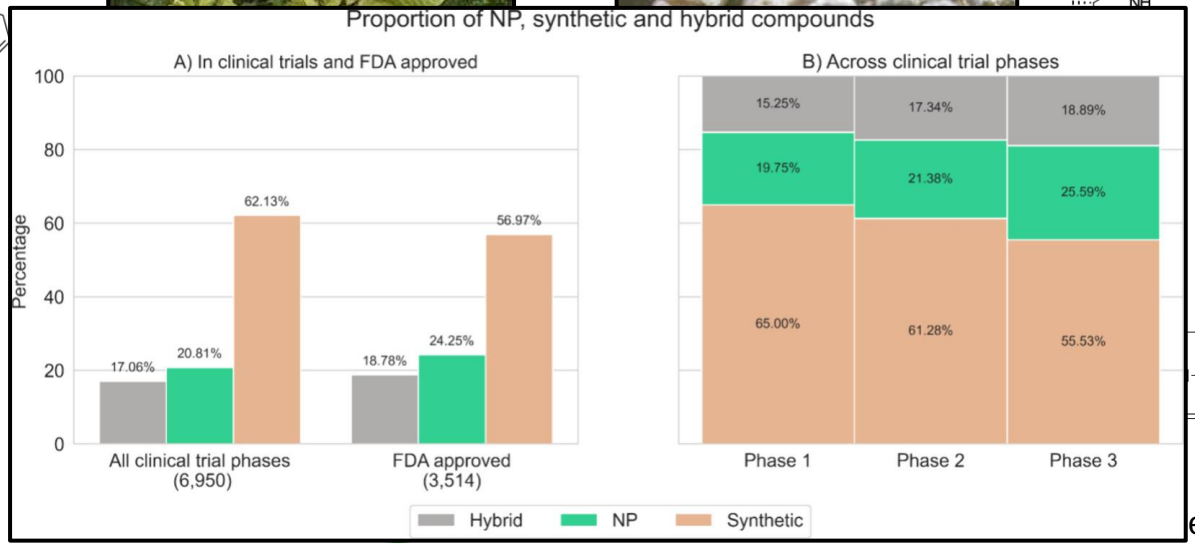


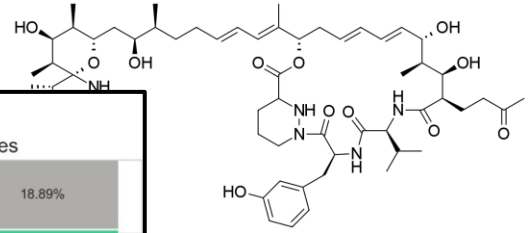


Natural Product Pharmacophores in the Clinic for Cancer

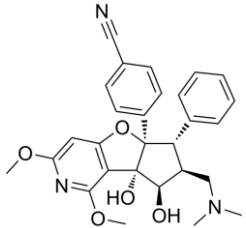


Rocaglamide

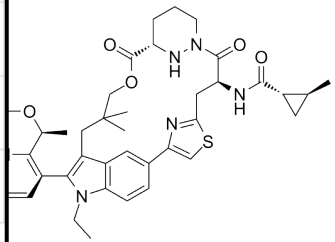




Sanglifehrin



Zotatifin/eFT226
eFFECTOR



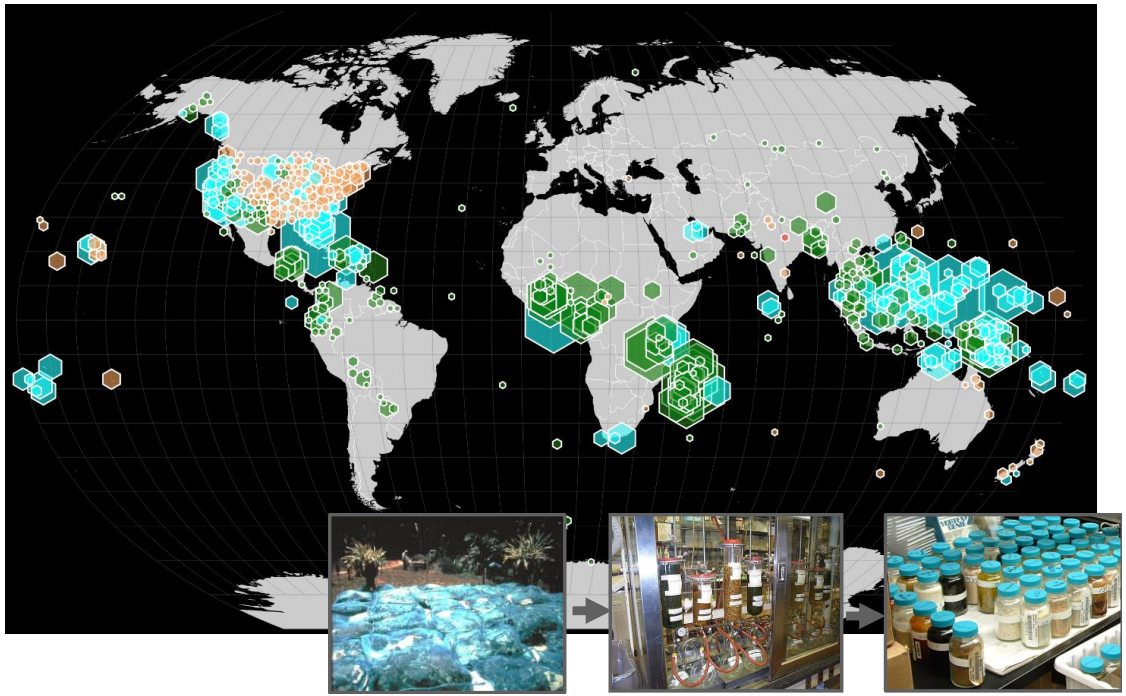
RMC-6236
evolution Medicines

Two new NP scaffolds for cancer therapy have been reporting positive initial results in the clinic.
Natural products and their derivatives have a record of success in clinical trails

Sedrani, *et al*. *JACS,* 2003; Lu King et al. *Chem Soc Chem Commun* 1982;
Domingo-Fernandez *et al*. J. Nat. Prod. 2024.

NCI Natural Product Collections

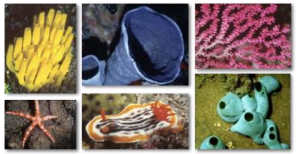
The NCI has one of the world's largest, most diverse collections of natural product extracts (>200,000 extracts).



Plant Extract Library

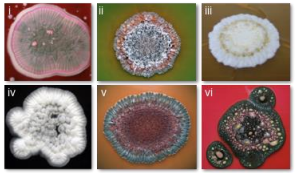
- **~161,000** extracts (organic + aqueous)
- **~44,000** plants, including 81,400 raw materials (leaves, roots, fruit, etc.) collected from Africa and Madagascar; North, Central and South America; and Southeast Asia.

Marine Extract Library



- **~41,000** extracts (organic + aqueous)
- **~20,500** organisms collected from the Indo-Pacific region.

Microbial Extract Library

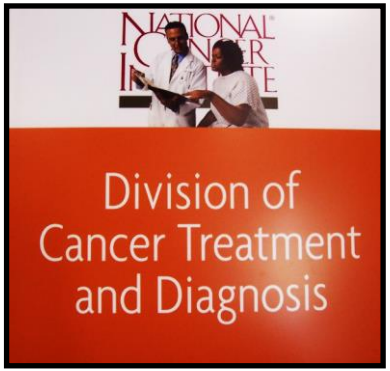


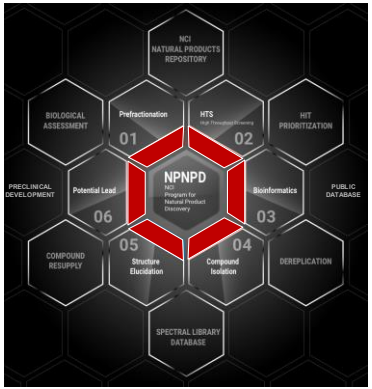
- **~30,000** extracts (organic + aqueous)
- **~26,000** organisms collected from US

- **New Collection**: 20,000 Fungal strains from USA (Univ. of Oklahoma)

NCI Program for Natural Products Discovery

The NCI Program for Natural Products Discovery (NPNPD) is a joint effort of the Division of Cancer Treatment and Diagnosis and the Center for Cancer Research.



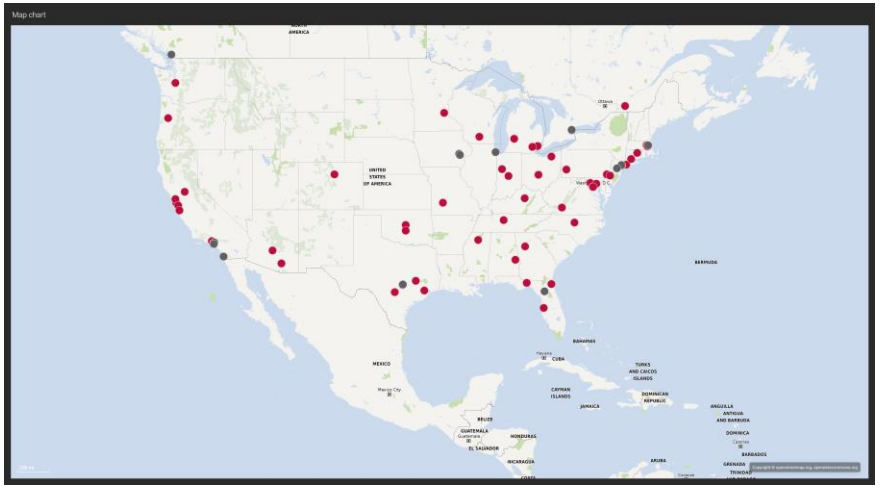


The NPNPD is designed to facilitate both intramural and extramural research and address current challenges in natural product-based drug discovery.

The NPNPD is funded by the Cancer Moonshot Program.

NCI NPB Agreements for Pre-fractionated Samples

- >680,000 fractions so far produced from NCI crude extracts
- Pre-fractionated library of 500,000 natural product samples publicly released
- >9,000,000 wells shipped to screening centers so far
- Technology transfer of methods and automated systems to groups worldwide
- >70 MTAs signed with industry, government, and academic screening centers



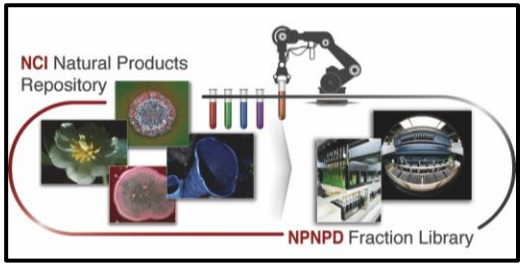
NPB/NPNPD Collaborations North America

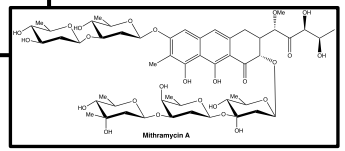
NPB/NPNPD Collaborations Worldwide

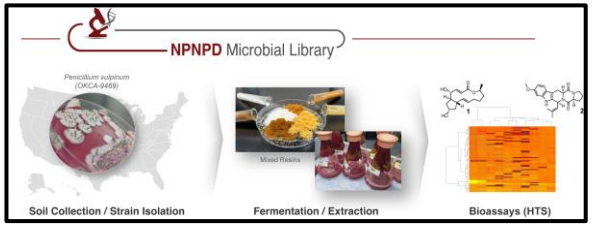
https://dtp.cancer.gov/organization/npb/npnpd_prefractionated_library.htm

NCI Natural Product Collections Available to the Public

- **Crude Natural Product Extract Library**
  - >200,000 Crude Natural Product Extracts
- **Pre-fractionated Natural Product Library**
  - >500,000 partially-purified fractions released to the public
- **Traditional Chinese Medicinal Plant Library**
  - Collaboration with Harvard, Beijing Univ., Hong Kong Baptist Univ. and the NCI Office of Cancer Complementary and Alternative Medicine
- **Pure Natural Product Library**
  - Plate of >400 pure natural products (many with known cytotoxicity)
- **New U.S. Soil Fungi Library**
  - Obtained through contract with University of Oklahoma citizen science program
  - >22,000 fungi accessioned, cryovialed; currently being grown, extracted and pre-fractionated

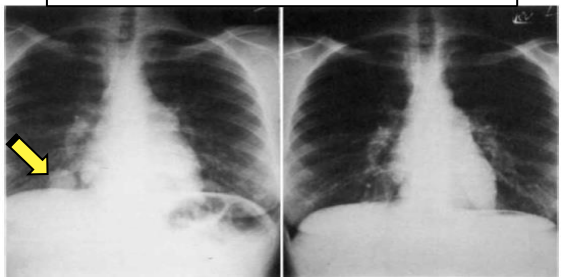






Molecular Targets Program/Pediatric Oncology Branch
EWS-FLI1 HTS Identifies Mithramycin

Treatment of Ewing Sarcoma Patient in 1964



56,000 Compounds

Natural Products Provided by NPB/DTP

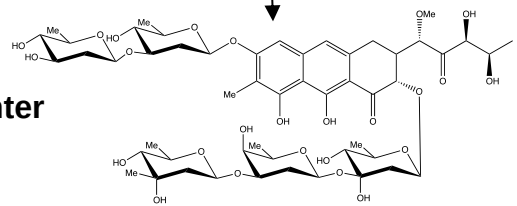
Primary Screen

EWS-FLI1 Target Promoter Luciferase Reporter (*NR0B1)

200 Compounds

Screen for Change in Gene Signature of EWS-FLI1

Secondary Screen



Mithramycin

Mithramycin tested in the NIH clinical center

- Drug provided by DTP/DCTD repository
- bolus injections too toxic for use
- needed less toxic analog

Mithramycin Blocks EWS-FLI1

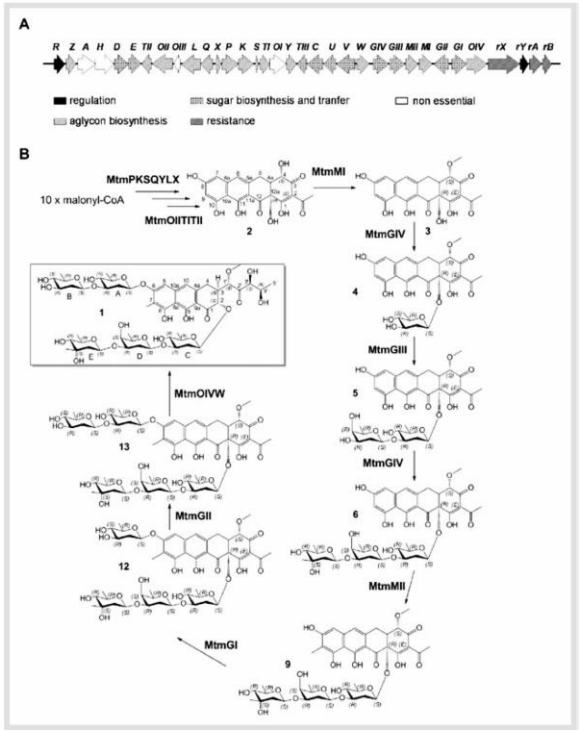
- Promoter
- mRNA
- Protein
- Genome wide reversal of gene signature of EWS-FLI1

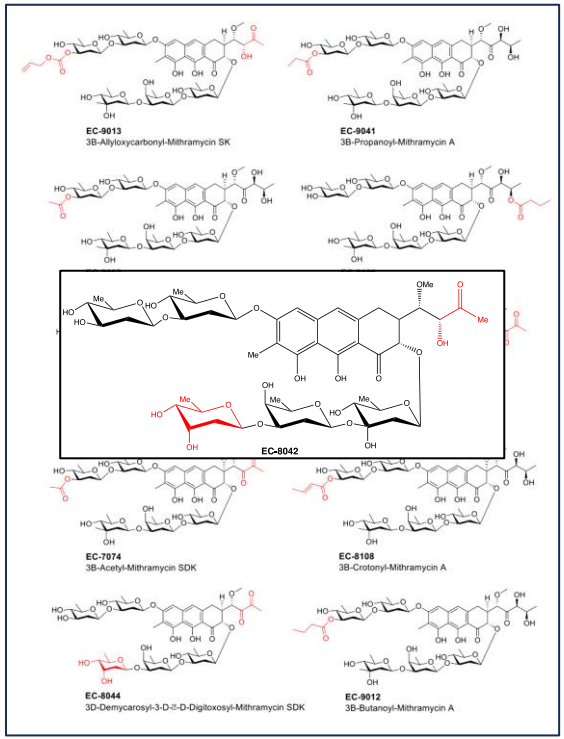
Grohar, P.J. et al. *J. Natl Cancer Inst* 2011.

Evaluation of Engineered Mithramycin Analogs (EntreChem)

Mithramycin is produced by *Streptomyces argillaceus*

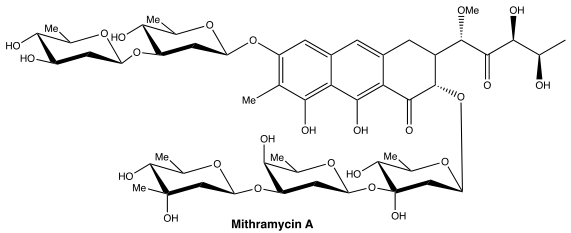
- Analogs (mithralogs) produced by combinatorial biosynthesis (gene inactivation and sugar modification)

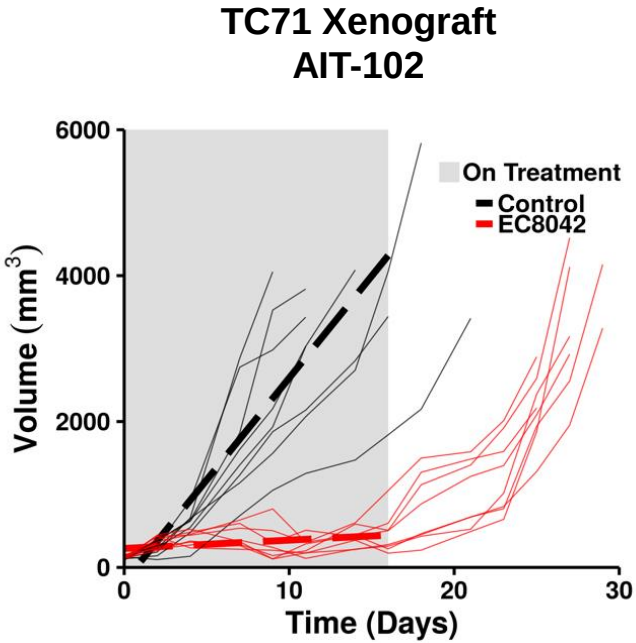




	Intraperitoneal	Intravenous
EC-code	MTD (mg/kg)	MTD (mg/kg)
MMA	1.5	2
EC7073		<4
EC7092		<4
EC8042	200	64
EC8043	50	*n.d.
EC8044	6.25	<4
EC8062	1.5	<4
EC8063	12.5	<4
EC8071	12.5	<4
EC8072		<8
EC8074	3.13	<4
EC8073	25	<64
EC8105		**<4**
EC8106		<4
EC8108		8
EC7072		32
EC9012		<4

EC-8042/AIT-102 Showed Improved Results in TC71 Xenograft





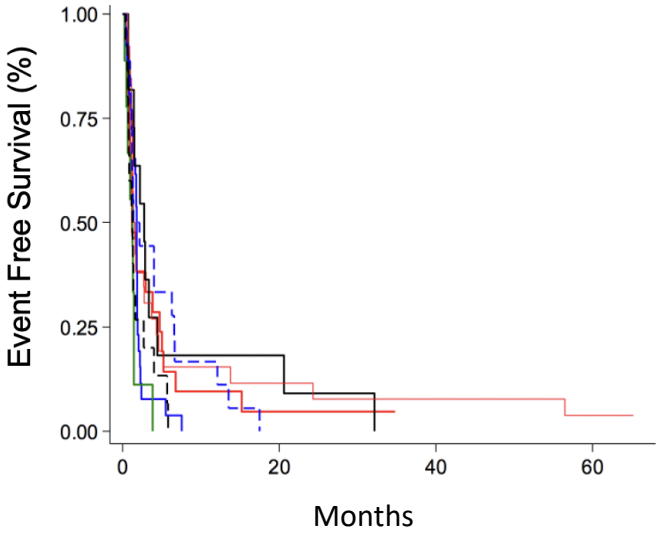
- New mithramycin analog: demycarosyl-3D-β-D-digitoxosylmithramycin SK (EC-8042) was discovered
- EC-8042 renamed AIT-102 and now being developed by OrphAI
- Showed good activity in Ewing sarcoma xenograft studies at doses farther away from MTD

At 1/10th MTD

Osgood et al. *Clin Cancer Research, 2016.*

How can we move these drugs more effectively to patients?

Last Five Phase II Studies in Ewing Sarcoma in COG



<u>Approach to improve outcomes in clinical trials</u>

(1) Focus on the right target(s)

(2) Mechanism of target suppression for a **<u>drug</u>**
- Natural products through the lens of modern genomics

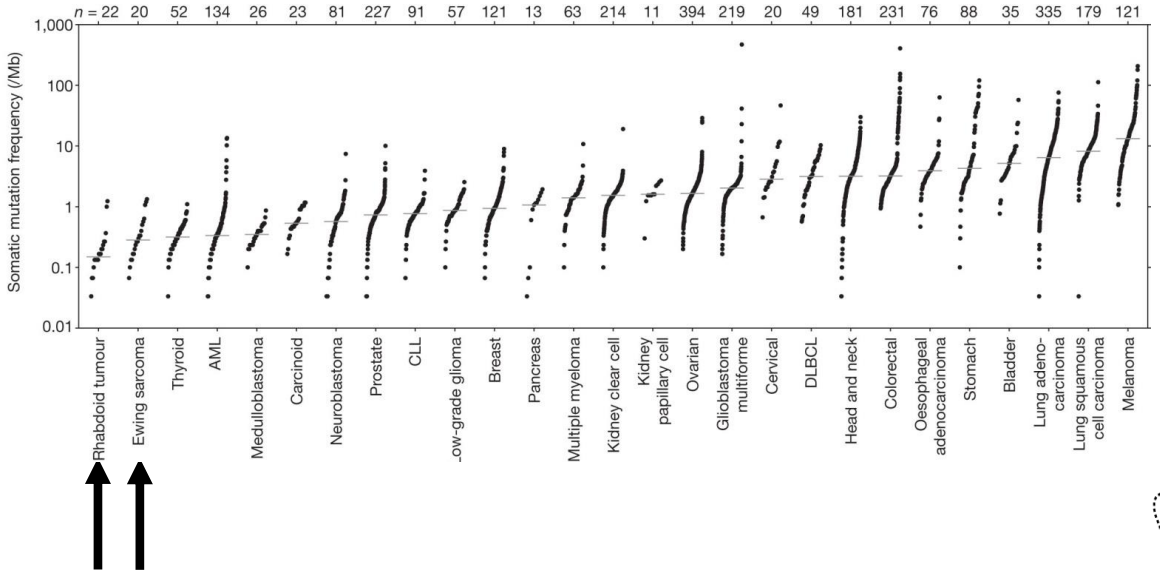
(3) Mechanism to determine drug exposure (Conc.*time) to achieve MOA
- Favor drugs that work far below the MTD
- Optimize schedule of administration for a target

Bottom Line: Need all three: the right target, drug, and schedule!!!

Focusing on the Right Target: Ewing Sarcoma and Rhabdoid Tumor Have Low Mutation Burden & Clear Oncogenic Driver

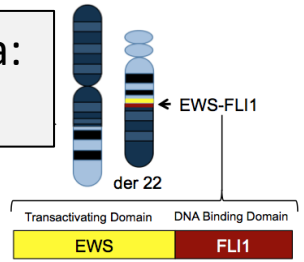
Somatic mutation frequencies of human cancer

Pediatric → Adult



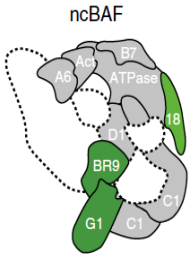
Lawrence MS *et al. Nature* (2013) 499(7457): 214-216

Ewing Sarcoma: EWS-FLI1



- Constitutively active TF
- Dependence known for 25 years
- (Multiple Independent Studies)
- Few recurrent cooperative mutations
- The ideal drug target

Rhabdoid Tumor: SMARCB1 Loss

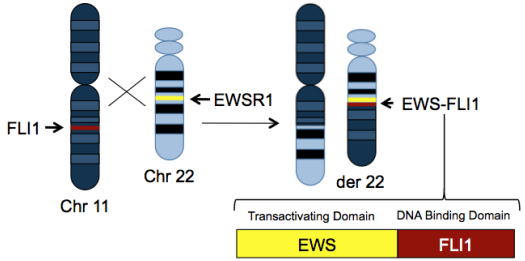


- ATP Chromatin Remodeler
- Lowers Affinity for Chromatin
- Redistribution in Genome
- Alters EZH2 (H3K27me3)
- Dependence on ncBAF

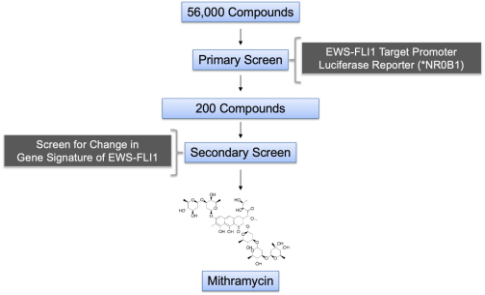
Michel,Kadoch et al. 2018 Nat. Cell Biol.

Unbiased Approaches Identify the Mithramycins as Important Compounds for These Targets

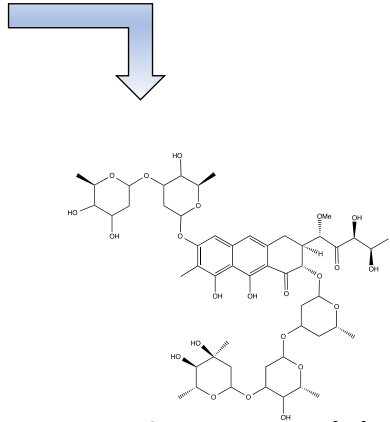
EWS-FLI1



50,000 Compound Screen for Inhibitors

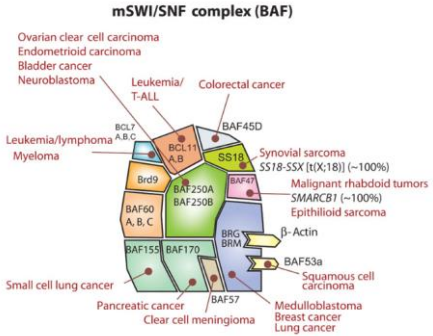


Grohar, P.J. et al. ***J. Natl Cancer Inst*** (2011) 103 (12); 962-978

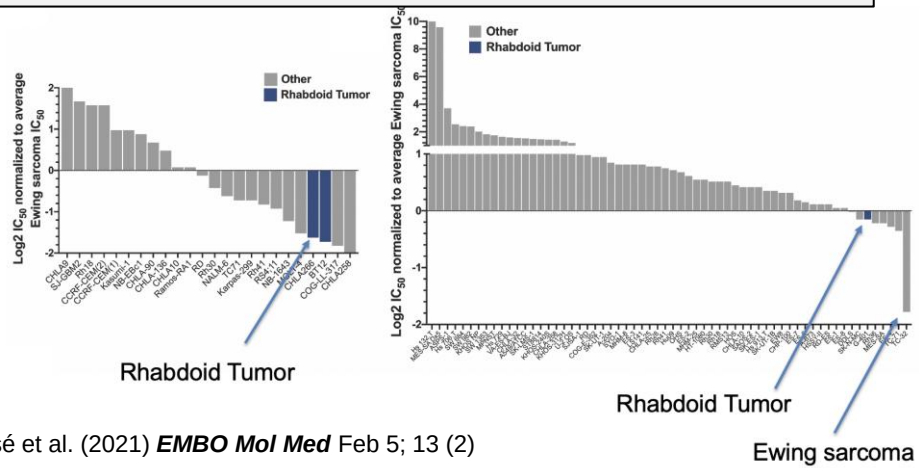


Mithramycin(s)

SMARCB1 Deleted SWI/SNF



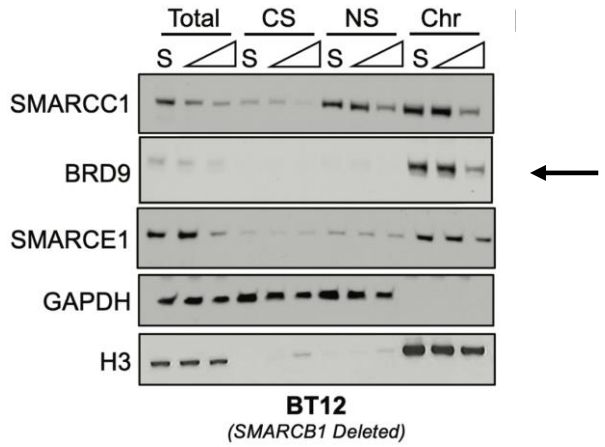
Multiple Cell Line Screens: Hypersensitivity



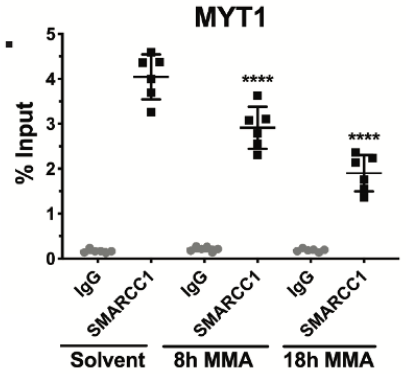
Chassé et al. (2021) ***EMBO Mol Med*** Feb 5; 13 (2)

Mechanism of Action in Rhabdoid Tumor

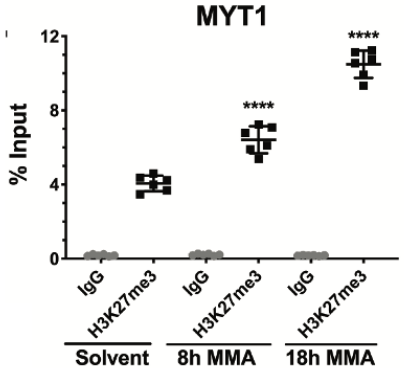
Blocks binding of mutated SWI/SNF (lower affinity/minor groove binder)



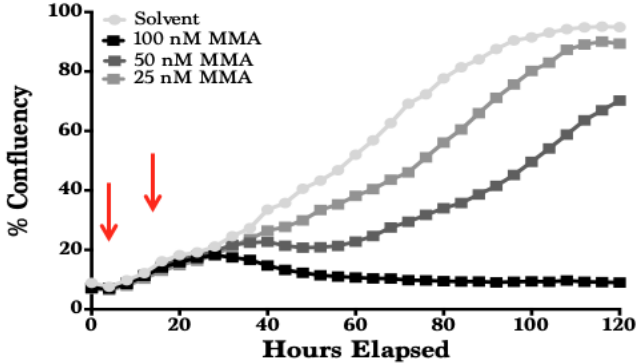
SMARCC1 binding



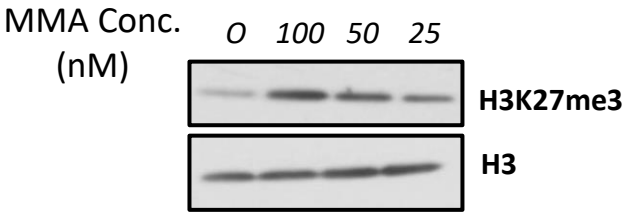
H3K27me3



Loss of Viability



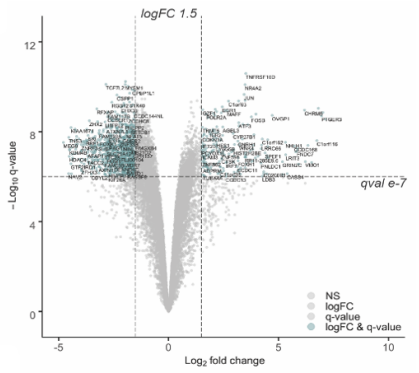
Epigenetic Switch: Gain of H3K27me3



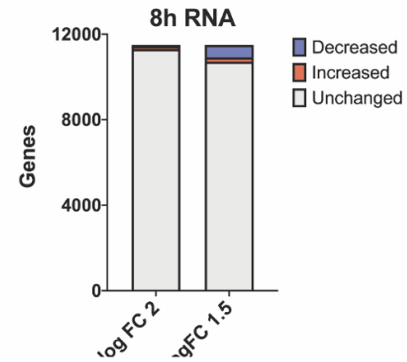
Chassé et al. (2021) **EMBO Mol Med** Feb 5; 13 (2)

Mithramycin Does Not Cause a General Blockade in Transcription: Reversal of **Oncogenic Transcriptome**

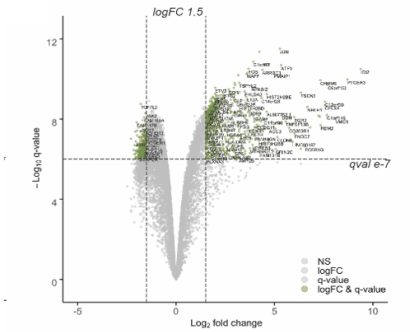
8 hours of exposure



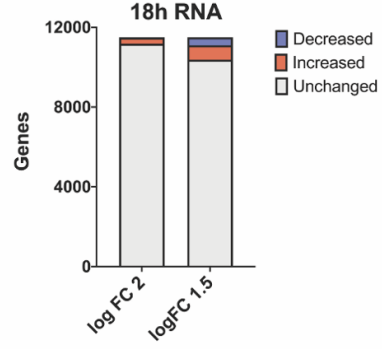
# of Changing Transcripts



18 hours of exposure



# of Changing Transcripts



Chassé et al. (2021) **EMBO Mol Med** Feb 5; 13 (2)

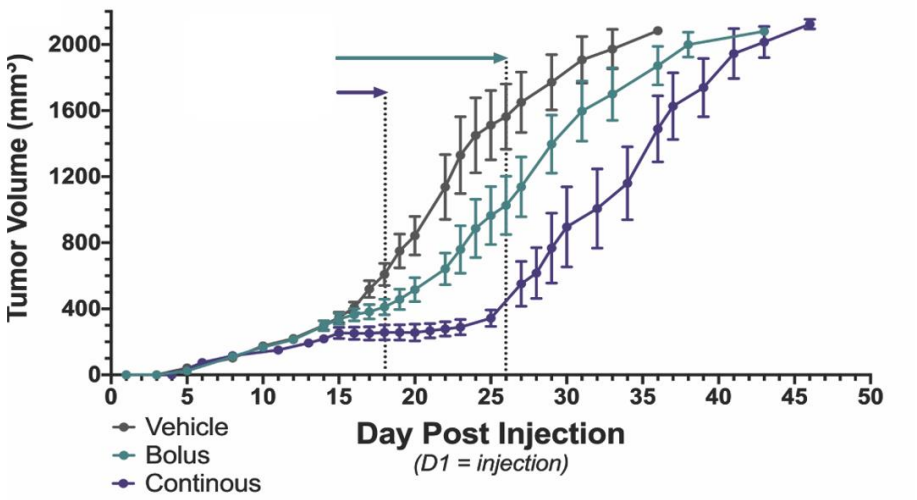
Loss of Self-renewal

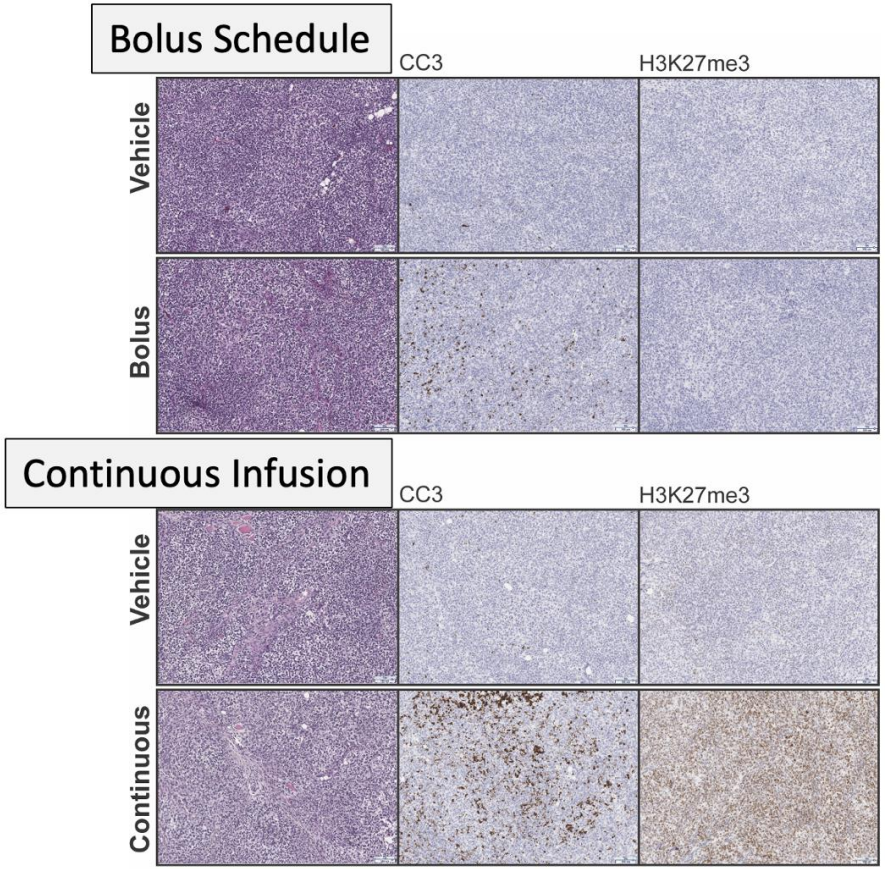
PATHWAY	GENE RANKS	NES	p-value	q-value
UV RESPONSE DOWN		-2.61	1.3e-03	1.4e-02
MITOTIC SPINDLE		-2.24	1.2e-03	1.4e-02
HEDGEHOG SIGNALING		-1.95	3.2e-03	1.4e-02
TGF BETA SIGNALING		-1.86	2.8e-03	1.4e-02
WNT BETA CATENIN		-1.64	1.7e-02	3.4e-02
ESTROGEN RESPONSE		-1.40	4.4e-02	7.4e-02
G2M CHECKPOINT		-1.37	2.8e-02	4.9e-02
IL2 STAT5 SIGNALING		-1.31	7.7e-02	1.2e-01
NOTCH SIGNALING		-1.28	1.7e-01	2.4e-01
ANDROGEN RESPONSE		-1.09	3.2e-01	4.2e-01

Gain of differentiation?

PATHWAY	GENE RANKS	NES	p-value	q-value
P53 PATHWAY		2.91	1.9e-03	4.6e-03
TNFA SIGNALING		2.67	2.0e-03	4.6e-03
ADIPOGENESIS		2.50	2.0e-03	4.6e-03
EMT		2.37	2.0e-03	4.6e-03
APOPTOSIS		2.18	2.0e-03	4.6e-03
DNA REPAIR		1.95	2.0e-03	4.6e-03
ALLOGRAFT REJECTION		1.89	2.0e-03	4.6e-03
MYOGENESIS		1.82	2.0e-03	4.6e-03
MYC TARGETS UP		1.74	2.0e-03	4.6e-03
GLYCOLYSIS		1.73	2.0e-03	4.6e-03

Recapitulating the Mechanism in Vivo: Importance of Schedule to Increase H3K27me3





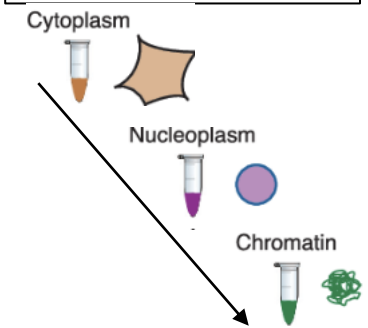
Mechanism Guides Schedule

Could not increase dose further; less toxic AIT102

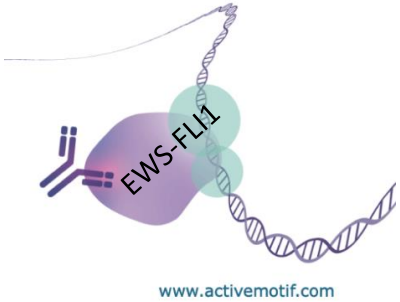
Mechanism in Ewing Sarcoma: Impact on EWS-FLI1 Binding and Transcription

EWS-FLI1 Binding To Chromatin

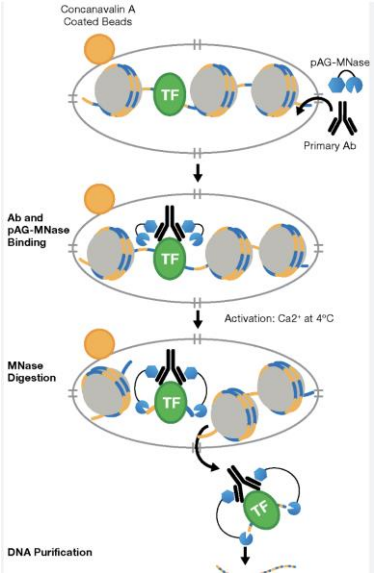
Chromatin Fractionation (Global Binding)



Chromatin Immunoprecipitation (Specific Loci)

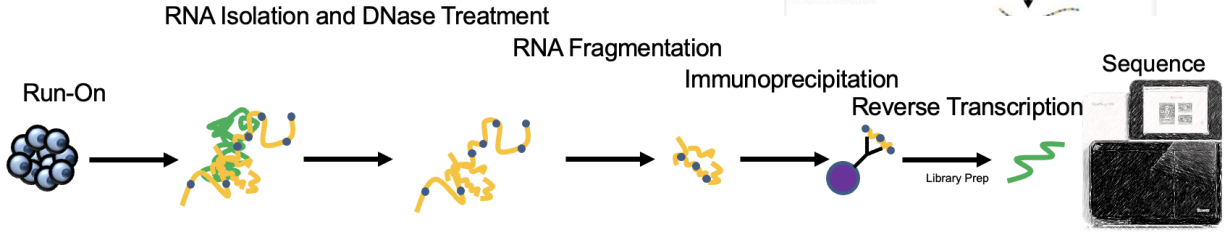


Cut and Tag (Genome Wide Specific Loci)

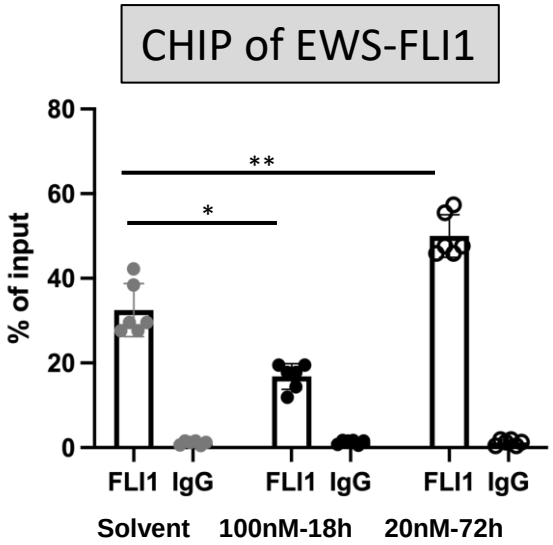


Impact Active Transcription

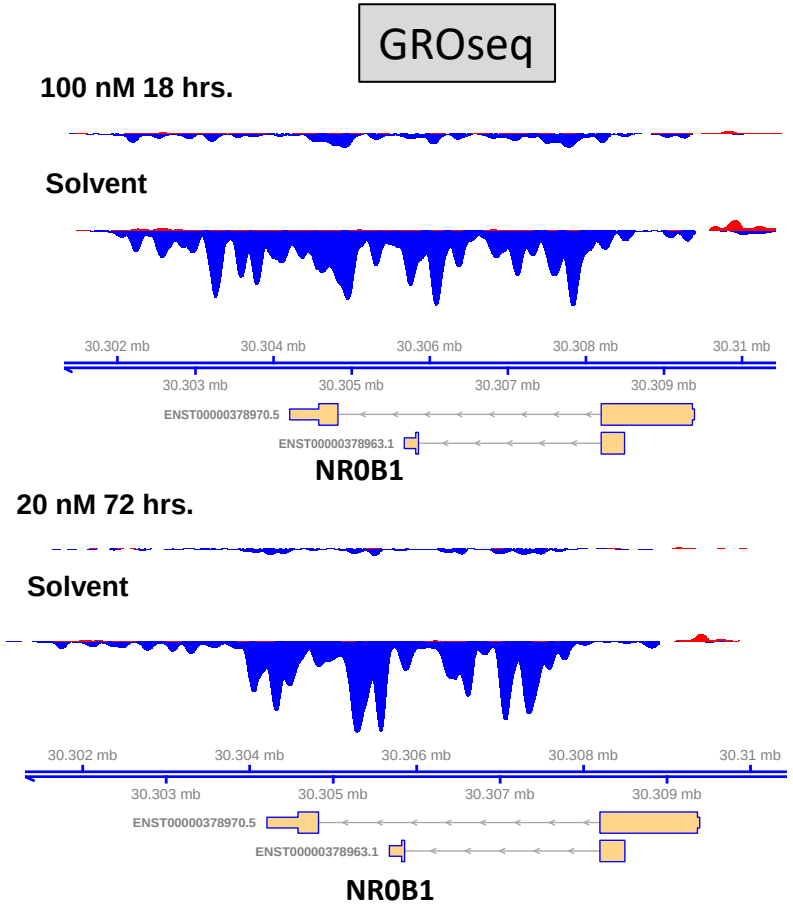
Global Run On (GROseq)



Mithramycin Alters EWS-FLI1 Binding and Blocks EWS-FLI1 Transcription: Definitive Evidence



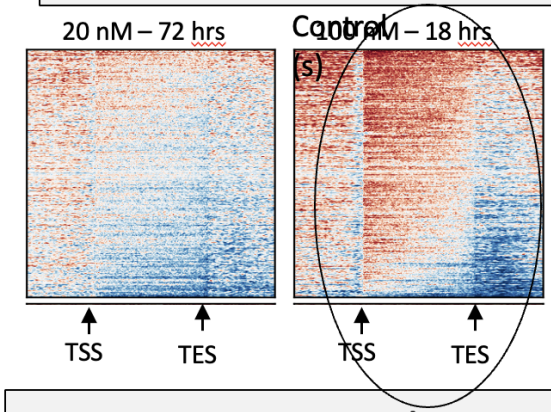
Cut/Tag; Chromatin fractionation not shown



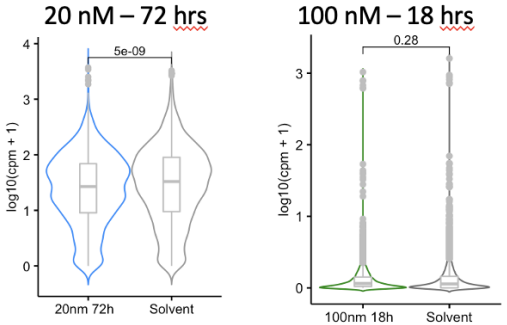
Levine, Kaufman, Flores et al 2024 *in preparation*

Low Dose Continuous More Specific: High Concentrations Impair RNAPII Processivity

EWS-FLI Induced Targets



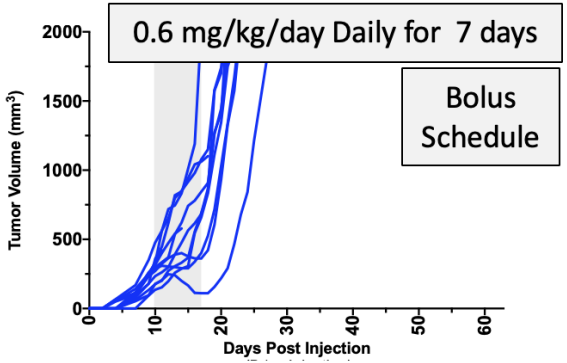
EWS-FLI1 Repressed Targets

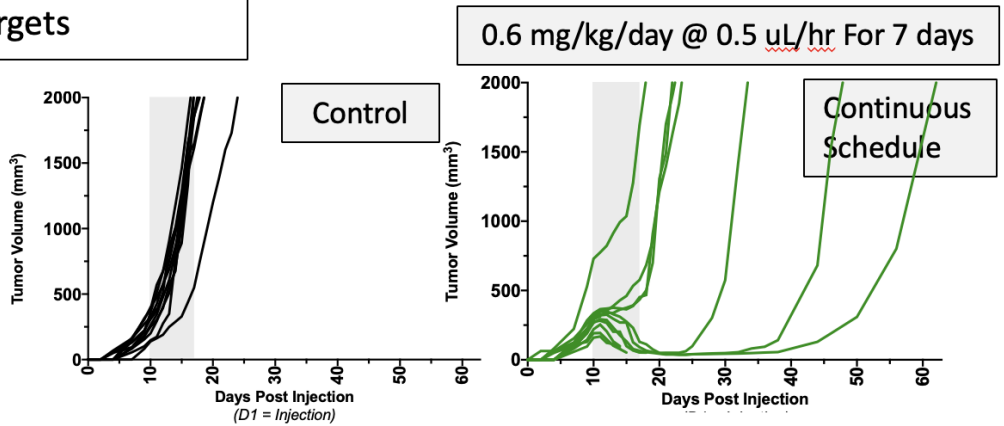


High MMA Concentrations Impair RNAPII processivity

- Less effective repression of EWS-FLI1 **induced targets** (medium and small targets)
- No induction of **EWS-FLI1 repressed** targets

More effective continuously in vivo



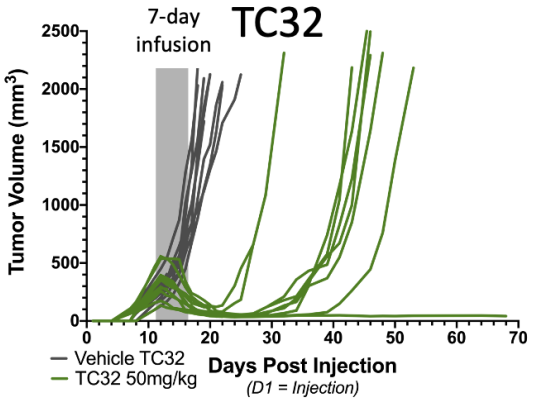


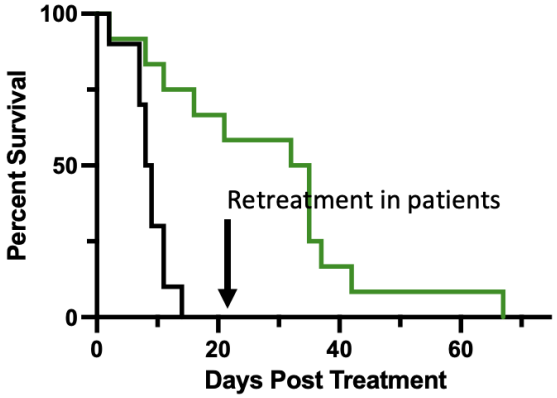
Levine, Kaufman, Flores et al 2024 *in preparation*

How about continuous AIT102 in RT/EWS??.....

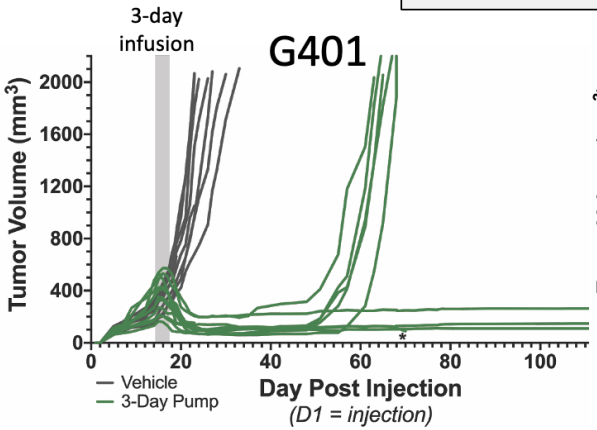
The Right Target, Drug (AIT-102), Mechanism Defined Schedule

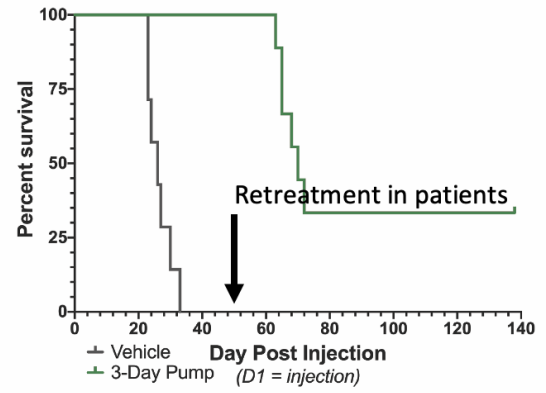
Ewing Sarcoma

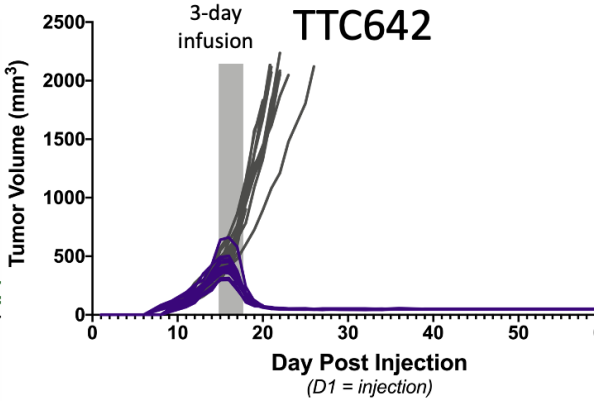


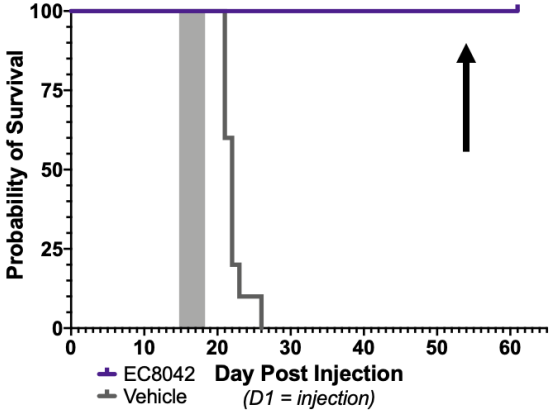


Rhabdoid Tumor

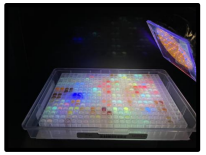


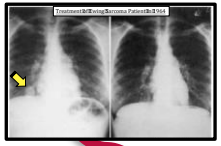


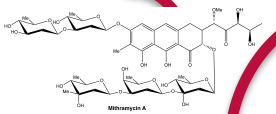




NCI Translating Natural Product Discoveries Toward the Clinic







Cancer Chemother Pharmacol (2017) 80:645–652
DOI 10.1007/s00280-017-3382-x

CLINICAL TRIAL REPORT

A phase I/II trial and pharmacokinetic study of mithramycin in children and adults with refractory Ewing sarcoma and EWS–FLI1 fusion transcript

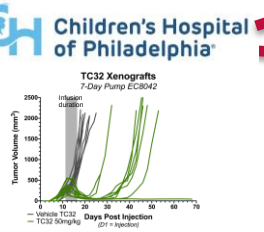
Patrick J. Grohar[1,2] · John Glod[1] · Cody J. Peer[3] · Tristan M. Sissung[2] · Fernanda I. Arnaldez[1] · Lauren Long[1] · William D. Figg[2] · Patricia Whitcomb[1] · Lee J. Helman[1] · Brigitte C. Widemann[1]

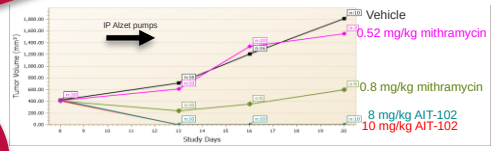
Article

EMBO Molecular Medicine

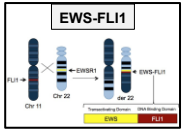
Mithramycin induces promoter reprogramming and differentiation of rhabdoid tumor

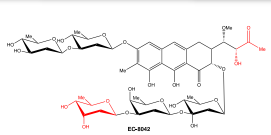
Maggie H Chasse[1], Benjamin K Johnson[1], Elissa A Boguslawski[1], Katie M Sorensen[1], Jessica E Rosien[2], Min H Kang[3], C Patrick Reynolds[3], Lyong Heo[3], Zachary B Madaj[3], Ian Beddows[3], Gabrielle E Foxa[1], Susan M Kitchen-Goosen[3], Bart O Williams[3], Timothy J Triche Jr[3] & Patrick J Grohar[1,4,5,*]

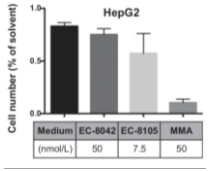


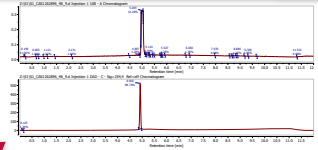


CCR/MTP & POB EWS-FLI-1 Screen







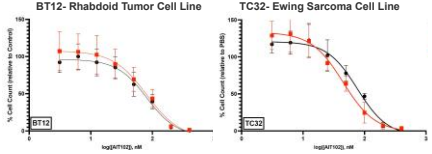


entrechem

NExT Application

Bankruptcy

OrphAI THERAPEUTICS



RED = New AIT-102
Black = Old Batch of AIT-102

Approved NExT Development Project