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MCL-1 INHIBITORS FOR THE TREATMENT OF CANCER

Steve Fesik, Ph.D.
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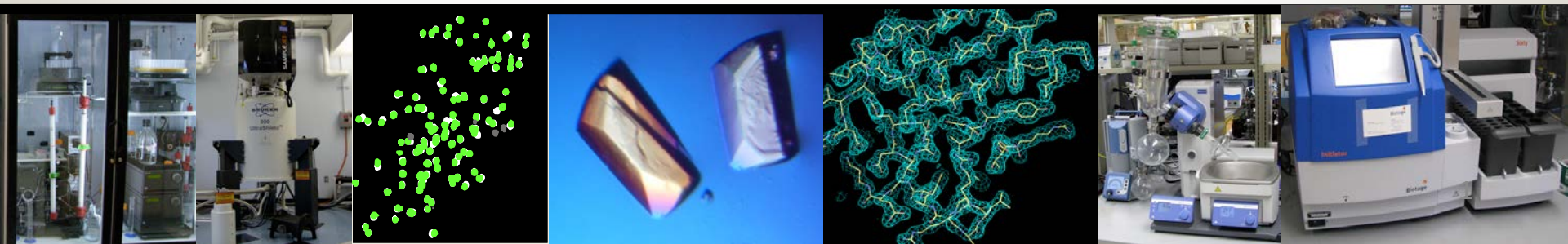
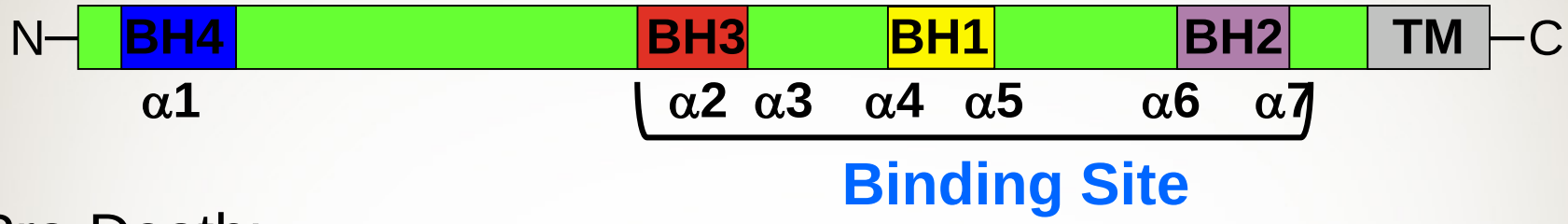
Orrin H. Ingram, II Chair in Cancer Research



Bcl-2 Family Proteins

Pro-Survival: Bcl-2, Bcl-xL, Bcl-w, Mcl-1, A1

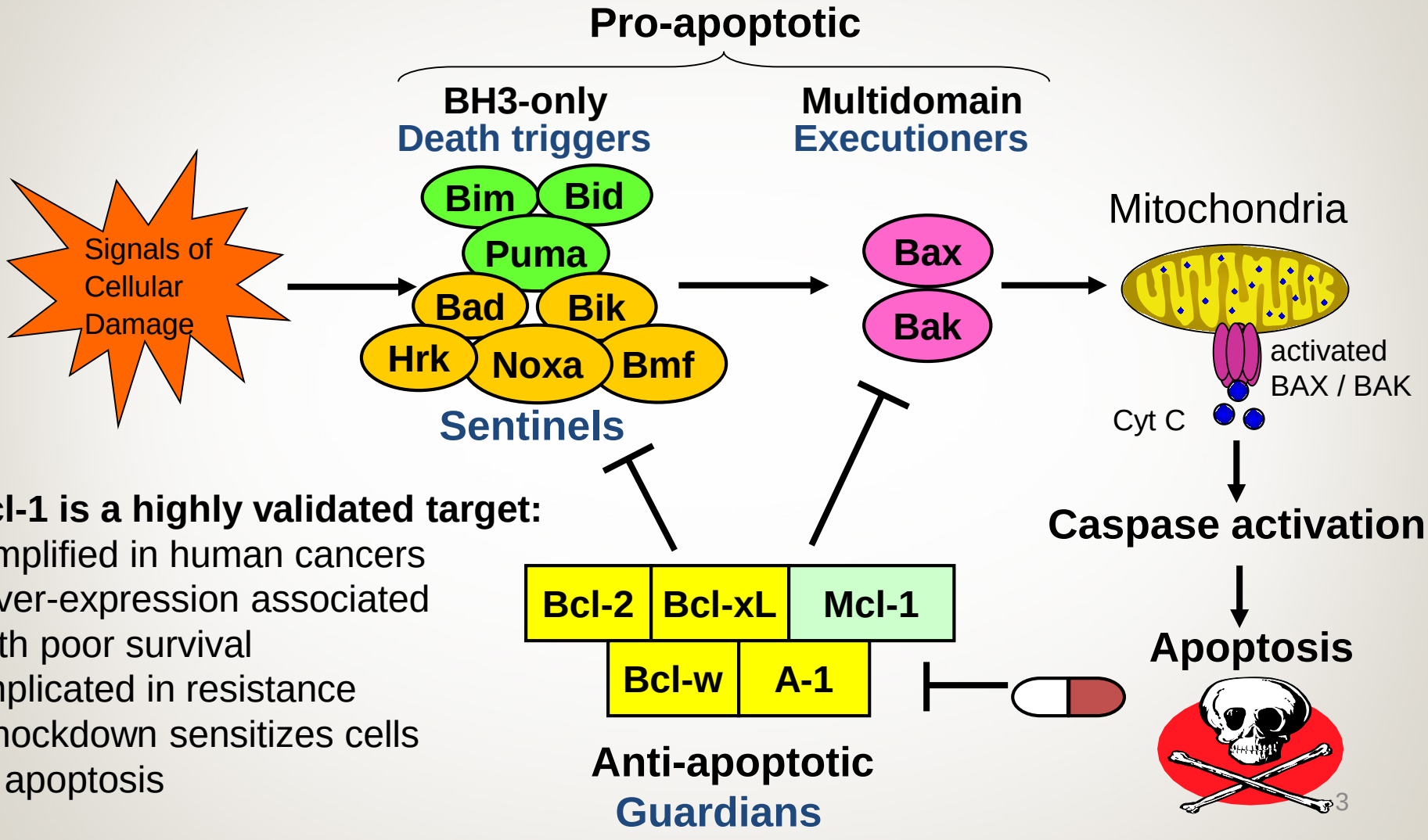


Pro-Death:

Effector (Bax, Bak)

BH3-only (Bid, Bim, Bad, Noxa, Puma, Bik, Hrk, Bmf)

BCL-2 FAMILY PROTEINS REGULATE APOPTOSIS



Mcl-1 is a highly validated target:

-Amplified in human cancers

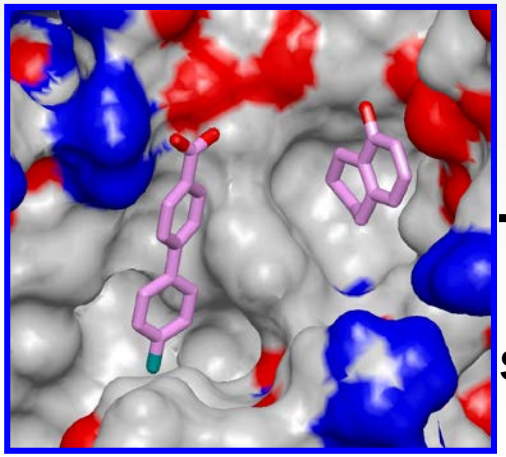
-Over-expression associated with poor survival

-Implicated in resistance

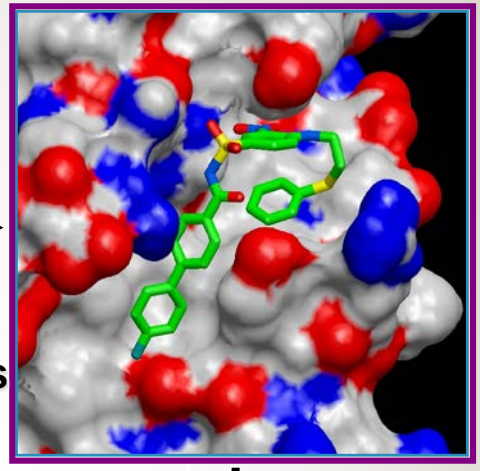
-Knockdown sensitizes cells to apoptosis

Validation of the Approach

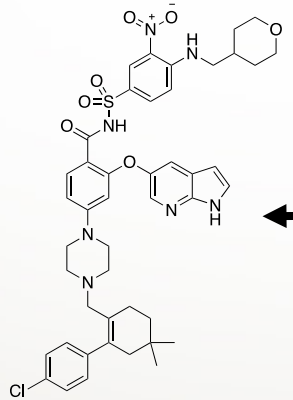
SAR by NMR



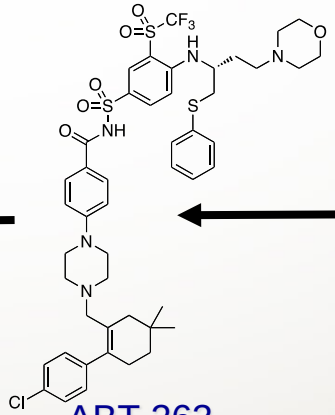
HT Organic Synthesis



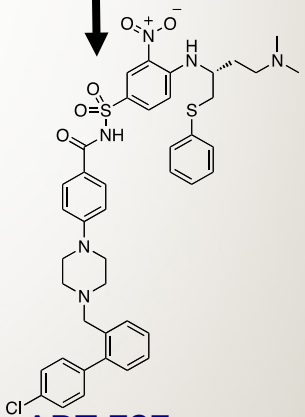
Structure-based design
Med Chem



ABT-199
Oral sel Bcl2



ABT-263
Oral Bcl2/BclxL



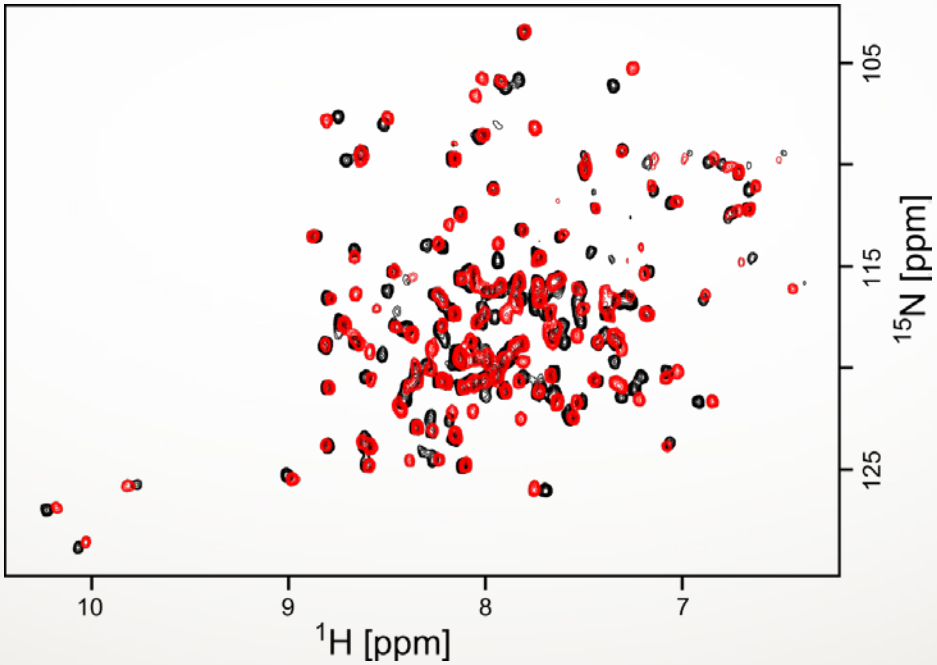
ABT-737
IV Bcl2/BclxL

Shuker et al.,Science 274, 1531 (1996)

Oltersdorf et al.,Nature 435, 677 (2005), Tse et al., Cancer Res 68, 3421 (2008), Souers et al., Nat Med (2013)

Vanderbilt Mcl-1 Program

- Screened proprietary fragment library of ~15,000 compounds
- Identified over 130 fragments that bind (Kd<500 uM) to Mcl-1
- Identified 13 structurally distinctive series



Friberg et al., J Med Chem 56, 15 (2013)

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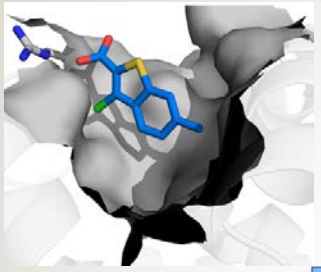
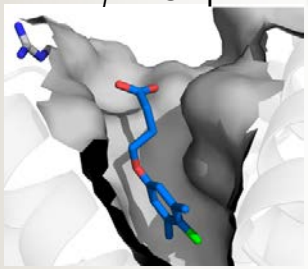
Medicinal Chemistry Milestones

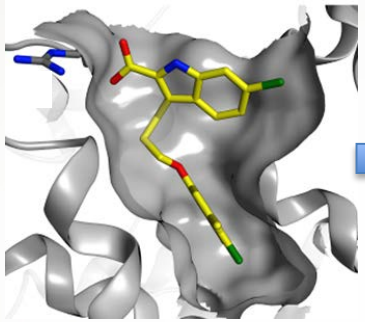
Hit to Lead | **Lead Optimization**



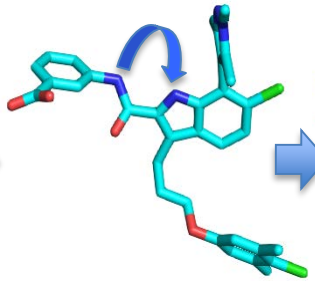
K_i = 131 μM



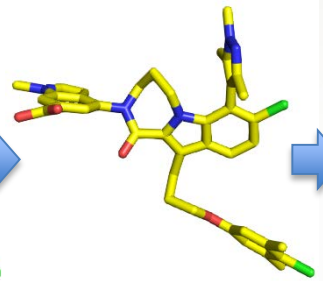
K_i = 60 μM



Mcl-1 K_i = 55 nM

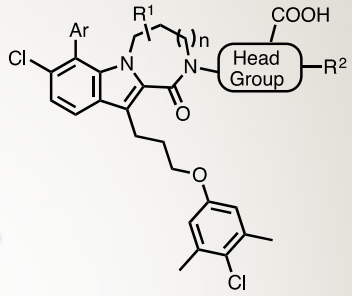


Mcl-1 K_i = 23 nM



Mcl-1 K_i = 0.34 nM
H929 GI_{50} = 1.5 μM

Lead Compound (9/15)



VU0661013

VU0661013

- Mcl-1 K_i = 0.13 nM (TR-FRET)
- Selective cellular activity
  - H929 GI_{50} = 250 nM (Mcl-1 Sensitive line)
  - AMO-1 GI_{50} = 140 nM (Mcl-1 Sensitive line)
  - K562 GI_{50} > 12.5 μM (Mcl-1 insensitive line)
- Target-based on-mechanism activity
  - Caspase activation, JC-1/BH3 profiling, co-IP, multiplex PD apoptosis assays

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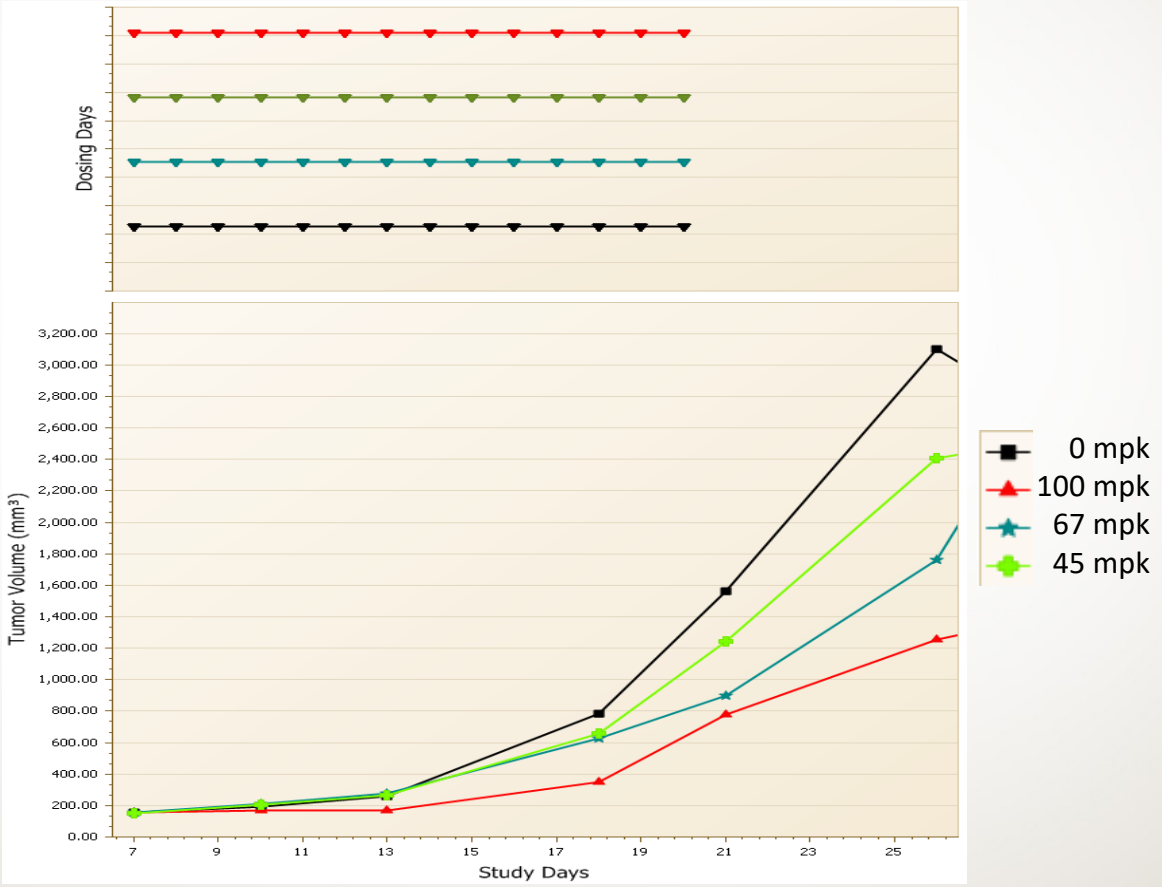
Dose dependent Inhibition of AMO1 Tumor Growth *in vivo*

QD dosing by IP for 14 days



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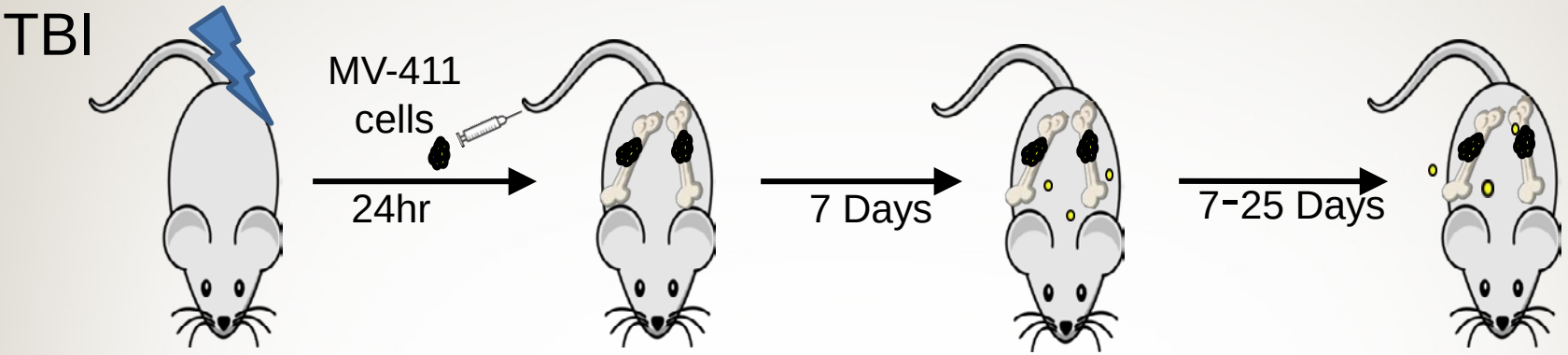
VU013 in human transgenic mouse models of heme malignancies



NSGS
(express hSCF, hGM-CSF, hIL-3)

Day 7
- 10mg/kg (n=5)
- 25 mg/kg (n=5)
- 75 mg/kg (n=5)
- Vehicle (n=5)

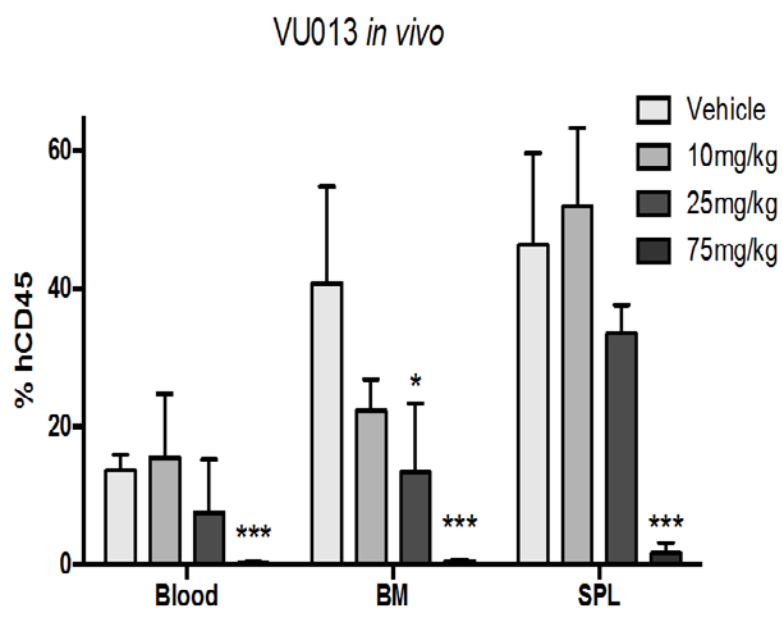
Daily i.p.

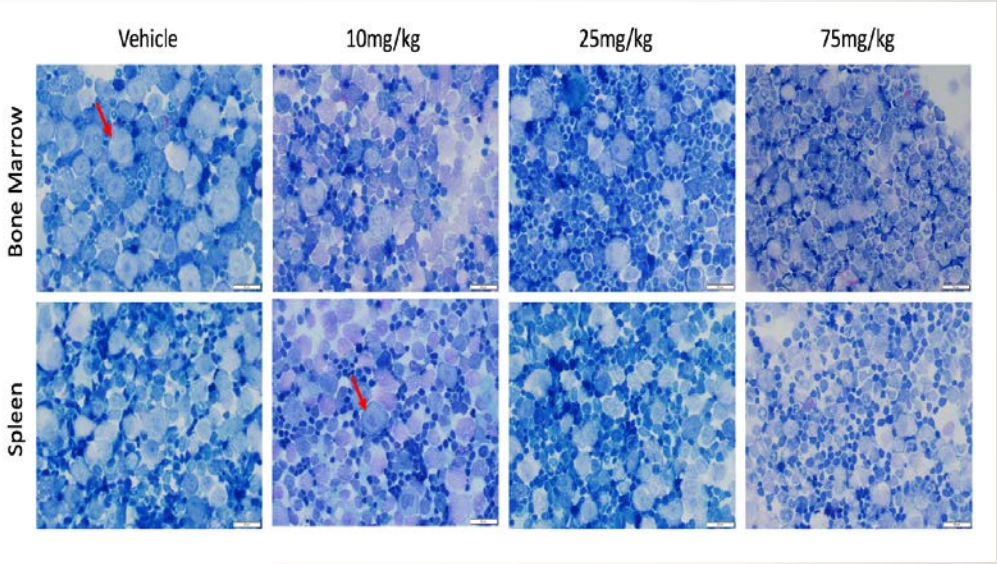
Weekly flow cytometric detection of hCD45 chimerism until vehicle group moribund at Day ~25

Advantages:

- Systemic model that mimics the human disease
- Homing of cells to the bone marrow

MCL-1 Inhibition significantly inhibits the *in vivo* expansion of leukemia cells





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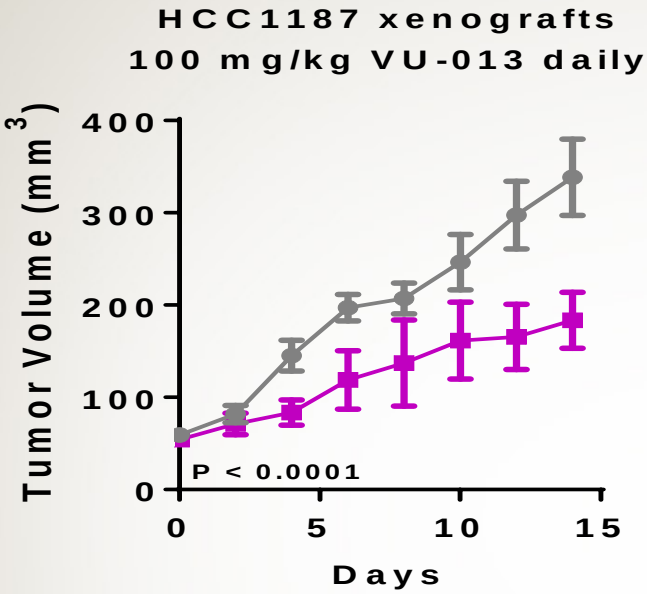
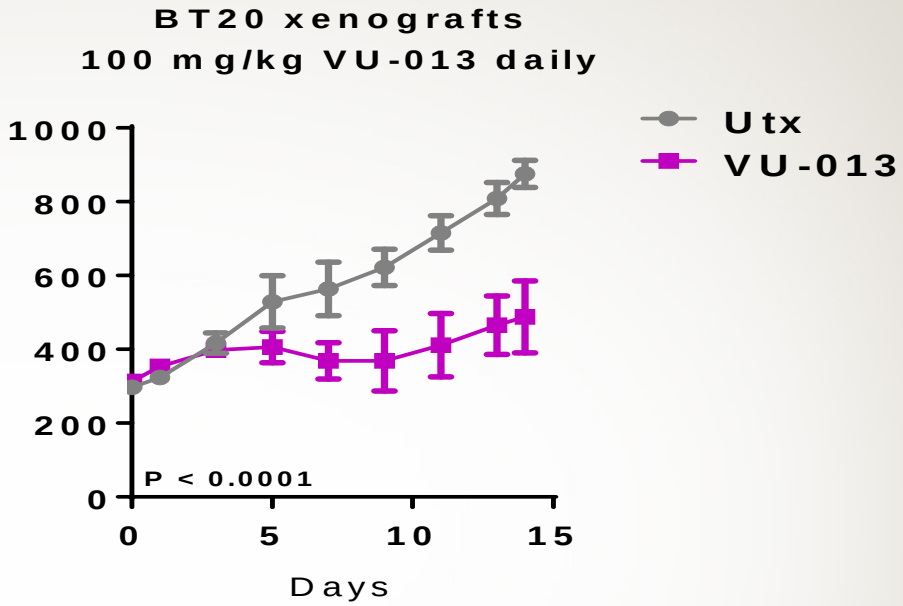
VU661013 Decreases Tumor Growth

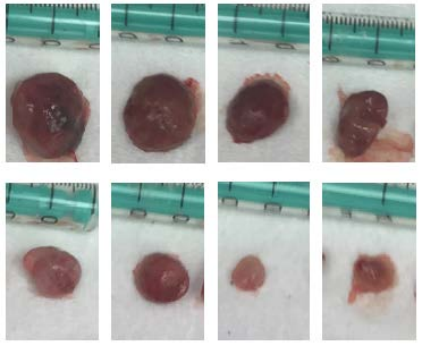


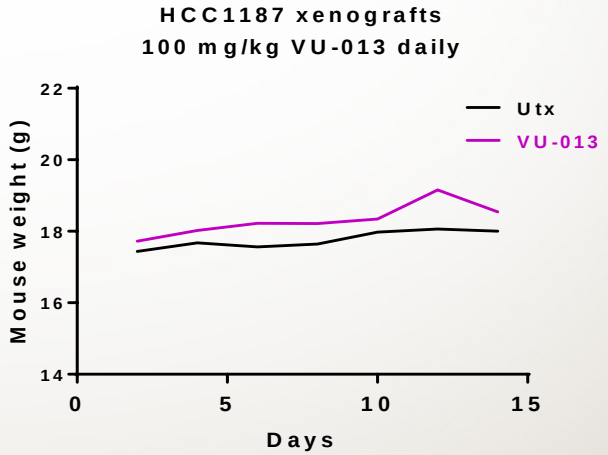


HCC1187 treated14 days

Vehicle
N = 4

VU-013
N = 6





No effect in body mass of *nu/nu* mice

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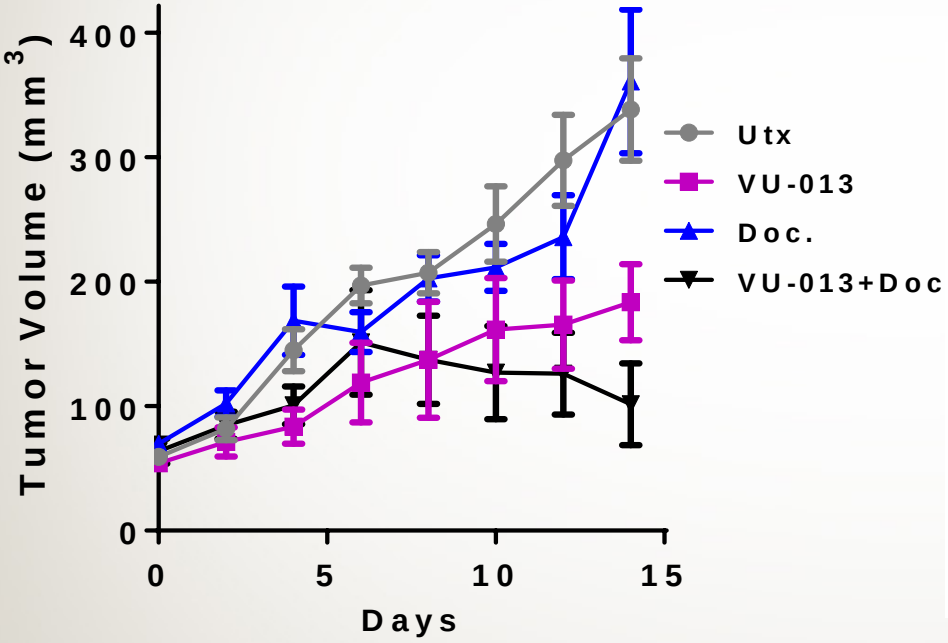
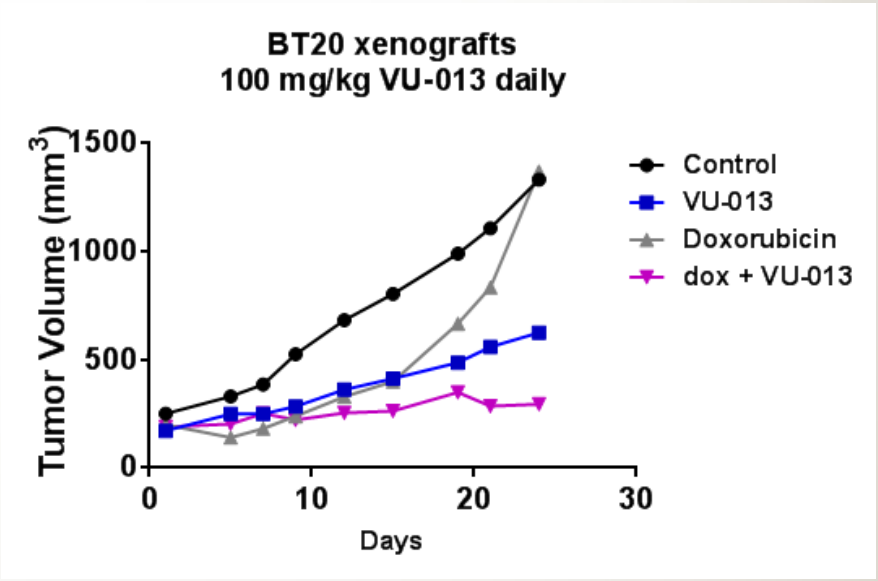
VU013 Cooperates with Docetaxel to Decrease TNBC Growth

HCC1187 xenografts
100 mg/kg VU-013 daily
2 mg/kg docetaxel once weekly





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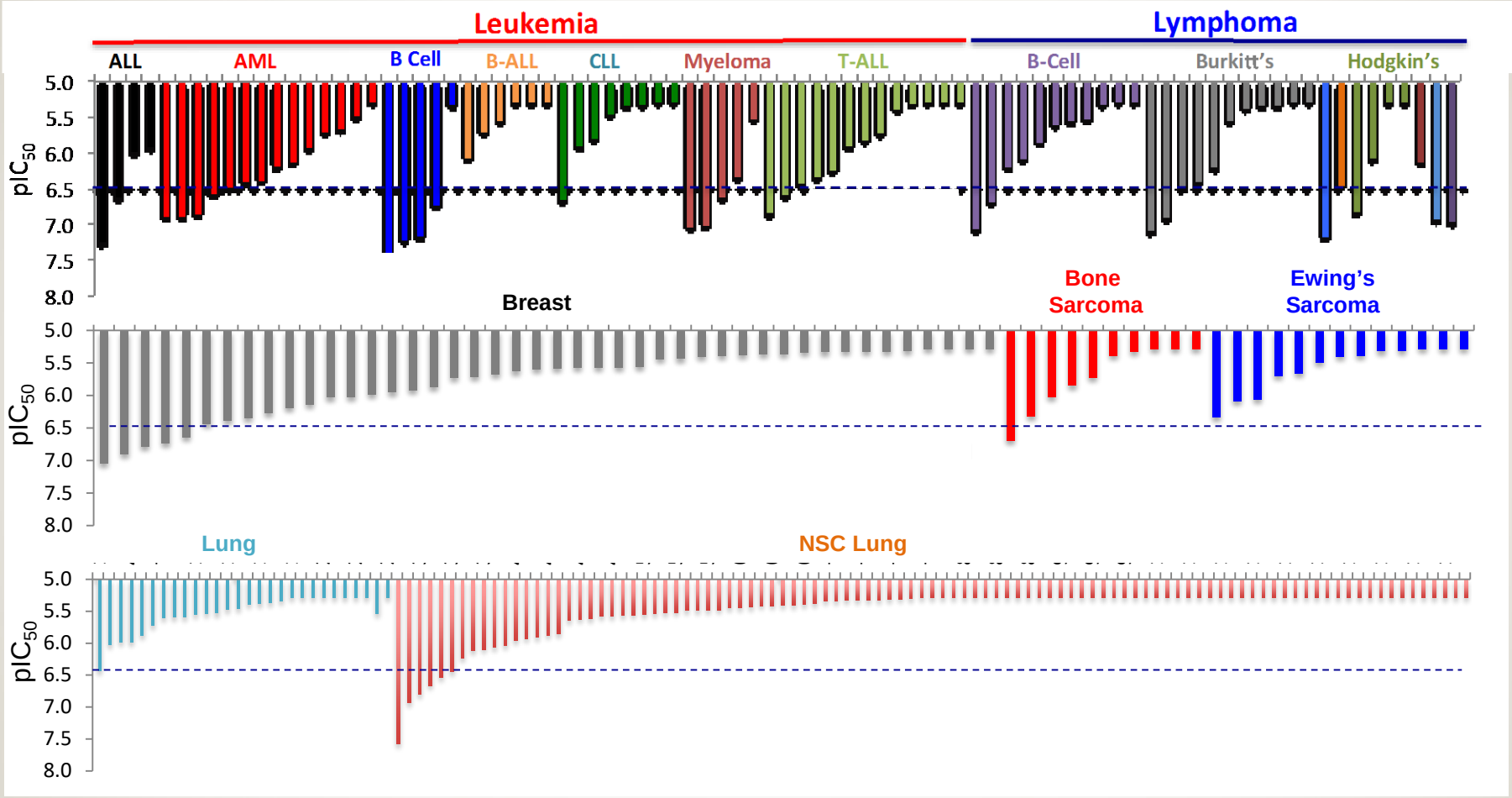
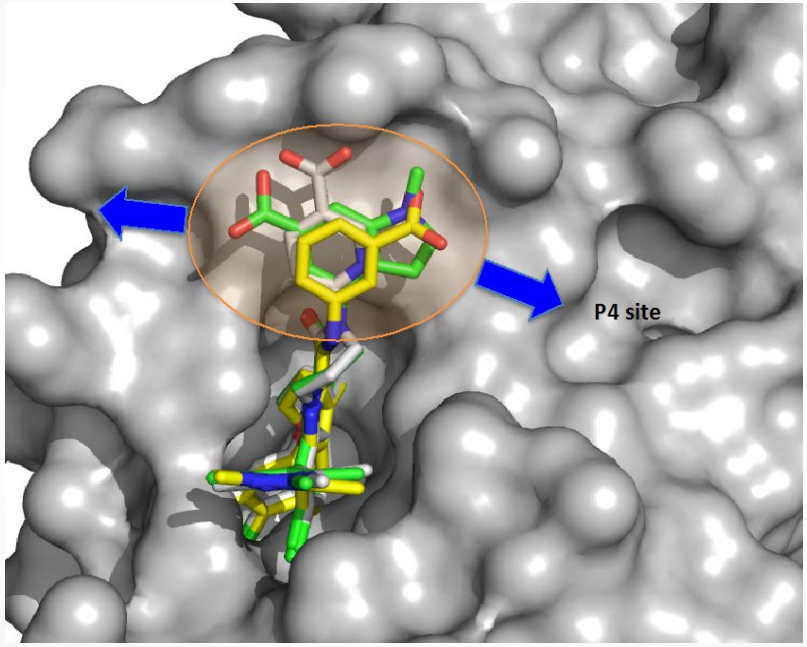
Cancer Cell Lines Screening Using VU013



- >740 cancer cell lines screened at **MGH**, NCI-60, and VU using VU013 as a single agent
- VU013 exhibits the highest potency against hematologic malignancies.
- High activity also observed in breast and NSC lung cancers.

Current Chemistry Strategies

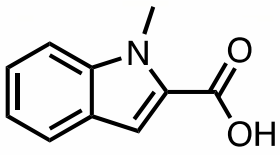


Binding Affinity Enhancement from Fragment Indole Hit

Current best binders

Compound	K_i (pM)
810930	10 ± 8
812326	35 ± 8
814119	13 ± 3
814148	41 ± 6

n = 6



K_i = 160 µM
LE = 0.40

16 million fold ↑ affinity

810930

K_i = 10 pM
LE = 0.28

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Current Best in COOH Series Mcl-1 Inhibitors

NSC ID	786006	791717	791406	791749	795579	796296	797045	797385	798601	798717	799602
VU ID	661013	811446	810998	811526	817411	817431	817530	822387	823802	823898	824092
FRET Bak K_i (nM)	0.13	0.075	0.049	0.079	0.050	0.082	0.021	0.041	0.064	0.051	0.094
FRET + 1% FBS (nM)	0.40	0.19	0.15	0.25	0.14	0.13	0.039	0.078	0.077	0.10	0.14
H929 Prol. GI_{50} (µM)	0.25	0.14	0.15	0.17	0.11	0.030	0.10	0.058	0.048	0.054	0.059
H929 013 index[1]		**2.1**	**1.9**	**1.0**	**2.7**	**5.0**	**3.4**	**3.5**	**4.6**	**3.3**	**2.4**
AMO1 Prol. GI_{50} (µM)	0.15	0.039	0.037	0.063	0.058	0.027	0.094	0.035	0.039	0.046	0.065
AMO1 013 index[1]		**4.3**	**4.5**	**2.7**	**2.9**	**4.2**	**2.3**	**2.3**	**3.4**	**3.1**	**1.7**
K562 Prol GI_{50} (µM)	>12	>12	>12	**>12**	**>12**	**>9.5**	**>12**	**>9.9**	**5.5**	**>7.7**	**6.1**
PD Effect	low	high	medium	high	high	high	high	high	In que.	In que.	
Rat PK IP (@3mpk)											
CL (F) (ml/min/Kg)	34	51	19		27	36	57	61	37		
C_{max} (nM)	626	227	501		510	391	187	221	380		
$T_{1/2}$ (h)	3.0	3.6	1.8		2.8	2.9	3.5	2.9	3.1		
MRT (h)	3.5	3.9	4.3		3.5	3.8	4.5	3.7	3.7		
T_{max} (min)	10	30	60		45	20	60	30	45		
AUC (nM*h)	2041	1269	3334		2430	1799	1080	1061	1758		

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Emerging Sub-Classes of Mcl-1 Inhibitors

	COOH	Zwitterlonic		Neutral		Basic	
NCI ID	786006	794614	795901	790603	794429	795339	793242
VU ID	661013	810584	814131	810499	816542	816794	813798
FRET Bak K_i (nM)	0.13	0.059	0.073	0.69	0.36	0.44	0.58
FRET + 1% FBS (nM)	0.44	0.16	0.11	0.95	0.49	0.34	0.50
H929 Prol. GI_{50} (µM)	0.30	0.19	0.10	0.15	0.25	0.11	0.20
H929 013 index[1]		**1.5**	**2.4**	**1.2**	**1.0**	**2.5**	**1.2**
AMO1 Prol. GI_{50} (µM)	0.14	0.10	0.11	0.15	0.15	0.090	0.13
AMO1 013 index[1]		**1.6**	**1.6**	**1.0**	**1.1**	**1.8**	**1.1**
K562 Prol GI_{50} (µM)	>10	**>12**	**>11**	>12	**>12**	**>12**	**8.7**
PD Effect		high	medium	low	low	low	Low-med.
Rat PK IP (@3mpk)							
CL (F) (ml/min/Kg)	34	48	70	35			15
C_{max} (nM)	626	306	192	111			335
$T_{1/2}$ (h)	3.0	2.9	3.4	8.3			5.6
MRT (h)	3.5	3.6	4.2	11			7.3
T_{max} (min)	10	30	60	60			120
AUC (nM*h)	2041	1296	951	1927			4055

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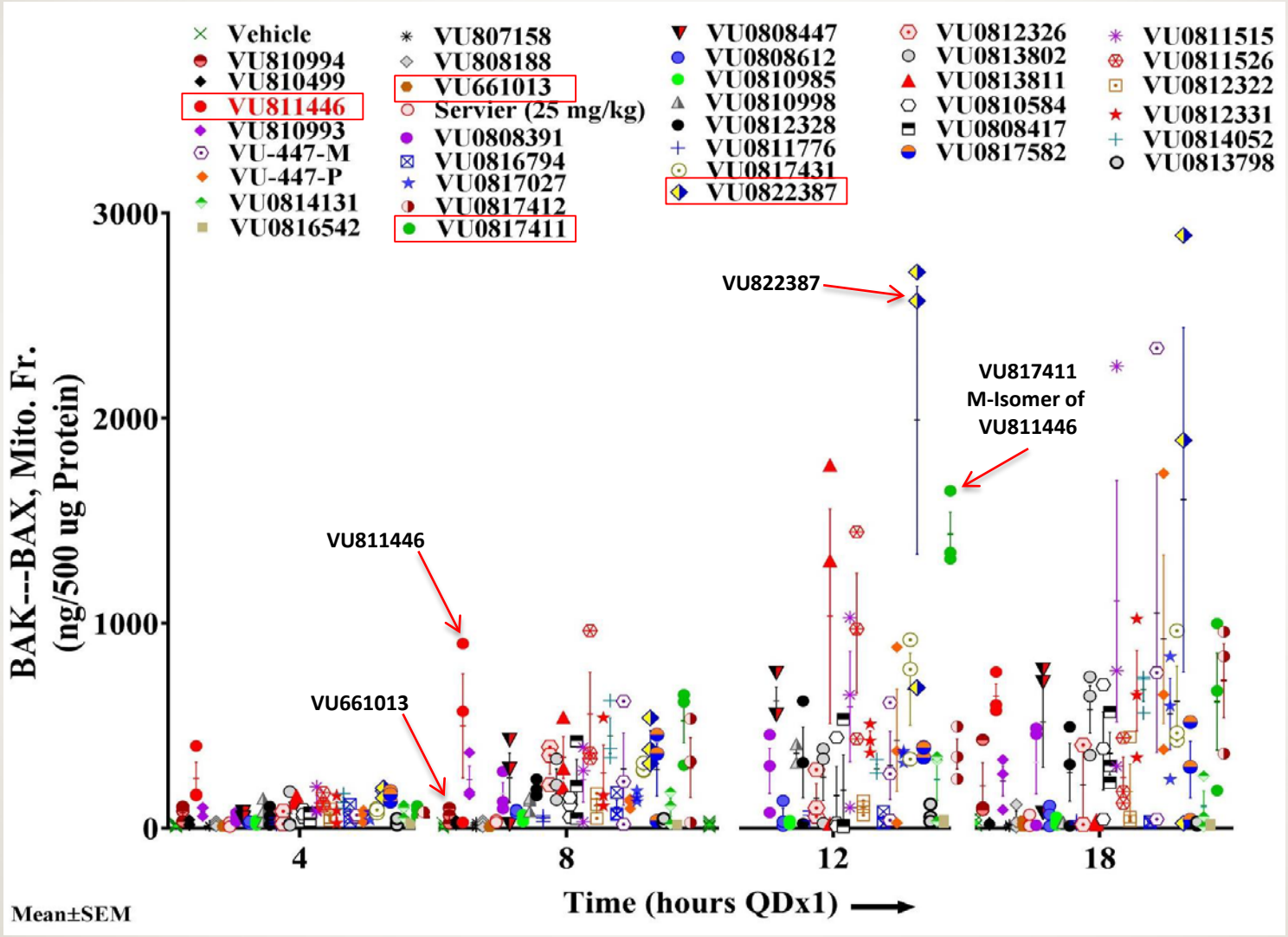
PK/PD Time Course Studies



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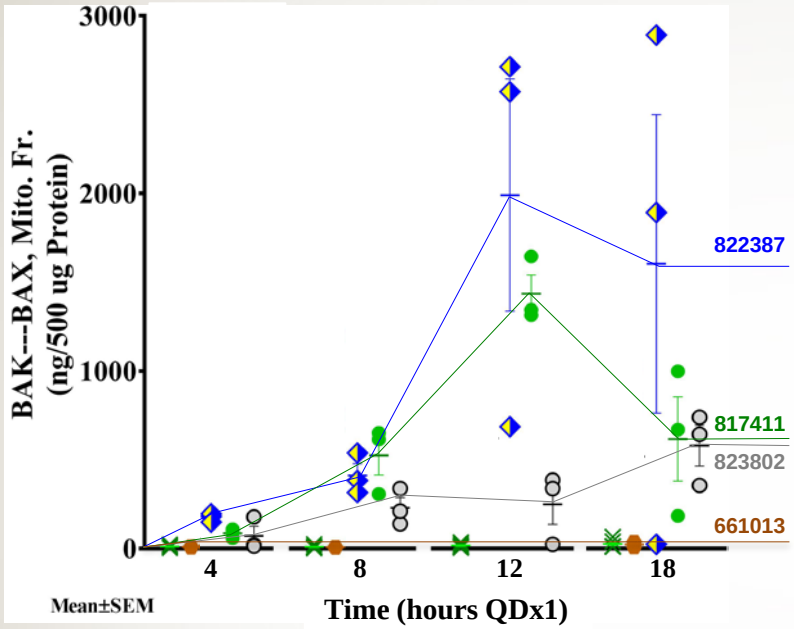
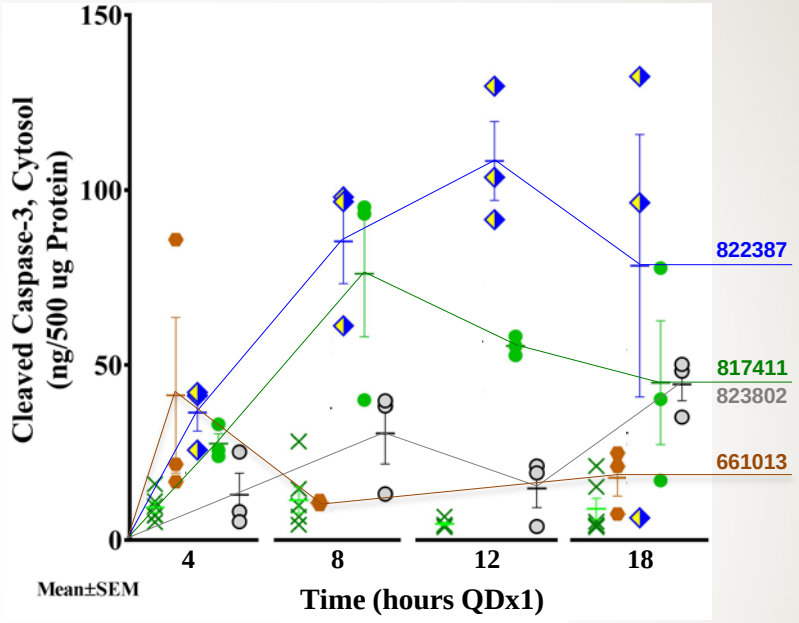
PK/PD Time Course Studies

BAK-BAX Heterodimer Levels



Cleaved Caspase-3 Levels



- Primary PK/PD screen
- Single IP dose @ 100 mg/kg in AMO-1 xenograft mouse model
- Plasma/tumor exposure & biomarker levels determined at 4 time points
- No BAK-BAX heterodimer response for VU661013

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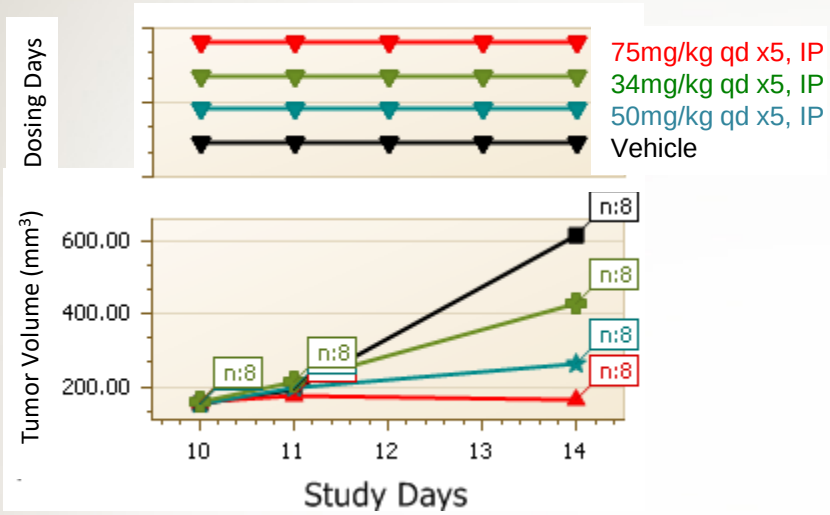
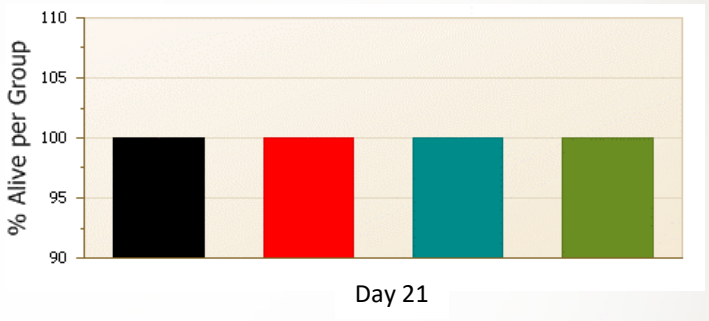
Multi-Dose *In Vivo* Tumor Regression Study of VU817411

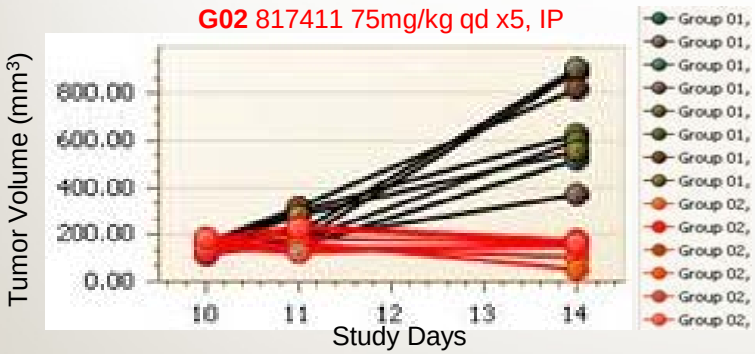
Average (Day 10-14)



Survival Rate



Individual Data



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Summary of Progress

Medicinal Chemistry (VU, NCGC)

- Filed 5 patents for 4 Series
- > 2475 New compounds synthesized

Structural Biology (VU)

- 64 X-ray co-crystal structures solved

Cell Biology and Assay Development (VU)

- FPA Binding assays: Bim6 (+ 10% FBS), TR-FRET (Bak, +1% FBS)
- Proliferation assay in Mcl-1 sensitive (H929, AMO-1, OPM2 etc.) and insensitive (K562) cell lines
- On-mechanism activity assays *(Caspase 3/7 Glo, JC-1, Mcl-1::Noxa, Bim IP)*
- *In vivo* efficacy models: H929, AMO-1 (MM), HCC1187, BT20 (TNBC) Xenograft, MV411(AML) Primagraft

DMPK & Animal Efficacy Model (VU, DCTD/DTP/BTB/PADIS)

- 440 compounds tested in eADME Screen (NCGC)
- PK studies conducted: 22 Compounds in CD-1 mouse (NCI); 102 compounds in rat (VU)
- *In vivo* efficacy studies conducted
- Discovered patient selection biomarkers and PD biomarkers

Animal Safety Assessment Model (DCTD/DTP/TPB)

- *In vitro* cardiac myocytes assay
- Multiple dosing MTD studies

Goal: Select clinical candidate ASAP

- IV once per week, oral agent QD as backup

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Acknowledgements

- Current Fesik Lab Members

Medicinal Chemistry
Taekyu Lee
Alex Waterson
Shaun Stauffer
Rocco Gogliotti
James Salovich
Changho Han
J. Chris Tarr
Jason Abbott
KyuOk Jeon
Jonathan Macdonald
Subrata Shaw
Naga Veerasamy

Cell / Cancer Biology
Jiqing Sai
John Sensintaffar
Tammy Sobolik
Joannes Yuh
Allison Arnold
Jennifer Howes

Structural Biology
Ed Olejniczak
Jason Phan
Feng Wang
Bin Zhao
Qi Sun
Evan Perry

- Former Fesik Lab Members

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- Collaborators

Joe Opferman, Tony Letai, Larry Boise
Gary Sulikowski, Kwangho Kim, Plamen Christov
Michael Savona, Haley Ramsey, Melissa Fischer
Carlos Arteaga, Rebecca Cook
Pierre Massion, Jonathan Lehman

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Funding

- Vanderbilt University
- GO grant, PI Larry Marnett (Inhibitors of TNBC targets)
- Pioneer Award (Drug Discovery for challenging targets)
- Lustgarten Foundation (Pancreatic cancer drug discovery)
- Lustgarten Investigator Award (K-Ras inhibitors)
- **NExT Program (Mcl-1 Inhibitors)**
- NExT Program (MLL Inhibitors)
- RO1 (RPA inhibitors)
- Career Development Award/Lung SPORE
- Breast Cancer SPORE (Mcl-1 Inhibitors)
- GI Cancer SPORE (K-Ras Inhibitors)
- BI collaboration (K-Ras inhibitors)
- Rittenberg, Kleberg, James Family, Martell & Baggett Foundations
- Other: postdoctoral fellowships, training grants, etc