

Guiding leukemia therapy with mitochondria

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Dana-Farber Cancer Institute
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Disclosure Information

AACR Special Conference on Hematologic Malignancies: Translating Discoveries to Novel Therapies

Anthony Letai

I have the following financial relationships to disclose:

Consultant for: AbbVie, Tetralogic

- and -

I will discuss the following off label use and/or investigational use in my presentation:

ABT-199 in CLL and AML

Preview

- Why conventional chemo works and why we should care.
- Assigning BCL-2 inhibitors.
- Overcoming resistance by superior selection of targeted therapies.

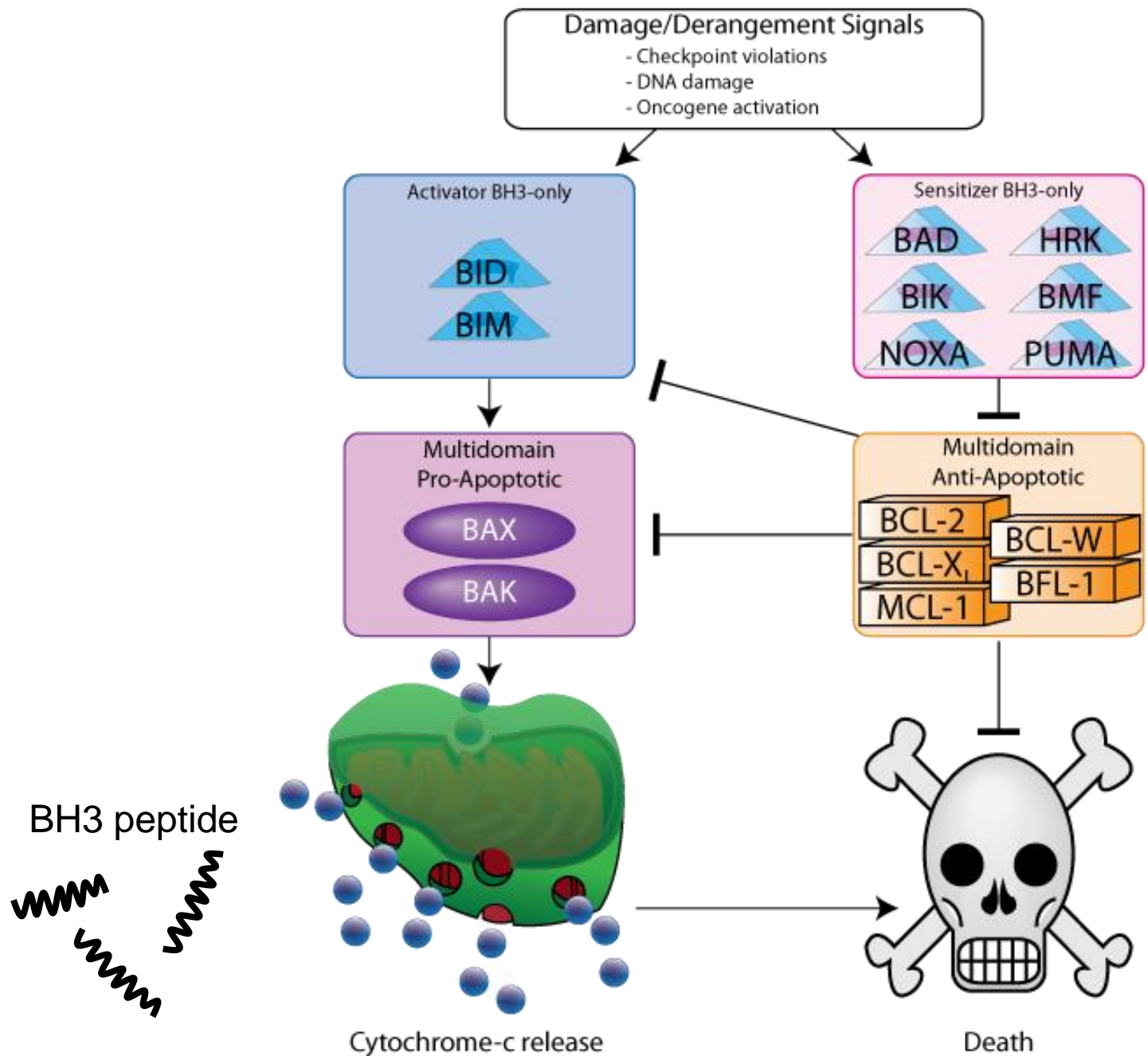
We should care a lot about conventional chemotherapy

Cures by conventional chemo =
Liters of beer drunk in
Germany in a year



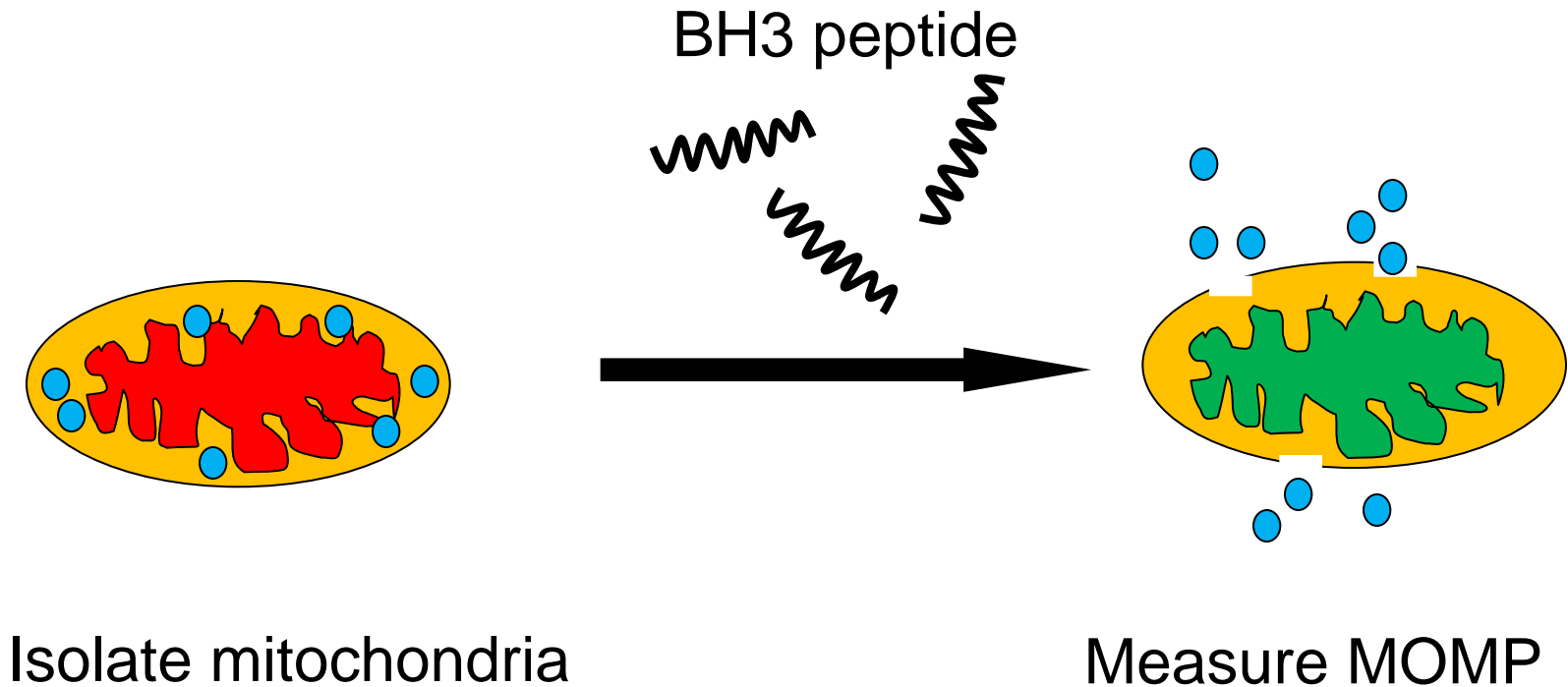
Cures by novel targeted therapies =
Liters of beer drunk in
Antarctica in a year





Certo et al,
Cancer Cell 2006

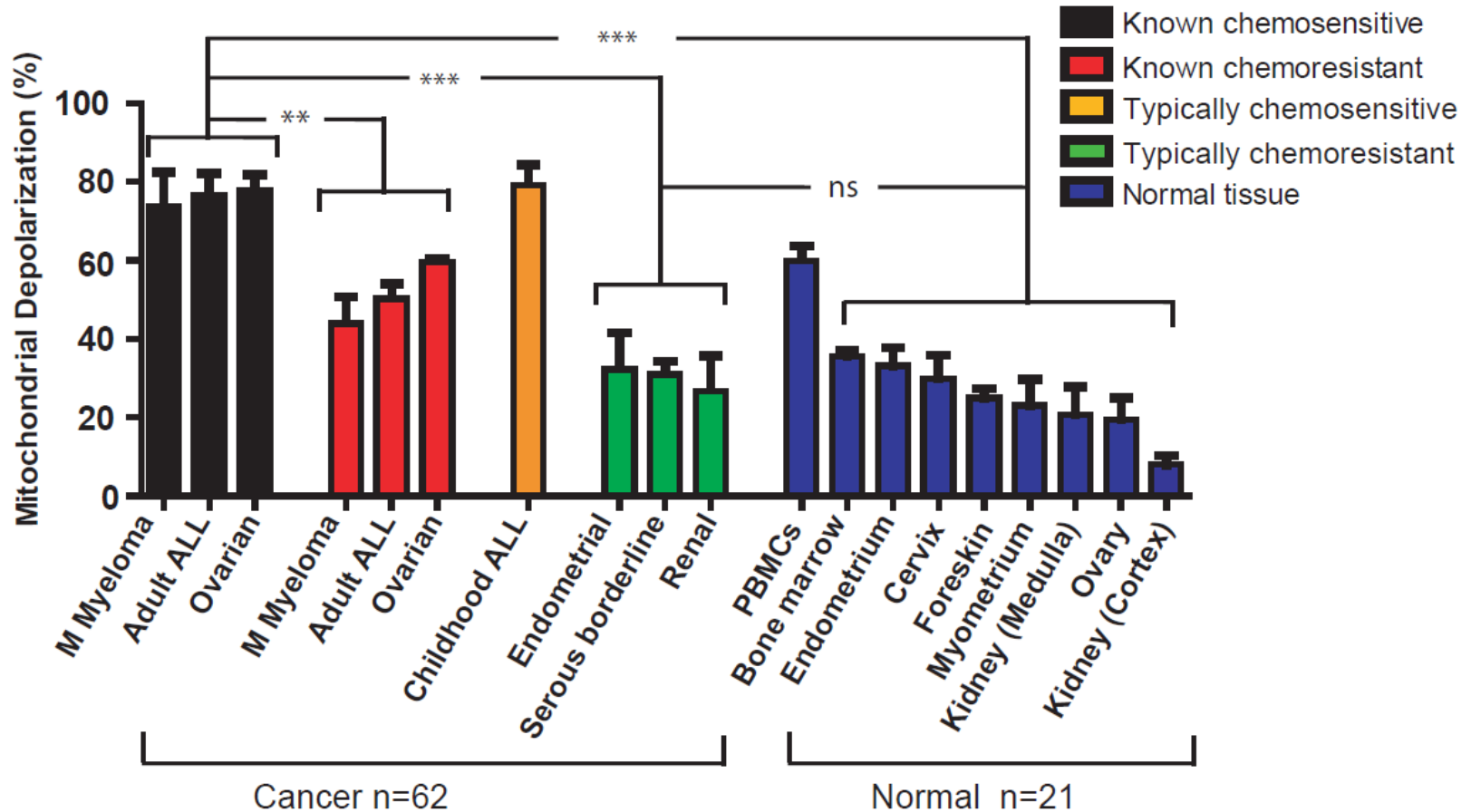
A functional test of readiness to undergo apoptosis: BH3 profiling



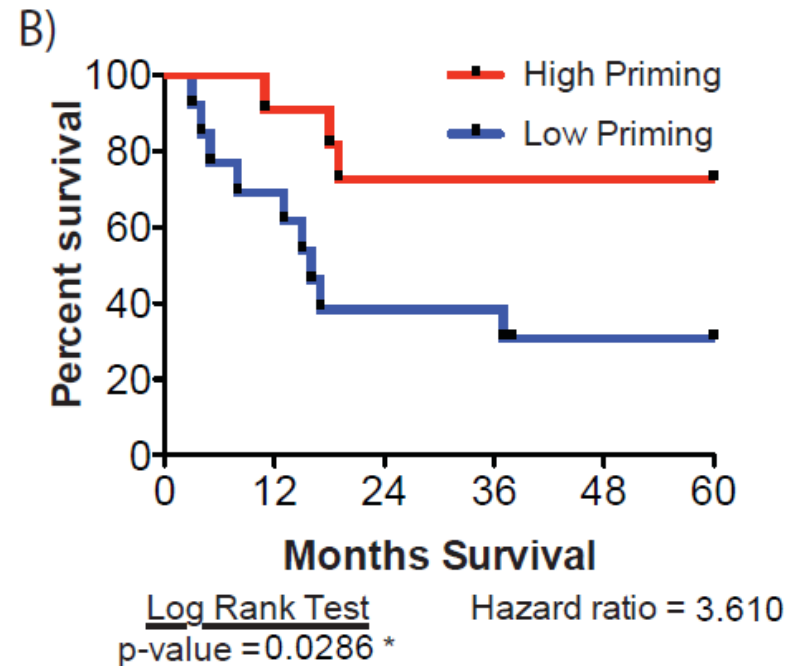
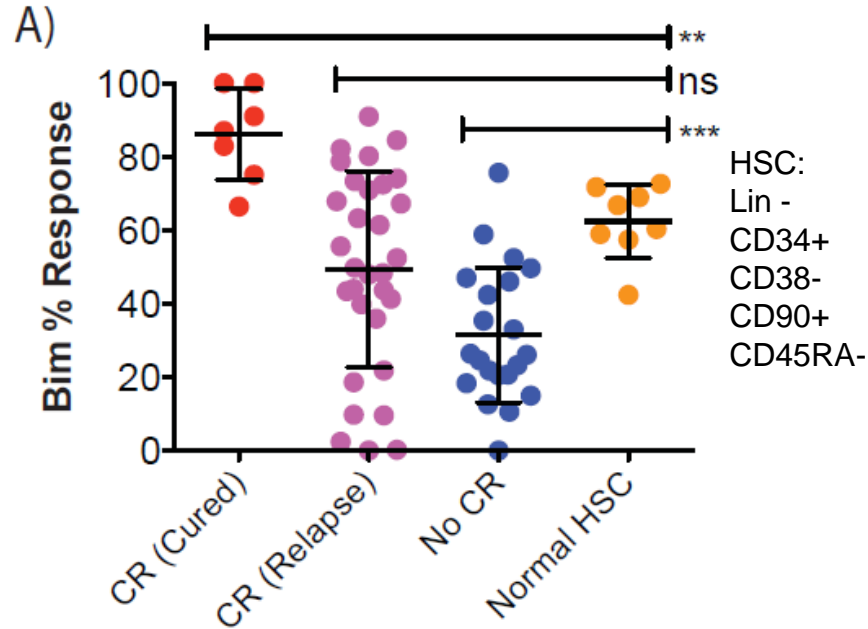
Promiscuous peptides measure overall priming

	Promiscuous			Selective		
	Bim	Bid	Puma	Bad	Noxa	Hrk
Bcl2						
BclXL						
Bclw						
Mcl1						
Bfl1						

Why chemo works - differential priming



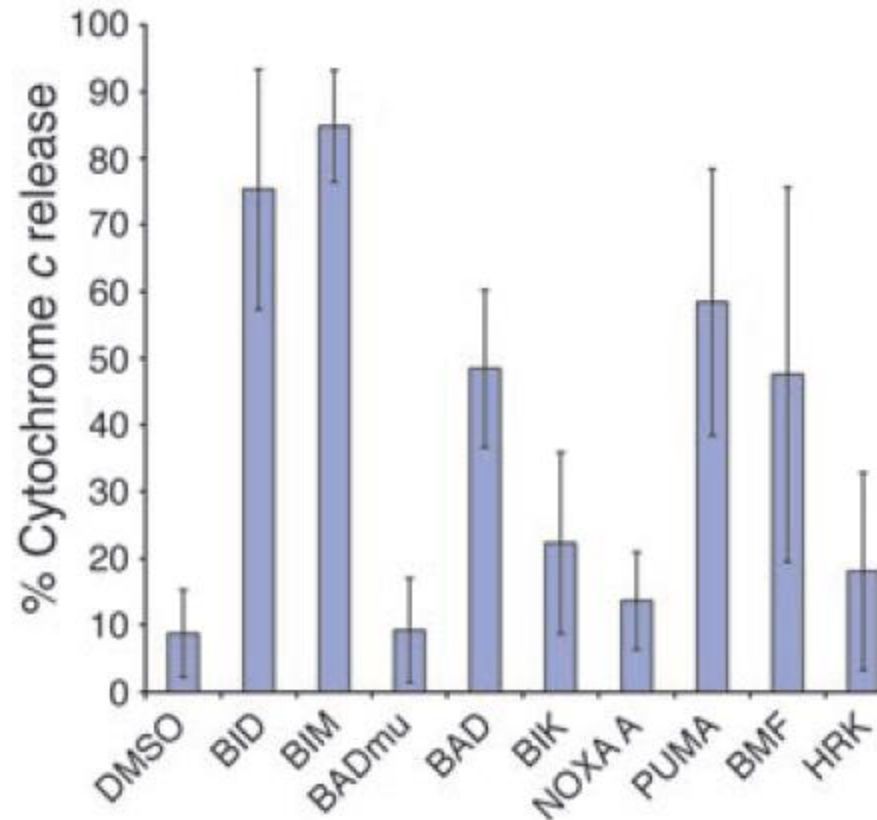
Priming sets therapeutic index in AML



Selective peptides measure dependence on individual anti-apoptotic proteins

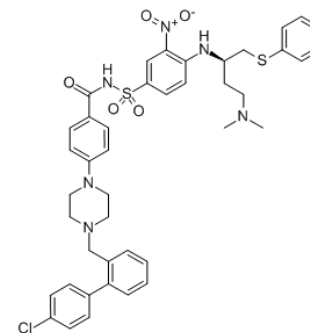
	Promiscuous			Selective		
	Bim	Bid	Puma	Bad	Noxa	Hrk
Bcl2						
BclXL						
Bclw						
Mcl1						
Bfl1						

CLL is a BCL-2 dependent disease

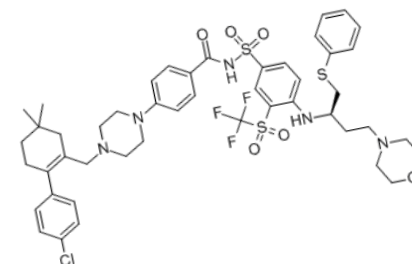


The Abbott drugs

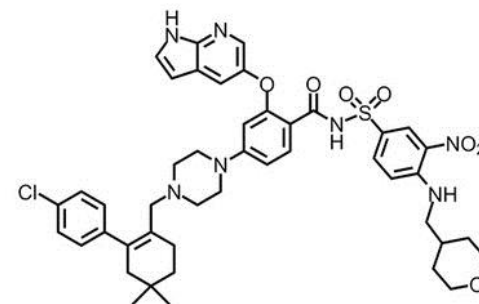
• **ABT-737.** Tool. BCL-2, BCL-XL, BCL-w
Thrombocytopenia.



• **ABT-263.** In vivo. BCL-2, BCL-XL, BCL-w.
Thrombocytopenia.
Oral drug.



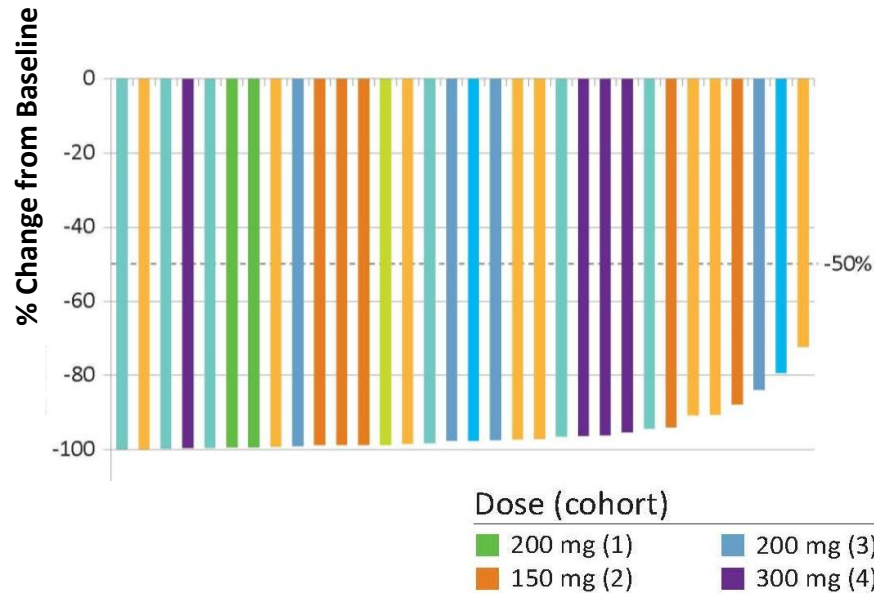
• **ABT-199.** In vivo. BCL-2.
No thrombocytopenia.
Oral drug.



Best Percent Change from Baseline in Lymphocyte Count and Bone Marrow Infiltrate

Lymphocyte Count

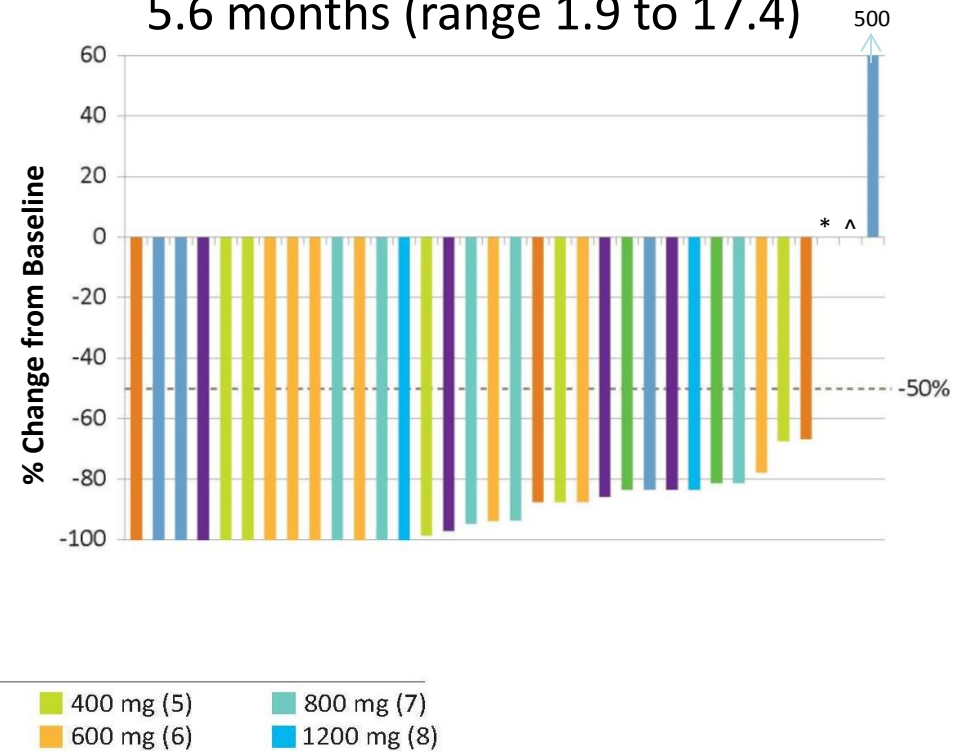
Median Time to 50% Reduction:
12 days (range 1 to 43)



Data represents patients with lymphocyte count $>5 \times 10^9/L$ at baseline.
N = 32 evaluable

Bone Marrow Infiltrate

Median Time to 50% Reduction:
5.6 months (range 1.9 to 17.4)



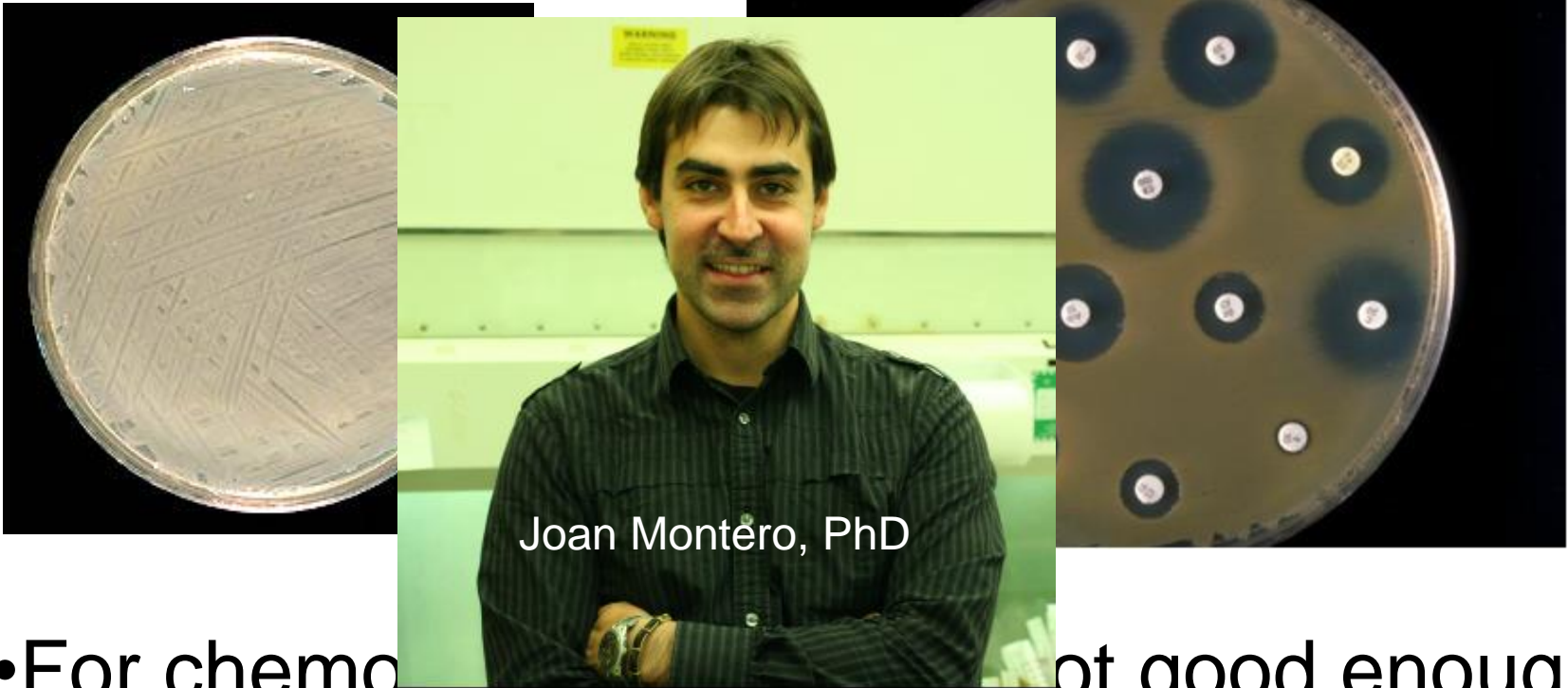
*Patient had 70% infiltrate at baseline and at Week 24.
^Patient did not have CLL infiltrate at baseline.
N = 34 evaluable

Anti-tumor activity of ABT-199 was observed in all tumor compartments.

Other BCL-2 dependent blood cancers identified by BH3 profiling

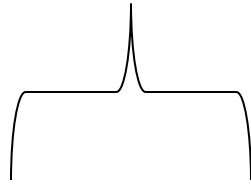
- B-ALL- Del Gaizo Moore et al, Blood 2008
- AML – Vo et al, Cell 2012; Pan, Hogdal et al, Cancer Discovery 2014
 - In clinical trials.
- ETP-ALL – NiChonghaile et al, Cancer Discovery 2014
- To be continued.....

“What drug(s) do I give this patient?”



- For chemo, ex vivo studies not good enough.
- Drugs too slow – days required for death.
- Consistent culture of cancer cells too difficult.

Dynamic BH3 profiling 8-24 hours



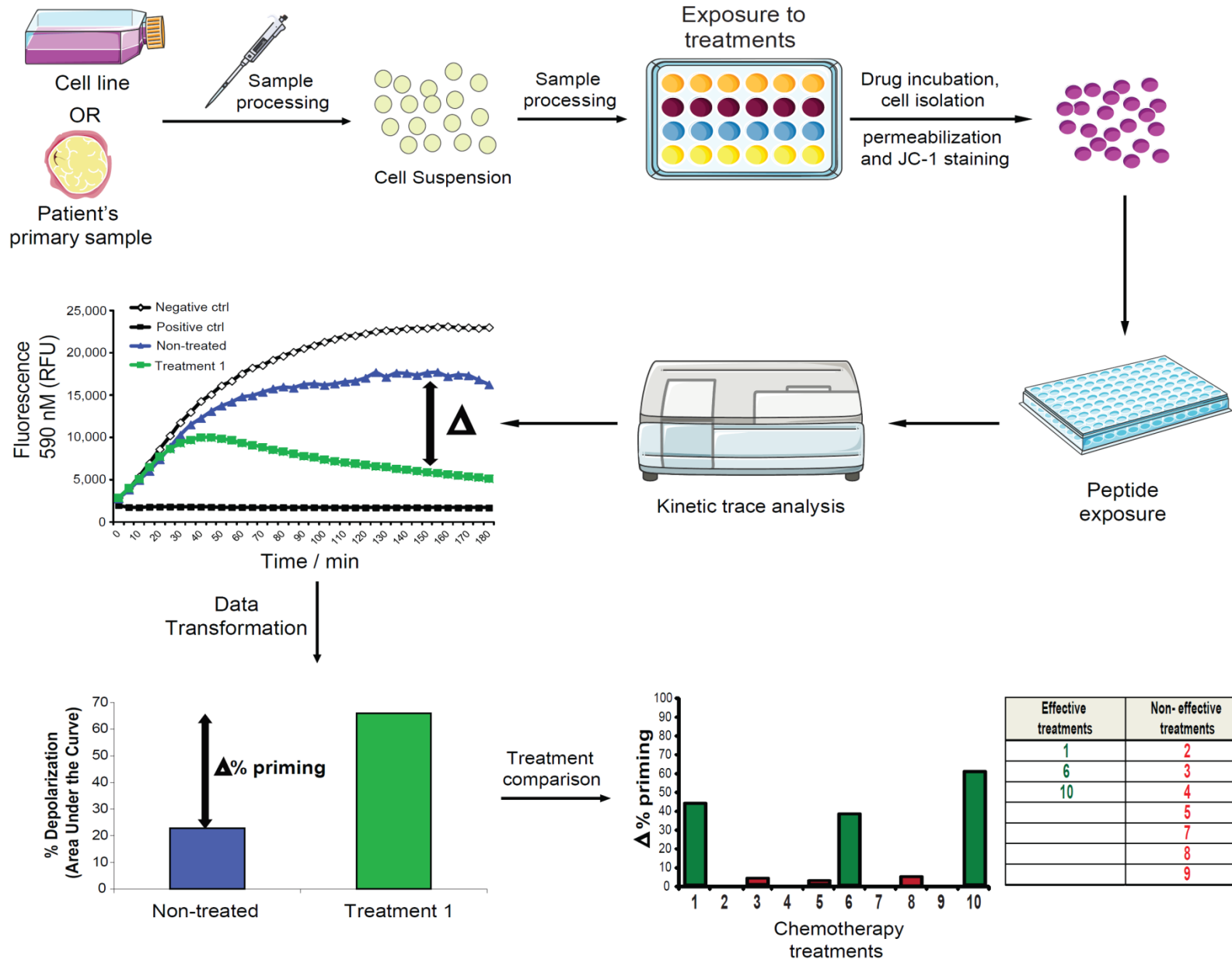
Right
treatment



APOPTOSIS
48-96 h



Dynamic BH3 profiling



Is Dynamic BH3 Profiling generally applicable?

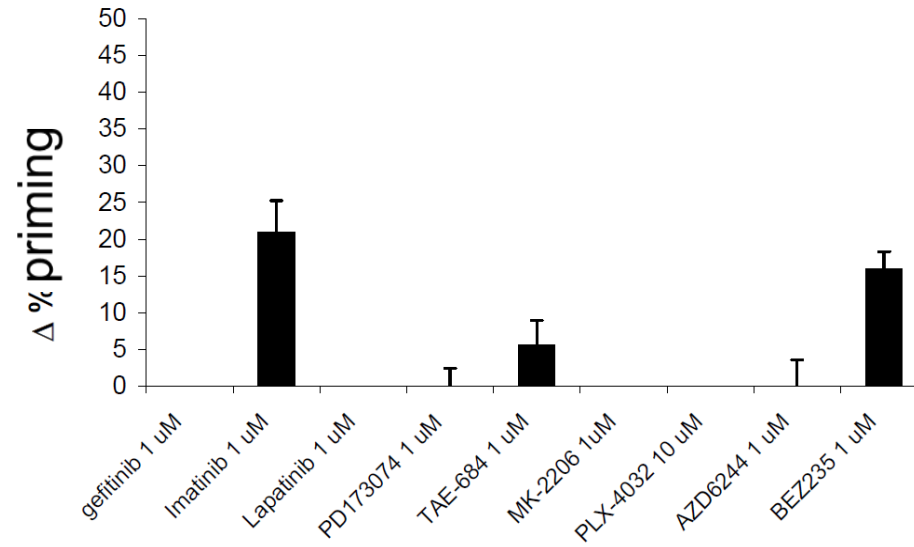
Cell line

Treatment

Hematological malignancies	<ul style="list-style-type: none">• K562 (CML)• DHL6 (DLBCL)• LP1 (MM)• DHL4 (DLBCL)• AML3 (AML)	<ul style="list-style-type: none">• Gefitinib (EGFR inh)• Imatinib (BCR-ABL inh)• Lapatinib (HER2 inh)• PD173074 (FGFR inh)• TAE-684 (EML4-ALK inh)• MK-2206 (Akt inh)• PLX-4032 (BRaf V600E inh)• AZD6244 (MEK inh)• BEZ235 (PI3K/mTOR inh).
Solid Tumors	<ul style="list-style-type: none">• Sk5 mel (Melanoma)• MCF7 (Breast Cancer)• PC9 (NSCLC)• HCT116 (Colon)• MDA-MB-231 (Breast)	

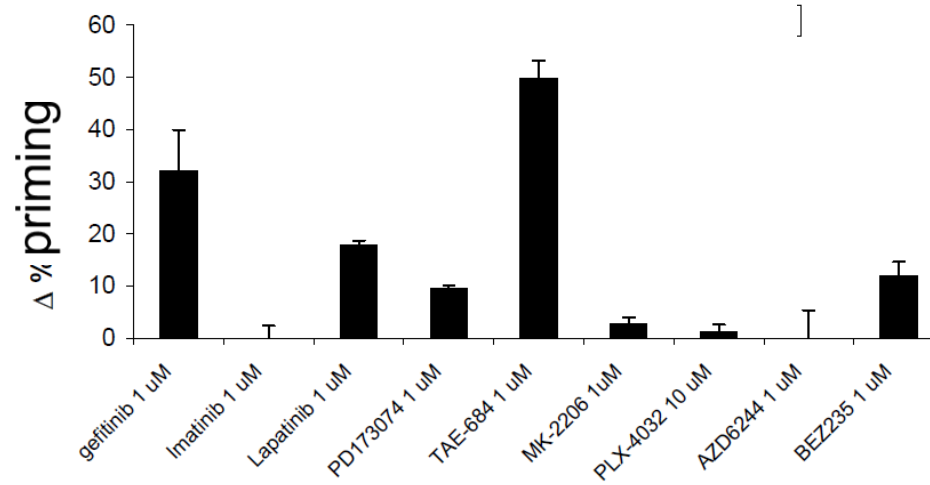
K562 (CML)

Dynamic BH3
profiling 16 h



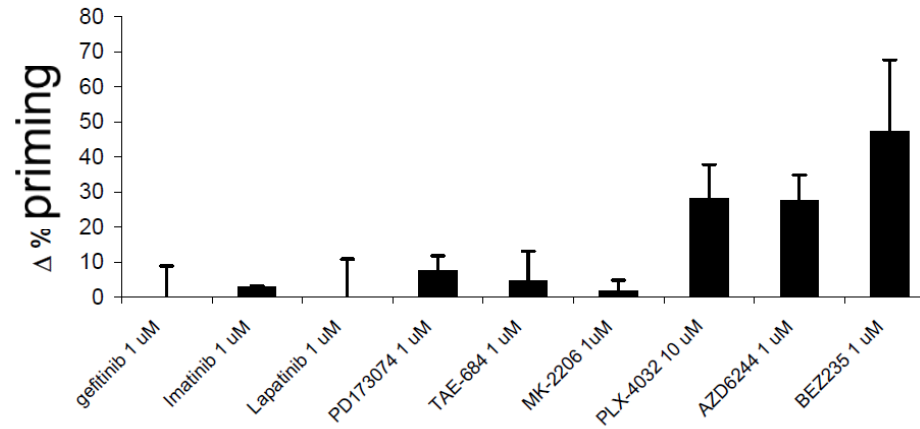
PC9 (NSCLC)

Dynamic BH3
profiling16 h



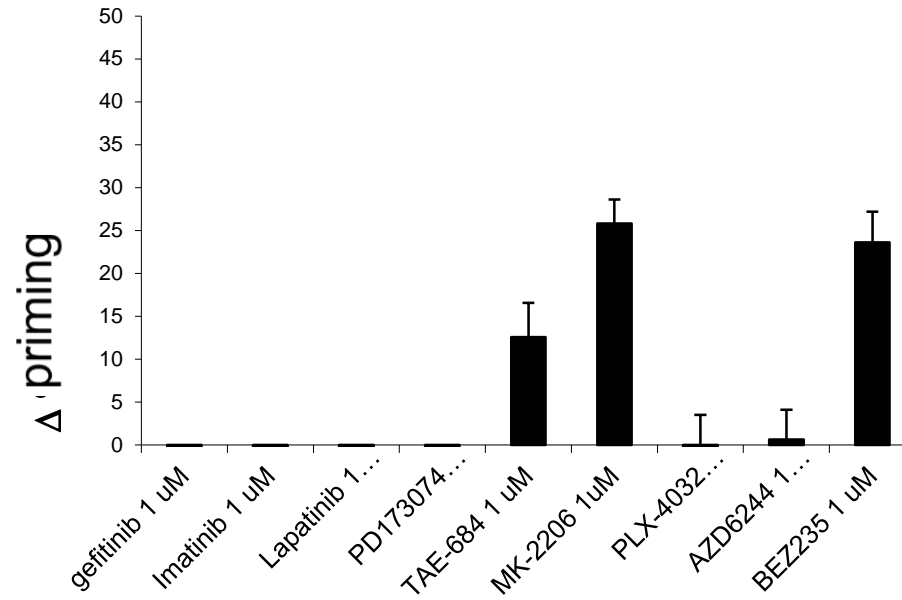
Sk5mel (melanoma)

Dynamic BH3
profiling16 h

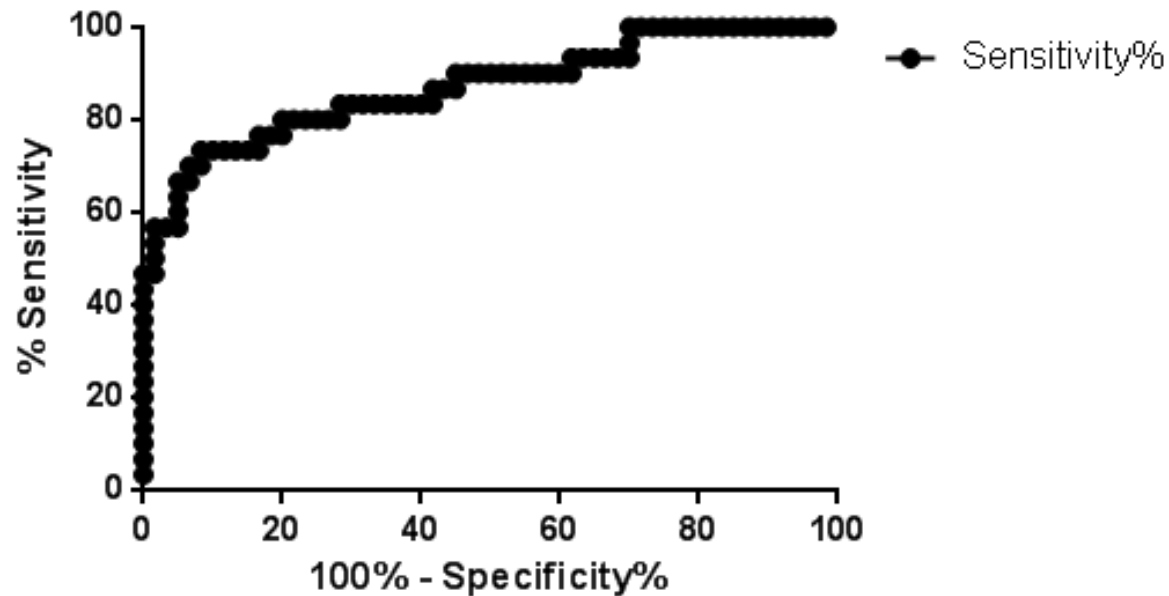


DHL4 (DLBCL)

Dynamic BH3
profiling16 h

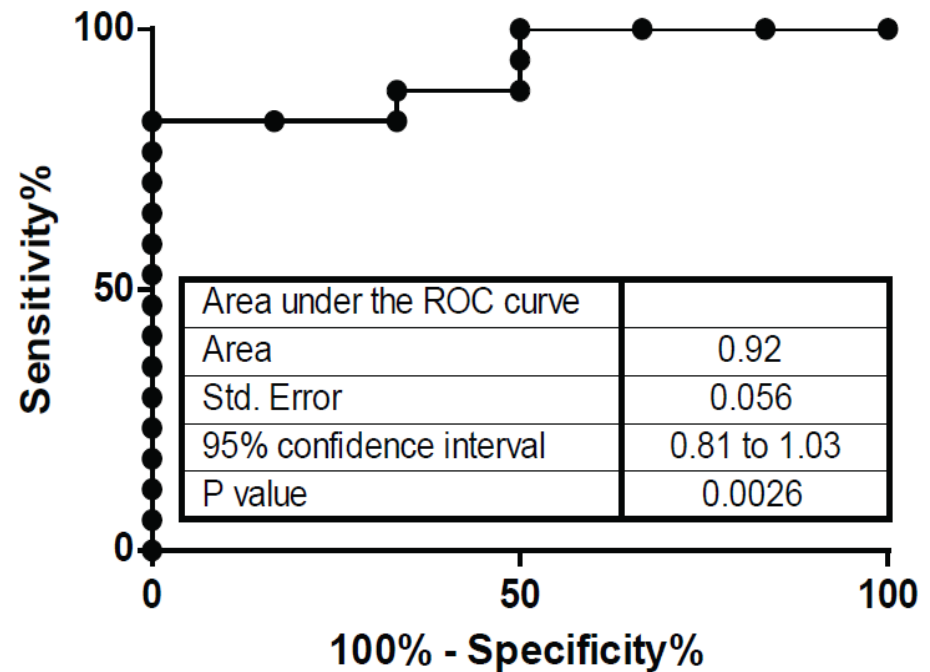
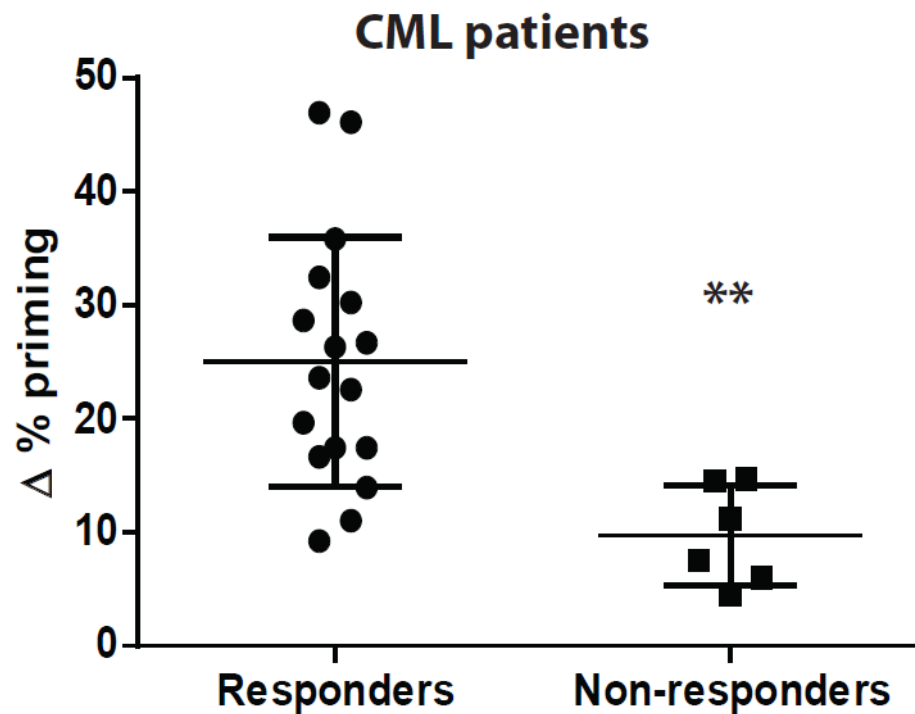


Dynamic BH3 profiling is a good binary predictor of cytotoxicity



Area under the ROC curve	
Area	0.8706
Std. Error	0.04284
95% confidence interval	0.7866 to 0.9545
P value	< 0.0001

Dynamic BH3 profiling predicts imatinib response in CML patients



Treatment: Imatinib 16h

Main points

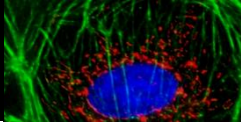
- Chemo preferentially kills primed cancer cells.
- BH3 profiling does not tell you about genes and sequences, but it tells you what will work.

Future

- Pick selective targeted agents to prime cancer cells.
- Finish them off with chemo.



Acknowledgements



Lab of Anthony Letai, MD PhD

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- ☐ Triona Ni Chonghaile
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- ☐ Jennifer Guerriero
- ☐ Leah Hogdal
- ☐ Elif Isik
- ☐ Joan Montero
- ☐ Richard Oakley
- ☐ Luv Patel
- ☐ **Jeremy Ryan**
- ☐ Cyrille Touzeau



Join us!! Post-doc wanted!!

☐ Collaborators

- ☐ Shenandoah Robinson MD (BCH)
- ☐ Michelle Hirsch (BWH)
- ☐ Ronny Drapkin (DFCI)
- ☐ Rich Stone (DFCI)
- ☐ Dan Deangelo (DFCI)
- ☐ Michelle Kelliher (UMass)
- ☐ Justine Roderick (UMass)
- ☐ Alejandro Gutierrez (DFCI)

☐ Sources of Funding.



- ☐ Women's Cancers Program at DFCI



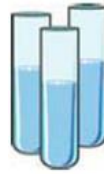
- ☐ American Cancer Society Postdoctoral Fellowship
- ☐ NIH/NCI
- ☐ Gabrielle's Angel Foundation
- ☐ Leukemia and Lymphoma Society

BH3 Profiles: Three Different Ways

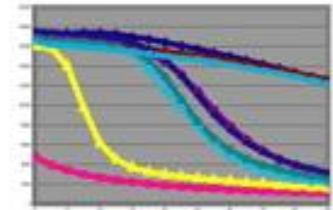
JC-1 Plate Reader Assay



Single Cell
Suspension of
Cancer Cells



Permeabilize cells, add
molecules or peptides,
add JC-1 dye

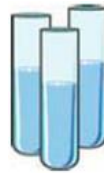


Kinetic Trace

Intracellular FACS Assay



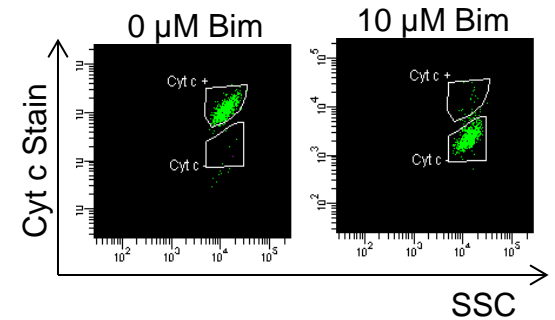
Single Cell
Suspension of
Cancer Cells



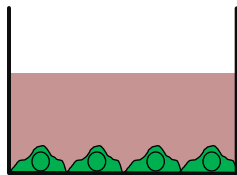
Permeabilize
cells and add
BH3 Peptide



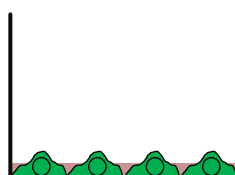
Fix cells and
stain with cyto
c antibody



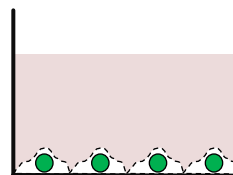
Microscopy Assay



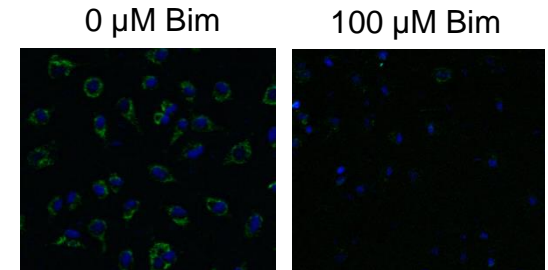
Cells plated in
advance



Media removal



Add DTEB &
molecules or peptides



Ryan and Letai, Methods 2013