Pediatric Preclinical Testing Program (PPTP) evaluation of the Bcl-2 inhibitor ABT-263

Malcolm A. Smith, *John M. Maris*, *Stephen T. Keir*, *Henry S. Friedman*, *Richard B. Lock*, *Hernan Carol*, *Mayamin Tajbakhsh*, *Richard Gorlick*, *E. Anders Kolb*, *Nino Keshelava*, *C. Patrick Reynolds*, *Christopher Morton*, *Peter J. Houghton*.

Disclosure Information

AACR 2007

Malcolm A. Smith

I have no financial relationships to disclose.

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I will not discuss off label use and/or investigational use in my presentation.

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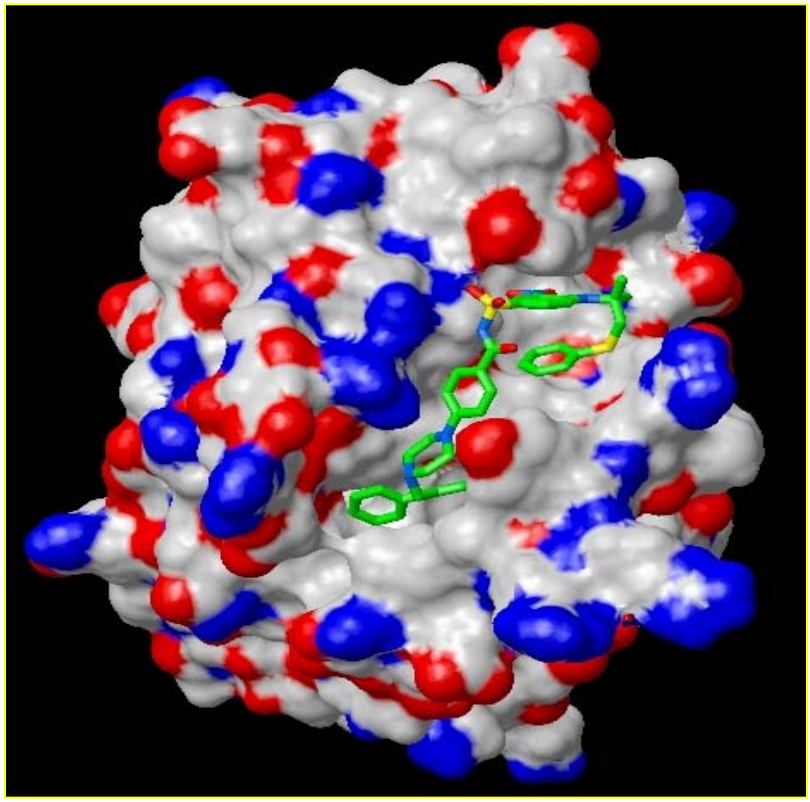
ABT-263 Introduction

- Orally bioavailable, BH3 mimetic small molecule inhibitor of Bcl-2 family proteins
- Binds with high affinity to multiple anti-apoptotic Bcl-2 family proteins including Bcl-X_L, Bcl-2 and Bcl-w.
- Like the structurally related compound ABT-737, ABT-263 displays potent cytotoxicity against selected cell lines derived from small cell lung carcinomas and lymphoid malignancies



Oltersdorf, et al. Nature 2005:435(7042):677-681

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Pediatric Preclinical Testing Program (PPTP)

- Research contract for testing new agents using *in vitro* and *in vivo* panels of childhood cancers:

– St. Jude Children's Research Hospital	Dr. Peter Houghton
– Children's Hospital of Philadelphia	Dr. John Maris
– Albert Einstein College of Medicine	Drs. Richard Gorlick & Andy Kolb
– Duke University Medical Center	Drs. Steve Keir & Henry Friedman
– Children's Cancer Institute Australia	Dr. Richard Lock
– Children's Hospital of Los Angeles	Dr. Patrick Reynolds

- *In vivo* panel with 60 xenograft lines & *in vitro* panel with 27 cell lines
- Able to test 10-12 new agents per year against the PPTP childhood cancer panels

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Testing of Agents by the PPTP

- Stage 1 Testing –
  - Single agent *in vitro* testing
  - *In vivo* efficacy testing at MTD or at dose recommended by sponsor as optimal
- Stage 2 Testing – May include one or more of the following:
  - Dose-response for selected sensitive lines
  - Pharmacokinetics
  - Evaluation of target modulation and other pharmacodynamic endpoints
  - Combinations (e.g., with standard chemotherapy agents)

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PPTP *In Vitro* Testing

- PPTP *in vitro* panel = 23 lines for Stage 1 testing
- DIMSCAN testing: semiautomatic fluorescence-based digital image microscopy system that quantifies viable cell numbers (Keshelava, et al. Methods Mol.Med., *110*: 139-153, 2005).
- 96 hour exposure at 9 concentrations spanning 4 logs (1.0 nM to 10 μM with replicates of 6 per data point).
- Data analyzed by fitting a non-linear regression sigmoidal dose-response model to the relative fluorescence values *vs*. the concentration.

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PPTP *In Vivo* Testing Procedures – Solid Tumor

- Solid tumor procedures:
  - 10 control & 10 treated mice bearing SC tumors for each xenograft
  - Initiate treatment when the tumors 0.2–0.5 cm^3.
  - Tumor diameters measured once weekly intervals with digital vernier calipers.
  - Assuming tumors to be spherical, volumes were calculated from the formula (π/6)×d3, where d represents the mean diameter
- "Event" defined as 4-fold increase in tumor volume

Houghton, et al. Pediatr Blood Cancer. 2006 Oct 25; [Epub ahead of print]

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PPTP *In Vivo* Testing Procedures Acute Lymphoblastic Leukemia

- NOD-SCID mice (8 for treatment group and 8 for control group) inoculated with 3-5 x 10^6 mononuclear cells purified from the spleens of secondary recipient mice.
- Engraftment monitored weekly by flow cytometry, and treatment initiated when the proportion of human $CD45^+$ cells in the peripheral blood > 1%.
- The proportion of human $CD45^+$ cells in the peripheral blood monitored weekly throughout the course of treatment.
- Event defined as 25% $CD45^+$ cells in peripheral blood

Liem NL, et al. Blood 2004:103(10):3905-3914

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Objective Response Assessment – Solid Tumors

Response	Definition	Score
Progressive Disease 1	>25% increase in tumor volume, and TGD value of ≤1.5	0
Progressive Disease 2	>25% increase in tumor volume, and TGD value of >1.5	2
Stable Disease	≤25% increase, and <50% regression	4
Partial Response	>50% regression	6
Complete Response (CR)	<0.1cm3 tumor volume	8
Maintained CR	<0.1cm3 at the end of study	10

*TGD = tumor growth delay

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Objective Response Assessment – Leukemia

Response	Definition	Score
Progressive Disease 1	CD45% never < 1%, Events before end of study, and TGD* value ≤1.5	0
Progressive Disease 2	CD45% < 1%, Events before end of study, and TGD value >1.5	2
Stable Disease	CD45% < 1%, and No events before end of study	4
Partial Response	CD45% <1% for only 1 week	6
Complete Response (CR)	CD45% <1% for 2 consecutive weeks	8
Maintained CR	CD45% <1% for the last 3 weeks of study	10

*TGD = tumor growth delay

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ABT-263 *In Vitro* Activity

- ABT-263 active against ~ 1/2 of the 23 PPTP cell lines
- EC_{50} values ranged from 0.06 μM for RS4;11 (ALL cell line with MLL gene rearrangement) to > 10 μM for 10 cell lines
- Median EC_{50} for *in vitro* panel was 1.8 μM
- Trend for lower EC_{50} values for the ALL panel compared to the remaining PPTP cell lines (median EC_{50} 0.4 μM vs > 10 μM, p=0.04).

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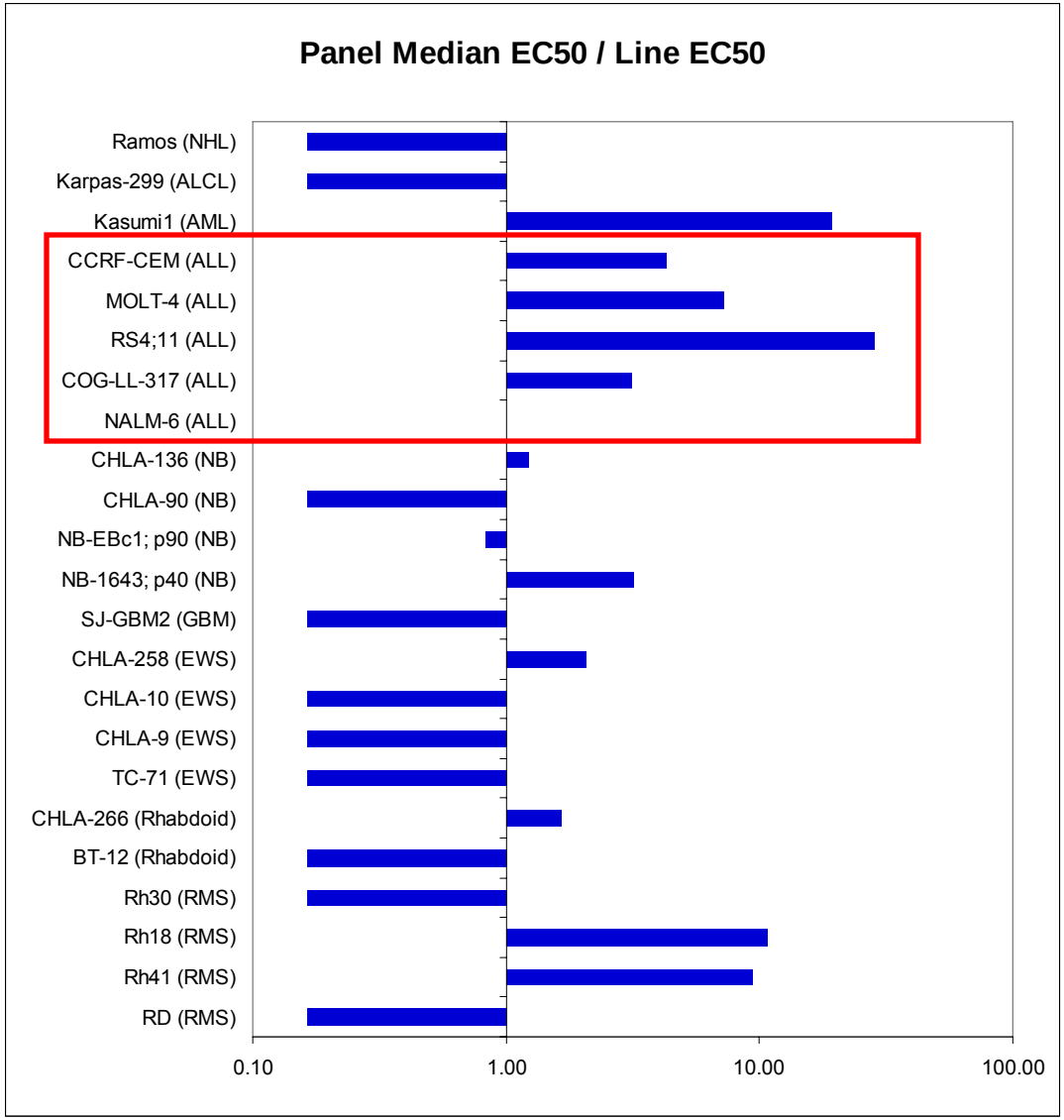
COMPARE-Like Graph for ABT-263 *In Vitro* Activity



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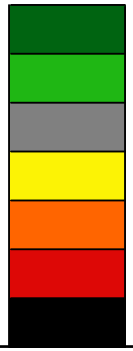
ABT-263 In Vivo Testing Results

- Testing at dose of 100 mg/kg administered orally daily x 21 days
- Solid tumor panel had no objective responses: best response was PD2
- ALL panel had 3 xenografts with CRs and 2 with PD2
- Two T-cell ALL xenografts achieved complete responses that were maintained 3 weeks after the last dose of ABT-263.



Line	Histology	Heat Map
BT-29	Rhabdoid	
KT-14	Rhabdoid	
KT-10	Wilms	
KT-11	Wilms	
KT-13	Wilms	
SKNEP	Ewings	
EW5	Ewings	
EW8	Ewings	
TC-71	Ewings	
CHLA258	Ewings	
Rh28	ALV RMS	
Rh30	ALV RMS	
Rh30R	ALV RMS	
Rh41	ALV RMS	
Rh18	EMB RMS	
BT-28	Medulloblastoma	
BT-45	Medulloblastoma	
BT-46	Medulloblastoma	
BT-50	Medulloblastoma	
BT-44	Ependymoma	
GBM2	Glioblastoma	
BT-39	Glioblastoma	
D645	Glioblastoma	
D456	Glioblastoma	
NB-SD	Neuroblastoma	
NB-1771	Neuroblastoma	
NB-1691	Neuroblastoma	
NB-EBc1	Neuroblastoma	
CHLA-79	Neuroblastoma	
NB-1643	Neuroblastoma	
OS-1	Osteosarcoma	
OS-2	Osteosarcoma	
OS-17	Osteosarcoma	
OS-33	Osteosarcoma	
OS-31	Osteosarcoma	
ALL-2	ALL B-precursor	
ALL-4	ALL B-precursor	
ALL-8	ALL T-cell	
ALL-16	ALL T-cell	
ALL-17	ALL B-precursor	
ALL-19	ALL B-precursor	
	Median	

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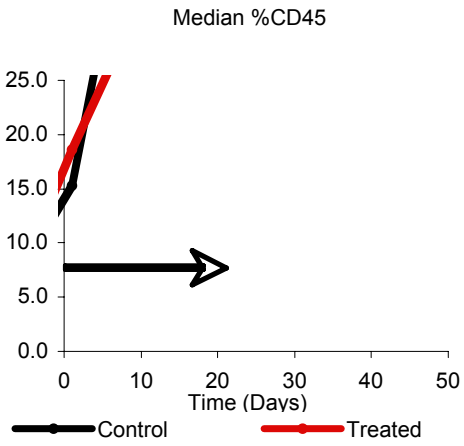
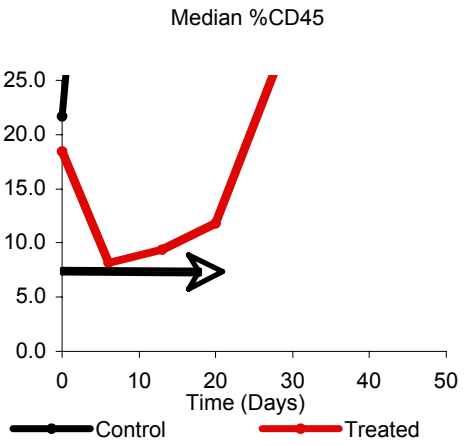
Progressive Disease (PD) 1 for ALL-2 and PD2 for ALL-4 and ALL-17

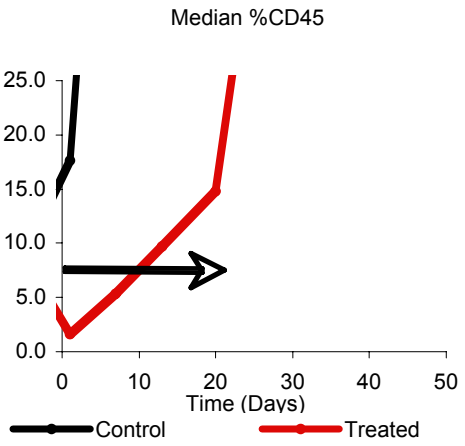
ALL-2



ALL-4



ALL-17



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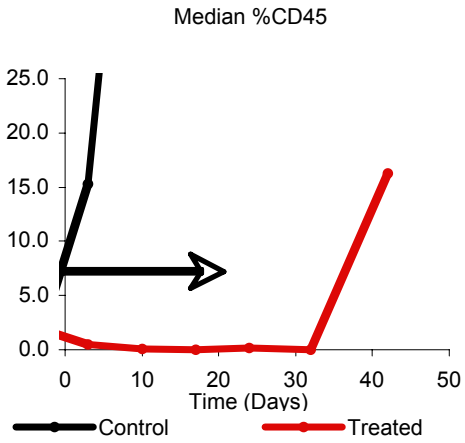
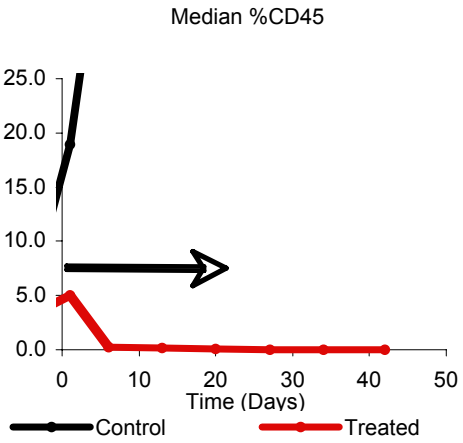
Complete Response (CR) and Maintained CRs for ALL-19, ALL-8 and ALL-16

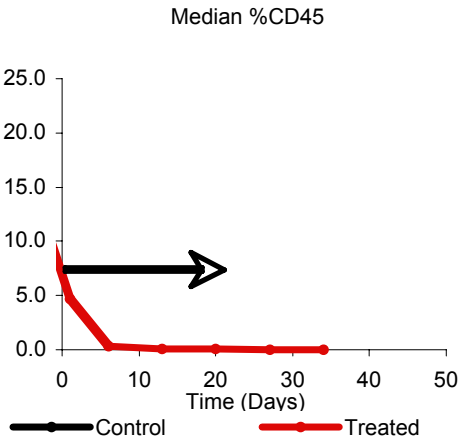
ALL-19



ALL-8



ALL-16



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Characteristics of ALL Panel

Line	Immunophenotype	Disease Status	CR1 (mos)	Status
ALL-2	B-precursor	Relapse	30	DOD
ALL-3	B-precursor	Diagnosis	38	CR2
ALL-4	B-precursor (Ph+)	Diagnosis	10	DOD
ALL-7	B-precursor	Diagnosis	7	DOD
ALL-8	T-cell ALL	Relapse	17	DOD
ALL-16	T-cell ALL	Diagnosis	120+	CR1
ALL-17	B-precursor	Diagnosis	25	CR2
ALL-19	B-precursor	Relapse	4	DOD
ALL-10	B-precursor	Diagnosis	85+	CR1
ALL-11	B-precursor	Diagnosis	137+	CR1

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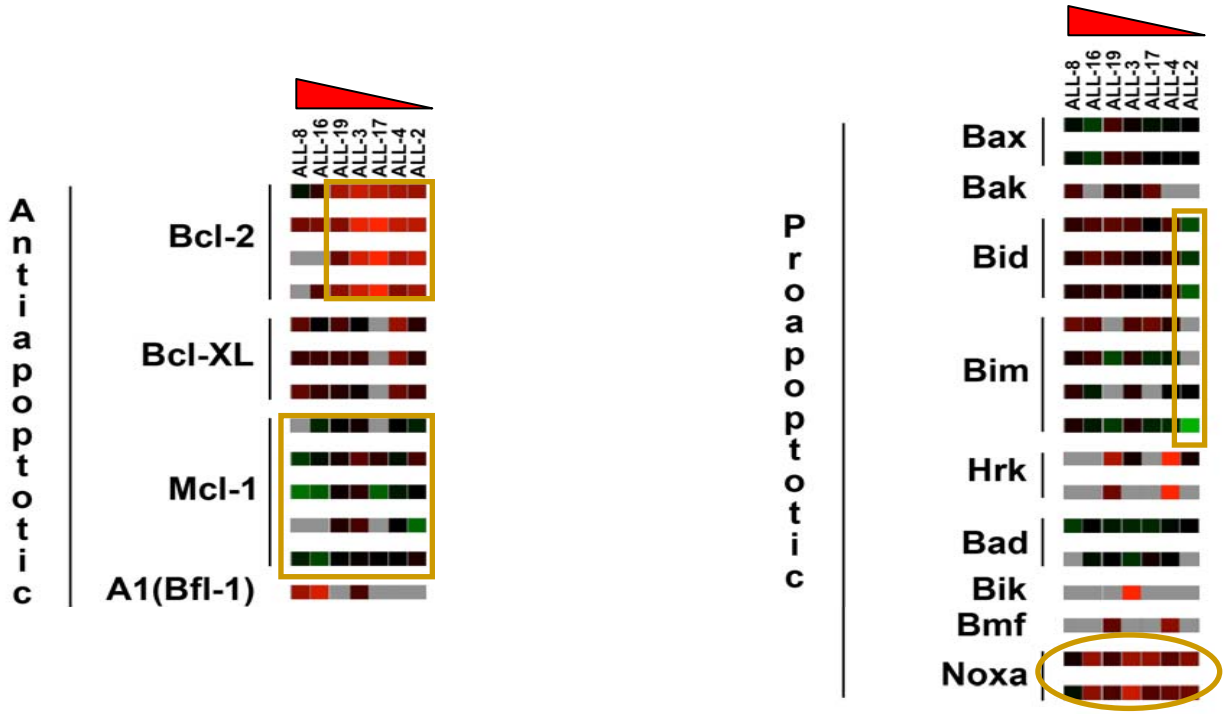
Bcl-2 Family Gene Expression – ALL Panel



- Bcl-2 expression elevated in B-precursor ALL xenografts
- Mcl-1 expression generally low in the ALL xenografts
- Noxa expression high in most ALL xenografts
- ALL-2 only xenograft with no response to ABT-263:
  - Lower expression of Bid and Bim than other ALL xenografts

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Conclusions

- ABT-263 shows little single agent *in vivo* activity against the PPTP's solid tumor panels.
- ABT-263 has remarkable *in vivo* activity against several of the PPTP's ALL xenografts.
- The single agent anti-leukemia activity observed for ABT-263 supports its rapid clinical evaluation for children with ALL.
- Future preclinical work will explore:
  - Combinations of ABT-263 with standard chemotherapy agents and with molecularly targeted agents that affect apoptosis, and
  - Biological characteristics associated with sensitivity & resistance to ABT-263.

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