



Pediatric Preclinical Testing Program (PPTP)

November 2013



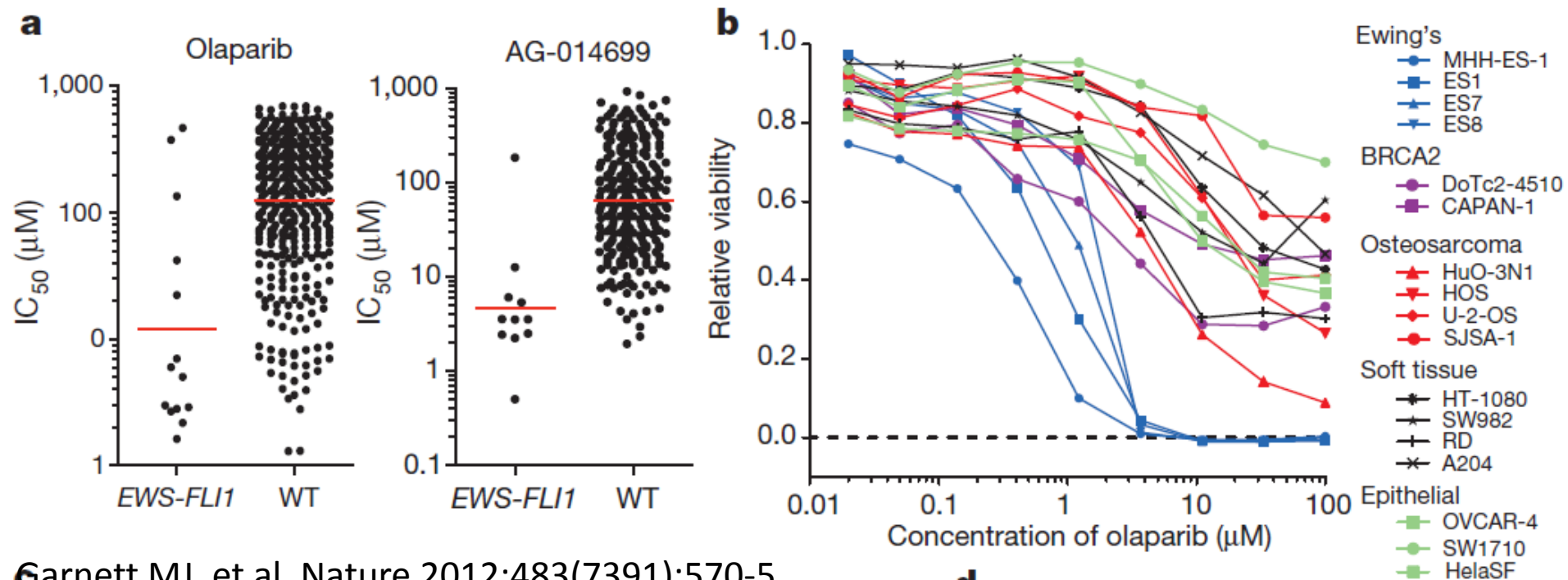
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Cancer Therapy Evaluation Program
National Cancer Institute, U.S.A.

Pediatric Oncology Drug Development

- Pediatric drug development is challenging
 - Limited pharmaceutical company interest
 - Limited number of clinical trials that can be conducted
 - Many anticancer agents entering pipeline
 - Critical need for effective prioritization
- Role of the PPTP
 - Provide evidence to support the presence or absence of a **therapeutic window** for specific agents against selected diseases

Example of Difficulty of Assessing Therapeutic Window: Ewing sarcoma cell lines are sensitive to PARP inhibition

- A. EWS-FLI1-translocation-positive cell lines show lower IC_{50} values to olaparib and AG-014699 compared to non-EWS-FLI1 cell lines.
- B. Dose-response curves to olaparib after 6 days of constant drug exposure. Cell lines are classified according to tissue subtype.



In Vivo Testing

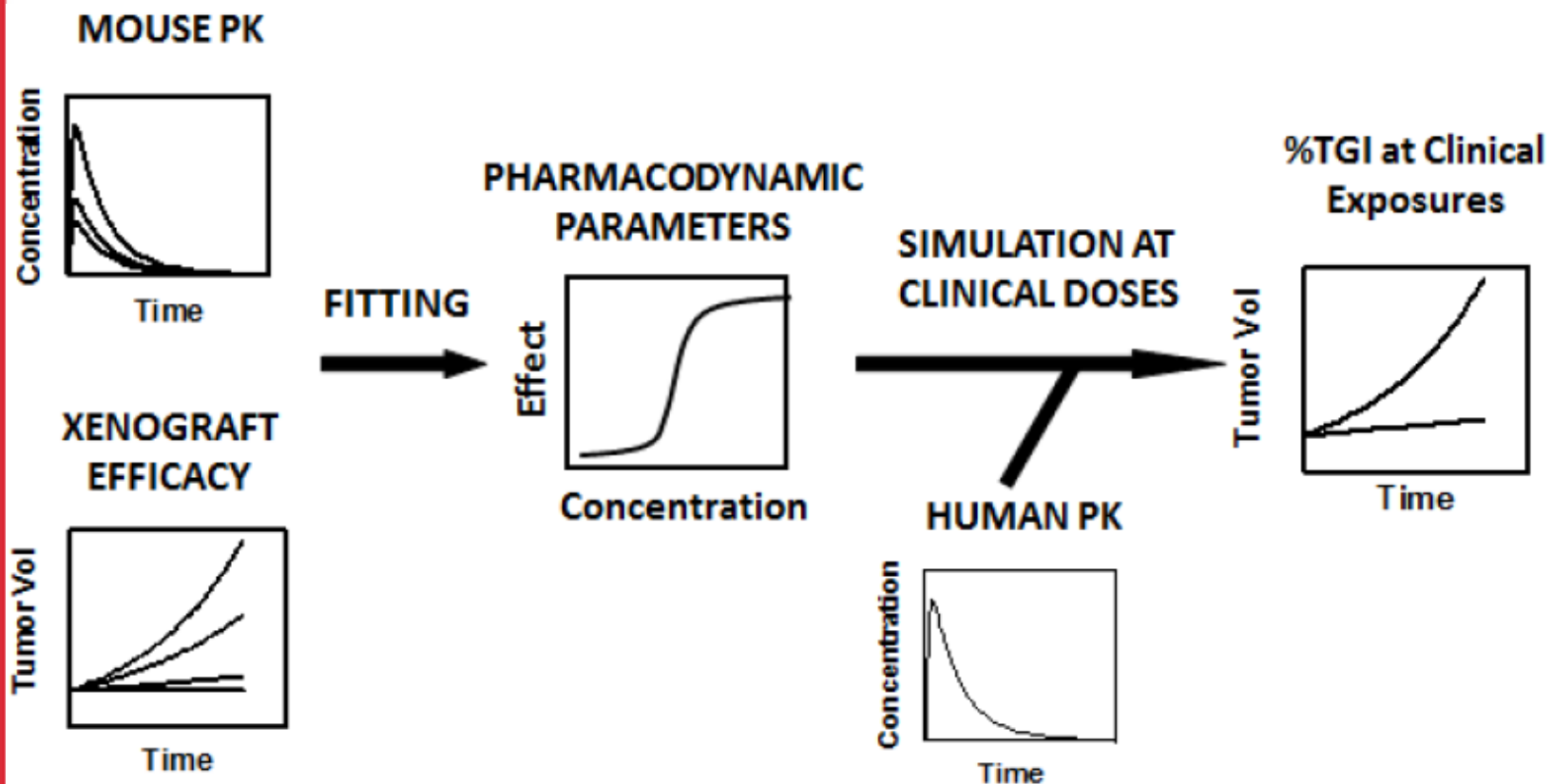
- Allows assessment of anticancer activity in relationship to systemic exposures that animals tolerate
- Pediatric preclinical testing has an advantage over adult cancer testing in that tolerable human systemic exposures are often known by the time testing occurs

Clinical Cancer Research

Anti-tumor activity of targeted and cytotoxic agents in murine subcutaneous tumor models correlates with clinical response

Harvey Wong, Edna F. Choo, Bruno Alicke, et al.

Clin Cancer Res Published OnlineFirst May 30, 2012.



The Critical Need for Incorporating Pharmacokinetics into Preclinical Testing

- “A significant correlation ($r = 0.91$, $P = 0.0008$) was observed between simulated xenograft/allograft TGI driven by human pharmacokinetics and clinical response but not when TGI observed at maximum tolerated doses in mice was correlated with clinical response ($r = 0.36$, $P = 0.34$).”
 - Wong H, et al. Antitumor activity of targeted and cytotoxic agents in murine subcutaneous tumor models correlates with clinical response. *Clin Cancer Res* 2012;18(14):3846-3855.
- Recent PPTP examples of incorporation of PK include PR-104 and eribulin.

Raise standards for preclinical cancer research

C. Glenn Begley and Lee M. Ellis propose how methods, publications and incentives must change if patients are to benefit.

29 MARCH 2012 | VOL 483 | NATURE | 531

- 53 'landmark' studies in hematology and oncology for which independent validation attempted.
 - Scientific findings confirmed in **only 6 (11%) cases**.
 - Some non-reproducible preclinical papers spawned an entire field, with 100s of secondary publications.
 - Some of the research triggered a series of clinical studies.
- Conclusion: The inability of industry and clinical trials to validate results from the majority of publications on potential therapeutic targets suggests a general, systemic problem.

PPTP Steps to Ensure Reliability of Results

- Standard testing protocols
- Blinded testing
- Standard analytic metrics for defining activity
 - Tumor regression (objective response)
 - Time to event
- Multiple models for each histotype studied
- Molecular characterization of models to confirm identify and biological similarity to clinical specimens
- Presentation/publication of all testing results

Pediatric Preclinical Testing Program

- Research contract with Dr. Peter Houghton as Principle Investigator and with 6 testing sites.
- Primary focus on in vivo testing with standard panels of 4-8 xenograft lines per histotype
- Initiated testing in 2005
- More than 50 companies with which PPTP has established collaborations
- More than 80 executed MTAs
- More than 50 publications of testing results

Molecular/Biological Characterization

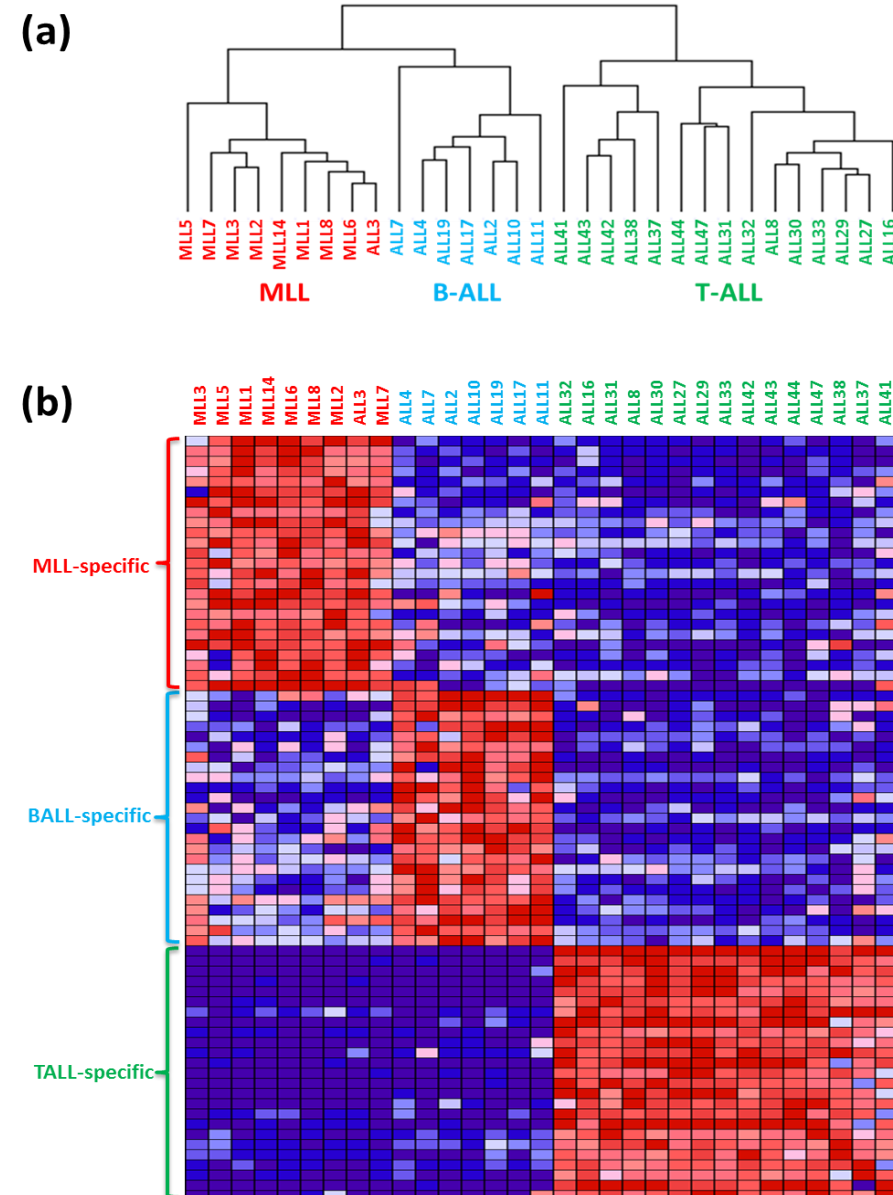
- Majority of models are patient derived xenografts not subjected to in vitro culture
- Gene expression profiles (cDNA & Affymetrix arrays and Illumina arrays)
- SNP analysis using Affymetrix GeneChip Human Mapping array
- Tissue arrays for immunohistochemical testing
- Data available through PPTP web site
- Sequencing of cell lines and xenografts in 2013 through collaboration with Office of Cancer Genomics

Agents Transitioned (or to be transitioned) to the Clinic

- In clinical evaluation:
 - Alisertib (MLN8237)
 - NTX-010
 - Selumetinib
 - Rapalog plus standard chemotherapy
 - IGF-1R antibodies
- In development:
 - Eribulin
 - BMN 673 plus temozolomide
 - Glembatumumab vedotin
- Future/Pending development:
 - SAR3419
 - MDM2 inhibitor
 - Bcl2 inhibitor
 - Lorvotuzumab mertansine (IMGN901)

Acute Lymphoblastic Leukemia Panel

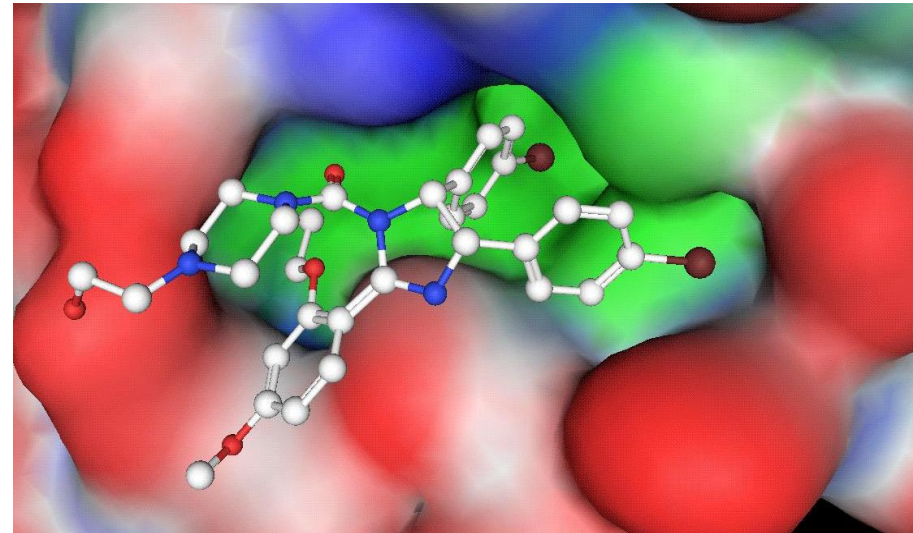
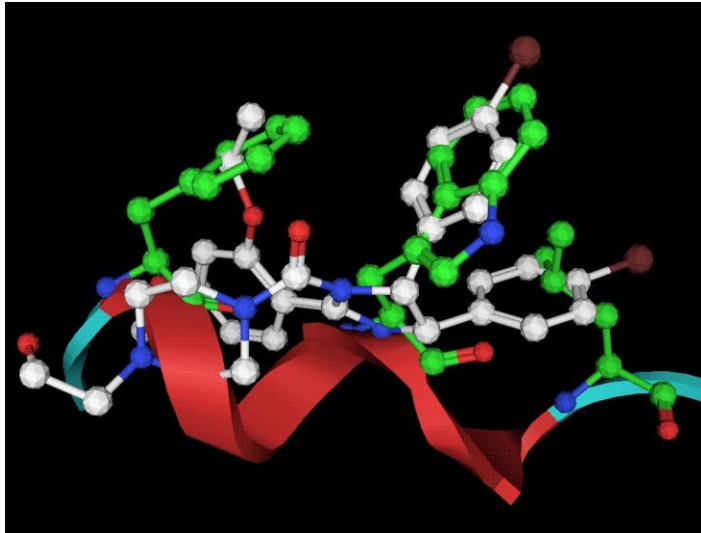
- Standard panel of 8 lines:
 - Bcr-Abl ALL (1)
 - T-cell ALL (2)
 - MLL ALL (1)
 - B-precursor ALL (4)
- Expanded panels
 - MLL
 - JAK-mutated ALL / Ph-like ALL
 - T-cell (including ETP)



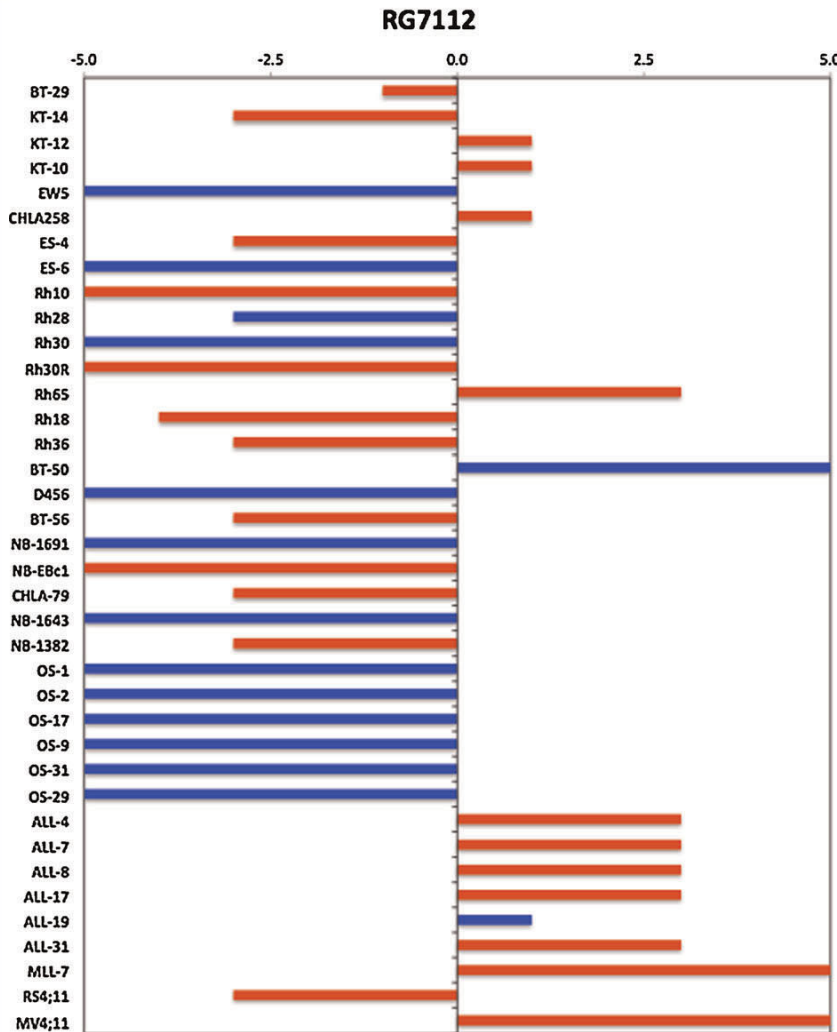
MDM2 Inhibitor RG7112

HDM2 Antagonists Bind to the p53-Binding Site on HDM2

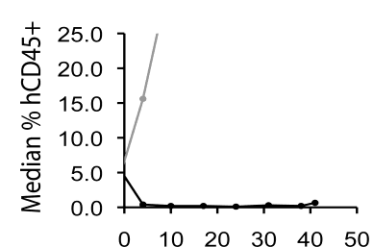
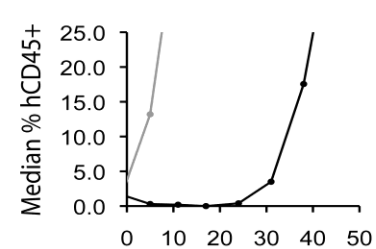
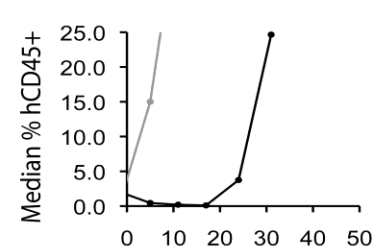
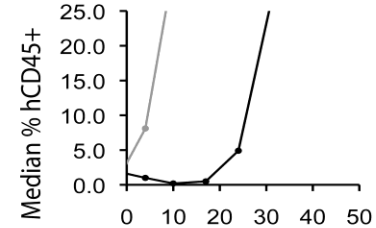
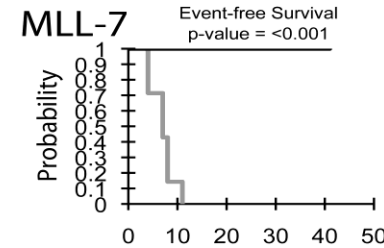
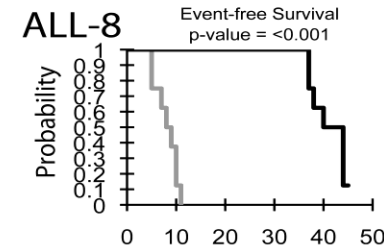
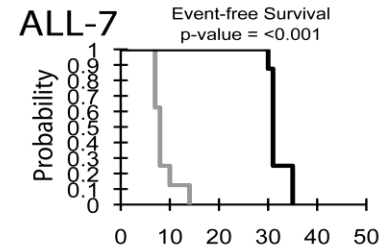
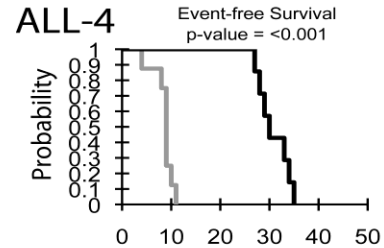
- Overlay of Nutlin-2 with HDM2 binding residues of p53
- Derived from crystal structure Of HDM2 – Nutlin-2 complex



RG7112 ALL Activity

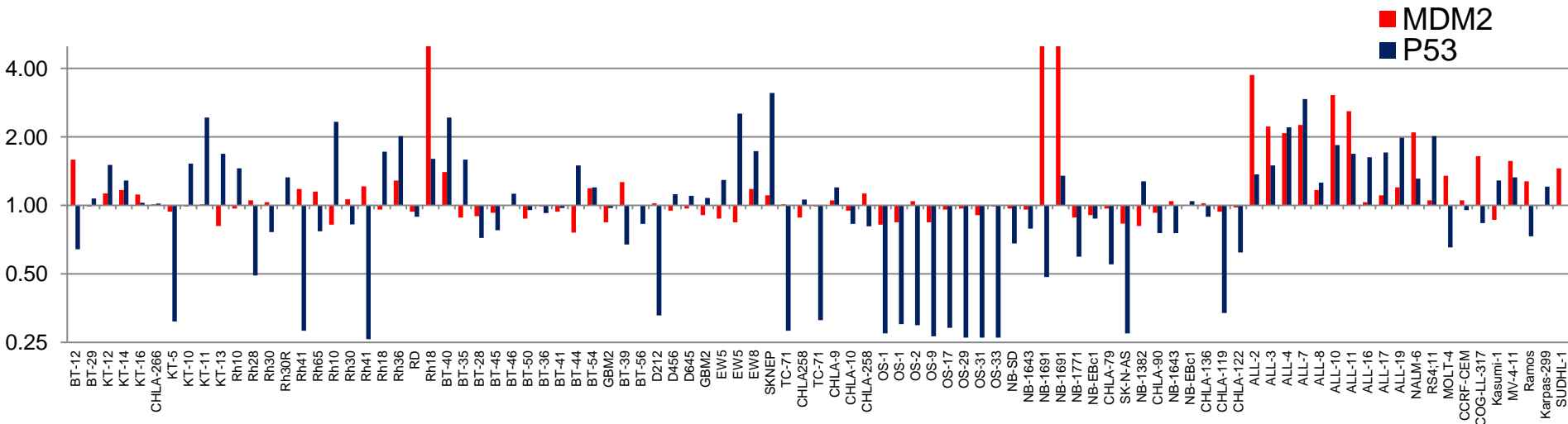


- 100 mg/kg daily for 14 days followed by 4 weeks of observation



MDM2 and P53 Expression

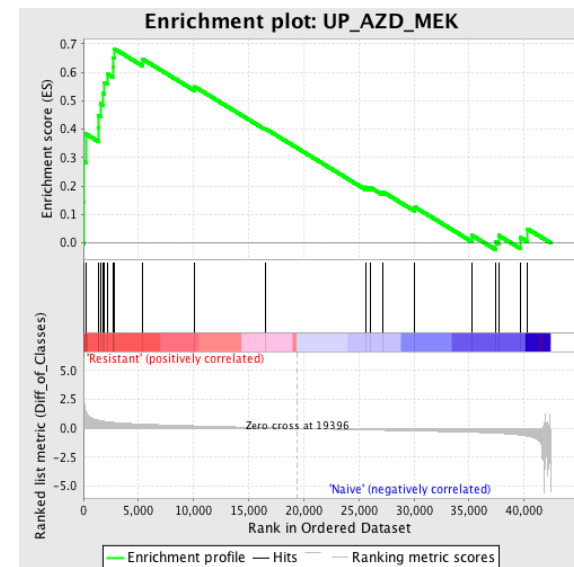
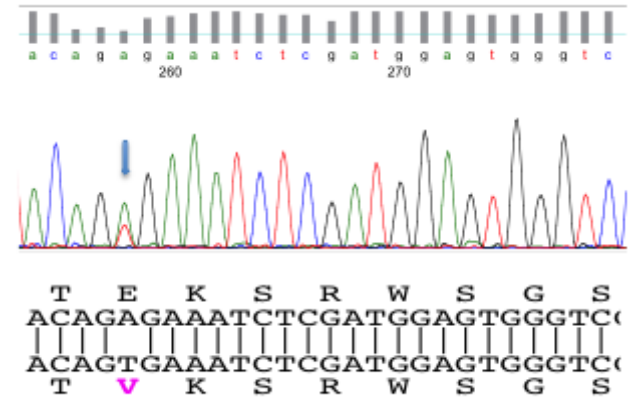
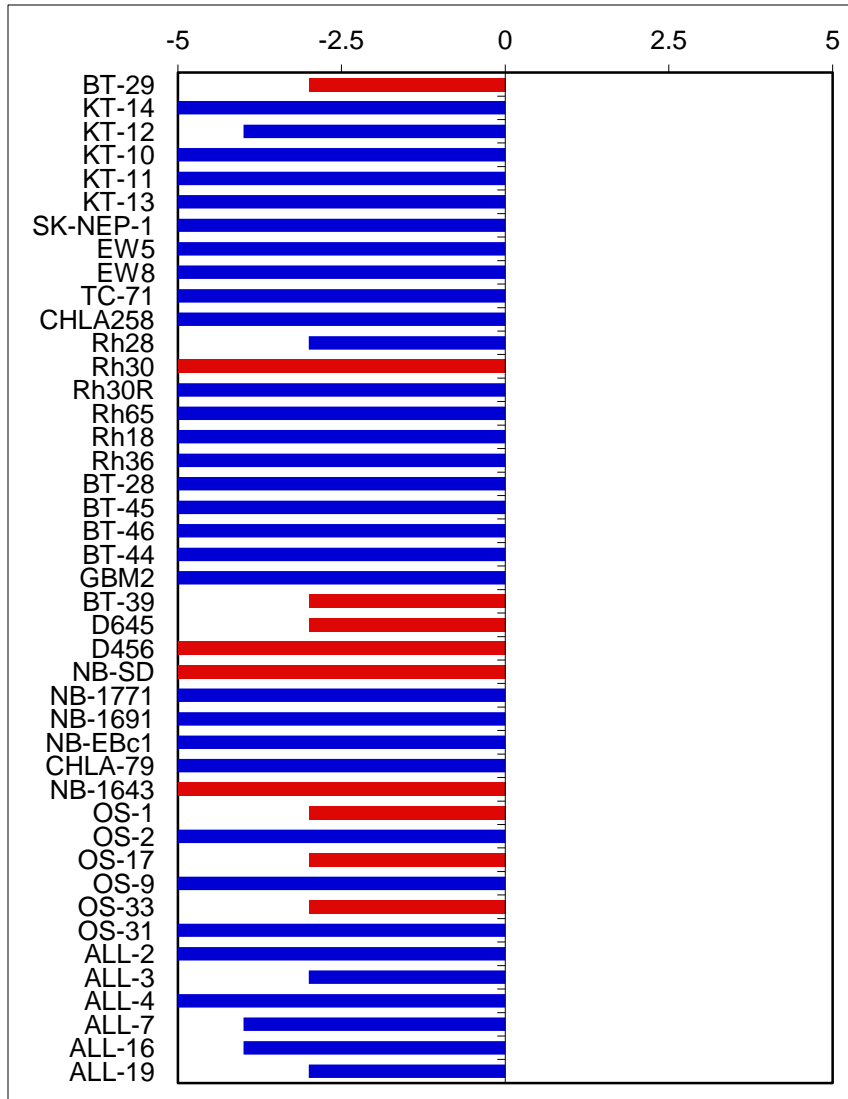
MDM2 (217373_x_at) and TP53 (201746_at)



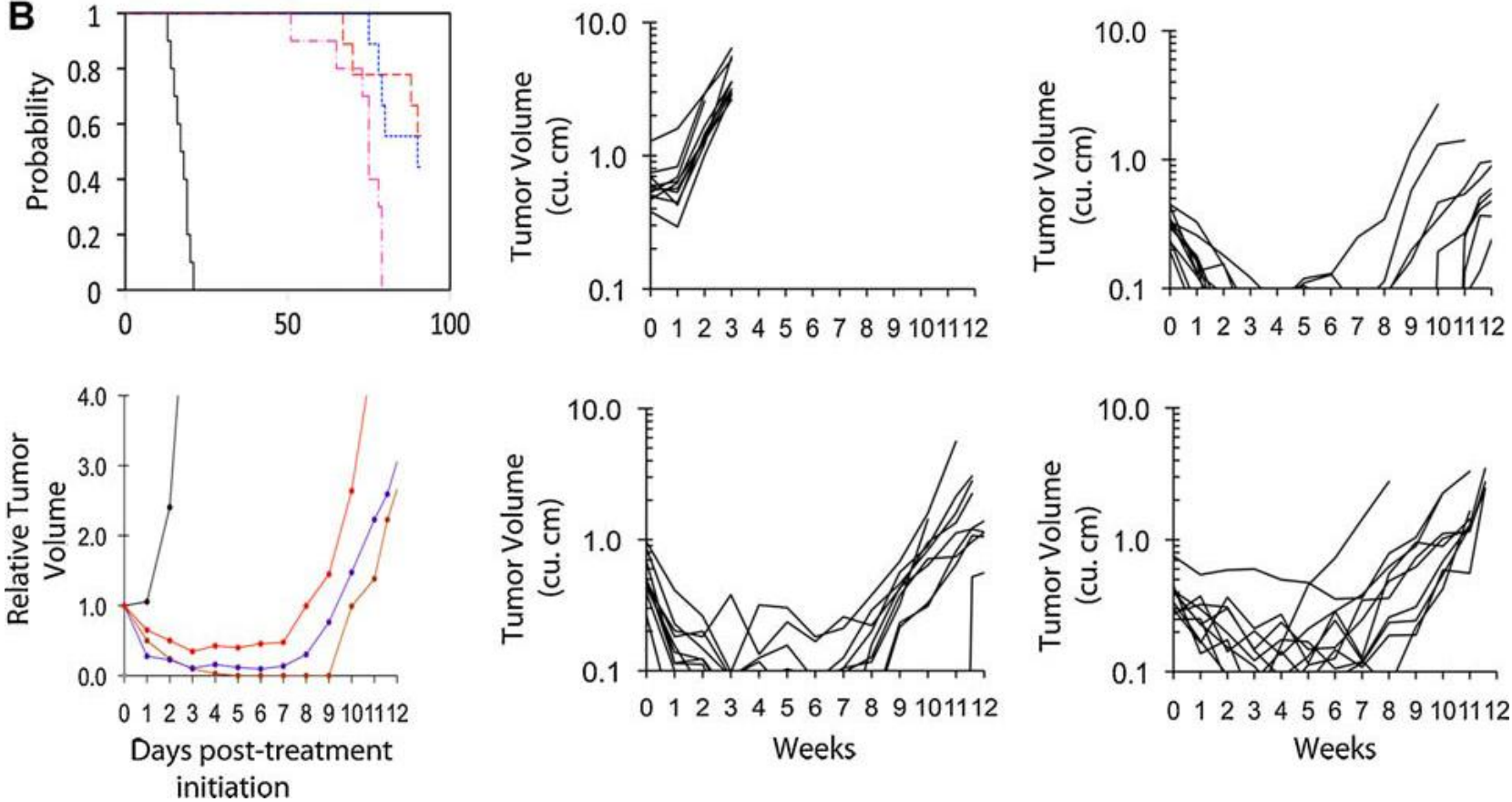
- The osteosarcoma xenografts were p53 WT, but had very low p53 expression and low MDM2 expression. They did not respond to RG7112. *in vivo*.
- The ALL xenografts expressed the highest levels of p53 and MDM2 among the PPTP panels and showed the most consistent *in vivo* responses to RG7112.
- Two PPTP xenografts have MDM2 amplification, Rh18 and NB-1691, and both showed high MDM2 expression. Neither responded to RG7112.

Selumetinib – MEK Inhibitor

The MEK inhibitor selumetinib (AZD-6244) has limited activity in the PPTP screen



Selumetinib (AZD6244) against a low-grade astrocytoma xenograft (BT-40) with the BRAF V600E mutation



Pediatric Development of Selumetinib

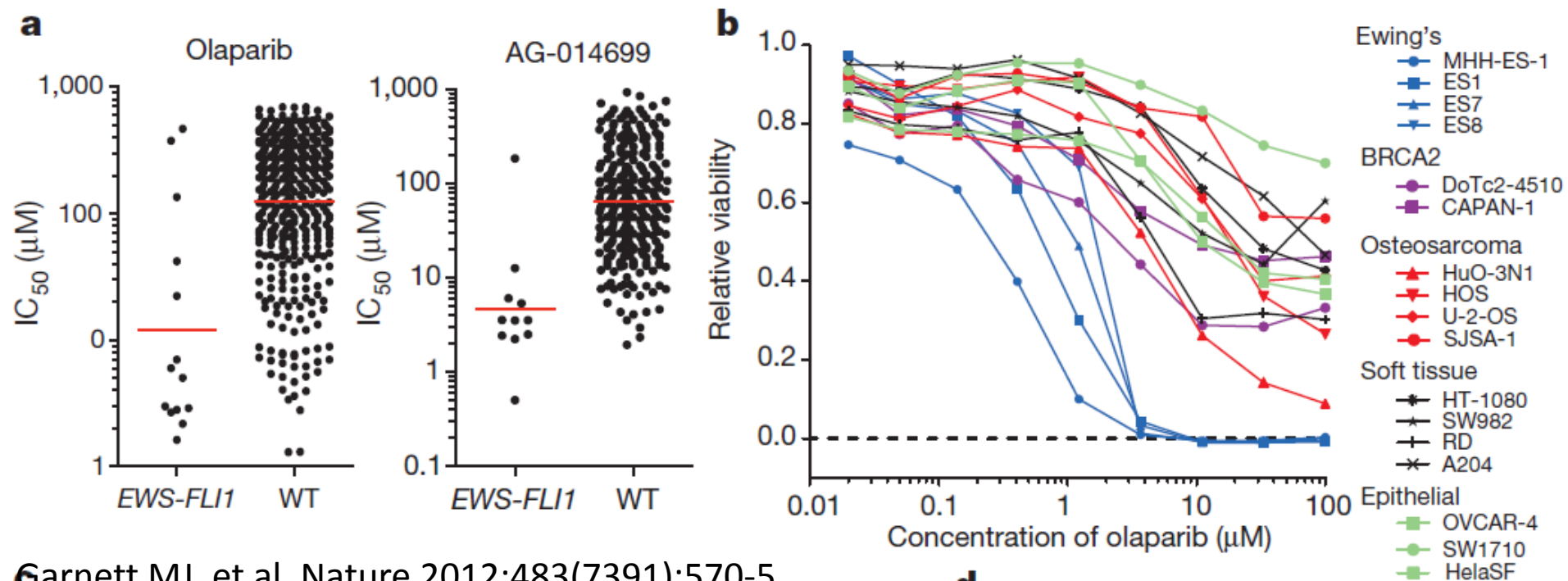
- Pediatric development of selumetinib influenced by PPTP results.
- Phase 1 study by Pediatric Brain Tumor Consortium (PBTC) restricted to children with refractory low grade astrocytomas (LGAs).
- Phase 2 expansion proceeding focusing on patients with BRAF-mutated LGA.
- Phase 1 results to be presented as “late breaking” abstract at Society for Neuro-Oncology Meeting.

BMN 673 plus temozolomide

Ewing sarcoma cell lines are sensitive to PARP inhibition

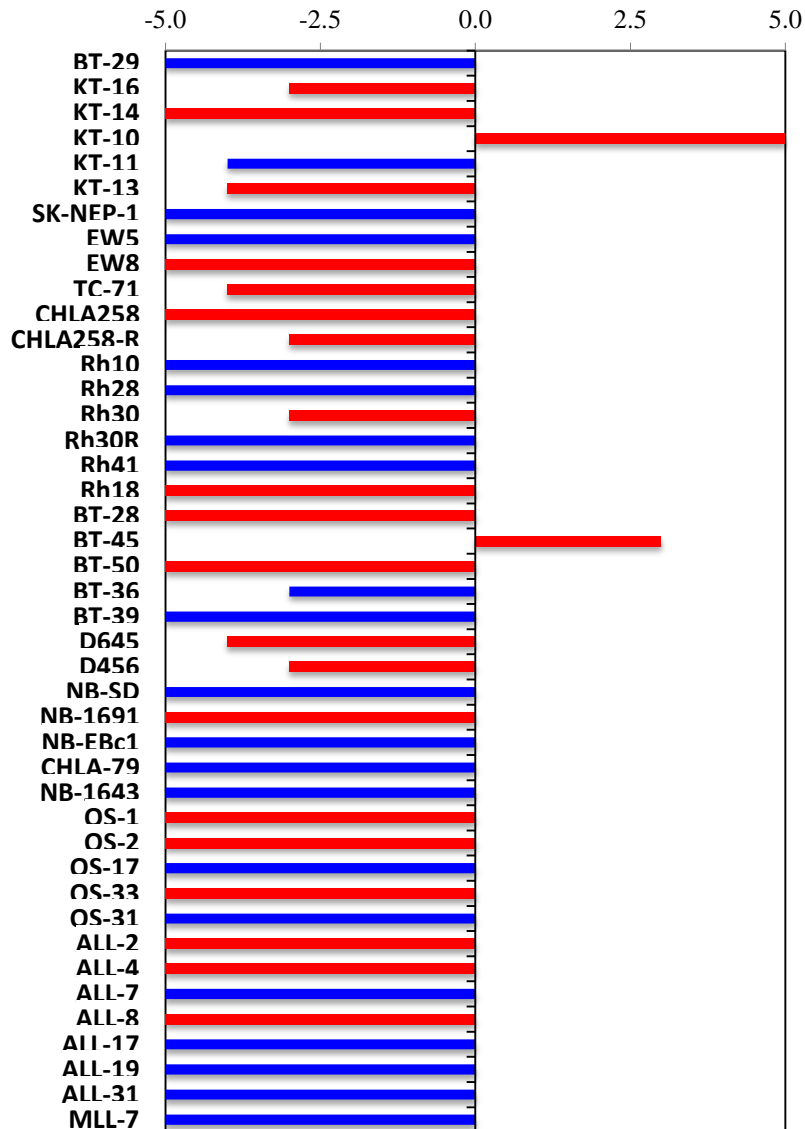
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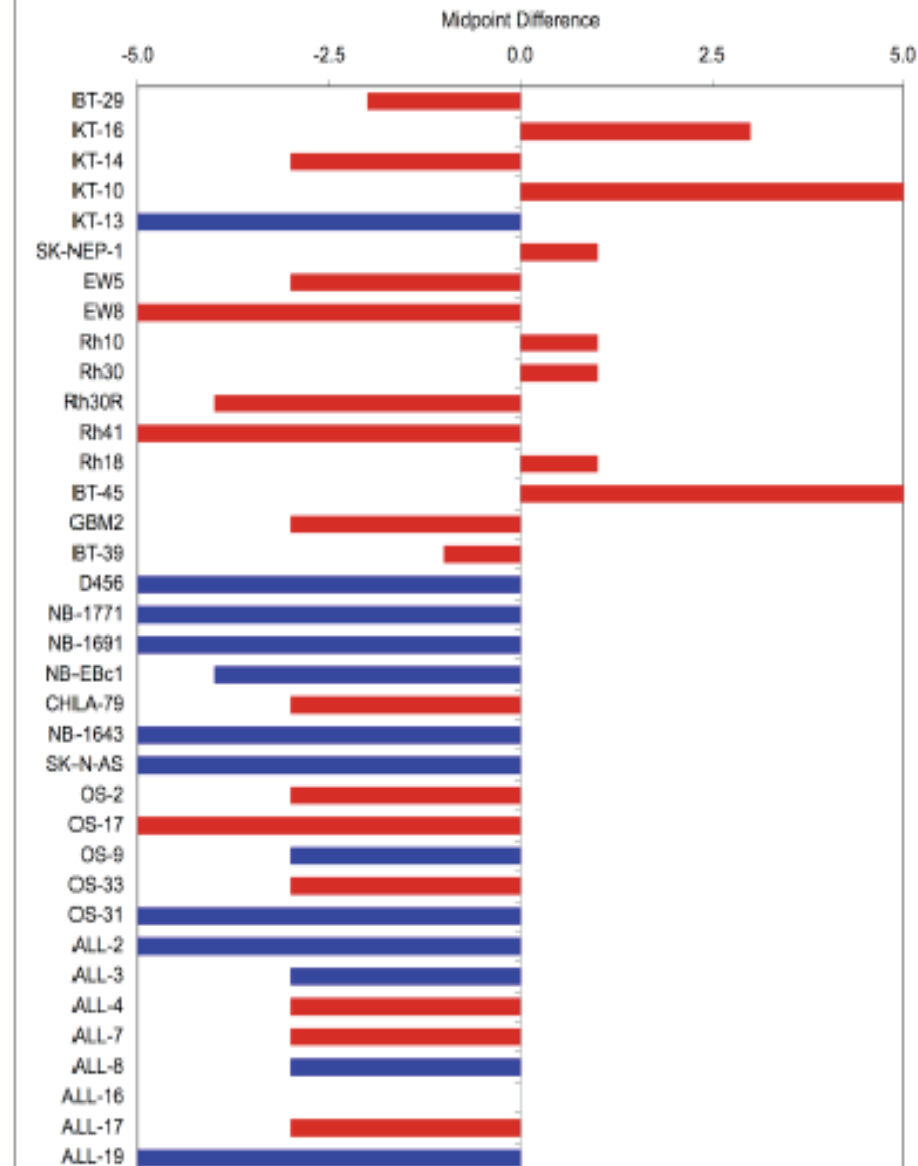


Cisplatin and BMN 673 Single Agent in Vivo Activity

BMN 673

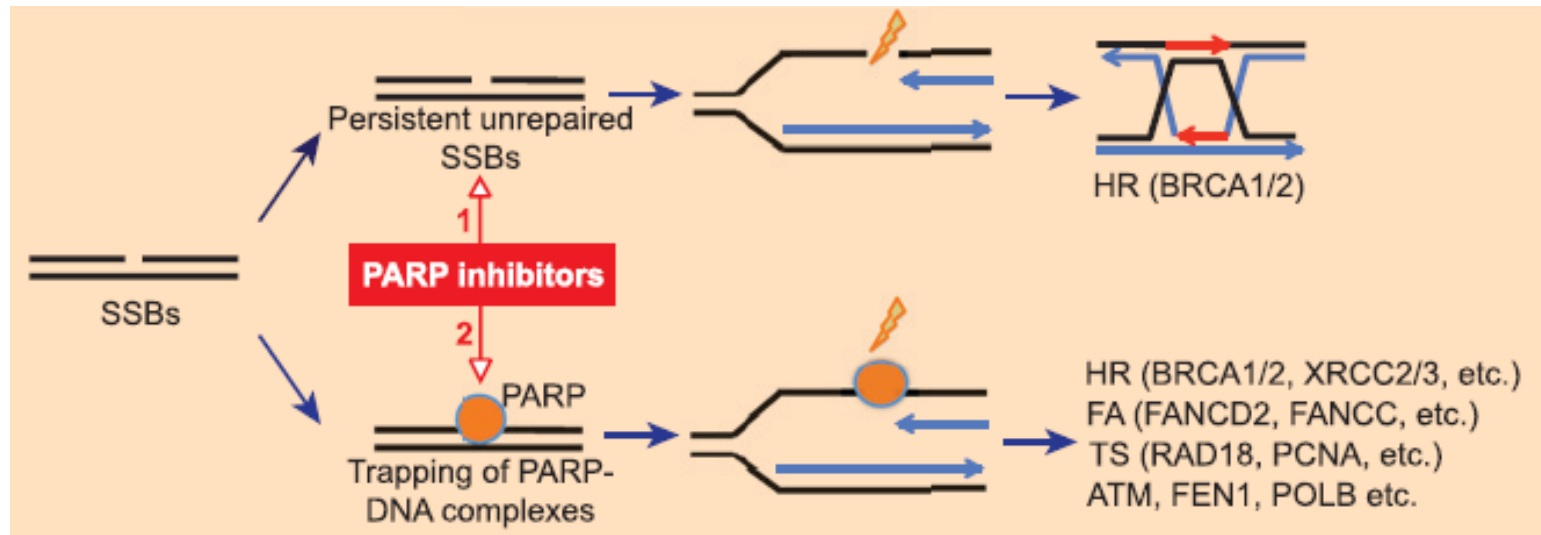


Cisplatin

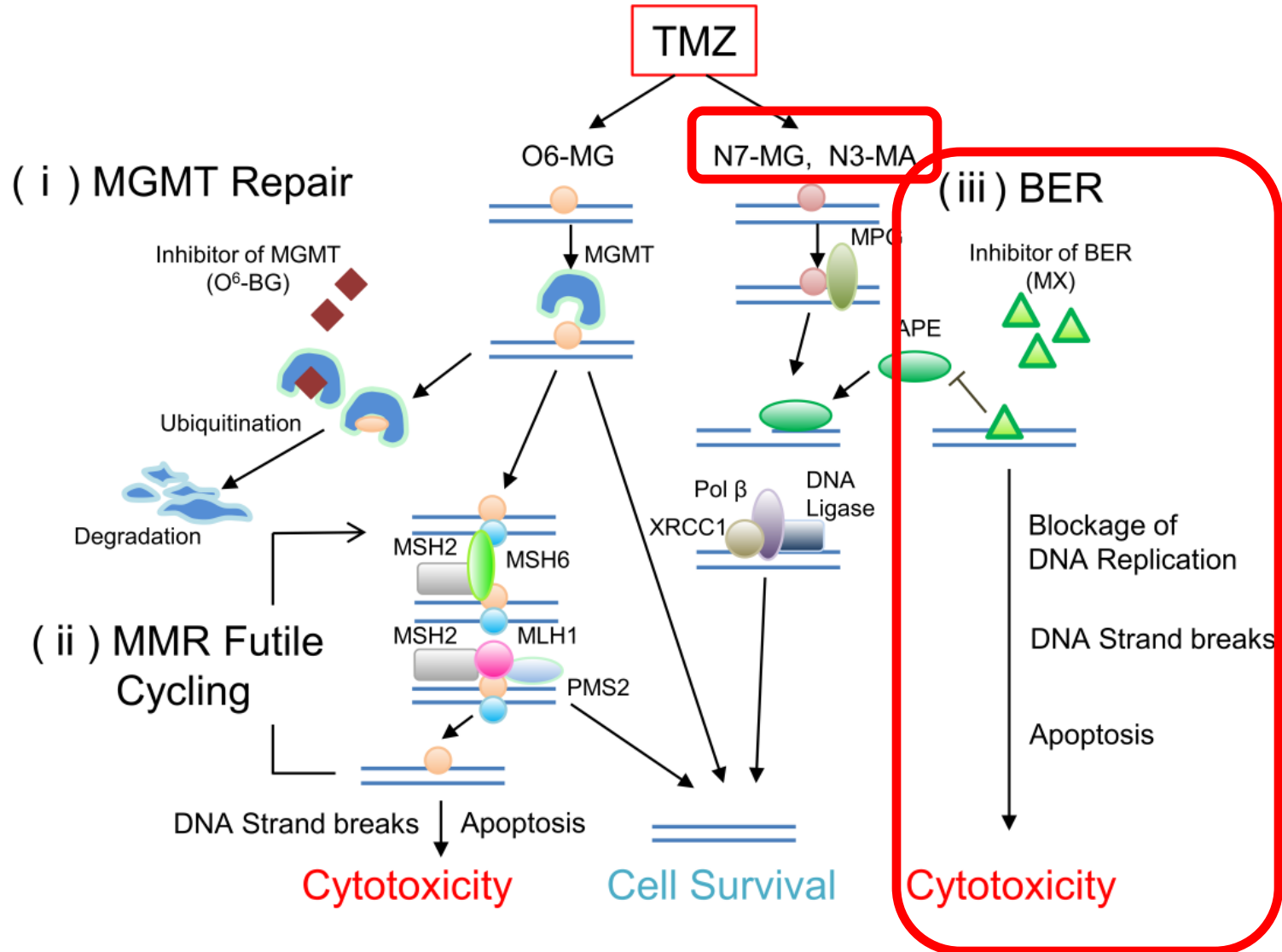


Dual Cytotoxic Mechanisms of PARP Inhibitors

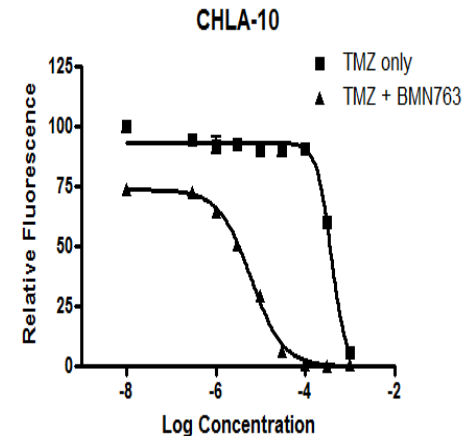
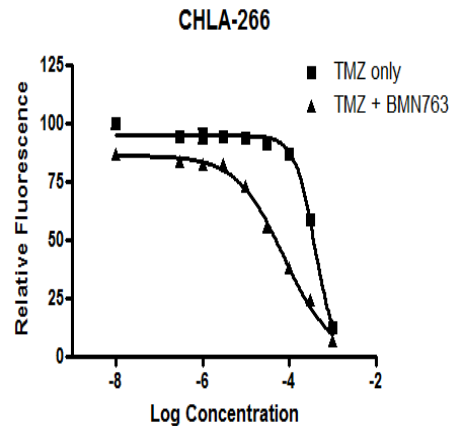
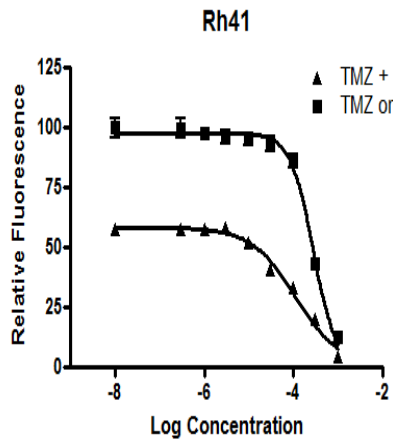
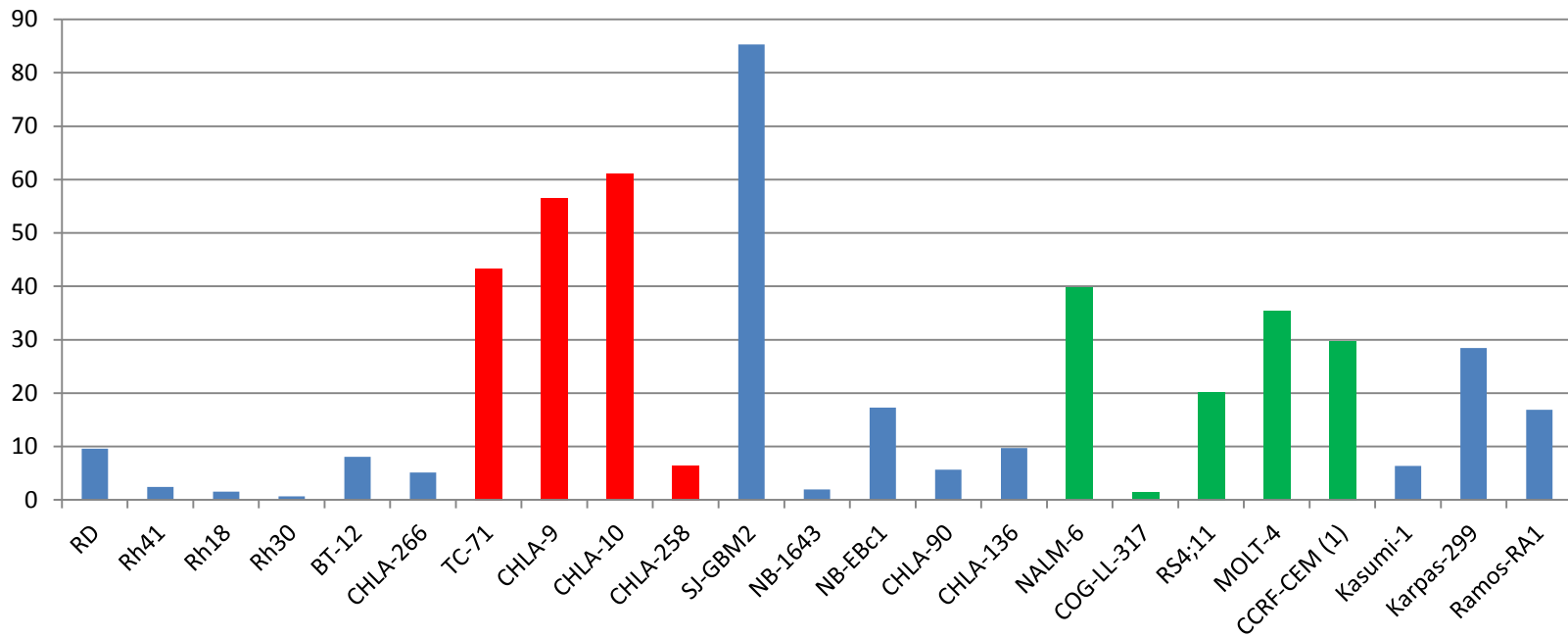
- Catalytic inhibition (upper pathway) interferes with the repair of SSBs, leading to replication fork damage that requires HR repair.
- Trapping of PARP–DNA complexes also leads to replication fork damage but uses additional repair pathways including Fanconi pathway (FA), template switching (TS), ATM, FEN1 (replicative flap endonuclease), and polymerase β .



PARP Inhibitors Converting TMZ-Induced N7-MG and N3-MA into Lethal Lesions



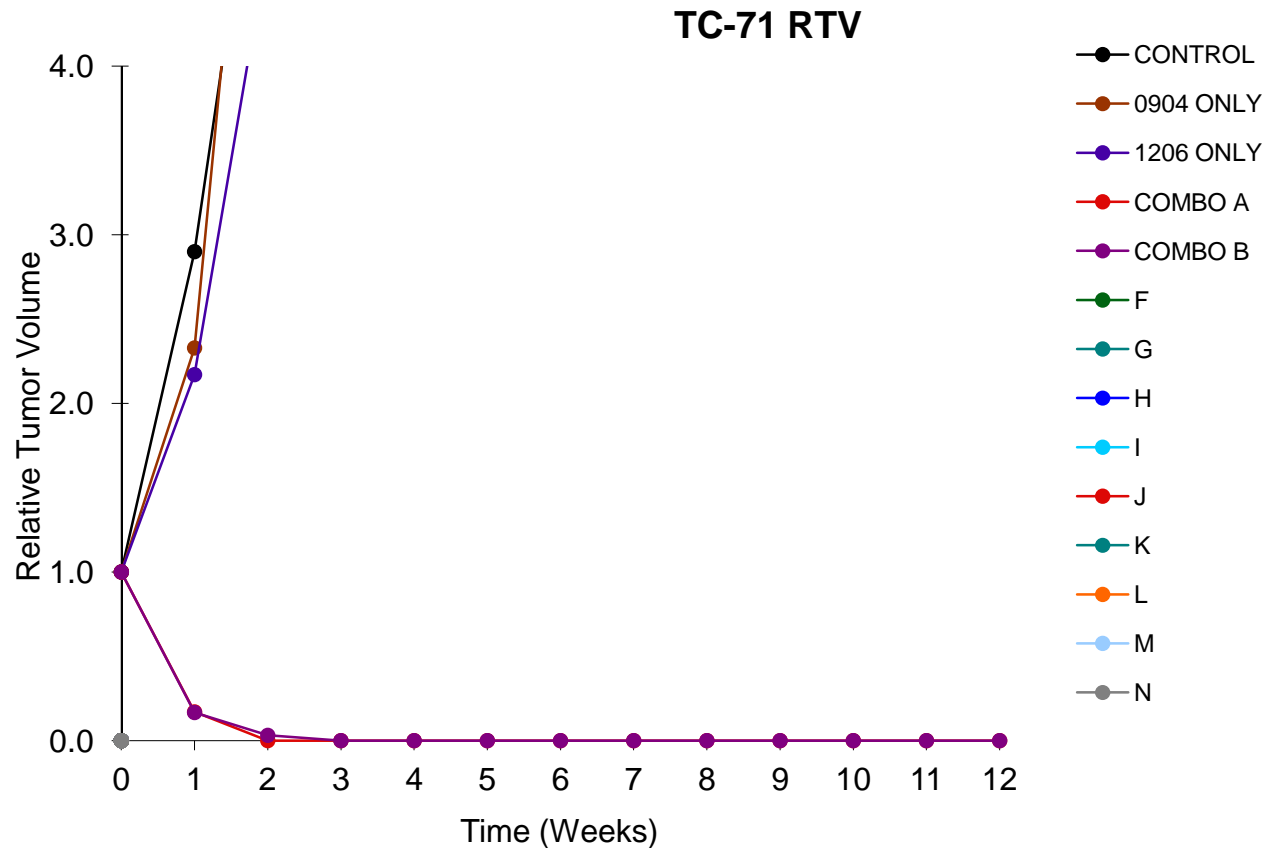
Fold Potentiation of TMZ IC_{50} Values by BMN 673 (10 nM)



Legend for TMZ + BMZ673 Combination Studies (Dose/Schedules)

- **0904 Only**: Temozolomide (TMZ) at 30 mg/kg/dose daily x 5 days
- **1206 Only**: BMN 673 at 0.25 mg/kg/dose BID x 5 days
- **Combo A (High-dose TMZ)**: TMZ at 30 mg/kg/dose daily x 5 days plus BMN 673 at 0.1 mg/kg/dose BID x 5 days
- **Combo B (High-dose BMN 673)**: TMZ at 12 mg/kg/dose daily x 5 days plus BMN 673 at 0.25 mg/kg/dose BID x 5 days

TC-71 (Ewing Sarcoma): Response to BMN 673 and Temozolomide



- Pediatric phase 1 trial of BMN 673 plus low dose temozolomide in development.

Agents with Limited Tumor-Regression Activity against Pediatric Preclinical Models

- Notch pathway inhibitors (GSI)
- Hsp90 inhibitors
- Ibrutinib for B-precursor ALL
- MEK inhibitors (excepting LGA)
- AKT inhibitors
- TOR kinase inhibitors
- Bcl-2 inhibitors (solid tumors)
- Arsenic trioxide (Ewing sarcoma)
- Cytarabine (Ewing sarcoma)

Contract (RFP) vs Cooperative Agreement (RFA)?

- The RFP mechanism initially selected because the objective of the PPTP:
 - to systematically perform testing of selected agents using a standard testing protocol and
 - to quickly make these data available to the childhood cancer research community.
- Given this objective, a contract mechanism was felt to be appropriate and most conducive to maintaining the tight timelines required for a large in vivo testing program testing up to 10 agents per year.

Individually Competing Each Tumor Testing Site?

- The advantage of this approach is enhanced competition.
- The challenge is that the program requires a considerable degree of central coordination (e.g., agent distribution, information distribution, data analysis, preparation of reports, etc.).
- Need to consider mechanism for supporting both coordination activity and competition of individual sites.

Future Plans

- Enhancing capabilities for evaluating CNS tumors
- Increasing efficiency and economy:
 - More selective testing based on molecular characterization
 - Consolidation of non-CNS solid tumor testing sites
- Enhancing options for output of data for bioinformatic analysis for non-PPTP researchers
- Increased focus on combination testing
- Evaluating pediatric specific agents

PPTP Funding History

Funding History Obligation by Year

FY2010	\$2,938,868
FY2011	\$2,791,925
FY2012	\$2,700,000
FY2013	\$2,700,000
FY2014	\$ TBD

Conclusion

- PPTP is unique resource
- PPTP activities not replicated within industry or academia
- PPTP results enhance efficiency of childhood cancer clinical research:
 - Limiting lines of nonproductive research
 - Focusing attention on promising areas
- More than ever reliable and robust preclinical data are needed given the broad range of potential therapeutic agents and the increasing challenges associated with clinical development of agents for children with cancer

Back-up Slides

Increasing Competition in Site Selection

- Are best sites / best models being employed for testing for each disease panel??
- Overall contract is an open competition
- Requirement for applicant to describe selection process for subcontracts and include:
 - Solicitation for subcontract sites
 - Criteria for selection of sites
 - Review and selection process
- Annual review of sites by External Advisory Committee and NCI with option for requiring change in testing sites

Preclinical-Clinical Comparisons

- Dasatinib is only active in vivo at standard doses against a BCR-ABL ALL xenograft.
- Gamma-secretase inhibitors that block Notch pathway signaling are ineffective against solid tumor models as well as against T-ALL xenografts with Notch1 mutations.
- Standard agents such as vincristine, cyclophosphamide, and topotecan show patterns of activity that are consistent with their major clinical patterns of activity.
- Monoclonal antibodies to IGF-1R induce regressions as single agents against a minority of Ewing sarcoma xenografts.
- The MEK inhibitor selumetinib is effective against BRAF-mutated low-grade astrocytoma.
- The addition of rapamycin to standard chemotherapy agents (a vinca alkaloid and cyclophosphamide) is more effective than chemotherapy alone for rhabdomyosarcoma.

PPTP Combination Testing

- Therapeutic enhancement: combination significantly better than either single agent used at their MTD
- mTOR inhibitor plus standard cytotoxic agents.
 - Therapeutic enhancement commonly observed for cyclophosphamide (CPM) and vincristine
 - Able to give each at their single agent MTDs with rapamycin
- PPTP results led to COG ARST0921 randomized phase 2 clinical trial for children with relapsed RMS in 1st relapse.
 - Vinorelbine/CPM plus either temsirolimus or bevacizumab

Published Online First on January 6, 2010 as 10.1158/1535-7163.MCT-09-0952

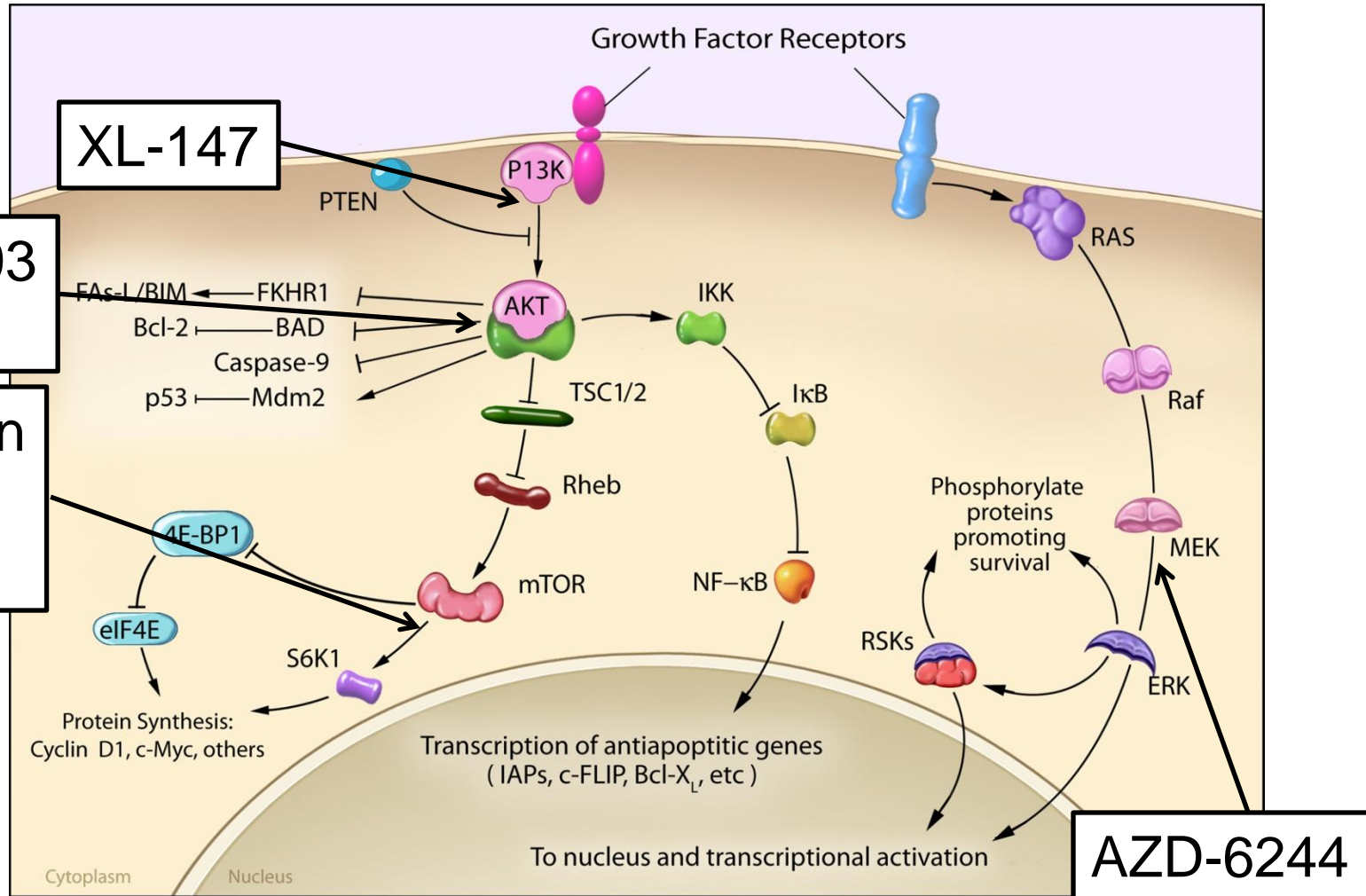
Research Article

Stage 2 Combination Testing of Rapamycin with Cytotoxic Agents by the Pediatric Preclinical Testing Program

Peter J. Houghton¹, Christopher L. Morton², Richard Gorlick³, Richard B. Lock⁴, Hernan Carol⁴, C. Patrick Reynolds⁵, Min H. Kang⁵, John M. Maris⁶, Stephen T. Keir⁷, E. Anders Kolb⁸, Jianrong Wu², Amy W. Wozniak², Catherine A. Billups², Larry Rubinstein⁹, and Malcolm A. Smith¹⁰

Molecular
Cancer
Therapeutics

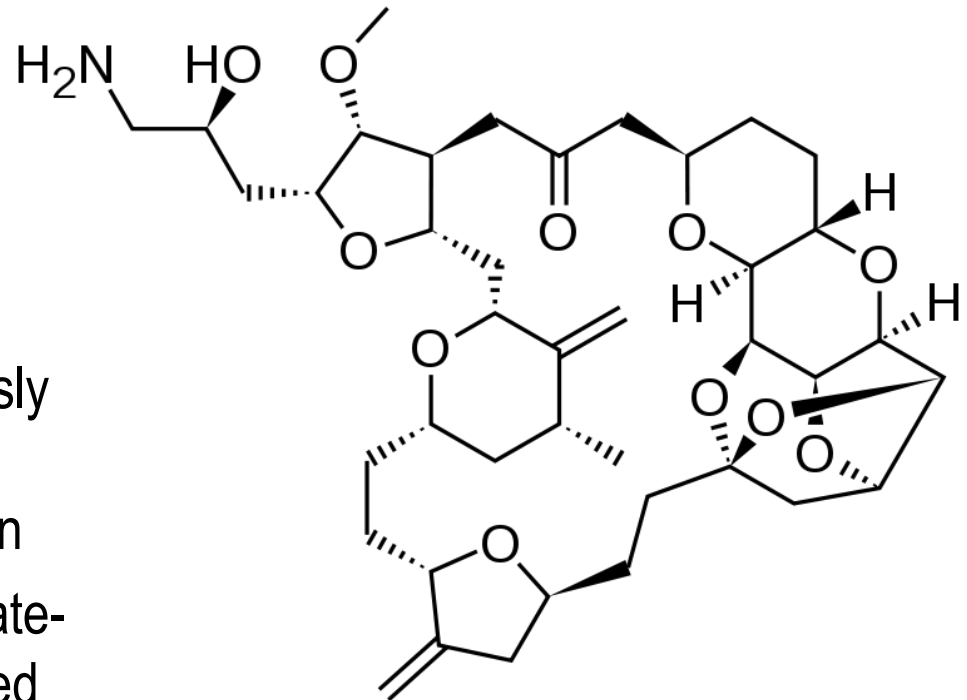
Inhibitors of the PI3K and MAPK Pathways



Eribulin (novel tubulin-binding agent)

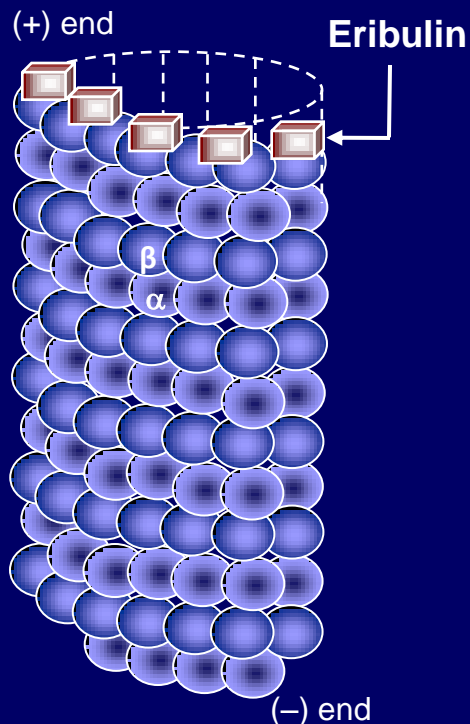
Eribulin Mesylate

- Synthetic analogue of halichondrin B
- Microtubule inhibitor with a binding site different from current agents
- Administered intravenously without reconstitution as a 2 - 5–minute infusion
- Approved in the US for late-line treatment of advanced breast cancer

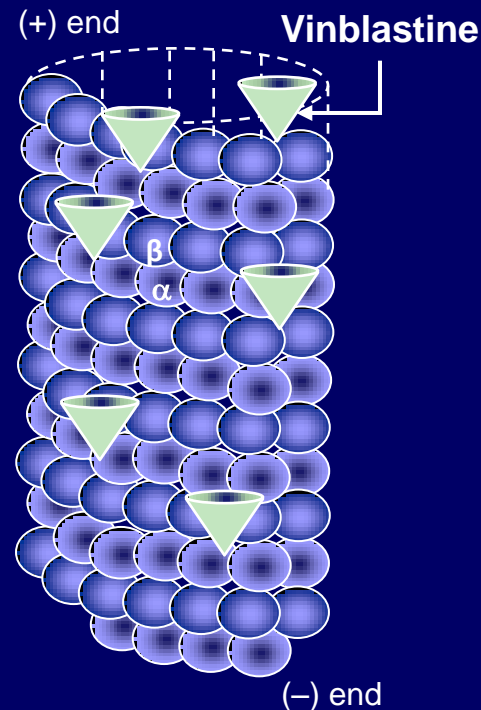


Eribulin Binding Site Differs From Other Microtubule Inhibitors

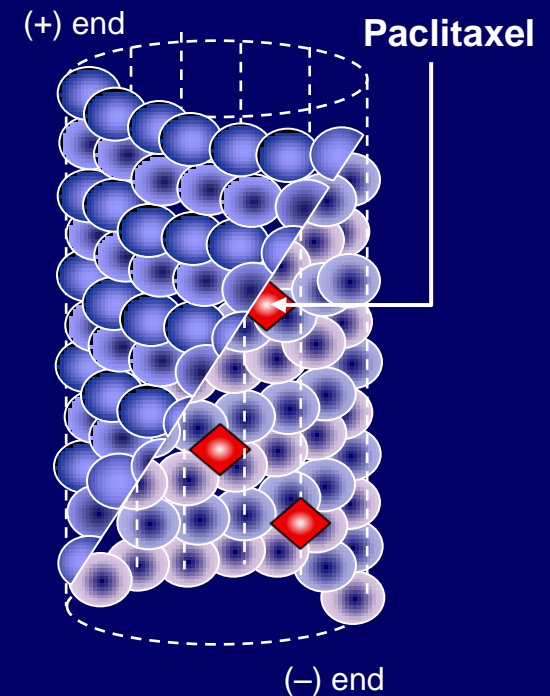
- Eribulin binds to (+) ends



- Vinblastine binds to (+) ends and along sides



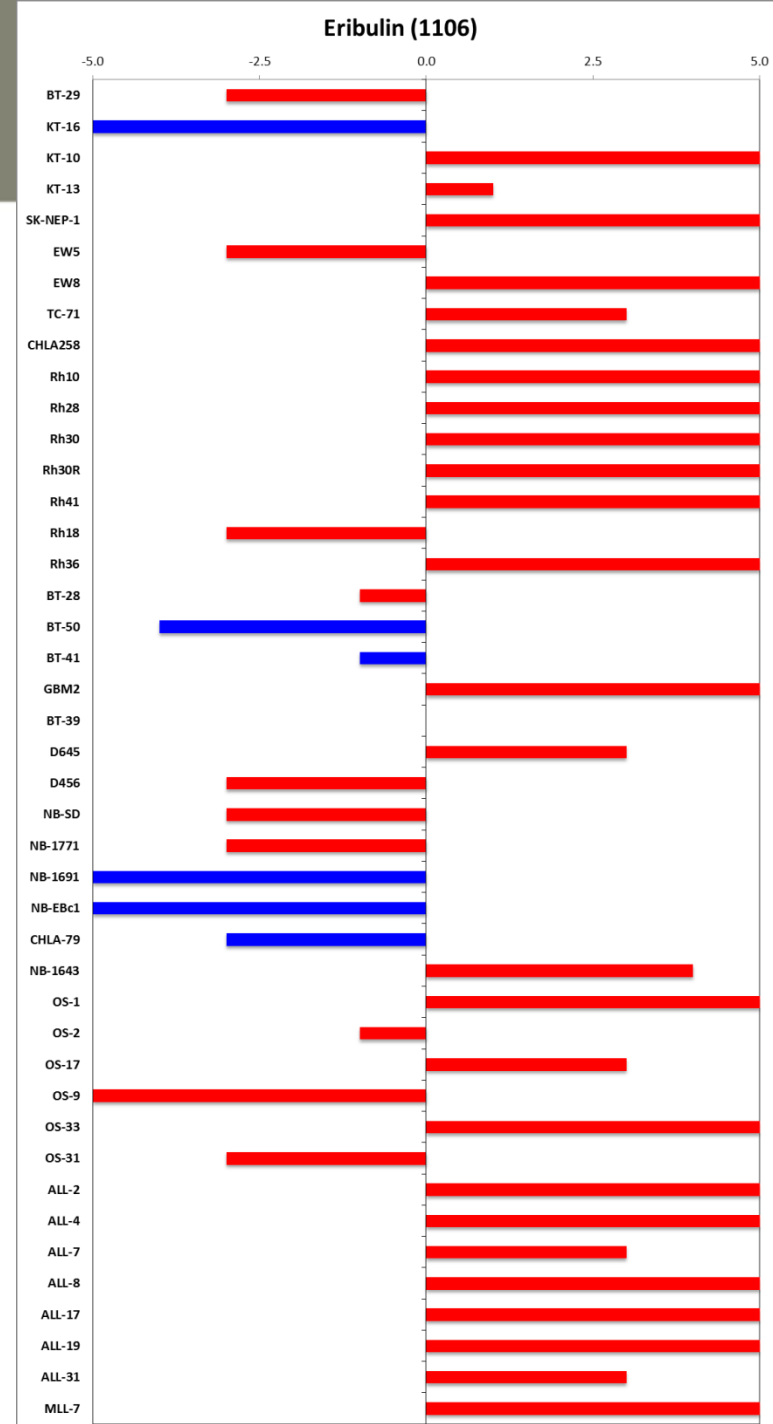
- Paclitaxel and docetaxel bind to β subunits at inside surface



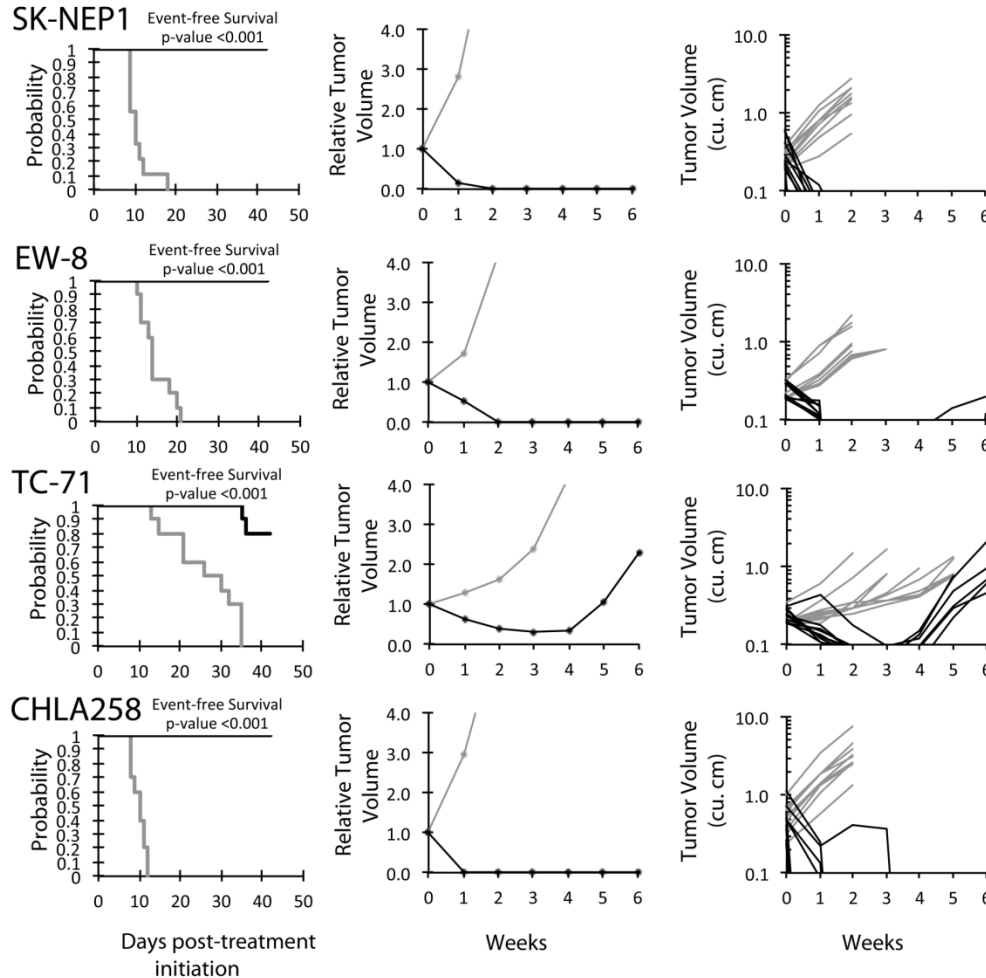
Eribulin is active against drug-resistant cells that harbor β -tubulin mutations associated with taxane resistance.

Eribulin in Vivo Activity

- 24 of 30 (80%) solid tumor models evaluable for the EFS T/C activity metric demonstrated EFS T/C > 2.0, with 7 lines showing intermediate activity and 17 showing high activity.
- CR/MCRs:
 - 4 of 5 evaluable Ewing xenografts,
 - 6 of 7 RMS xenografts,
 - 2 of 4 glioblastoma xenografts, and
 - 3 of 6 evaluable osteosarcoma xenografts.
 - 8 of 8 ALL



Examples of Eribulin Activity Against Ewing Sarcoma Xenografts



- Used 1 mg/kg Q4D x 3 schedule

Mouse versus Human Systemic Exposures

- Comparison of mouse PK (1 mg/kg IP) and human PK (1.4 mg/m² IV)

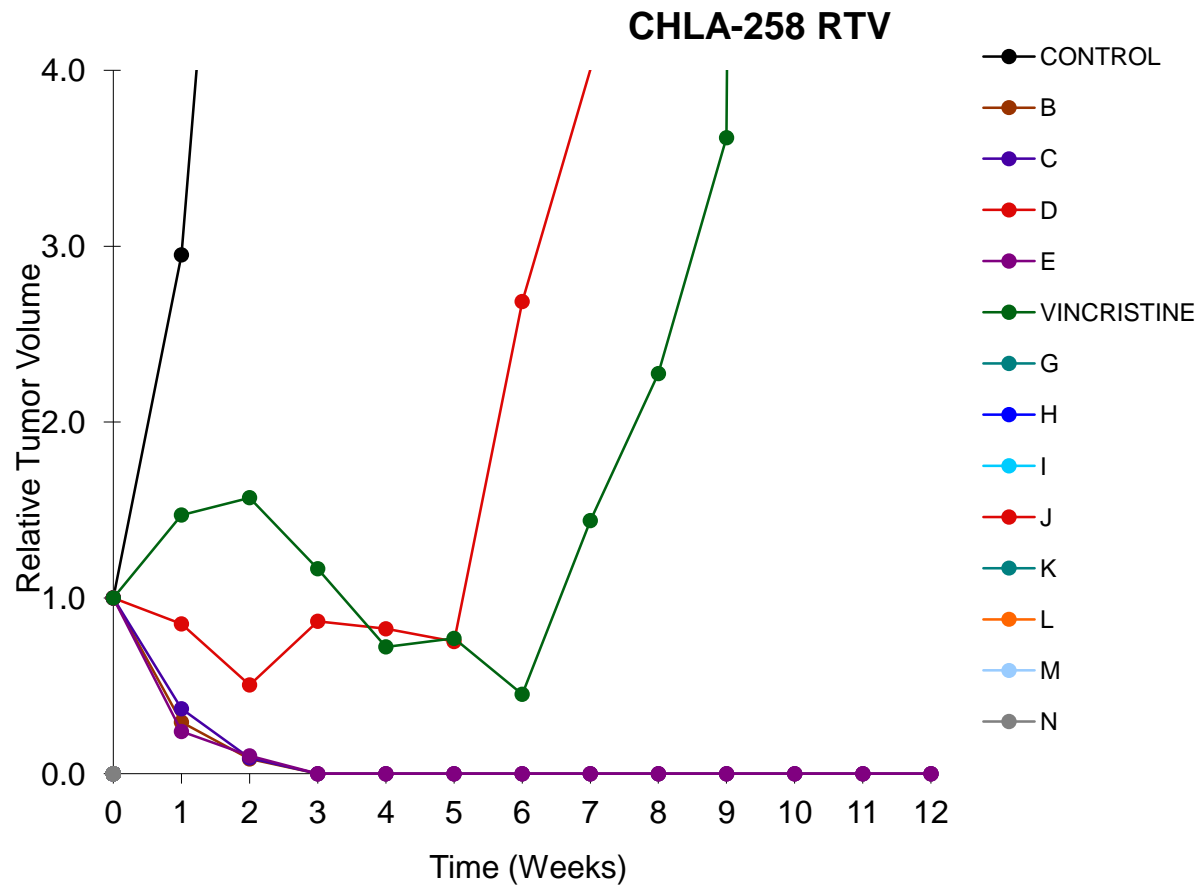
- Mouse

Dose (mg/kg)	Route	Tissue	Cmax (ng/mL)	Cmax/D (ng/mL/D)	tmax (h)	t1/2 (h)	AUC0-t (ng·h/mL)	AUC0-inf (ng·h/mL)	AUC0-inf/D (ng·h/mL/D)
1	i.p.	plasma	1032.354	1032.354	0.167	3.76	651.627	657.629	657.629

- Human

Study	N	AUC (ng·hr/ml)
Goel (1)	9	856
Devriese (2)	9	971
Devriese (3)	11	757
Devriese (4)	6	600
Mukohara (5)	6	673
Weighted average	41	790

Eribulin Dose-Response

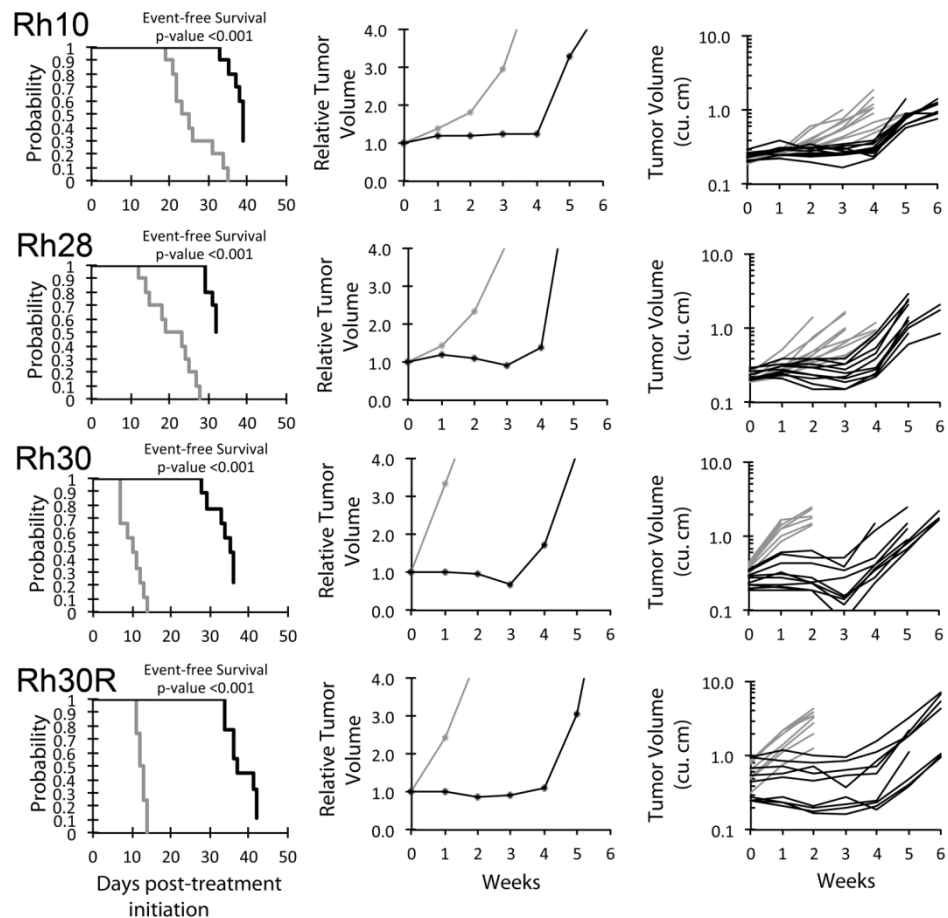
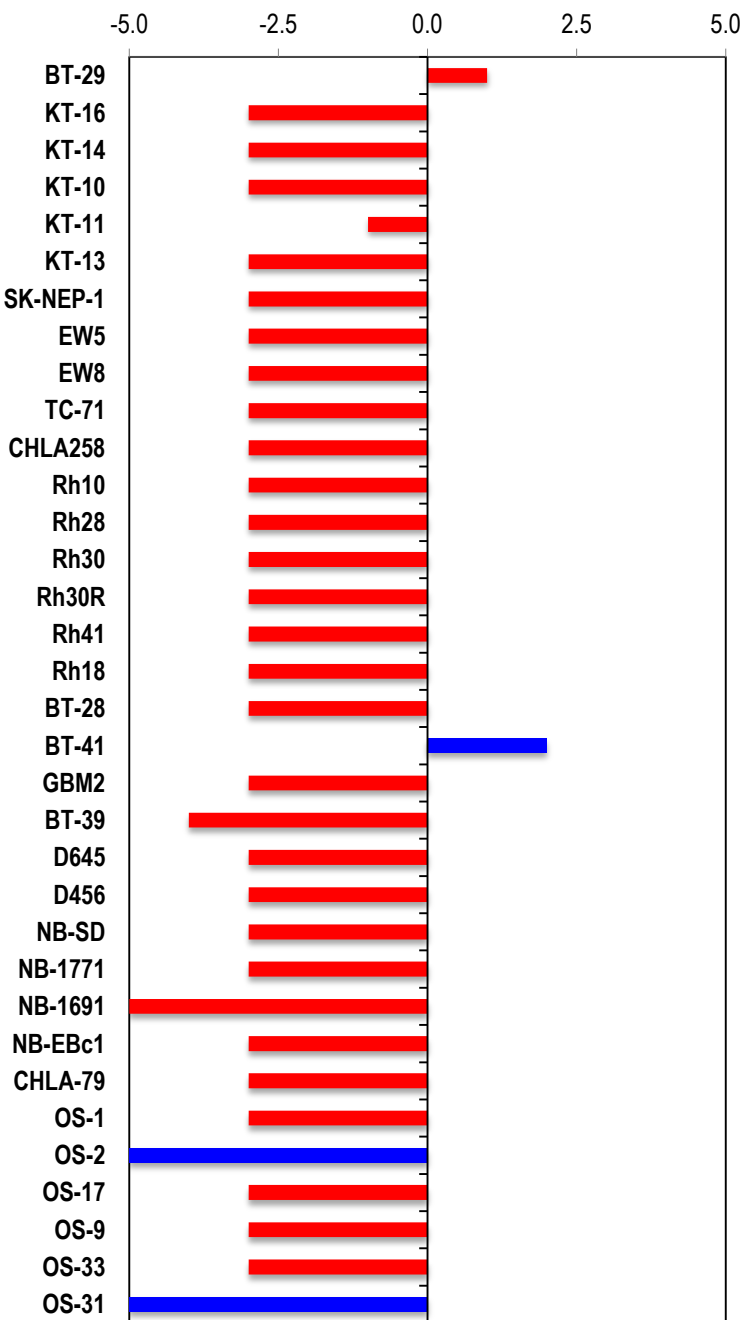


- **B** = 1 mg/kg q7d X 3 **C** = 0.5 mg/kg q7d X 3
- **D** = 0.25 mg/kg q7d X 3 **E** = 1 mg/kg q7d X 2
- **Vincristine** = 1 mg/kg weekly x 6

VEGFR2-Targeted Agents

Cabozantinib (1112)

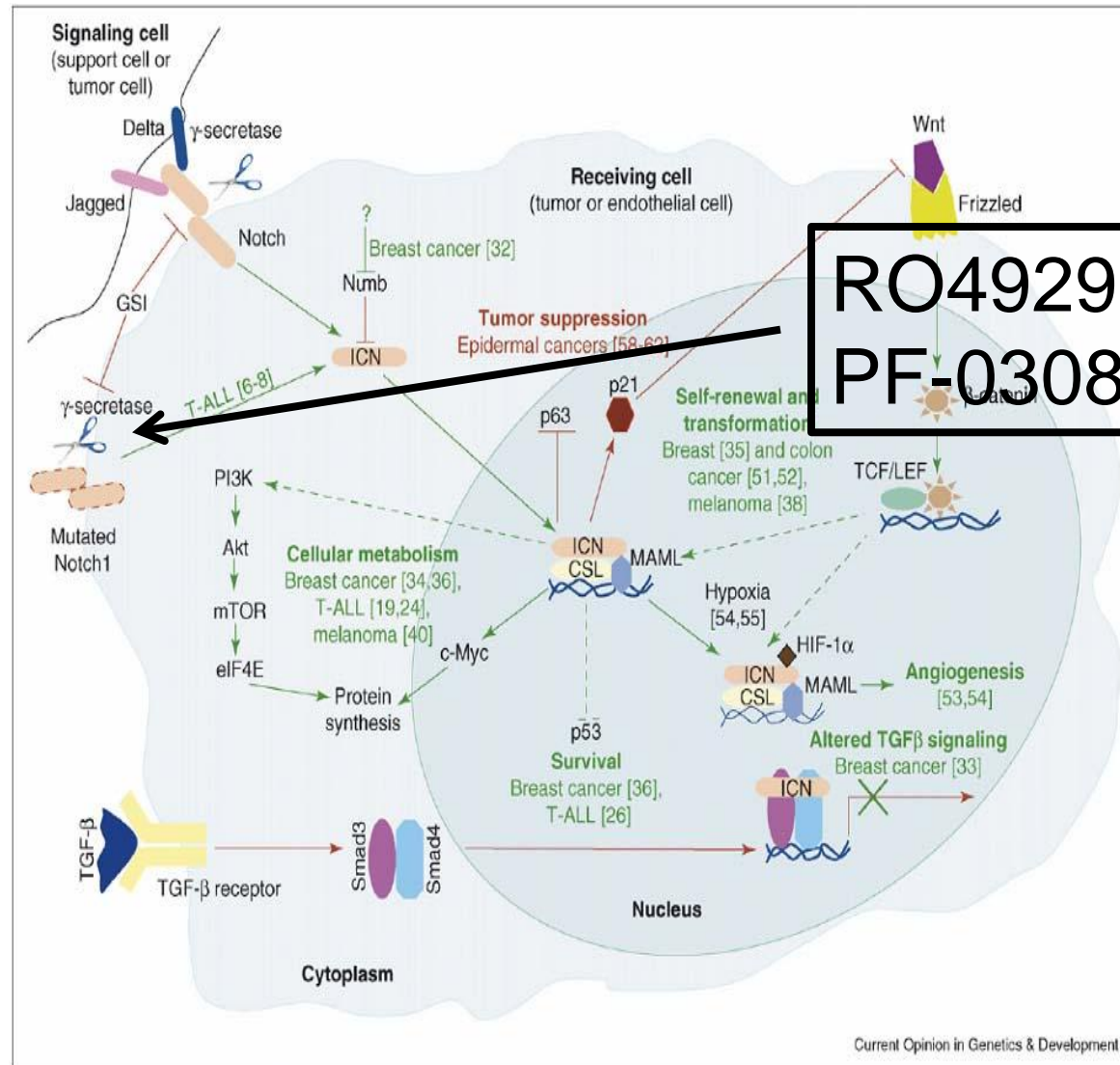
Cabozantinib in Vivo Results



Dose/Schedule: 30 mg/kg x 21 days

Notch Pathway Inhibitors

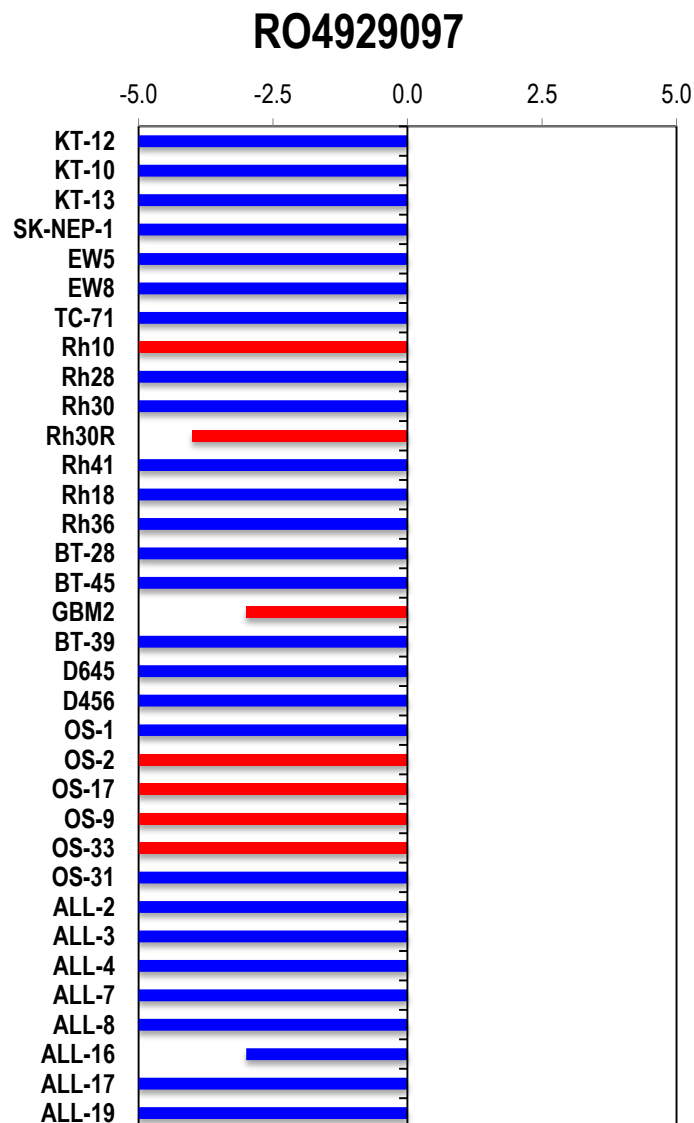
Notch Pathway Activation and T-cell ALL



RO4929097
PF-03084014

Limited in Vivo Activity for Notch Inhibitors

- Lack of in vivo activity against PPTP xenografts for the gamma-secretase inhibitor (GSI).
- Tested second GSI (PF-03084014) and observed little activity against multiple T-cell ALL xenografts with Notch1 mutations.



Antibody-Drug Conjugates

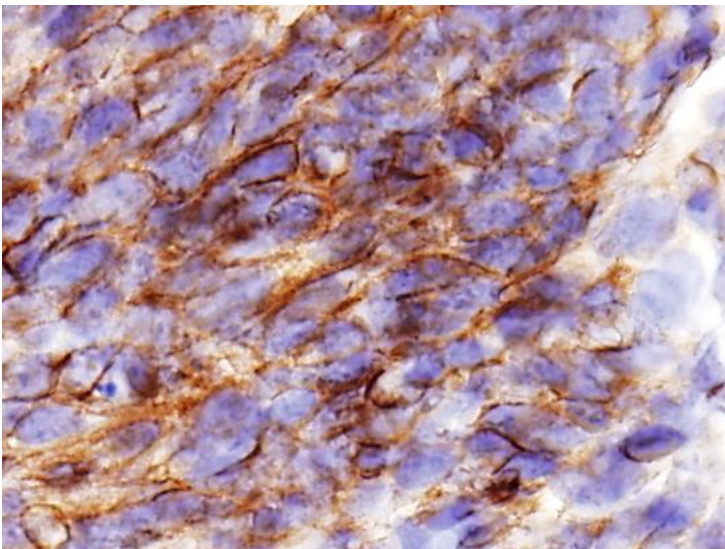
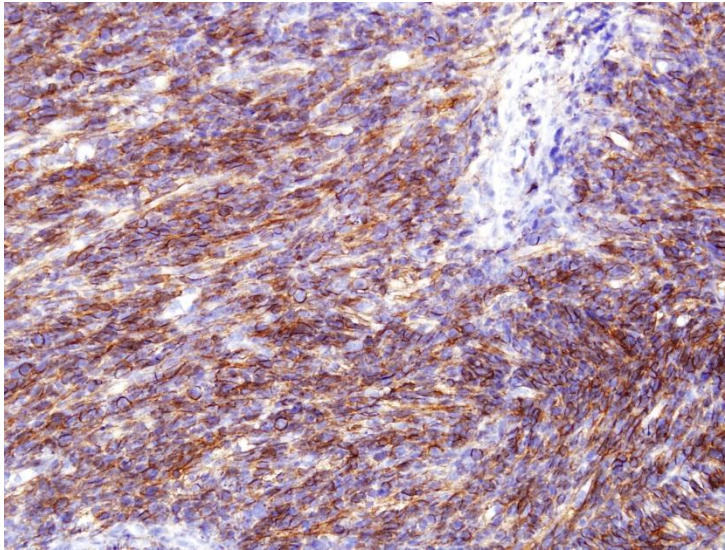
CD56 Expression on NCI Pediatric Tumor Xenografts

Tumor Line	N	<u>IHC Score</u>				
		3+ - 3 Homo	3 Hetero	2-3 Hetero or Homo	< 2 Hetero	0
Brain	9	4	0	3	1	1
Kidney*	4	3	0	1	0	0
Neuroblastoma	7	5	1	1	0	0
Osteosarcoma	4	0	2	1	1	0
Rhabdomyosarcoma	7	5	0	2	0	0
Totals	31	17	3	8	2	1

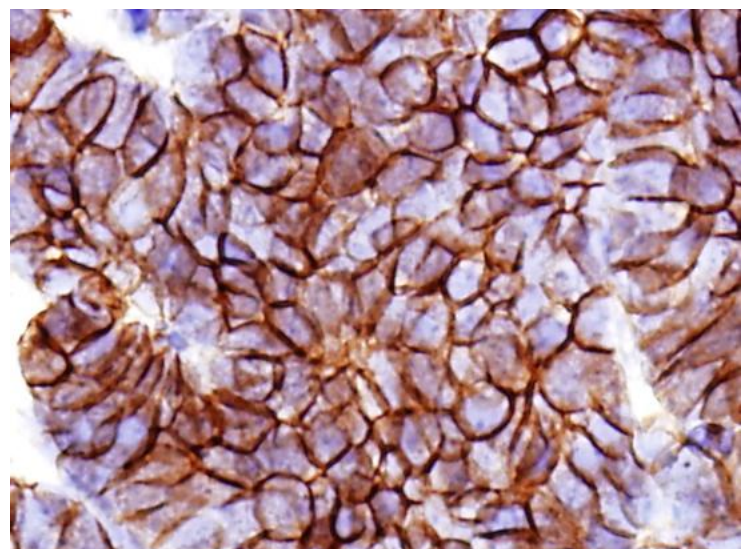
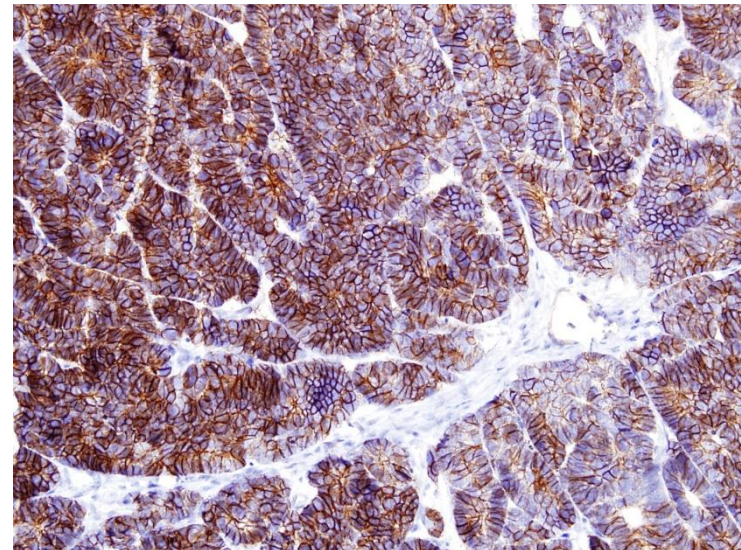
*3 of 3 Wilms tumor xenografts 3-3+ homogeneous staining

CD56: Homogeneous Staining Pattern

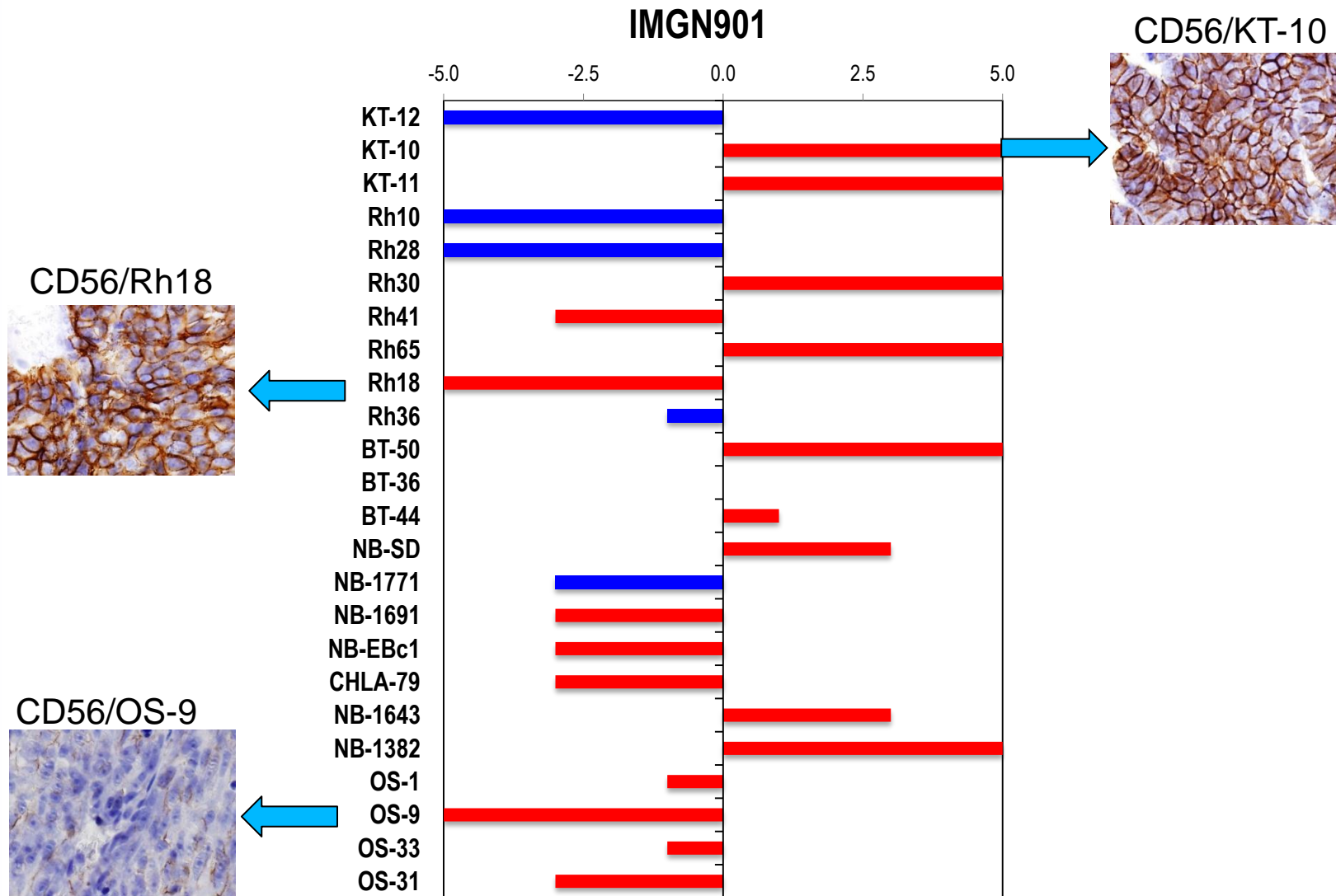
- **KT-5**



KT-10



IMGN901 Response and CD56 Expression



- Each of the 9 xenografts achieving an objective response showed homogeneous staining by IHC for CD56 with expression levels of 2-3, 3 or 3+.

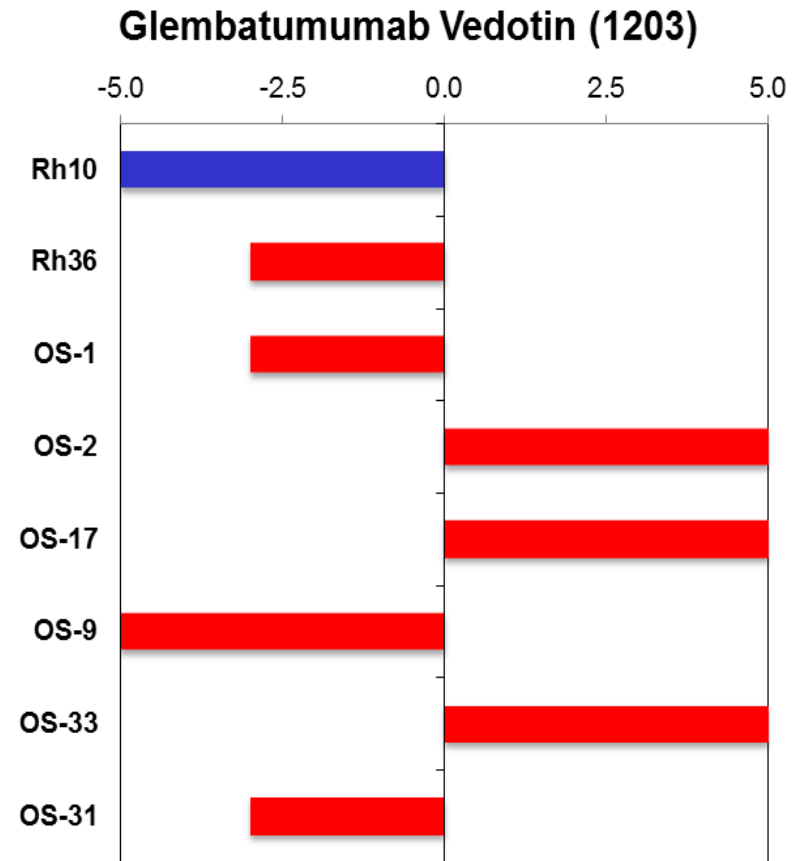
Antibody-Drug Conjugates

GPNMB as a Cancer Therapy Target

- Over-expressed in a number of cancer types
 - Melanoma, breast cancer, NSCLC, lymphoma
- Overexpression correlated with poor prognosis in breast cancer
 - High tumor expression of GPNMB specifically correlated to poor prognosis in TNBC
- Membrane expression accessible to antibody therapy, efficiently internalized for antibody-drug-conjugate approaches
- Glembatumumab vedotin is ADC targeting GPNMB expressing cancers under development by Celldex

Glembatumumab Vedotin

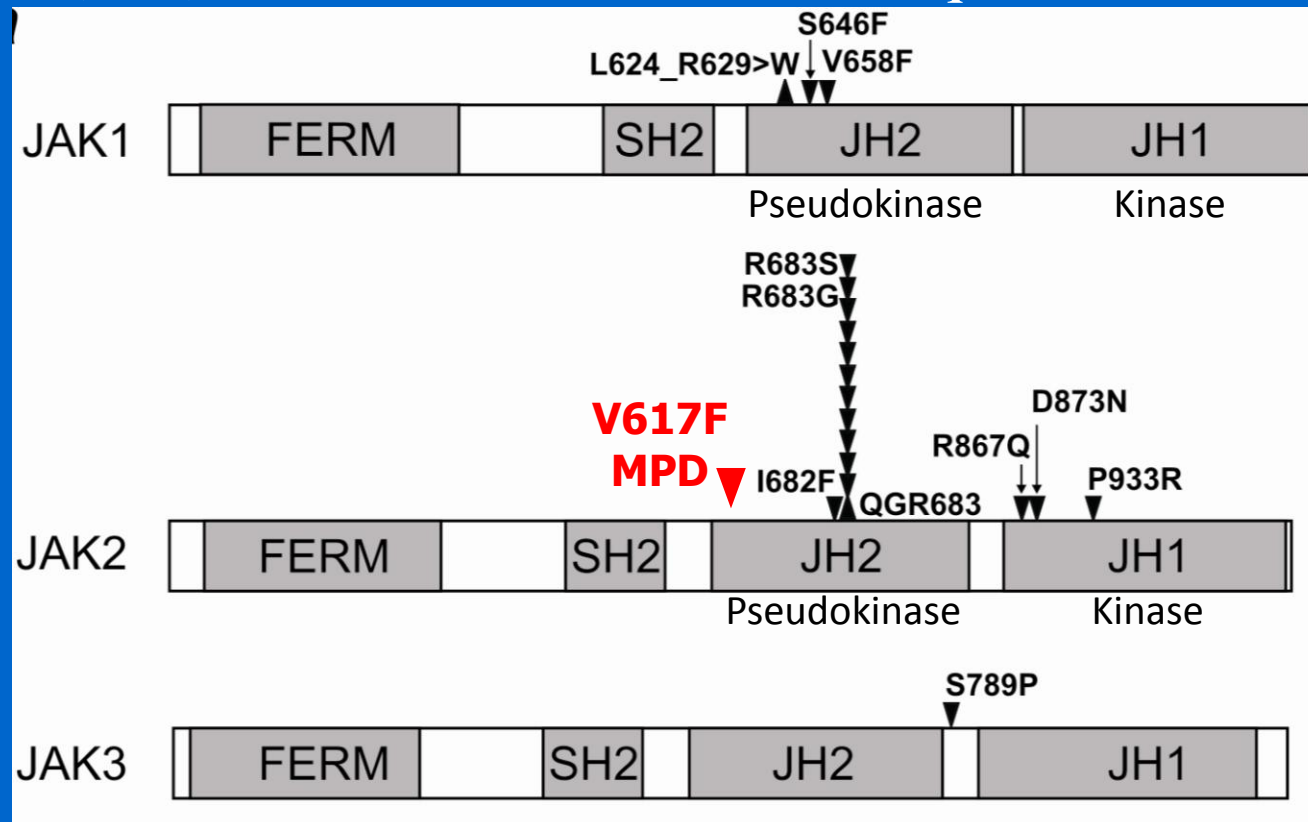
- An antibody-auristatin conjugate that targets cells expressing GPNMB.
- Glembatumumab vedotin induces remissions in GPNMB-expressing osteosarcoma, but not in rhabdomyosarcoma.
- Pediatric clinical trial being planned for patients with osteosarcoma.



JAK Inhibitors

JAK mutations in “BCR-ABL1-like” ALL

- JAK2 (n=16): 10 R683G; 3 non-R683G pseudokinase domain; 3 kinase domain
- JAK1 (n=3): 3 pseudokinase domain
- JAK3 (n=1): uncertain functional consequences



Testing JAK-STAT Pathway Inhibitors for ALL

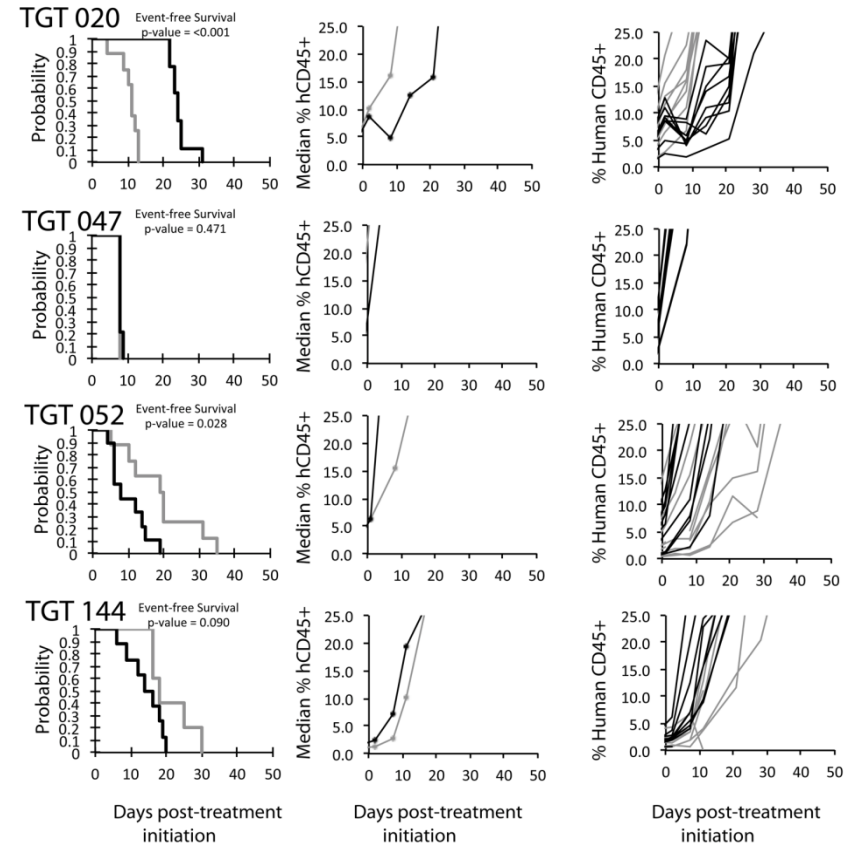
- Pick ALL models with relevant mutations from xenografts established by direct transplantation into NOD-SCID mice

ALL-10 (JAK1 V658)	TARGET-047 (JAK2 R683)
TARGET-144 (JAK1 L624)	TARGET-020 (JAK2 R867)
TARGET-038 (JAK2 I682)	TARGET-174 (JAK2 P933)

- Evaluate role of different mutations in effecting response to therapy
- Illustrates the emerging “standard of care” for evaluating molecularly targeted agents

Going against the Paradigm: Limited activity of JAK inhibitor against JAK-mutated ALL xenografts

- AZD1480 evaluated against 6 ALL xenografts with JAK1 or JAK2 mutations
- No objective responses (CR or PR) observed
- Similar results observed for ruxolitinib by different research team.
- Similar to lack of effect of JAK inhibitors on MPN malignant clone.
- JAK-translocations potentially different in their response to JAK inhibitors.



Criteria for Agents for PPTP Evaluation

- The agent should generally be one for which clinical testing in children is considered a potential priority, with testing able to begin within 12 to 24 months. Satisfactorily addressing this criterion will generally imply an active development plan for the agent for adult cancers and a willingness to consider pediatric evaluations of the agent.
- The agent should have plausible relevance to the treatment of childhood cancers, based on current understanding of the mechanism of action of the agent and current understanding of the biology of childhood cancers.
- Agents with molecular targets or mechanisms of action that have not been previously addressed by the PPTP will be prioritized higher than agents whose molecular targets have previously been addressed by the PPTP.
- Sufficient quantity of agent available for testing.

Sensitivity, Specificity, and Prevalence

- Assume 10% prevalence of true actives
- Negative test results are likely to be true
- Increasing sensitivity & specificity leads to increased probability of success for positive result.
- False positives remain relatively common even with reasonably reliable testing program.

	Sensitivity	Specificity	PPV	NPV
Scenario 1	50%	50%	10%	90%
Scenario 2	90%	20%	11%	95%
Scenario 3	80%	80%	31%	97%
Scenario 4	90%	90%	50%	99%

Two-Stage Process for Drug Evaluation

