

Towards Personalized Medicine: Establishment and In Vivo Characterization of a Low Passage Tumor Xenograft Predictive of Clinical Response

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ABSTRACT

Human tumor xenograft models originating from high passage, commercially available cell lines are routinely used in anticancer drug development. While information from these studies is often useful in a preclinical setting, clinical relevance is often limited likely due to genetic alterations and adaptations from successive, long-term in vitro and in vivo passages.

In an attempt to develop more clinically relevant preclinical models, we and others have successfully established low passage xenograft models from patient tumors engrafted into nude mice. However, whether these models are more predictive of clinical response is unclear. To address this question, we established a low passage tumor model from a non-small cell lung patient with an available clinical history which included platinum resistance. Based on these data, we carried out three in vivo studies evaluating past, current, and prospective therapies focused on targeted therapy combinations. Consistent with clinical results, the tumor model was insensitive to platinum regimens including cisplatin alone or an oxaliplatin/pemetrexed combination. However, cisplatin resistance was partially reversed with co-administration of the novel endoribonuclease, ranpirnase. Treatment with a sorafenib/bevacizumab regimen resulted in impressive tumor growth inhibition which was further enhanced with irinotecan including tumor regressions in half of the treated mice. Interestingly, the donor patient responded to this triple therapy with a 50% maximal remission and continued with stable disease on a sorafenib/bevacizumab regimen.

Taken, together, these results demonstrate this model as predictive for clinical response of this patient. In addition, these data suggest low passage xenograft models may serve as a useful tool to better predict human response to test agents in a preclinical setting. Additional studies are currently underway to further validate and refine the use of these models in oncology drug development.

MATERIALS AND METHODS

- Male CD-1 nude mice (Charles River) were subcutaneously implanted with 3x3 mm tumor fragments harvested from host animals. Once tumors reached a designated volume range, animals were pair-matched into control and treatment groups and dosing initiated; each subject was followed individually throughout the experiment.
- Mice were weighed and tumor measurements taken twice weekly by Vernier caliper.
- Tumor volume was calculated by conversion of 2-D caliper measurements using the equation:

$$\text{Tumor Volume (mm}^3\text{)} = \text{Width}^2 \text{ (mm)} \times \text{Length (mm)} \times 0.52$$

- At study completion, percent maximum weight loss (nadir) and drug-related deaths are reported; percent weight change versus Day 1 is followed throughout the study in control and treatment groups.
- At study completion, mean final tumor volume of each treated group was compared with vehicle control and a tumor growth inhibition (TGI) value calculated using the equation:

$$1 - \frac{(\text{Final Volume}-\text{Initial Volume (Treated)})}{(\text{Final Volume}-\text{Initial Volume (Control)})} \times 100$$

- Animals reporting partial or complete tumor responses (PR/CR) were not included in final TGI calculations; however, activity trend (AT) calculated at each tumor measurement for each treatment group included PR/CR animals. A tumor regression value (%TR) was reported for PR animal(s) while mice lacking palpable tumors (< 3x3 mm³) at study completion were designated CR.
- Statistical analyses was carried out between treated and control groups comparing final tumor volume using a two-tailed One-Way Analysis of Variance (ANOVA) followed by the Dunnett multiple comparisons test.

RESULTS

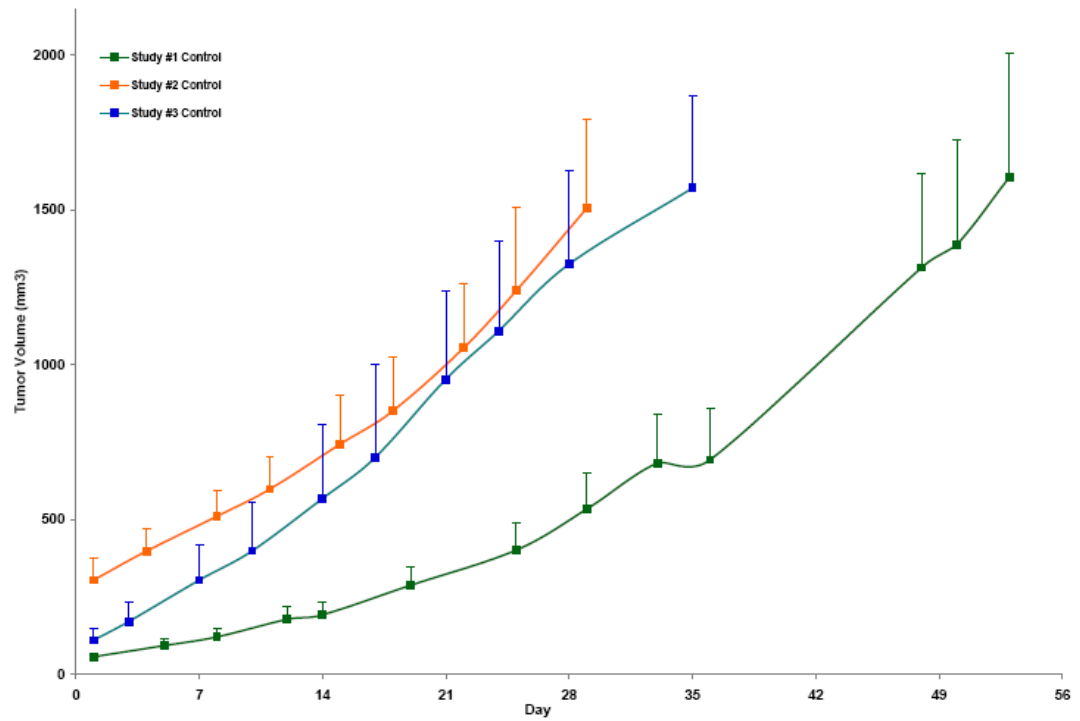
BML-1 Human NSCL Tumor Model (Study #1)												
GROUP	N	DOSE (MG/KG)	ROUTE	SCHEDULE	FINAL TUMOR VOLUME (MM ³)			WEIGHT NADIR		DRUG-RELATED DEATHS		
					MEAN ± SEM	%TGI	#PR/#CR	%TR	% CHANGE	DAY	TOTAL	DAY (#)
Control	9	No Treatment	--	--	1607 ± 398	--	--	--	--	--	0	--
Sorafenib	5	30 mg/kg	PO	QDx10	266 ± 87	86%	--	--	--	--	0	--
Bevacizumab		10 mg/kg	IP	Q3Dx10								
Irinotecan		10 mg/kg	IP	Q7Dx3								
Sorafenib	5	20 mg/kg	PO	QDx10	176 ± 28	92%	--	--	--	--	0	--
Bevacizumab		10 mg/kg	IP	Q3Dx10								
Sunitinib	5	15 mg/kg	PO	QDx21	299 ± 138	87%	--	--	--	--	0	--
Bevacizumab		10 mg/kg	IP	Q3Dx10								
Oxaliplatin	5	2.5 mg/kg	IP	QDx5	2350 ± 552	--	--	--	--	--	0	--
Pemetrexed		75 mg/kg	IP	QDx5								
Sirolimus	5	8 mg/kg	PO	QDx14	2194 ± 903	--	--	--	--	--	0	--
Erlotinib		15 mg/kg	PO	QDx14								

BML-1 Human NSCL Tumor Model (Study #2)												
GROUP	N	DOSE (MG/KG)	ROUTE	SCHEDULE	FINAL TUMOR VOLUME (MM ³)			WEIGHT NADIR		DRUG-RELATED DEATHS		
					MEAN ± SEM	%TGI	#PR/#CR	%TR	% CHANGE	DAY	TOTAL	DAY (#)
Control	4	No Treatment	--	--	1506 ± 287	--	--	--	--	--	0	--
Sorafenib	4	40 mg/kg	PO	QDx29	131 ± --	99%	2/0	76%	-6%	18	0	--
Bevacizumab		10 mg/kg	IP	Q3Dx10								
Sorafenib	4	80 mg/kg	PO	QDx29	150 ± --	96%	2/0	38%	-8%	11	0	--
Bevacizumab		10 mg/kg	IP	Q3Dx10								
Sunitinib	4	25 mg/kg	PO	QDx29	-- ± --	--	3/0	47%	-2%	11	0	--
Bevacizumab		10 mg/kg	IP	Q3Dx10								
Ranpirnase	4	2.5 mg/kg	IV	Q3Dx6	1174 ± 464	28%	0/0	--	-20%	18	0	--
Ranpirnase	4	2.5 mg/kg	IV	Q3Dx6	-- ± --	--	2/0	28%	-35%	22	2	11, 24
Sorafenib		40 mg/kg	PO	QDx29								
Ranpirnase	4	2.5 mg/kg	IV	Q3Dx6	-- ± --	98%	3/0	28%	-29%	18	0	--
Bevacizumab		10 mg/kg	IP	Q3Dx10								
Ranpirnase	4	2.5 mg/kg	IV	Q3Dx6	463 ± --	76%	2/0	52%	-38%	18	1	18
Cisplatin		3 mg/kg	IP	QDx5								
Ranpirnase	4	2.5 mg/kg	IV	Q3Dx6	-- ± --	--	0/0	--	-32%	11	4	4, 12, 12, 13
Docetaxel		13 mg/kg	IV	Q2Dx3								
Ranpirnase	4	2.5 mg/kg	IV	Q3Dx6	1200 ± 58	36%	0/0	--	-29%	18	2	1, 1
Irinotecan		100 mg/kg	IP	Q7Dx3								
Ranpirnase	4	2.5 mg/kg	IV	Q3Dx6	609 ± 144	71%	0/0	--	-32%	18	1	22
Cetuximab		10 mg/kg	IV	Q3Dx10								
Ranpirnase	4	2.5 mg/kg	IV	Q3Dx6	150 ± --	98%	0/0	--	-34%	18	3	9, 12, 19
Sunitinib		25 mg/kg	PO	QDx29								
Ranpirnase	4	2.5 mg/kg	IV	Q3Dx6	770 ± 176	61%	0/0	--	-29%	18	0	--
Erlotinib		30 mg/kg	PO	QDx29								

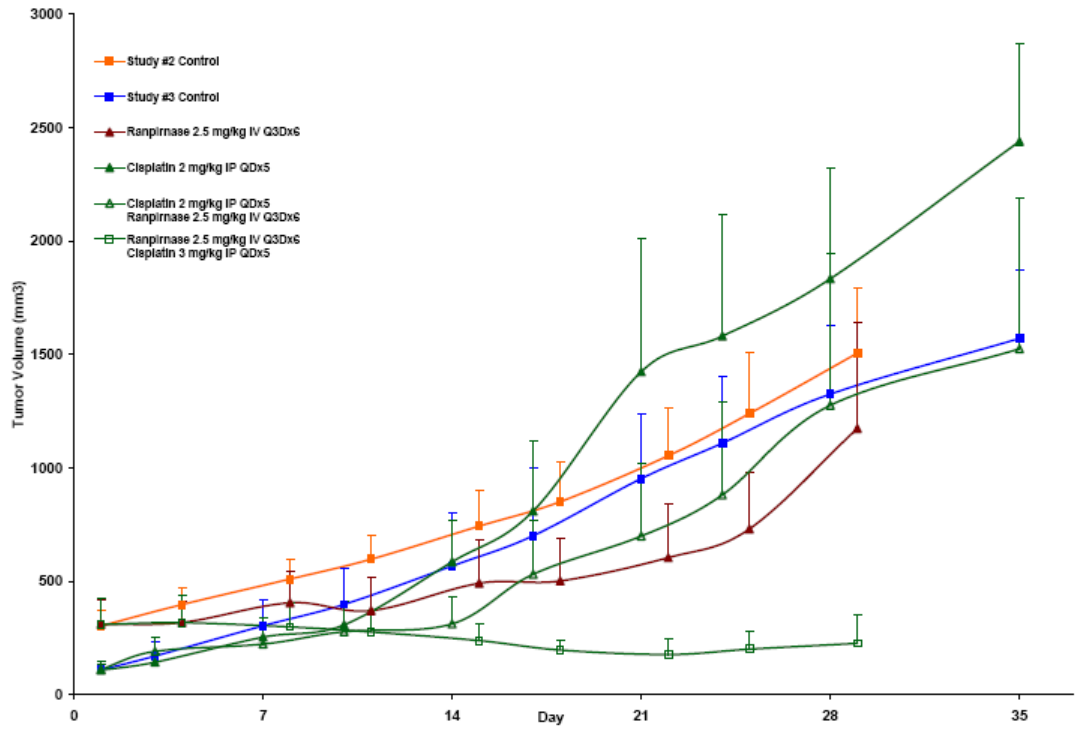
BML-1 NSCL Tumor Model (Study 3)

GROUP	N	DOSE (MG/KG)	ROUTE	SCHEDULE	FINAL TUMOR VOLUME (MM ³)			WEIGHT NADIR			DRUG-RELATED DEATHS	
					MEAN ± SEM	%TGI	#PR/#CR	%TR	% CHANGE	DAY	TOTAL	DAY (#)
Control	7	No Treatment	--	--	1538 ± 678	--	--	--	-3%	7	0	--
Cisplatin	4	2 mg/kg	IP	QDx5	1619 ± 573	--	0/0	--	-8%	7	0	--
Sorafenib	4	40 mg/kg	PO	QDx21	923 ± 585	43%	0/0	--	-4%	7	0	--
Bevacizumab	4	10 mg/kg	IP	Q3Dx10	291 ± 90	87%	0/0	--	-3%	7	0	--
Cisplatin Ranpirnase	4	2 mg/kg 2.5 mg/kg	IP IV	QDx5 Q3Dx6	882 ± 408	46%	0/0	--	-20%	14	0	--
Sorafenib Ranpirnase	4	40 mg/kg 2.5 mg/kg	PO IV	QDx21 Q3Dx6	762 ± 275	53%	0/0	--	-23%	14	0	--
Bevacizumab Ranpirnase	4	10 mg/kg 2.5 mg/kg	IP IV	Q3Dx10 Q3Dx6	293 ± 56	87%	0/0	--	-23%	14	0	--
Sorafenib Bevacizumab	4	80 mg/kg 10 mg/kg	PO IP	QDx21 Q3Dx10	224 ± 38	92%	0/0	--	-6%	7	0	--

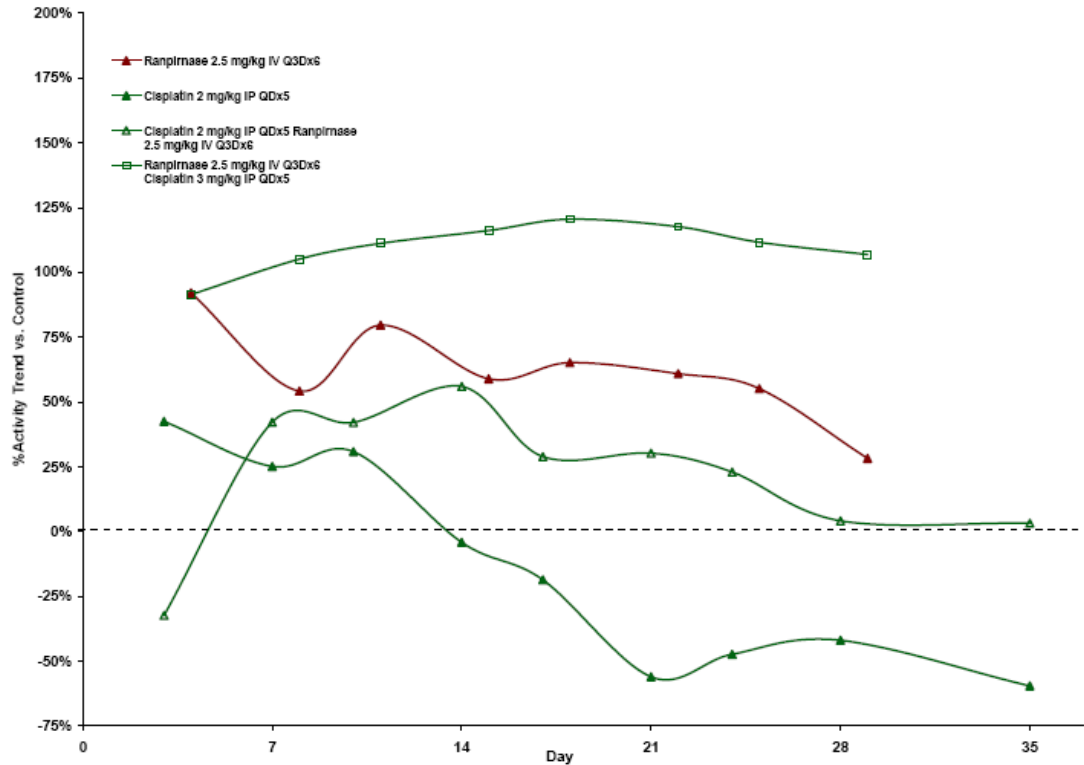
Mean Tumor Volume Curves: Studies 1-3



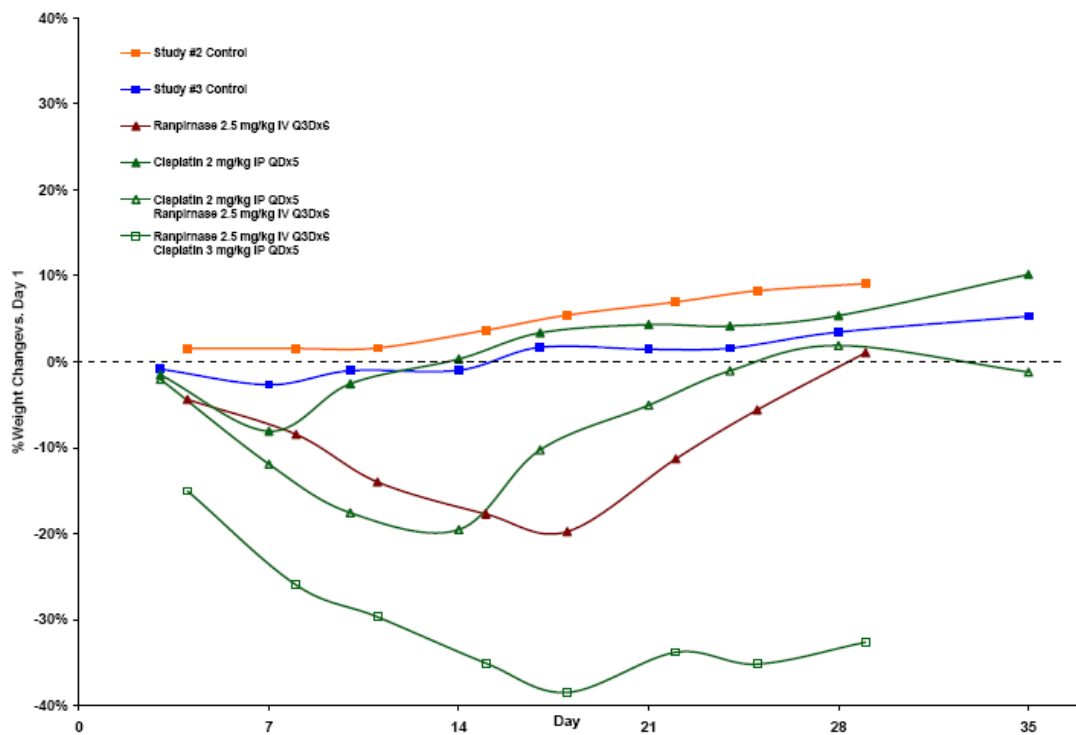
Mean Tumor Volume: Cisplatin +/- Ranpirnase



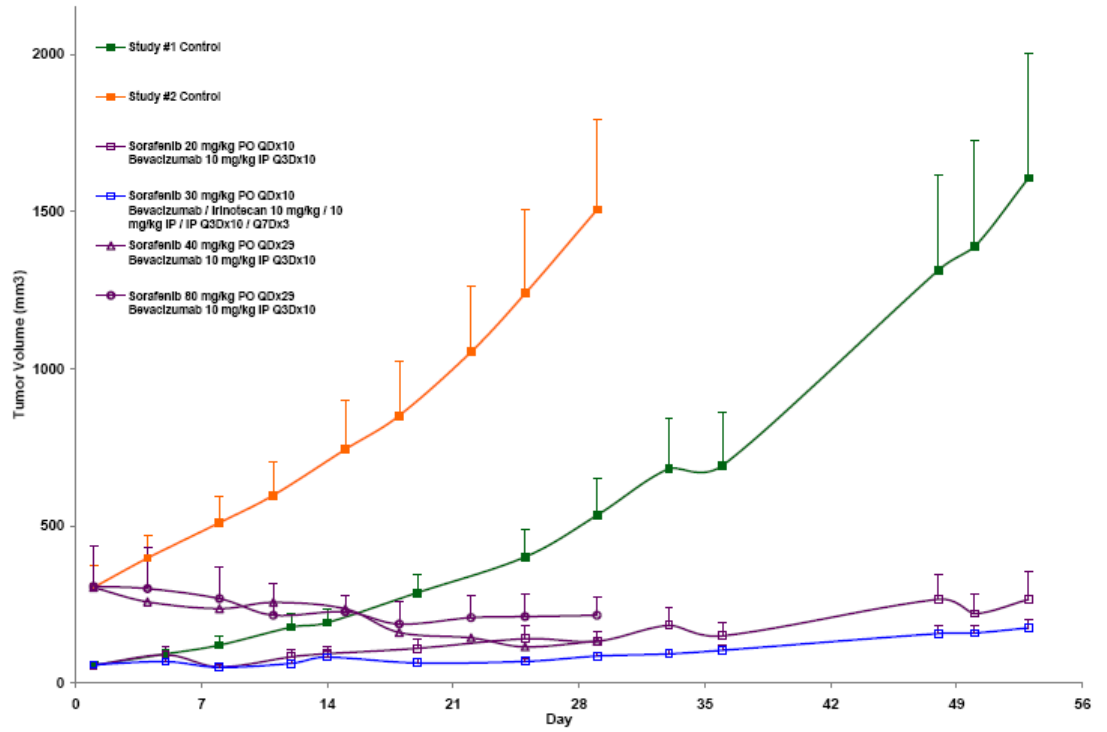
Mean Activity Trend: Cisplatin +/- Ranpirnase



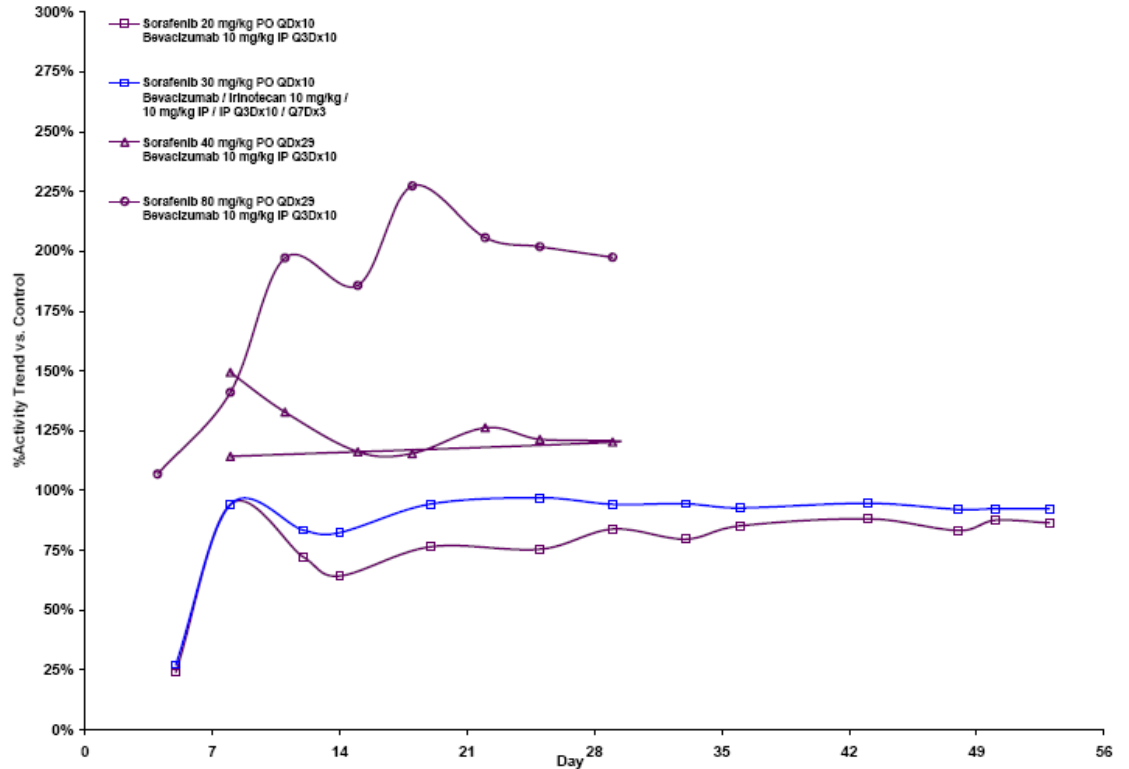
Mean Percent Weight Change: Cisplatin +/- Ranpirnase



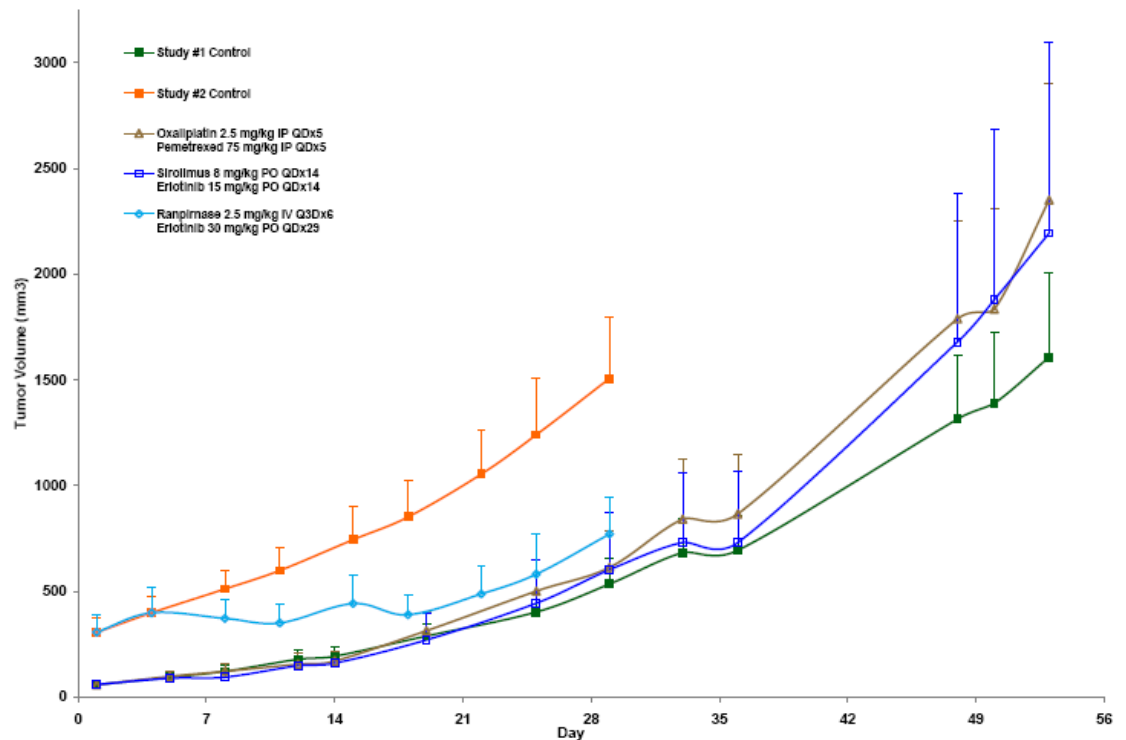
Mean Tumor Volume: Bevacizumab/Sorafenib +/- Irinotecan



Mean Activity Trend: Bevacizumab/Sorafenib +/- Irinotecan



Mean Tumor Volume: Additional Combination Groups



SUMMARY

- Growth characteristics of the BML-1 model were consistent through three studies with slight variations noted when experiments were initiated at mean tumor volumes 100 mm³.
- BML-1 demonstrated consistent drug sensitivity or resistance through three studies suggesting this model as reproducible for evaluation and comparison of test and standard agents.
- This model demonstrated drug sensitivity and resistance consistent with clinical observations of the patient donor:
 - Consistent with patient clinical results, the tumor model was insensitive to platinum regimens including cisplatin alone or an oxaliplatin/pemetrexed combination
 - Model treatment with a sorafenib/bevacizumab regimen resulted in impressive tumor growth inhibition which was further enhanced with irinotecan including tumor regressions in half of the treated mice. The donor patient responded to the sorafenib/bevacizumab/irinotecan triple therapy with a 50% maximal remission and continued with stable disease on a sorafenib/bevacizumab regimen

This suggests that this low passage tumor model platform is useful in identifying efficacious therapies for individualized treatment.

- Ranpirnase sensitized the BML-1 model to cisplatin and to a lesser extent erlotinib.