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## Initial in vivo testing of a multitarget kinase inhibitor, regorafenib, by the Pediatric Preclinical Testing Consortium

Douglas J. Harrison<sup>1</sup>, Jonathan D. Gill<sup>1</sup>, Michael E. Roth<sup>1</sup>, Wendong Zhang<sup>1</sup>, Beverly Teicher<sup>2</sup>, Stephen Erickson<sup>3</sup>, Greg Gatto<sup>3</sup>, Raushan T. Kurmasheva<sup>4</sup>, Peter J. Houghton<sup>4</sup>, Malcolm A. Smith<sup>2</sup>, Edward Anders Kolb<sup>5</sup>, Richard Gorlick<sup>1</sup>

<sup>1</sup>Division of Pediatrics, University of Texas MD Anderson Cancer Center, Houston, Texas

<sup>2</sup>Cancer Therapeutics Evaluation Program, National Cancer Institute, Bethesda, Maryland

<sup>3</sup>Global Health Technologies, RTI International, North Carolina

<sup>4</sup>Greehey Children's Cancer Research Institute, University of Texas Health Science Center at San Antonio, San Antonio, Texas

<sup>5</sup>Division of Pediatric Hematology/Oncology, Nemours/Alfred I. duPont Hospital for Children, Wilmington, Delaware

### Abstract

**Background:** Regorafenib is a small molecule multikinase inhibitor that inhibits multiple kinases including BRAF, KIT, PDGFRB, RAF, RET, and VEGFR1–3.

**Procedures:** The in vivo anticancer effects of regorafenib were assessed in a panel of six osteosarcoma models, three rhabdomyosarcoma models, and one Ewing sarcoma model.

**Results:** Regorafenib induced modest inhibition of tumor growth in the models evaluated.

**Conclusion:** The overall pattern of response to regorafenib appears similar to that of the kinase inhibitor sorafenib, with pronounced slowing of tumor growth in some models, limited to the period of agent administration, being the primary treatment effect.

### Keywords

Ewing sarcoma; multitarget kinase inhibitor; osteosarcoma; patient derived xenograft; pediatric preclinical testing consortium; rhabdomyosarcoma

## 1 | INTRODUCTION

The outcome for pediatric patients diagnosed with refractory sarcoma has not changed in several decades despite extensive clinical investigation. The 5-year event-free survival

**Correspondence** Richard Gorlick, Division of Pediatrics, The University of Texas MD Anderson Cancer Center, Houston, TX77030, RGorlick@mdanderson.org.

### CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

### SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

(EFS) for pediatric patients with metastatic osteosarcoma (OS), rhabdomyosarcoma (RMS), and Ewing sarcoma (ES) is approximately 20%, while the outcomes for patients who sustain recurrent disease is worse, in some cases less than 10%, despite extensive treatment.<sup>1–5</sup> While the outcomes for pediatric patients with localized sarcoma have improved significantly, the outcomes for patients with refractory or metastatic disease remain poor, attesting to the urgent need for novel therapies moving forward.

Regorafenib is a small-molecule multitarget kinase inhibitor (MKI) developed by adding a fluorine atom to the phenyl ring of the receptor TKI sorafenib.<sup>6</sup> Regorafenib inhibits several kinases including BRAF, KIT, PDGFRB, RAF, RET, and VEGFR1–3 with higher potency than sorafenib.<sup>6</sup> It is approved by the FDA for the treatment of patients with metastatic colorectal cancer, gastrointestinal stromal tumors, and hepatocellular carcinoma.

The *in vivo* activity of sorafenib was reported by the Pediatric Preclinical Testing Program (PPTP), with its primary effect being tumor growth inhibition without regression.<sup>7</sup> Tumor growth inhibition was seen across a range of histotypes, with four of five OS xenografts studied showing more than a twofold extension in median time to event for sorafenib compared to control treatment. In this study, the *in vivo* effects of regorafenib were assessed in OS, RMS, and ES xenograft patient-derived xenograft (PDX) models.

## 2 | MATERIALS AND METHODS

The *in vivo* anticancer effects of regorafenib were assessed in a panel of six OS models (OS2, OS9, OS31, OS33, OS36, OS60), two alveolar RMS models with *PAX3-FOXO1* (Rh30, Rh41), an embryonal RMS model (Rh18), and one ES model with *EWSR1-FLI1* (EW5). PDX models were heterotopically injected into the flanks of CB17SC *scid*<sup>-/-</sup> female mice (Taconic Farms, Germantown, NY). Regorafenib solution (5 mg/mL) was administered by oral gavage at a dose of 30 mg/kg/day given daily for 21 consecutive days to cohorts (n = 10). Tumor volumes were measured as previously described, and a control cohort of mice (n=10) that was administered vehicle was utilized for each PDX model.<sup>8</sup> Full molecular and pathologic descriptions of the models used have been previously published.<sup>9,10</sup> During testing, all mice were maintained under barrier conditions, and experiments were conducted using protocols approved by the Institutional Animal Care and Use Committees at MD Anderson Cancer Center and Greehey Children's Cancer Research Institute. Regorafenib's activity was evaluated using standard Pediatric Preclinical Testing Consortium (PPTC) measures (see Supporting Information Materials for details), including the ratio of median time to event for the treated and control groups (EFS T/C), minimum relative tumor volume (minRTV), and objective response categories. The exact long-rank test (Proc StatXact for SASR®) was used to compare EFS distributions between treatment and control groups. *P*-values were two-sided and were considered statistically significant if <.05.

## 3 | RESULTS

Regorafenib at a dose of 30 mg/kg/day was tested against six OS, three RMS, and one ES xenografts. Regorafenib was well tolerated across both the OS models (0–9.8% avg. weight lost) and the other sarcoma models (0–13.9% avg. weight lost). Across 120 tested mice,

three experienced toxic death. Regorafenib significantly prolonged time to event in all 10 sarcoma models tested ( $P < .05$ ) (Table 1 and Figure 1). There were no objective responses observed. Three out of 10 models demonstrated EFS T/C values  $> 2$  (1/6 OS, 2/3 RMS, and 0/1 ES) (Table 1). These models (OS33, Rh30, and Rh41) were thus classified as having a progressive disease 2 (PD2) response. All other models experienced a response of PD1 (Table 1).

Most models showed pronounced slowing of tumor growth compared to control during the 21 days of regorafenib treatment, with tumor growth generally approximating control rates soon after completion of regorafenib treatment (Figure 1). minRTV in treated mice was significantly lower than that in control mice for all models except for OS2 (Table 1/Supporting Information Figure 1). minRTVs ranged from 0.74 to 1.60, with OS60-SJ, Rh30, and Rh41 exhibiting some evidence of minor tumor regression (minRTV  $< 1$ ) and with all other models exhibiting minRTV  $> 1$  (indicating an absence of tumor regression in these models).

## 4 | DISCUSSION

Multitargeted kinase inhibitors have undergone evaluation in multiple in vitro and in vivo sarcoma models. The PPTP demonstrated sorafenib to have tumor growth inhibition activity across a range of sarcoma models, including osteosarcoma.<sup>7</sup> Slowing of tumor growth as a primary treatment effect was also observed by the PPTP for sarcoma xenografts for sunitinib,<sup>11</sup> pazopanib,<sup>12</sup> and cabozantinib.<sup>13</sup> The higher potency with which regorafenib inhibits several kinase targets as compared to sorafenib rendered it an intriguing molecule for further study within the PPTC.<sup>6</sup>

Regorafenib induced inhibition of tumor growth in the pediatric sarcoma models evaluated. The overall pattern of response to the regorafenib against the PPTC sarcoma models appears similar to that of sorafenib, with pronounced slowing of tumor growth in some models that is generally limited to the period of agent administration. These results are similar to those previously reported by Daudigeos-Dubus, et al for regorafenib for ES, RMS, and neuroblastoma xenograft models,<sup>14</sup> and they are similar to preclinical results reported for other MKIs tested against pediatric preclinical models.<sup>11–13</sup>

Regorafenib has shown activity in multiple adult cancers, including colorectal cancer,<sup>15</sup> gastrointestinal stromal tumor,<sup>16</sup> and hepatocellular carcinoma.<sup>17</sup> Three recently published clinical trials documented modest activity for regorafenib in adults with refractory bone and soft tissue sarcomas. Regorafenib improved disease stabilization as compared to placebo in a phase 2, randomized, double-blind, placebo-controlled study of adult patients with metastatic progressive OS.<sup>18</sup> A second randomized double-blind phase 2 study found that regorafenib improved time to progression compared to placebo (3.6 months vs 1.7 months, respectively) in adults with progressive, metastatic OS.<sup>19</sup> In a clinical trial of adults with doxorubicin refractory soft tissue sarcoma, regorafenib improved progression-free survival as compared to placebo for the nonadipocytic sarcoma subtypes studied.<sup>20</sup> These data for regorafenib are consistent with reports of slowing of tumor progression in patients

with OS treated with other MKIs including sorafenib,<sup>21</sup> pazopanib,<sup>22</sup> cabozantinib,<sup>23</sup> and lenvatinib.<sup>24</sup>

One challenge for moving regorafenib into the upfront treatment of OS (particularly for pediatric patients for whom methotrexate is typically given) is that there is a risk of increased methotrexate-related adverse reactions with concurrent treatment with the two agents as methotrexate is a BCRP substrate and regorafenib is a BCRP inhibitor. The preclinical activity seen in pediatric sarcoma xenografts, in conjunction with the modest activity seen in large randomized clinical trials of adult patients with refractory carcinomas as well as the activity seen in smaller studies of adults with bone or soft tissue sarcomas, support consideration of the evaluation of MKIs like regorafenib in OS.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## Abbreviations:

<b>ES</b>	Ewing sarcoma
<b>MKI</b>	multitarget kinase inhibitor
<b>OS</b>	osteosarcoma
<b>PDX</b>	patient-derived xenograft
<b>PPTC</b>	Pediatric Preclinical Testing Consortium
<b>RMS</b>	rhabdomyosarcoma

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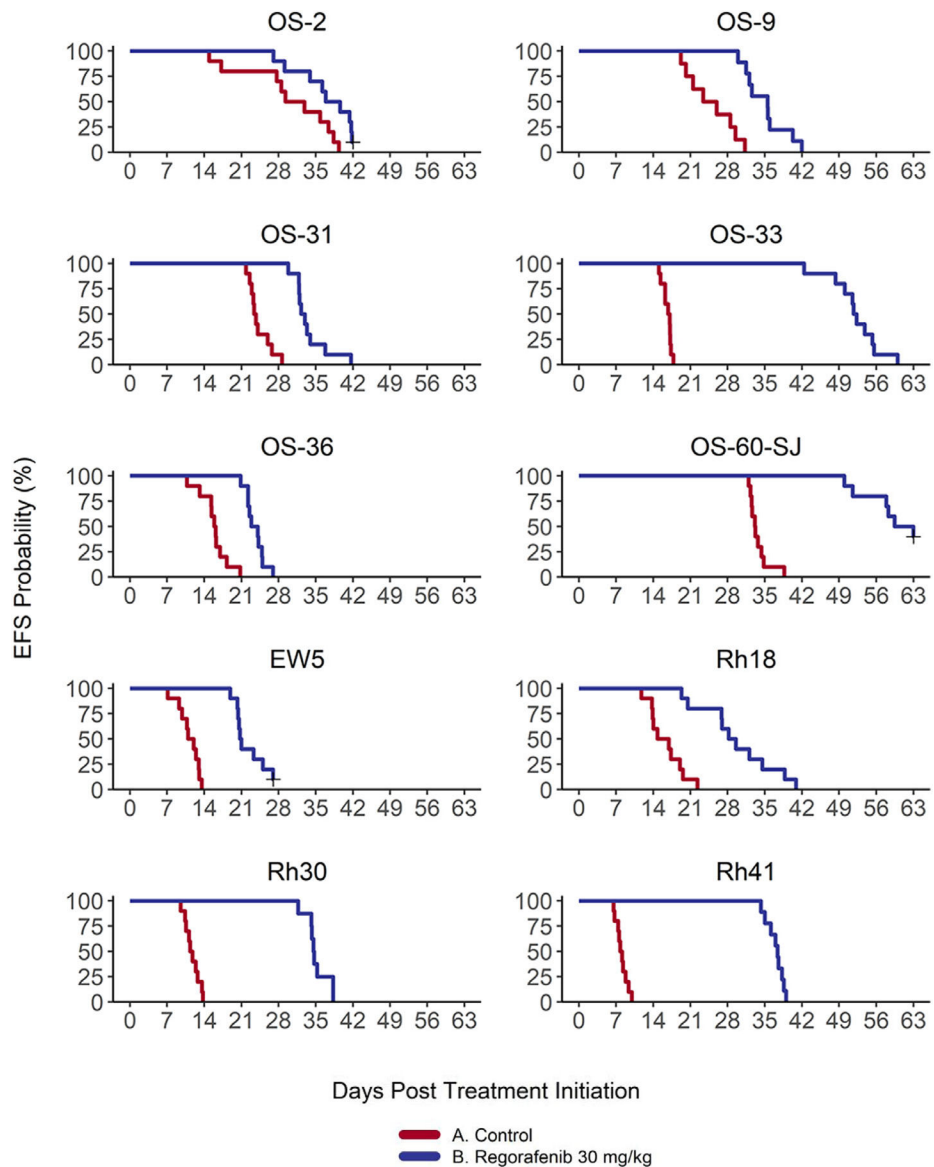
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**FIGURE 1.**

Event-free survival to regorafenib across all PDX models. Regorafenib induced significant improvements in event-free survival (EFS) compared to control in 100% (10/10) of the sarcoma models tested with significantly prolonged time to event in all models ( $P < .05$ ). Mice still alive at the conclusion of the experiment, but whose tumors had not yet quadrupled are considered right-censored and are indicated by a plus sign (+)

Regorafenib as single agent versus control for all PDX models

**TABLE 1**

Model	KM med (days)	EFS T-C (days)	EFS T/C	P-value Gehan-Wilcoxon	minRTV mean ± SD	minRTV, P-value	Objective response measure
OS-2	38.2	7.1	1.23	.046	1.175 ± 0.221	.075	PD1
OS-9	35.5	10.8	1.44	<.001	1.040 ± 0.047	<.001	PD1
OS-31	32.6	9.0	1.38	<.001	1.062 ± 0.108	<.001	PD1
OS-33	52.0	35.1	3.07	<.001	1.065 ± 0.064	<.001	PD2
OS-36	23.5	7.4	1.46	<.001	1.557 ± 0.149	.002	PD1
OS-60-SJ	61.2	28.0	1.85	<.001	0.840 ± 0.279	<.001	PD1
EW-5	20.9	9.4	1.82	<.001	1.597 ± 0.355	<.001	PD1
Rh-18	28.9	13.0	1.82	<.001	1.491 ± 0.225	.035	PD1
Rh-30	34.6	23.1	2.99	<.001	0.949 ± 0.253	<.001	PD2
Rh-41	37.4	29.4	4.69	<.001	0.739 ± 0.226	<.001	PD2

Three out of 10 sarcoma models demonstrated EFS T/C values > 2 (1/6 OS, 2/3 Rh, 0/1 EW). Treated mice from models OS33, Rh30, and Rh41 experienced an objective response of PD2 (progressive disease 2), and treated mice from all other models experienced an objective response of PD1. For sorafenib compared to control treatment. In this study, the in vivo effects of regorafenib were assessed in OS, RMS, and ES xenograft patient-derived xenograft (PDX) models.