

## Pre-clinical studies of EC2629, a highly potent folate- receptor-targeted DNA crosslinking agent

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### Supplementary Information:

**Supplementary Table S1. Relative potencies of base drugs and associated folate-based SMDCs on KB cells**

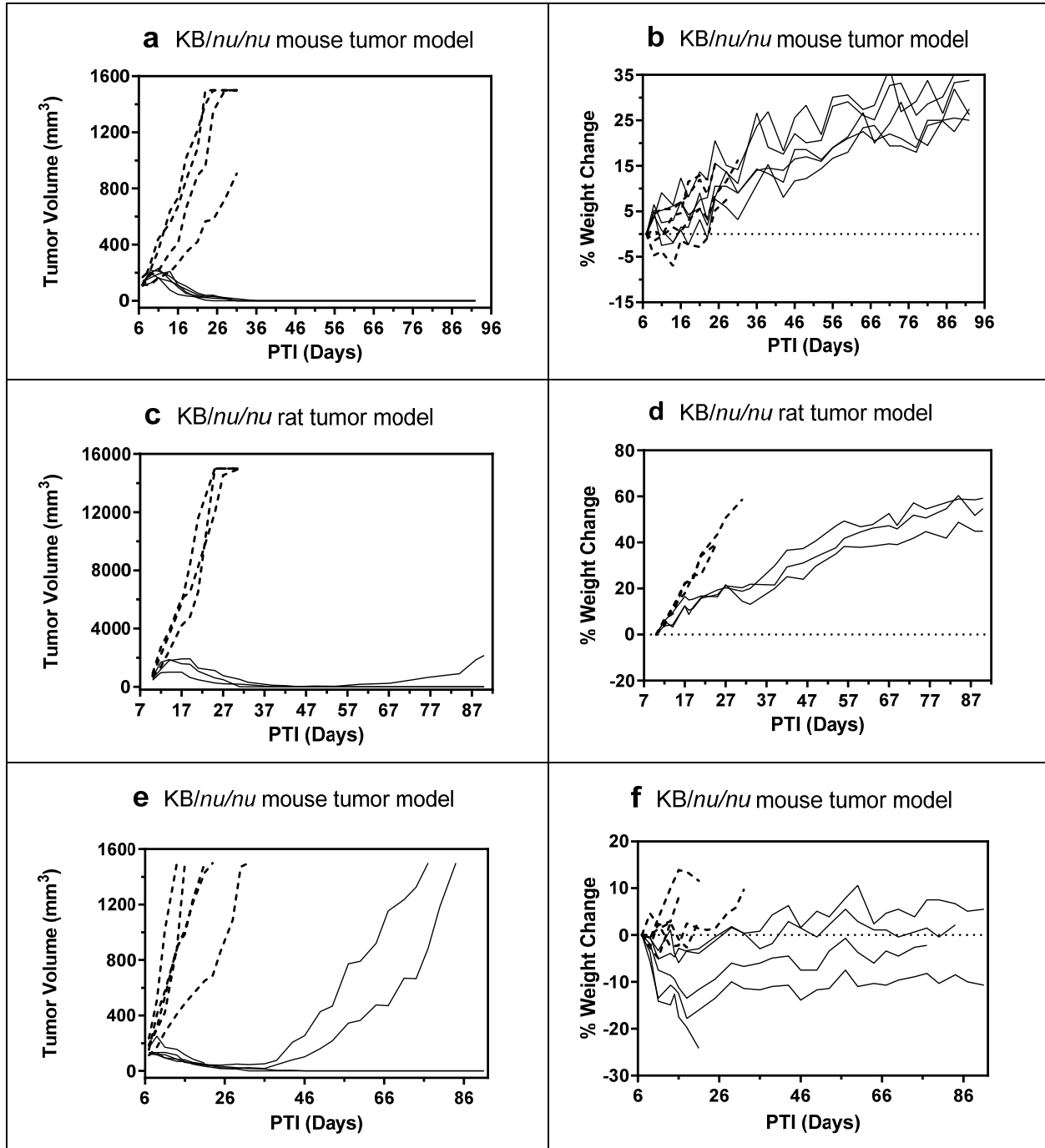
Base Drug			SMDC		
Name	IC <sub>50</sub> (pM)	Relative Potency	Name	IC <sub>50</sub> (pM)	Relative Potency
DAVLBH	35,000	1	EC145	9,000	1
EC0347	2500	14	EC1456	1500	6
EC2491	15	2333	EC2629	52	173

**Supplementary Table S2: Activity and response for each of the graphs in figures 3, 4 and 5.** SD, stable disease defined as volume regression < 50% and increase in volume of < 10% ; PR, partial response defined as volume regression >50% but with measurable tumor (>2 mm<sup>3</sup>) remaining at all times; CR, complete response defined as a disappearance of measurable tumor mass (<2 mm<sup>3</sup>) at some point within 90 days after tumor implantation; cures were defined as CRs without tumor regrowth within the 90-day study time frame.

<b>Figure # : Tumor model</b>	<b>Treatment Regimen</b>	<b>n</b>	<b>SD</b>	<b>PR</b>	<b>CR</b>	<b>Cures</b>
<b>Fig 3a: KB/ nu/nu mice</b>	<b>Control mice</b>	4	0	0	0	0
	<b>EC2629 0.3 μmol/kg SIW x 2</b>	5	0	0	0	5
<b>Fig 3c: KB/ nu/nu rats</b>	<b>Control rats</b>	3	0	0	0	0
	<b>EC2629 0.15 μmol/kg SIW x 2</b>	3	0	1	2	0
<b>Fig. 3e: KB/ nu/nu mice</b>	<b>Control mice</b>	5	0	0	0	0
	<b>SJG-136 0.3 mg/kg SIW x 2</b>	5	0	2	0	2
<b>Fig. 4a: KB-PR/ nu/nu mice</b>	<b>Control mice</b>	5	0	0	0	0
	<b>Paclitaxel 20 mg/kg, TIW x 2</b>	5	1	0	0	0
	<b>EC2629 0.5 μmol/kg SIW x 2</b>	5	0	0	0	5
<b>Fig. 4b: KB-CR/ nu/nu mice</b>	<b>Control mice</b>	4	0	0	0	0
	<b>Cisplatin 3 mg/kg, BIW x 2</b>	5	0	0	0	0
	<b>EC2629 0.5 μmol/kg SIW x 2</b>	5	0	0	0	5
<b>Fig. 5a: ST502/ nu/nu mice</b>	<b>Control mice</b>	7	0	0	0	0
	<b>Eribulin mesylate 1 mg/kg SIW x 2</b>	7	1	1	0	0
	<b>EC2629 0.27 μmol/kg BIW x 2</b>	7	0	2	2	3
<b>Fig. 5b: ST738/ nu/nu mice</b>	<b>Control mice</b>	7	0	0	0	0
	<b>Eribulin mesylate 1 mg/kg SIW x 2</b>	7	5	2	0	0
	<b>EC2629 0.27 μmol/kg BIW x 2</b>	7	2	2	1	2

<b>Fig. 5c: ST040/ nu/nu mice</b>	<b>Control mice</b>	7	0	0	0	0
	<b>Paclitaxel 15 mg/kg SIW x 2</b>	3	0	0	0	0
	<b>EC2629 0.27 <math>\mu</math>mol/kg BIW x 2</b>	7	2	3	0	0
<b>Fig. 5d: ST070/ nu/nu mice</b>	<b>Control mice</b>	7	0	0	0	0
	<b>Paclitaxel 15 mg/kg SIW x 2</b>	7	0	0	0	0
	<b>EC2629 0.27 <math>\mu</math>mol/kg BIW x 2</b>	7	0	0	0	5

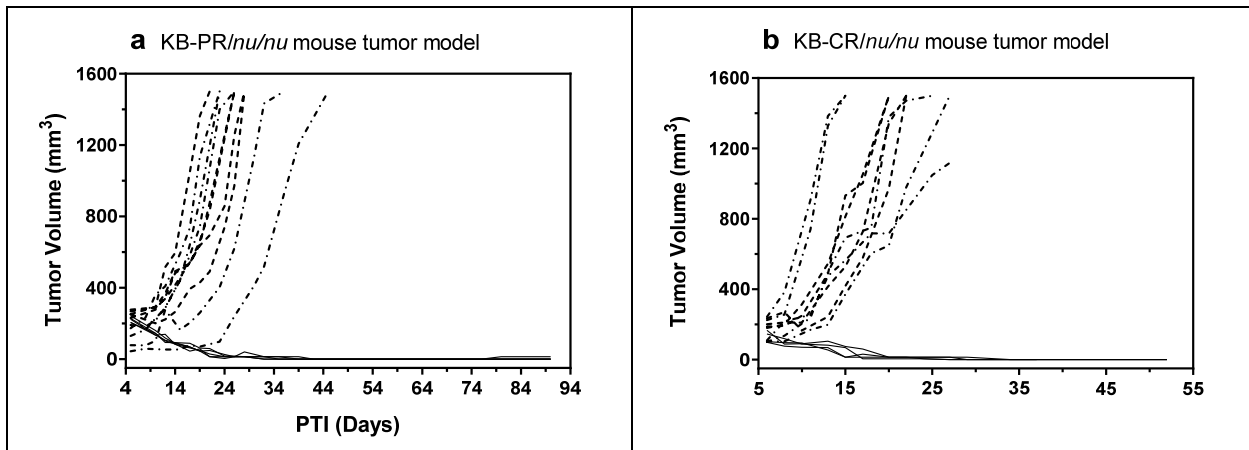
Supplementary Figure S1



Supplementary Figure S1 Antitumor (a, c, e) and weight change (b, d, f) effects of EC2629 (a, b, c, d) and SJG-136 (e, f) on FR expressing KB tumor *nu/nu* mice (a, b, e, f) and rat (c, d) models. KB tumor cells were inoculated subcutaneously into *nu/nu* mice ( $1 \times 10^6$  cells) and rats ( $5 \times 10^6$  cells) and therapy started on randomized animals with tumors in the 111–168 mm<sup>3</sup> (mice; n = 5) and 411–704 mm<sup>3</sup> (rats; n = 3) range. (---), untreated controls; (—), EC2629, 0.3

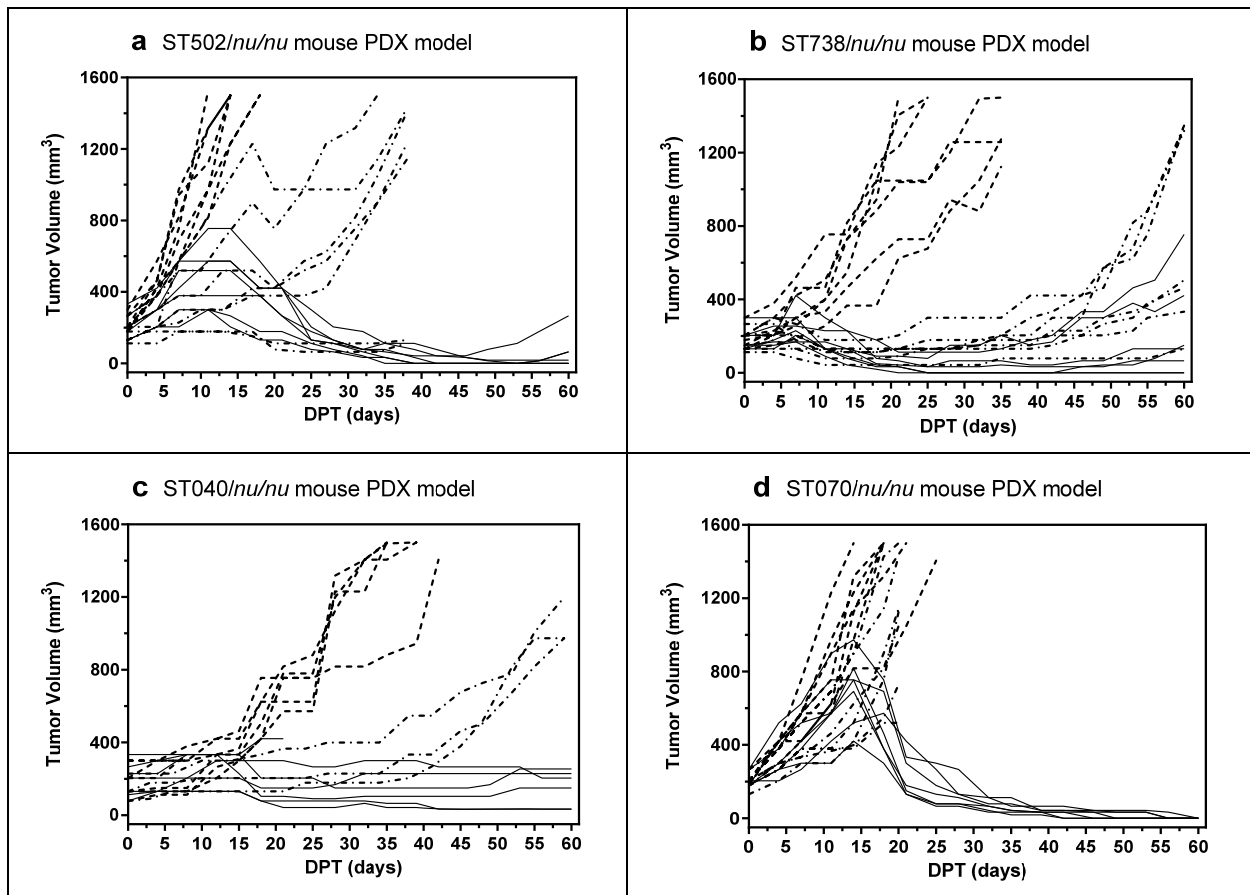
$\mu\text{mol/kg}$  (a, b) or  $0.15 \mu\text{mol/kg}$  (c, d) or SJG-136,  $0.3 \text{ mg/kg}$  (e,f) in 0.05% Tween-80, SIW x 2 weeks. Each curve represents the growth of a single tumor in an individual mouse.

### Supplementary Figure S2



**Supplementary Figure S2 Antitumor effects of EC2629 on FR expressing KB-PR (a) and KB-CR (b) tumors in *nu/nu* mice.** KB-PR (a) or KB-CR (b) tumor cells ( $1 \times 10^6$ ) were inoculated subcutaneously into *nu/nu* mice and therapy started on randomized animals with tumors in the  $205\text{-}243 \text{ mm}^3$  (a) and  $98\text{-}168 \text{ mm}^3$  (b) range. (---), untreated controls; (-.-.-) (a), paclitaxel,  $20 \text{ mg/kg}$ , TIW x 2 weeks (b) cisplatin,  $3 \text{ mg/kg}$ , BIW x 2 weeks; (—), EC2629,  $0.5 \mu\text{mol/kg}$ , SIW x 2 weeks. Each curve represents the growth of a single tumor in an individual mouse.

### Supplementary Figure S3



**Supplementary Figure S3 Antitumor effects of EC2629 on patient derived triple negative breast ST502 (a), ST738 (b), endometrial ST040 (c) and ovarian ST070 (d) xenograft models in *nu/nu* mice.** Tumor fragments harvested from host animals were inoculated subcutaneously into *nu/nu* mice and therapy started on randomized animals with tumors in the 112-333 mm<sup>3</sup> (a), 112 to 300 mm<sup>3</sup> (b), 78 to 333 mm<sup>3</sup> (c) and 131 to 266 mm<sup>3</sup> (d) range. (---), untreated controls; (-·-·-), eribulin mesylate, 1 mg/kg SIW x 2 weeks (a, b) or paclitaxel, 15 mg/kg, SIW x 2 weeks (c, d) (—), EC2629, 0.27 μmol/kg, BIW x 2 weeks (a,b,c,d). Each curve represents the growth of a single tumor in an individual mouse