

# Supplementary Materials: The Small Molecule Ephrin Receptor Inhibitor, GLPG1790, Reduces Renewal Capabilities of Cancer Stem Cells, Showing Anti-Tumour Efficacy on Preclinical Glioblastoma Models

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**Table S1.** Hazard ratios for the single therapies (subcutaneous xenografts).

Comparison	U87MG			U251			T98G		
	Hazard Ratio (HR)	CI95%	Significance	Hazard Ratio (HR)	CI95%	Significance	Hazard Ratio (HR)	CI95%	Significance
CTRL vs. GLPG1790	4.38	0.87–22.15	$p < 0.0001$	3.55	1.23–10.21	$p = 0.0004$	4.18	1.38–12.69	$p < 0.0001$
CTRL vs. RT	2.86	0.65–15.63	$p = 0.0012$	1.58	0.60–3.61	$p = 0.3060$ (NS)	2.94	0.99–8.73	$p = 0.0008$
CTRL vs. TMZ	4.67	1.35–12.59	$p < 0.0001$	5.73	1.49–15.02	$p < 0.0001$	2.12	0.83–5.42	$p = 0.0334$
CTRL vs. RT+TMZ	8.88	3.07–32.77	$p < 0.0001$	5.53	1.71–17.95	$p < 0.0001$	5.55	1.51–20.11	$p < 0.0001$
RT vs. GLPG1790	1.82	0.73–4.55	$p = 0.0962$ (NS)	2.87	1.06–7.82	$p = 0.0041$	2.23	1.14–4.36	$p = 0.0115$
RT vs. TMZ	4.46	1.44–13.85	$p < 0.0001$	4.77	1.50–15.17	$p < 0.0001$	1.04	0.43–2.50	$p = 0.9132$ (NS)
RT vs. RT+TMZ	5.20	1.64–16.53	$p < 0.0001$	5.57	1.71–18.14	$p < 0.0001$	3.68	1.73–7.85	$p < 0.0001$
GLPG1790 vs. TMZ	2.10	0.82–5.37	$p = 0.0427$	2.88	1.04–7.99	$p = 0.0027$	1.49	0.76–2.69	$p = 0.2561$ (NS)
GLPG1790 vs. RT+TMZ	3.81	1.31–11.09	$p = 0.0014$	5.12	1.62–16.17	$p < 0.0001$	2.37	1.20–4.68	$p = 0.0011$
TMZ vs. RT+TMZ	2.42	0.88–5.84	$p = 0.0407$	1.97	0.75–5.15	$p = 0.0983$ (NS)	2.69	1.341–5.41	$p = 0.0007$

**Table S2.** GLPG1790 reduced vasculogenesis.

Treatment	Angiogenic Vessels Counts/100× Microscopic Field					
	U87MG		U251		T98G	
	Mean ± SD	Statistics	Mean ± SD	Statistics vs. Vehicle	Mean ± SD	Statistics
Vehicle	25.12 ± 4.09		20.75 ± 2.87		18.87 ± 5.03	
GLPG1790	7.38 ± 2.72	$p < 0.0001$ *	6.12 ± 1.81	$p < 0.0001$ *	5.12 ± 1.55	$p < 0.0001$ *
RT	5.0 ± 1.93	$p = 0.636$ NS **	6.00 ± 1.20	$p = 0.8727$ NS **	7.75 ± 1.98	$p < 0.0001$ * $p = 0.0106$ **
TMZ	9.62 ± 2.13	$p = 0.0871$ NS **	8.37 ± 1.06	$p = 0.0137$ **	11.0 ± 3.38	$p < 0.0001$ * $p = 0.0005$ **

RT+TMZ	3.37 ± 1.30	$p = 0.0022^{**}$	3.87 ± 1.36	$p = 0.0137$	5.87 ± 2.59	$p < 0.001^{*}$ $p = 0.04936 \text{ NS}^{**}$
Vasculomimetic Vessels Counts/100× Microscopic Field						
Vehicle	16.45 ± 4.2		10.32 ± 2.87		12.25 ± 2.96	
GLPG1790	7.25 ± 1.5	$p < 0.0001^{*}$	4.45 ± 1.37	$p < 0.0001^{*}$	3.37 ± 3.24	$p < 0.0001^{*}$
RT	24.30 ± 6.2	$p < 0.001^{*}$ $p = 0.0136^{**}$	14.36 ± 4.40	$p = 0.2332 \text{ NS}^{*}$ $p < 0.0001^{**}$	15.87 ± 4.94	$p < 0.0001 \text{ NS}^{**}$
TMZ	5.50 ± 2.3	$p < 0.0001^{*}$ $p = 0.6446 \text{ NS}^{**}$	5.44 ± 1.54	$p = 0.2137 \text{ NS}^{**}$	12.0 ± 4.63	$p < 0.0001^{**}$
RT+TMZ	3.75 ± 3.2	$p < 0.0001^{*}$ $p = 0.1022 \text{ NS}^{**}$	3.87 ± 1.36	$p = 0.0137$	1.87 ± 1.88	$p = 0.2776 \text{ NS}^{**}$

\* vs. Vehicle, \*\* vs. GLPG1790.

**Table S3.** GLPG1790 reduced hypoxic areas.

Treatment	Necrosis (Arbitrary Score)					
	U87MG		U251		T98G	
	Mean ± SD	Statistics	Mean ± SD	Statistics vs. Vehicle	Mean ± SD	Statistics
Vehicle	1.0 ± 0.63		1.67 ± 0.82		1.17 ± 0.75	
GLPG1790	0.33 ± 0.52	$p = 0.0734 \text{ NS}^{*}$	0.55 ± 0.81	$p = 0.0346^{*}$	0.35 ± 0.45	$p = 0.0490^{*}$
RT	1.67 ± 0.82	$p = 0.0070^{**}$	2.0 ± 0.75	$p = 0.0114^{**}$	1.33 ± 0.90	$p = 0.4890^{**}$
TMZ	2.0 ± 0.63	$p = 0.0005$	2.35 ± 0.82	$p = 0.0033^{**}$	1.33 ± 0.70	$p = 0.0290^{**}$
RT+TMZ	2.33 ± 0.52	$p < 0.0001$	2.55 ± 0.55	$p = 0.0006^{**}$	2.17 ± 0.75	$p = 0.0006^{**}$
HIF2 Immunostain (Arbitrary Score)						
Vehicle	1.50 ± 0.76		1.57 ± 0.79		2.00 ± 0.82	
GLPG1790	0.50 ± 0.53	$p = 0.0086^{*}$	0.57 ± 0.79	$p = 0.0013^{*}$	1.14 ± 0.39	$p = 0.0408^{*}$
RT	2.285 ± 0.71	$p = 0.0004^{**}$	2.28 ± 0.76	$p = 0.0349^{**}$	2.43 ± 0.79	$p = 0.0060^{**}$
TMZ	1.88 ± 0.83	$p = 0.0022^{**}$	2.14 ± 0.90	$p = 0.0046^{**}$	2.14 ± 0.69	$p = 0.0152^{**}$
RT+TMZ	2.37 ± 0.52	$p = 0.0001$	2.64 ± 0.37	$p = 0.0014$	2.71 ± 0.49	$p = 0.0005$

\* vs. Vehicle, \*\* vs. GLPG1790.

**Table S4.** GLPG1790 reduced leucocytes infiltration.

Treatment	Granulocytes Infiltration (Arbitrary Score)					
	U87MG		U251		T98G	
	Mean ± SD	Statistics	Mean ± SD	Statistics vs. Vehicle	Mean ± SD	Statistics
Vehicle	4.60 ± 1.11		5.25 ± 1.39		3.87 ± 0.99	
GLPG1790	1.05 ± 0.73	$p = 0.0082^{*}$	2.00 ± 1.31	$p = 0.0003^{*}$	1.50 ± 1.33	$p = 0.0011^{*}$
RT	7.24 ± 1.33	$p < 0.0001^{**}$	6.87 ± 1.25	$p < 0.0001^{**}$	6.00 ± 1.51	$p < 0.0001^{**}$
TMZ	8.15 ± 1.00	$p < 0.0001^{**}$	7.50 ± 1.20	$p < 0.0001^{**}$	4.75 ± 1.67	$p = 0.0007^{**}$
RT+TMZ	8.62 ± 0.55	$p < 0.0001^{**}$	8.00 ± 1.20	$p < 0.0001^{**}$	7.62 ± 0.92	$p < 0.0001^{**}$
Lymphomonocytes Infiltration (Arbitrary Score)						
Vehicle	5.60 ± 1.10		4.00 ± 1.20		3.25 ± 2.19	
GLPG1790	1.00 ± 0.55	$p = 0.0001^{*}$	1.88 ± 1.46	$p = 0.0066^{*}$	1.25 ± 1.58	$p = 0.0548 \text{ NS}^{*}$
RT	7.20 ± 1.11	$p < 0.0001^{**}$	5.25 ± 1.83	$p = 0.0011^{**}$	4.13 ± 2.53	$p = 0.0165^{**}$
TMZ	7.85 ± 1.30	$p < 0.0001^{**}$	3.50 ± 1.93	$p = 0.0779 \text{ NS}^{**}$	3.38 ± 2.26	$p < 0.0471^{**}$

RT+TMZ      8.35 ± 0.80       $p < 0.0001$  \*\*      7.13 ± 1.13       $p < 0.0001$  \*\*      6.75 ± 0.71       $p < 0.0001$  \*\*

\* vs. Vehicle, \*\* vs. GLPG1790.

**Table S5.** Hazard ratios for the single therapies (U87MG orthotopic model).

Comparison	DFS			OS		
	Hazard Ratio (HR)	CI95%	Significance	Hazard Ratio (HR)	CI95%	Significance
CTRL vs. GLPG1790	2.85	1.05–7.74	$p = 0.0022$	3.15	1.13–8.76	$p = 0.0010$
CTRL vs. RT	1.67	0.68–4.14	$p = 0.1379$ (NS)	2.44	0.93–6.41	$p = 0.0147$
CTRL vs. TMZ	3.38	1.19–9.60	$p = 0.0003$	3.65	1.26–10.59	$p = 0.0001$
CTRL vs. RT+TMZ	5.22	1.64–16.59	$p < 0.0001$	5.35	1.67–17.18	$p < 0.0001$
RT vs. GLPG1790	1.81	0.73–4.53	$p = 0.1002$ (NS)	1.87	0.75–4.60	$p = 0.0883$ (NS)
RT vs. TMZ	2.11	0.83–5.39	$p = 0.0321$	2.78	1.03–7.49	$p = 0.0036$
RT vs. RT+TMZ	4.03	1.37–11.90	$p = 0.0001$	5.06	1.61–15.94	$p < 0.0001$
GLPG1790 vs. TMZ	1.52	0.62–3.73	$p = 0.2631$ (NS)	1.85	0.74–4.64	$p = 0.1128$ (NS)
GLPG1790 vs. RT+TMZ	3.79	1.31–11.01	$p = 0.0008$	4.22	1.41–12.60	$p = 0.0003$
TMZ vs. RT+TMZ	3.42	1.21–9.71	$p = 0.0022$	3.39	1.20–9.61	$p = 0.0020$

**Table S6.** Hazard ratios for the single therapies (CSCs5 cell orthotopic model).

Comparison	DFS			OS		
	Hazard Ratio (HR)	CI95%	Significance	Hazard Ratio (HR)	CI95%	Significance
CTRL vs. GLPG1790	2.46	0.93–6.45	$p = 0.0060$	4.15	1.37–12.55	$p < 0.0001$
CTRL vs. RT	1.15	0.48–2.77	$p = 0.6491$ (NS)	2.00	0.79–5.09	$p = 0.0733$ (NS)
CTRL vs. TMZ	3.11	1.12–8.64	$p = 0.0004$	4.30	1.40–13.17	$p < 0.0001$
CTRL vs. RT+TMZ	4.20	1.40–12.60	$p < 0.0001$	5.25	1.63–16.95	$p < 0.0001$
RT vs. GLPG1790	2.44	0.89–6.88	$p = 0.0192$	3.04	1.10–8.40	$p = 0.0027$
RT vs. TMZ	3.40	1.20–9.65	$p = 0.0006$	3.83	1.30–11.29	$p = 0.0002$
RT vs. RT+TMZ	4.61	1.49–14.26	$p < 0.0001$	5.27	1.63–17.04	$p < 0.0001$
GLPG1790 vs. TMZ	1.75	0.70–4.34	$p = 0.1421$ (NS)	1.40	0.58–3.42	$p = 0.4006$ (NS)
GLPG1790 vs. RT+TMZ	3.62	1.26–10.42	$p = 0.0008$	4.11	1.38–12.28	$p = 0.0002$
TMZ vs. RT+TMZ	3.14	1.27–8.73	$p = 0.0030$	3.89	1.33–11.43	$p = 0.0005$



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