Preclinical evaluation of novel CDK4/6 inhibitor GLR2007 in glioblastoma models

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Background

- Glioblastoma multiforme (GBM) is characterized by dysregulation of the cyclin-dependent kinase (CDK)4 and CDK6 pathway, which leads to hyperproliferation of tumor cells[1]
- To date, no clinical trials have demonstrated efficacy of CDK4/6 inhibitors in patients with GBM[2,3]
- The development of new therapies for GBM is constrained by the presence of the highly selective, semipermeable blood–brain barrier (BBB)[4,5]
- GLR2007 is an investigational CDK4/6 inhibitor with the potential for improved penetration across the BBB

Objectives

To investigate the activity, antitumor efficacy, and central nervous system distribution of GLR2007 in GBM cell lines and xenograft models

Methods

- *In vitro* assays were used to assess the activity of GLR2007
  - Inhibition of CDK4/cyclin D1 (CyD1) and CDK6/CyD1 enzymatic activity was assessed as half maximal inhibitory concentration (IC_{50}) and inhibition constant (K_i)
  - Cell cycle progression was analyzed in human GBM U87-MG cells
- Quantitative whole-body autoradiography was used to assess the tissue distribution of [^{14}C]GLR2007 in Sprague Dawley rats
- Orthotopic and subcutaneous human GBM cell line-derived xenografts in BALB/c nude mice were used to assess the antitumor efficacy of GLR2007

Table 1. Inhibition of CDK4/CyD1 and CDK6/CyD1 enzymatic activity (nM, mean ± standard deviation [SD])

	IC_{50}		K_i	
Test compound	**CDK4/CyD1**	**CDK6/CyD1**	**CDK4/CyD1**	**CDK6/CyD1**
GLR2007	0.22±0.02	0.53±0.07	<0.1	<0.08
Palbociclib	7.28±1.17	2.02±0.25	2.35±0.14	0.77±0.15

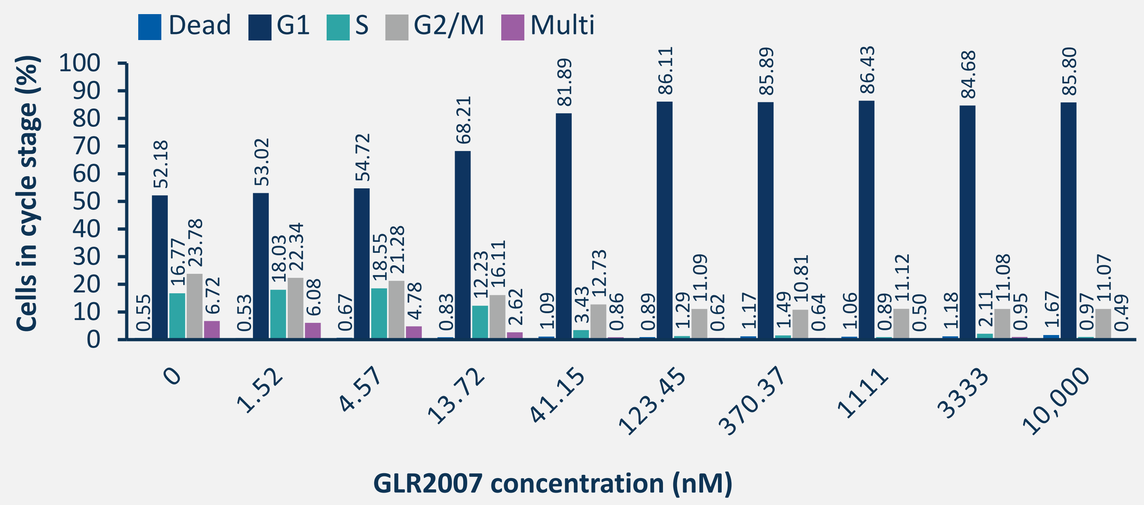
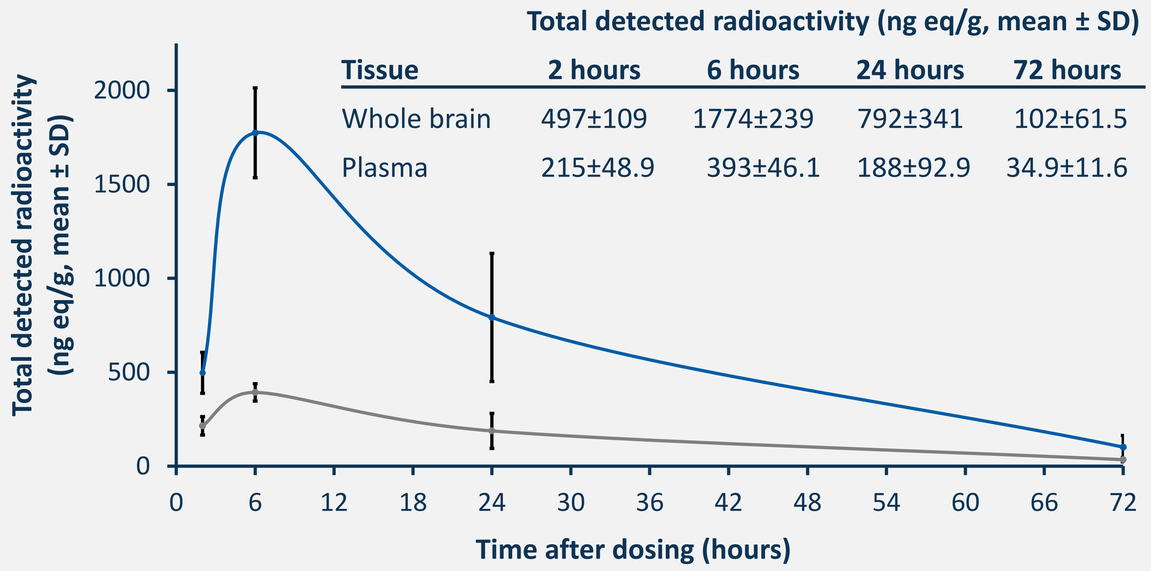


Figure 1. Percentages of U87-MG cells in each stage of the cell cycle following treatment with GLR2007 for 24 hours



Tissue	2 hours	6 hours	24 hours	72 hours
Whole brain	497±109	1774±239	792±341	102±61.5
Plasma	215±48.9	393±46.1	188±92.9	34.9±11.6

Figure 2. Total radioactivity (ng eq/g, mean ± SD) detected in brain tissue and plasma of Sprague Dawley rats up to 72 hours following dosing with 6 mg/100 µCi/kg [^{14}C]GLR2007

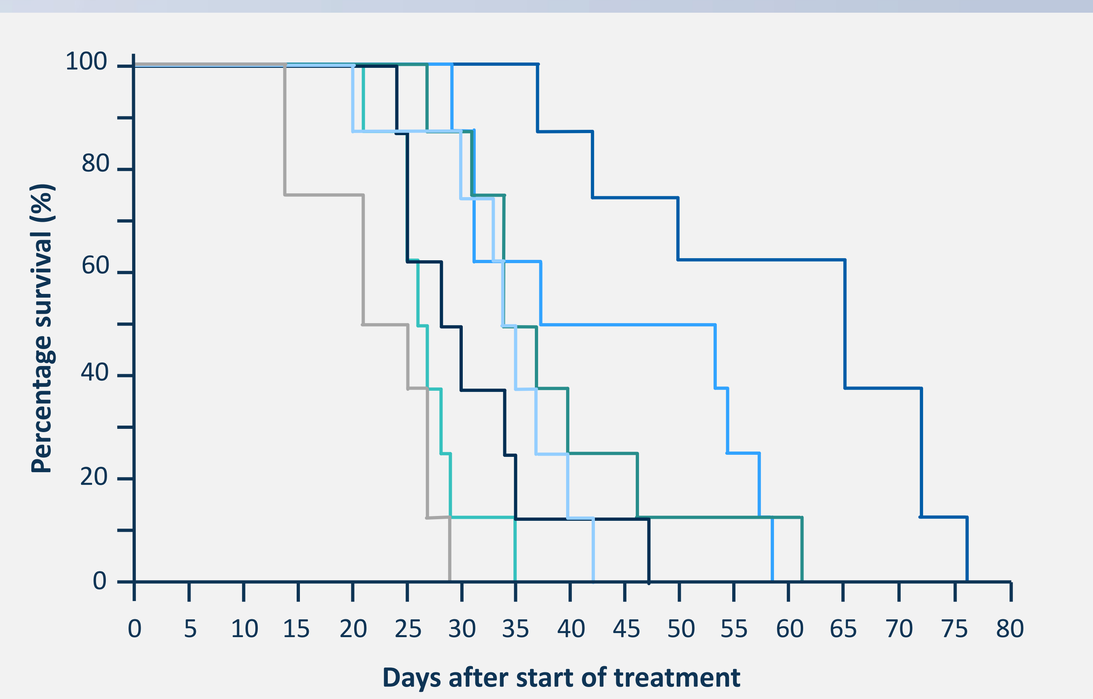
Results

- GLR2007 inhibited the activity of CDK4 and CDK6 at numerically lower IC_{50} and K_i values than palbociclib (**Table 1**)
- GLR2007 induced G1 arrest in human GBM cell line U87-MG at concentrations >13.72 nM (**Figure 1**)
- GLR2007 demonstrated penetration into the central nervous system, with total radioactivity levels in the brains of Sprague Dawley rats exceeding those in plasma by 2.3–4.5-fold from 2–6 h after dosing with [^{14}C]GLR2007 (**Figure 2**)
- In GBM xenograft models, tumor growth was inhibited and survival time increased in mice treated with GLR2007, versus vehicle control (**Table 2**, **Figure 3**)

Table 2. Tumor growth inhibition in mouse xenograft models

Xenograft model	Treatment	Dose (mg/kg)	Length of dosage (days)	Measure-ment day[a]	TGI[b] (%)	TGI *P* value[c]
Subcutaneous BN2289	GLR2007	5	28	21	4.9	<0.001
	GLR2007	25			39.4	<0.001
	GLR2007	50			56.4	<0.001
	Abemaciclib	25			24.9	<0.001
	Palbociclib	25			34.0	<0.001
Orthotopic U87-luc	GLR2007	25	42	21	86.3	0.588
	GLR2007	50			95.9	0.496
	Abemaciclib	25			79.0	0.666
Orthotopic U87-luc	GLR2007	6.25	35	28	78.8	0.028
	GLR2007	50			98.6	0.009

[a]Number of days after tumor cell inoculation on which tumor growth measurement was taken
[b]TGI, tumor growth inhibition (calculated as change in mean tumor volume from baseline for treatment group, as a percentage of change in vehicle group)
[c]Efficacy of treatment was evaluated by determining relative TGI versus vehicle control



Treatment	Increase in median survival time versus vehicle control (%)	*P* value
Vehicle control	–	–
Abemaciclib 50 mg/kg	15.2	0.2196
Abemaciclib 150 mg/kg	54.4	0.0002
GLR2007 12.5 mg/kg	50.0	0.0009
GLR2007 25 mg/kg	95.7	<0.0001
GLR2007 50 mg/kg	182.6	<0.0001
GLR2007 150 mg/kg	26.1	0.02

Figure 3. Kaplan–Meier survival curves of mouse orthotopic U87-luc xenograft models following treatment with a range of doses of GLR2007 or abemaciclib for 42 days

Conclusions

These preclinical studies suggest the potential value of GLR2007 for the treatment of GBM, supported by evidence that GLR2007 showed numerically greater antitumor efficacy than approved CDK4/6 inhibitors palbociclib and abemaciclib in GBM xenograft models and evidence of substantial central nervous system distribution.

References: 1. Schettini F, et al. *Front Oncol* 2018;8:608; 2. Miller TW, et al. *J Neurooncol* 2019;144:563–572; 3. Taylor JW, et al. *J Neurooncol* 2018;140:477–483; 4. Serwer LP, James CD. *Adv Drug Deliv Rev* 2012;64:590–597; 5. Taylor OG, et al. *Front Oncol* 2019;9:963

Conflicts of interest: Lei Yin, Zhenglin Yao, and Ang Yin are employees of Gan & Lee Pharmaceuticals; Yue Wang and Michelle Mazuranic are employees of Gan & Lee Pharmaceuticals USA Corp.

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