TG02, a brain penetrant multi-CDK inhibitor inhibits growth in MYC-driven glioblastoma

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Abstract

MYC is a central driver of tumorigenesis in many malignancies, including the universally lethal glioblastoma (GBM). However, developing direct inhibitors of MYC has proven challenging. One appealing alternative strategy to target MYC-driven cancers is to interfere with the signaling program necessary to facilitate MYC-dependent transcription. Cyclin-dependent kinase 9 (CDK9) has emerged as an attractive candidate through its function as a critical regulator in the transcriptional elongation of MYC and its target genes. Using a panel of patient-derived GBM cells, here we demonstrate that CDK9 inhibition with the brain-penetrant multi-CDK inhibitor, TG02, potently suppresses GBM cell growth. Importantly, the anti-GBM efficacy of TG02 strongly correlates with MYC expression and appears to be independent of methylation status, suggesting a critical role for CDK9 in MYC-driven GBMs. These preliminary results indicate that CDK9 may be an actionable therapeutic target in GBM with aberrant MYC signaling and, importantly, the clinical stage oral small molecule, TG02, is an appealing drug candidate for GBM with elevated MYC activity. Ongoing in vivo efficacy studies are evaluating TG02 in clinically relevant MYC-driven GBM mouse models.

Background

- Glioblastoma is the most lethal and the most malignant primary brain tumor with a median survival of 15 months
- MYC is known to be deregulated in a wide variety of cancers including glioblastomas and its constitutive expression is an established driver of tumorigenisis
- The MYC/MAX heterodimer lacks necessary binding pockets, which makes the MYC oncoprotein an undruggable driver
- MYC is known to promote transcriptional elongation by recruiting P-TEFb to RNA Polymerase II, and causing phosphorylation at Ser 2 (**Figure 1**)
- A cyclin-dependent kinase, CDK9, is required for the aberrant proliferation of MYC-overexpressing tumors. CDK9 promotes transcriptional elongation via phosphorylation of RNA Pol II
- TG02 is a novel multi-kinase inhibitor developed by Tragara Pharmaceuticals
- TG02 is blood-brain barrier penetrant, and exhibits a half maximal inhibitory concentration below 10nM for CDKs 1, 2, 3, 5, and 9

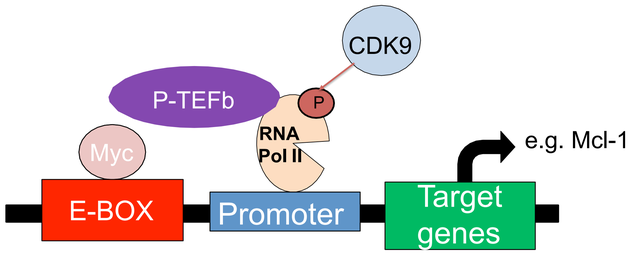


Fig.1 Myc promotes transcriptional elongation

Hypothesis and Approach

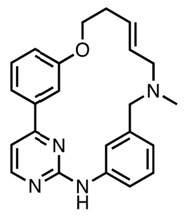
TG02 inhibits cell growth in GBM through CDK9-mediated transcription elongation in tumors with highly expressing Myc protein levels

- Determine a correlation between protein expression and half-maximal inhibitory concentration (IC50)
- Determine the effect of TG02 in a patient-derived orthotopic xenograft GBM model

A

Sphere		Genetic alteration					
HK157	N/A	CDKN2A					
HK301	N/A	CDKN2A	EGFR amp				
GBM39	M	CDKN2A	EGFR amp	PTEN			
GS014	U	EGFR vIII	PIK3CA	PTEN	CDKN2A	TERT	
GS104	U	EGFR amp	TP53	RB1	TERT		
GS025	M	EGFR amp	PIK3CA	CDKN2A	NOTCH	TERT	STAT4
GS027	M	PTEN	NF1	CDKN2A	TERT	MLL2	
GS100	U	EGFR vIII	PIK3C2B	MDM4	CDKN2A	TERT	
HK248	N/A	CDKN2A	PTEN	RB1	TP53		
GS05	U	PTEN	NF1	CDKN2A	TERT	BRCA2	SETD2
GS028	U	MET	PIK3C2B	PTEN	MDM4	CDKN2A	TERT
GS062	U	PTEN	CDKN2A	TERT	TSC1		
GS017	M	EGFR A289D/C636Y	PIK3C2B	MDM4	CDK4	TERT	
HK347	N/A	CDKN2A					
GS013	M	NF1	SETD2	LZTR			
GS075	M	PIK3C2B	PTEN	NF1	MDM4	CDKN2A	TERT

B



TG02

Target	IC50
CDK 9	3 nm
CDK 5	4 nm
CDK 2	5 nm
CDK 1	9 nm

Fig.2 A) Patient-derived neurospheres recapitulate GBM diversity **B)** TG02 is a potent multi-CDK inhibitor with IC50 in the low nm range

Primary GBM cells show variable sensitivity to TG02

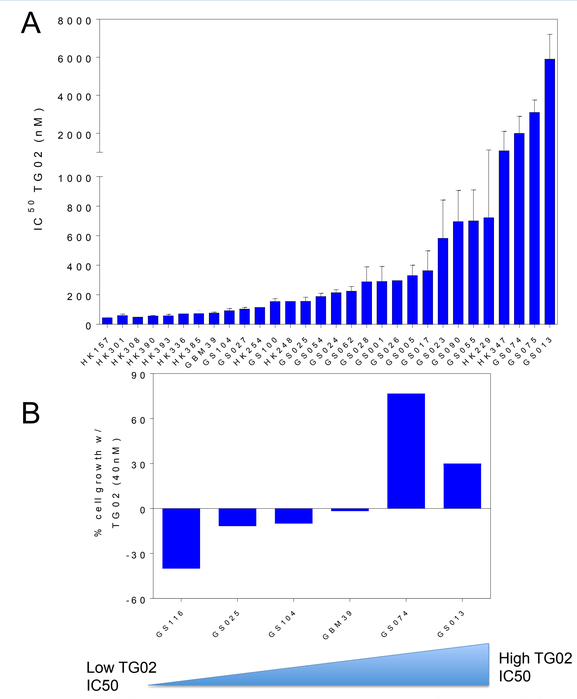


Fig.3 Primary GBM cells show variable sensitivity following TG0 treatment. **A)** Half-maximal inhibitory concentrations of 31 patient-derived GBM cell lines following 72 hours of TG02 treatment. **B)** Percent growth inhibition following 72 hours of 40nM TG02 treatment

MYC protein levels inversely correlate with TG02 IC50

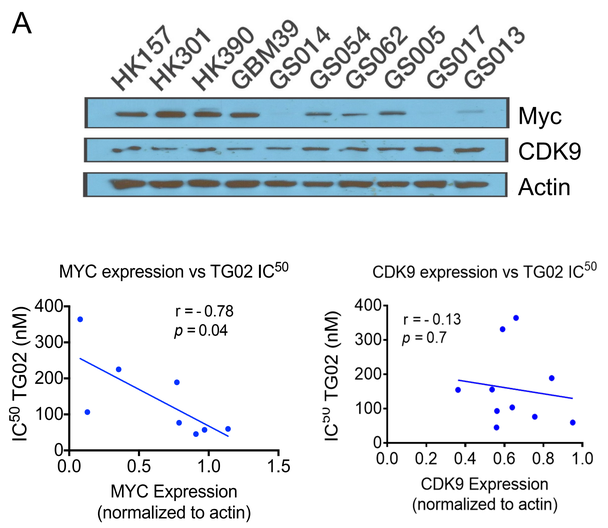


Fig. 4 Myc protein levels inversely correlate with IC50 of TG02**. A)** Western blot showing wide range of MYC and CDK9 protein levels in 10 patient-derived spheres **B)** MYC protein levels inversely correlate with TG02 IC50, but not CDK9.

TG02 inhibits CDK9 downstream signaling in GBM

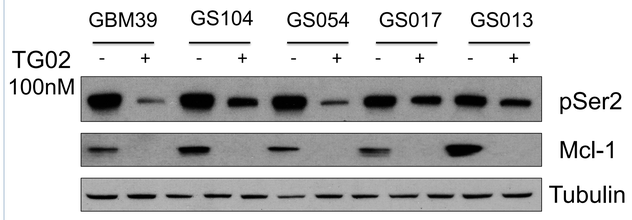


Fig. 5 Western blot showing effect of TG02 on phosphorylation of Ser2 RNA Pol II and Mcl-1 levels. Cells were treated with 100nM TG02 for 24 hours.

In vivo study design using PDOX model

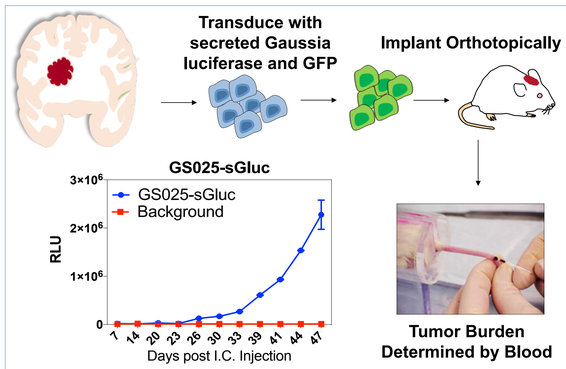


Fig.7 Study design for preclinical trial using patient-derived orthotopic models

TG02 treatment delays tumor growth of GBM PDOX model

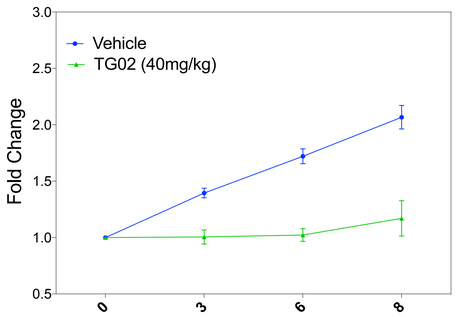
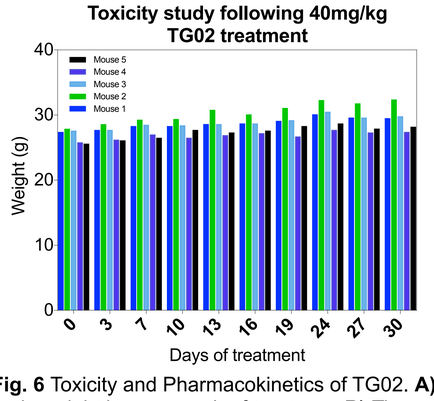


Fig.8 Orthotopic GBM tumors display growth inhibition following 8 days of TG02 treatment

Toxicity and Pharmacokinetics of TG02



	Plasma	Liver	Lung	Kidney	Heart	Brain
C_{max} (ng/mL or ng/g)	1029	21095	13618	5789	1513	2121
t_{max} (h)	0.5	0.5	1	0.5	4	0.5
$AUC_{0\text{-}last}$ (ng.h/mL or ng*h/g)	2523	38918	34751	17187	7137	6052
Tissue/plasma ratio	-	15.4	13.8	6.8	2.8	2.4

Fig. 6 Toxicity and Pharmacokinetics of TG02. **A)** Mice treated with 40mg/kg TG02 3 times/week did not display fluctuations in body weight in one month of treatment **B)** Tissue distribution of TG02 after a single administration under fed condition at 75 mg/kg.

Conclusions

- Inhibition of CDK9 with TG02 has potent, but heterogeneous activity in a subset of primary GBM samples
- Expression of MYC, but not CDK9, correlates with sensitivity
- Sensitivity to TG02 does not correlate with MGMT methylation status
- Preliminary results show activity of TG02 in an intracranial GBM model with high MYC expression

Future Directions

- Evaluate in vivo activity of TG02 in MYC high vs MYC low orthotopic patient-derived xenograft models

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