Synthetic luciferin, CycLuc1 improves bioluminescence imaging for intracranial glioblastoma xenografts

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Abstract

Background: Bioluminescence imaging (BLI) of firefly luciferase expressing cells is widely used for analysis of growth, metastasis and drug response in tumor xenografts. d-Luciferin, a commonly used luciferase substrate has limited brain distribution that may undermine BLI for intracranial xenografts. The synthetic luciferin, cyclic alkylamino-luciferin (CycLuc1) is a purported brain penetrant luciferase substrate not yet tested in intracranial xenografts. We compared BLI with CycLuc1 and d-luciferin in a glioblastoma (GBM) xenograft model.

Methods: GBM6 cells were lentivirally transduced to express LUC2=tdTomato fusion protein. *In vivo* BLI were performed in orthotopic and heterotopic xenografts established in athymic nude mice. Concentrations of d-luciferin and CycLuc1 were measured by LC-MS/MS.

Results: In a dose response analysis, CycLuc1 at 5 mg/kg provided robust and reproducible imaging. However, an increased photon flux was observed with 10 mg/kg, while escalation to 15 or 20 mg/kg provided marginal additional gains in photon flux. In a comparison of CycLuc1 (5 or 25 mg/kg) and d-luciferin (30 or 150 mg/kg, respectively) with serial, crossover BLI at both dose levels, CycLuc1 resulted in less variability and significantly higher photon flux as compared to d-luciferin in GBM6 intracranial xenografts (15 days after implantation). On average BLI with 25 mg/kg CycLuc1 resulted in ~8 fold greater photon flux from these early stage xenografts as compared to 150 mg/kg d-luciferin ($2.9\pm0.6\times10^6$ vs. $3.3\pm2.8\times10^5$ p/sec/cm^2, $p<0.001$). Interestingly, bioluminescence emission with CycLuc1 and d-luciferin were comparable in xenografts imaged 28 days after implantation. In samples harvested after last imaging session, there was no significant difference in substrate distribution in intracranial tumors (tumor to plasma ratios for CycLuc1 and d-luciferin were 0.012 ± 0.020 and 0.012 ± 0.015, respectively; p=0.89). These results suggest that intracranial tumors at early stage may comprise a relatively intact blood-brain barrier, which is disrupted at later stage. Consistent with the notion that sub-optimal delivery may interfere with d-luciferin mediated BLI in intracranial xenografts, photon flux in heterotopic tumors with d-luciferin or CycLuc1 was comparable ($4.9\pm4.6\times10^8$ vs. $1.3\pm0.8\times10^8$ p/sec/cm^2, p=0.10).

Conclusions: These findings demonstrate that CycLuc1 may be a superior BLI substrate as compared to d-luciferin for monitoring tumor growth of intracranial GBM xenografts.

Hypothesis and Objective

Hypothesis: Brain penetrant luciferase substrate can significantly improve quality and rigor of *in vivo* BLI for monitoring growth of *in vivo* GBM xenografts.

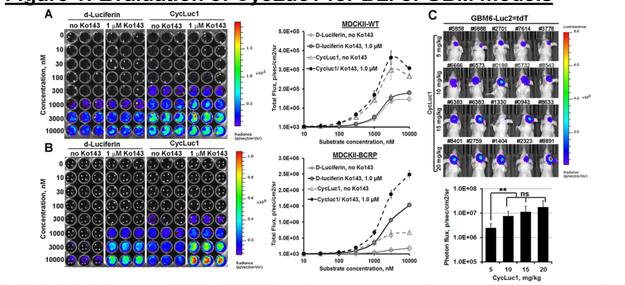
Objective: To compare CycLuc1 and d-luciferin mediated BLI in a GBM xenograft model refractory to several targeted therapies potentially due to partially intact blood-brain barrier.

Materials & Methods

- D-Luciferin was purchased from Sigma-Aldrich, dissolved in PBS, aliquoted and kept frozen at -80ºC. CycLuc1 was obtained from Glix Laboratories Inc., dissolved at 10X concentration in DMSO, aliquoted and kept frozen at -20ºC. Injectable d-luciferin aliquots were thawed to room temperature, while CycLuc1 was diluted in PBS immediately prior to imaging.
- MDCKII-WT, MDCKII-BCRP and GBM6 cells were lentivirally transduced to express LUC2=tdTomato fusion protein and stable transductants expressing luc2=tdT fusion gene were selected in 5 µg/mL puromycin. GBM6-Luc2=tdTomato cells were propagated as flank xenografts in athymic nude mice.
- Intracranial (orthotopic) xenografts of GBM6-Luc2=tdTomato cells were established by injecting 3×10^5 cells into the right hemisphere of the brain, and heterotopic xenografts were established by injecting 2×10^6 cells in the flank of mice. Mice were randomized in groups of 4-5 mice, and imaged weekly.
- For *in vitro* BLI with defined substrate concentrations cells were suspended in Hanks balanced buffer, treated with (or without KO143, BCRP inhibitor) and plated in 96-well plates.
- For *in vivo* BLI, animals were anesthetized by isoflurane inhalation, and maintained under anesthesia by continuous infusion of isoflurane until imaging was complete.
- BLI performed using IVIS Spectrum with open emission filter, exposure time 60s, medium binning, field of view 12.9 cm and f/stop 1. Unless stated otherwise, all images were captured 10 min after intraperitoneal injection with an indicated dose of the substrate, d-luciferin or CycLuc1. To compare light emission with each substrate, cross-over imaging was performed on same animals with 24-48 h gap was between cross-over imaging sessions.
- Images were analyzed by Living Image 4.3 software (PerkinElmer) specially designed for IVIS system, data presented as median ± sd, analyzed by paired t-test, $p<0.05$ considered significant and denoted with *.
- At terminal stage, animals were injected with 150 mg/kg d-luciferin and 10 mg/kg CycLuc1 just 10 min prior to euthanasia by CO_2 inhalation. Plasma and whole brain were extracted for analysis of substrate concentration by LC-MS/MS.

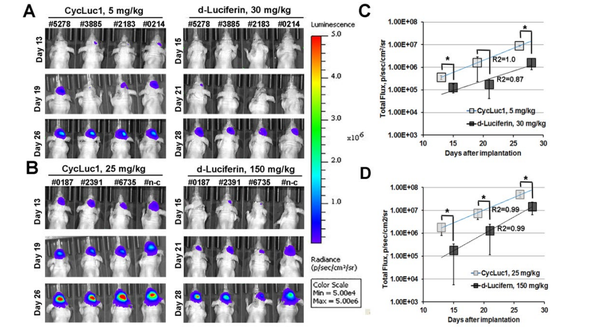
Results

Figure 1: Evaluation of CycLuc1 for BLI of GBM models



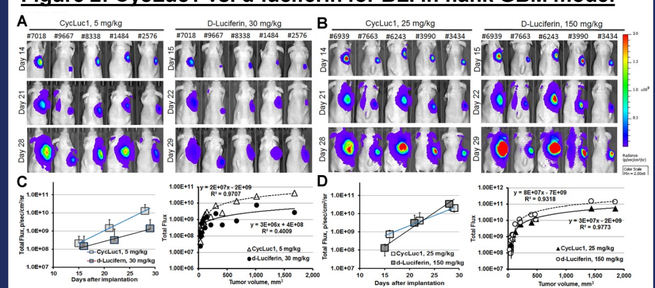
A. MDCKII-WT versus MDCKII-BCRP cells overexpressing Luc=tdTomato fusion were treated with (or without) BCRP inhibitor KO143 for 30 min, subjected to in vitro BLI using graded concentration of CycLuc1 or d-luciferin; B. dose response to determine optimal dose of CycLuc1 for in vivo BLI in orthotopic GBM xenografts.

Figure 2: CycLuc1 vs. d-luciferin for BLI in orthotopic xenografts



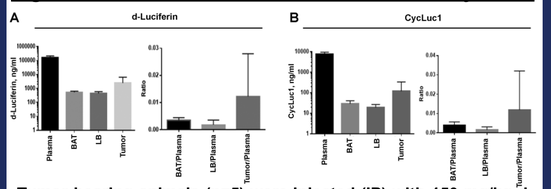
A-B. Monitoring of orthotopically implanted GBM cells by *in vivo* sequential and cross-over BLI with low (A) or high dose (B) of CycLuc1 or d-luciferin; C-D. Graphs showing median photon flux in each group and fitting of data in an exponential growth curve for the low (C) and high (D) dose BLI, respectively.

Figure 2: CycLuc1 vs. d-luciferin for BLI in flank GBM model



A-B. Monitoring of subcutaneously implanted GBM cells by *in vivo* sequential and cross-over BLI with low (A) or high dose (B) of CycLuc1 or d-luciferin; C-D. Graphs showing median photon flux ± SD in each group of animals and fitting of the data in an exponential growth curve (left), and correlation between phonon flux and tumor volume analyzed for linear model (right) for the low (C) and high (D) dose BLI, respectively.

Figure 4: Tissue distribution of d-luciferin & CycLuc1



Tumor bearing animals (n=5) were injected (IP) with 150 mg/kg d-luciferin (A), 5 mg/kg CycLuc1 (B), euthanized after 10 min. Extracted tissues: plasma, left brain (LB), brain around tumor (BAT) and tumor were stored in -80°C & later analyzed by LC/MS.

Discussion

- *In vitro* BLI in MDCKII-WT and MDCKII-BCRP cells suggest that Cyclucl and d-luciferin imaging both have significant efflux liability towards BCRP.
- Similarity in tissue distribution for the Cyclucl and d-luciferin suggest that blood-brain barrier suppresses overall delivery of both substrates, but it would not explain the differential signal output for BLI in orthotopically implanted GBM xenografts.
- Superior emission properties of CycLuc1 may be in part responsible for the stronger BLI signal in orthotopic GBM xenografts.
- Comprehensive analyses of transport mechanisms, regional distribution within tumor tissues and physio-chemical properties may reveal other factors that influence BLI in GBM xenografts.

Conclusions

- Both D-luciferin and CycLuc1 BLI signals are influenced by BCRP-mediated efflux, D-luciferin imaging suffers from a higher degree of efflux.
- High variability in bioluminescence emission from d-luciferin-mediated BLI in orthotopic xenografts an be suboptimal for monitoring tumor growth.
- Linear correlation between photon flux and tumor volume in heterotopic model suggest that low dose CycLuc1 is likely applicable to monitor growth of orthotopic tumors.
- Blood-brain barrier equally restricts delivery of CycLuc1 and d-luciferin.
- Regional differences in blood-brain barrier integrity within orthotopic GBM xenografts may not influence delivery or efficiency of BLI.
- Superior emission properties with low dose CycLuc1 is more suitable for BLI of deep tissues such as orthotopic GBM xenografts.