# PRECLINICAL DEVELOPMENT OF miR-10b ANTAGONIST FOR THE TREATMENT OF GLIOBLASTOMA

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## Abstract

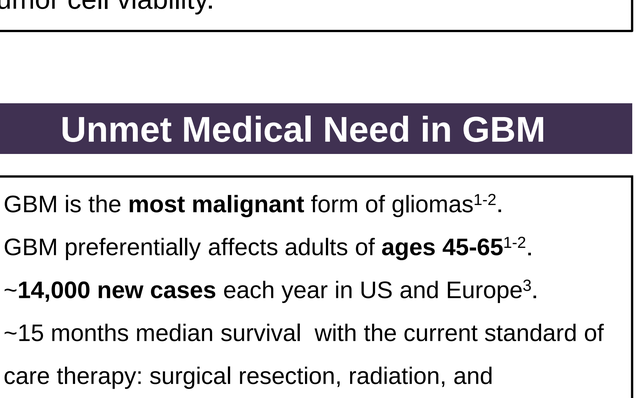
Glioblastoma (GBM) is the most aggressive primary brain cancer with a median survival of 15 months after diagnosis[1-4]. miR-10b is highly expressed in all GBM molecular subtypes whereas its expression in normal brain cells is nearly undetectable[5-6]. Herein, we report the status of the preclinical development of oligonucleotide antagonists of miR-10b for the treatment of GBM.

A library of 218 anti-miR-10b oligonucleotides with various lengths and chemical modifications was prepared and screened using a luciferase-based cellular miR-10b activity assay and liver slice assay (to assess potential off-target inflammatory effects). Compounds were profiled in vitro using multiple functional assays including selective inhibition of cell viability and induction of apoptosis comparing GBM cell lines and other cell lines lacking miR-10b expression. Nineteen compounds were selected for further evaluation in a xenograft mouse GBM model using human LN229 GBM cells injected intracranially. An anti-miR-10b lead compound exhibited consistent efficacy in vitro and in vivo in all screening assays. A single intratumoral injection of anti-miR-10b lead compound significantly increased median survival of tumor-bearing animals by 18%, while combination treatment with temozolomide (TMZ) extended median survival time by 159% (TMZ alone increased median survival by 27%). This anti-miR-10b lead compound exhibits favorable physiochemical properties and in vivo safety profile, which support its further development toward clinical testing. Preliminary mechanistic studies indicate that inhibition of miR-10b in GBM cell lines increased apoptosis/cell death-related and decreased proliferation-related gene expression and had synergistic inhibitory effects with TMZ on tumor cell viability.

## Unmet Medical Need in GBM

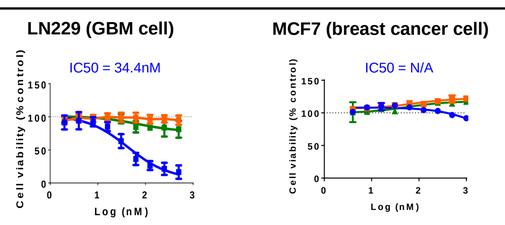
- GBM is the **most malignant** form of gliomas[1-2].
- GBM preferentially affects adults of **ages 45-65**[1-2].
- ~**14,000 new cases** each year in US and Europe[3].
- ~15 months median survival with the current standard of care therapy: surgical resection, radiation, and chemotherapy[3-4].
- Major treatment hurdles: drug delivery and molecular heterogeneity[3].

## miR-10b is Selectively Up-regulated in GBM




Left: GBM cell lines (LN229, T98G, U87, and GBM-6 Neuro luc) express higher miR-10b levels compared to those of non-GBM cell lines (MCF7, HepG2, human normal astrocytes, and cortical neuron HCN2 cells).
Right: GBM tumors provided by TD2 and collected from Regulus *in vivo* studies express high levels of miR-10b compared to those of normal brain tissues.

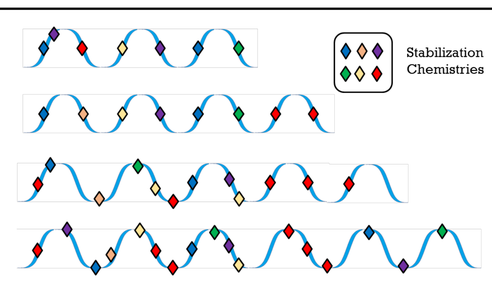
## Anti-miR-10b Specifically Decreases GBM Cell Viability




| Oligo# | Target | Sequence | Modification |
|---|---|---|---|
| RG362223 | miR-155 | CCCCTATCACAATTAGCATTAA | uniform 2'- |
| RG386666 | mismatch miR-122a | ACATACTCCTTTCTCAGAGTCCA | MOE in PS |
| RG348124 | miR-10b | CACAAATTCGGTTCTACAGGGTA | backbone |

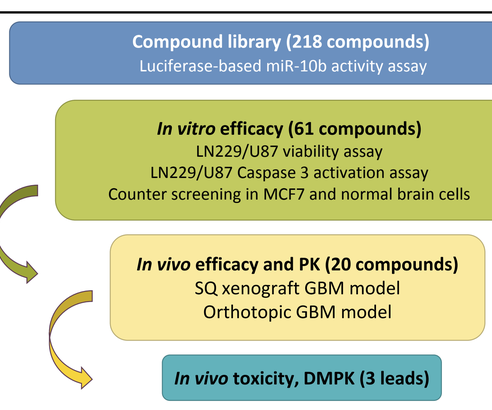
Anti-miR-10b tool compound RG348124, but not control compounds RG362223 and RG386666, specifically decreased cell viability in GBM cell lines LN229 (left), but not in breast cancer cells MCF7 that has minimal miR-10b expression (right).

## Design of Anti-miR-10b Library

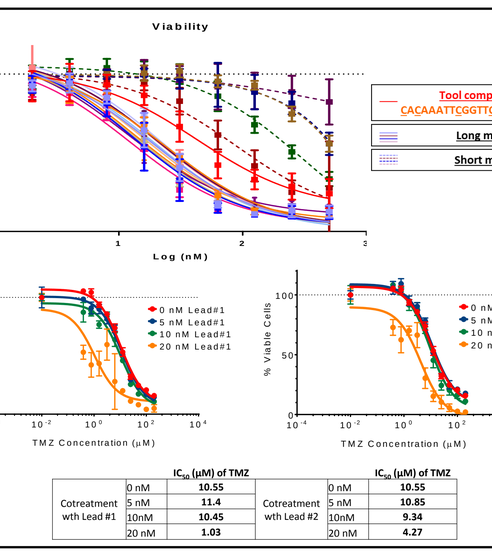



- Benchmark with tool compound: : CACAAATTCGGTTCTACAGGGTA
- The library includes 218 new anti-miR10b compounds.
- Length varies from 9 to 23 nt.
- Stabilization chemistries include phosphorothioate (PS) backbone modification and sugar modifications like 2'-O-methyl, 2'-O-methoxyethyl, 2'-fluoro, and (S)-constrained ethyl.
- Structure modifications include long miR, short miR, linker miR, seed miR, and proseed miR.

## Screening Cascade



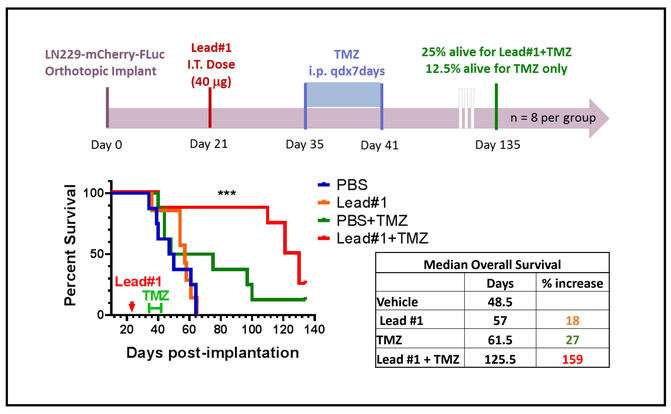

## *In Vitro* Efficacy of anti-miR-10b Lead Compounds




| | | IC50 (μM) of TMZ | | | IC50 (μM) of TMZ |
|---|---|---|---|---|---|
| Cotreatment wth Lead #1 | 0 nM | 10.55 | Cotreatment wth Lead #2 | 0 nM | 10.55 |
| | 5 nM | 11.4 | | 5 nM | 10.85 |
| | 10nM | 10.45 | | 10nM | 9.34 |
| | 20 nM | 1.03 | | 20 nM | 4.27 |

Top: Oligos with top efficacy from in vitro functional screen in LN229 GBM cell line. Red solid line: tool compound RG348124**.** Other solid lines: novel long miRs. Dotted lines: novel short miRs.
Bottom: Lead molecules significantly enhance the potency of TMZ, showing as decreased $IC_{50}$ of TMZ in LN229 viability assay after combination treatment.

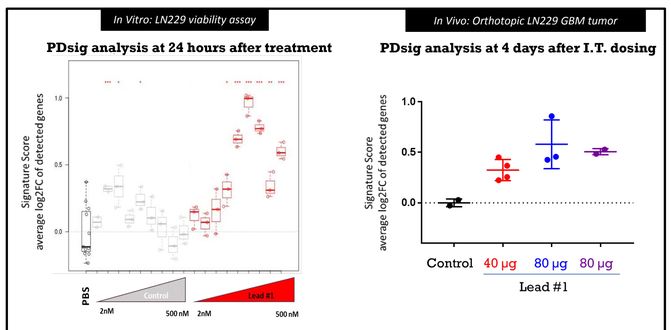
## *In Vivo* Efficacy of anti-miR-10b Lead




| Median Overall Survival | | |
|---|---|---|
| | Days | % increase |
| Vehicle | 48.5 | |
| Lead #1 | 57 | 18 |
| TMZ | 61.5 | 27 |
| Lead #1 + TMZ | 125.5 | 159 |

Top: Study design diagram. LN229 tumor cells were implanted orthotopically on Day 0 into nude mice. Mice were given intratumoral dosing of either 40 μg lead #1 or vehicle at Day 21, then followed by TMZ or vehicle treatment from Day 35-41. Overall median survival was recorded daily.
Bottom: Single agent treatment of Lead #1 resulted in ~18% increase of median survival in GBM mice compared with vehicle treatment (57 days vs. 48.5 days). In combination with TMZ, Lead #1 increased animal survival by ~159% (125.5 days vs. 48.5 days).

## On-target Pharmacodynamics Effect after Treatment with Lead #1




**PDsig of grouped 18 miR-10b downstream genes:**
**ATXN2, ATXN7, BCL6, BDNF, CRLF3, DAZAP1, DVL3, FXR2, GATAD2A, GCLM, GTF2H1, INO80D, MIEF1, NCOA6, NFE2L1, PDE4A, SMAD2, TET2**

A set of 18 miR-10b direct-target genes were identified by NGS analysis and selected as miR-10b PDsig genes. Treatment of LN229 cells *in vitro* with Lead #1 resulted in dose dependent de-repression of miR-10b PDsig (left). Consistently, treatment of orthotopic LN229 tumor with Lead #1 resulted in de-repression of miR-10b PDsig in *in vivo* (right).

## Summary Profile of Lead #1

| Category | Assays | Lead #1 |
|---|---|---|
| In Vitro Efficacy | LN229 viability assay | $IC_{50}$= 12.2nM |
| | U87 viability assay | $IC_{50}$= 24.8nM |
| | LN229 caspase3 activation assay | $EC_{50}$= 13.1nM |
| In Vitro Selectivity | MCF7 viability assay | $IC_{50}$= 65.5nM (7x) |
| In Vitro TI | HCN2 viability assay | $IC_{50}$= 85.9nM (TI~7) |
| In Vivo Efficacy | Single agent intratumoral dosing | 18 % median survival increase |
| | Combination with TMZ | 159 % median survival increase |
| In Vitro DMPK | % Metabolite in Mouse Liver | 13 |
| | % Metabolite in Mouse Brain | 0 |
| Physicochemical Property | Avg. Viscosity (cP) | 41.4 |
| | Osmo (mOsm/kg) | 316 |
| | pH | 8.2 |
| CNS Safety | MTD intra-cerebral dosing in B6 mice | 250 μg |
| | CNS TI | ~6 |
| Systemic Safety | Clinical laboratory changes | negative @ ≤300 mg/kg |
| | IFIT and OASL in liver | negative @ ≤300 mg/kg |
| | IFIT and OASL in kidney | negative @ ≤300 mg/kg |
| | Histopathology in liver and kidney | negative @ ≤300 mg/kg |
| | Systemic TI | >300 |

TI: therapeutic index

## References

1. Cihoric, N., et al., *Current status and perspectives of interventional clinical trials for glioblastoma - analysis of ClinicalTrials.gov.* Radiat Oncol, 2017. **12**(1): p. 1.
2. Touat, M., et al., *Glioblastoma targeted therapy: updated approaches from recent biological insights.* Ann Oncol, 2017. **28**(7): p. 1457-1472.
3. Weathers, S.P. and M.R. Gilbert, *Advances in treating glioblastoma.* F1000Prime Rep, 2014. **6**: p. 46.
4. El Fatimy, R., et al., *Genome Editing Reveals Glioblastoma Addiction to MicroRNA-10b.* Mol Ther, 2017. **25**(2): p. 368-378.
5. Gabriely, G., et al., *Human glioma growth is controlled by microRNA-10b.* Cancer Res, 2011. **71**(10): p. 3563-72.
6. Guessous, F., et al., *Oncogenic effects of miR-10b in glioblastoma stem cells.* J Neurooncol, 2013. **112**(2): p. 153-63.

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