Poster #1399

MEIRAGTx

AAV-mediated riboswitch-controlled delivery of anti-HER2 antibody suppresses HER2-positive tumorigenesis

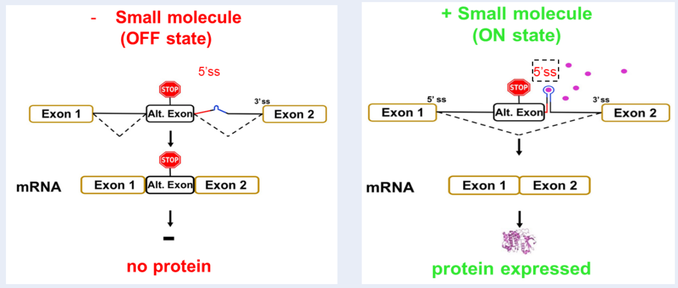
Authors:
George Wang, Zhaojing Zhong, Jae Gyun Oh, Samuel D Waksal, Alexandria J Forbes, & Xuecui Guo

Gene Regulation, MeiraGTx, New York

ABSTRACT

Controlled expression of delivered transgenes may be critical for optimized, safe and effective genetic medicines. AAV-mediated gene transfer is a promising therapy for many diseases. However, excessive amounts of transgene from unregulated vector may limit the breadth of applicability of gene therapy. A specific and precise mechanism for gene control via orally delivered small molecules with high dynamic range and gene expression at least as high as unregulated genes would provide a safe and effective gene therapy approach to a broad range of disease areas. Here, we present the development of regulated vectorized antibody genes, whose expression is controlled by riboswitch via oral small molecule inducers. Antibody vectorization is optimized for each antibody gene sequence to physiologically relevant levels of antibody production. Optimized vectorized antibody sequences are regulated using our proprietary synthetic mammalian riboswitch platform. In contrast to previously reported gene regulation systems that involve the use of exogenous protein components, our gene expression platform utilizes a synthetic mammalian riboswitch which is an RNA element that contains an aptamer as sensor for small molecule ligand/inducer. In our aptamer riboswitch system, aptamer/ligand binding alters transgene splicing, turning gene expression on or off in a dose dependent fashion. In the absence of the small molecule inducer *in vitro*, antibody gene with riboswitch cassette does not express antibody protein, whereas in the presence of small molecule inducer, antibody is robustly produced with a precise dose response to the small molecule. When antibody gene with riboswitch was delivered in AAV to mice, orally dosed small molecule induced antibody expression in a dose responsive fashion to the oral inducer. Expression subsequently diminishes and returns to baseline level following withdrawal of the small molecule inducer. We also demonstrate a function dose response in a tumor model of one of our optimized vectorized regulated antibody constructs. Our data indicate that our synthetic mammalian riboswitch works efficiently *in vivo* and can provide precise control of therapeutic antibody expression by controlling the dose of orally administered small molecule.

Aptamer-modulated alternative splicing riboswitch

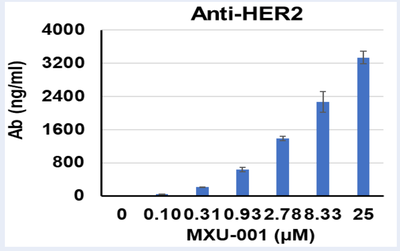


Schematics of synthetic riboswitch

Aptamer is inserted in the downstream intron of an Intron-AltExon-intron cassette within the cDNA

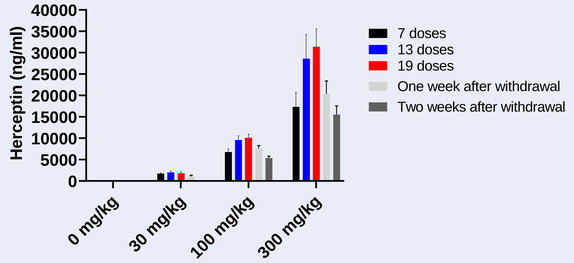
In the absence of small molecule (Left panel):	In the presence of small molecule (right panel):
Alt. exon 5′ ss is accessible	Alt. exon 5′ ss is sequestered
Alt. exon is included	Alt. exon is skipped
No protein expressed	Protein expressed

Riboswitch-regulated anti-HER2 antibody *in vitro*



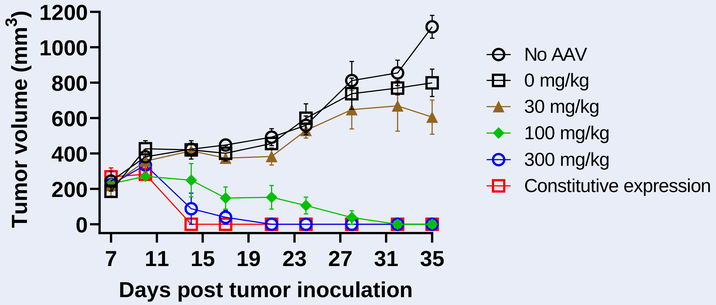
- Schematics of therapeutic antibody expression construct with riboswitch cassette being inserted in the antibody coding sequence.
- Antibody constructs were transfected into HEK 293 cells.
- Transfected HEK 293 cells were treated with small molecule inducer MXU-001 at different concentrations.
- Antibody expression was evaluated by ELISA.
- Antibody expression was induced in response to riboswitch inducer in a dose dependent manner.

Riboswitch-regulated expression of anti-HER2 antibody via orally dosed inducer *in vivo*



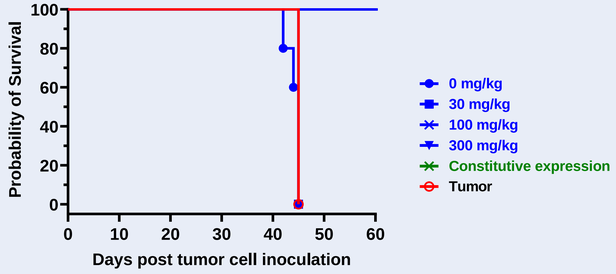
- AAV vectors with anti-HER2 Ab gene containing riboswitch were injected into mouse thigh muscle.
- 4 weeks post AAV delivery, HER2+ Calu-3 lung cancer cells were implanted *s.c.* into B6.Rag1 mice.
- 1 week post tumor cell inoculation, mice were dosed orally with MXU-001 at different doses, 6 doses/week.
- Blood samples were collected during MXU-001 treatment and after its withdrawal.
- Mouse serum anti-HER2 Ab levels were evaluated by ELISA.

AAV-delivered, riboswitch-controlled anti-HER2 antibody inhibits HER2⁺ tumor growth *in vivo*



- AAV vectors with anti-HER2 Ab gene containing riboswitch were injected into mouse thigh muscle.
- 4 weeks post AAV delivery, HER2+ Calu-3 lung cancer cells were implanted *s.c.* into B6.Rag1 mice.
- 1 week post tumor cell inoculation, mice were dosed orally with MXU-001 at different doses, 6 doses/week.
- Tumor size was measured twice weekly.

AAV-delivered, riboswitch-controlled anti-HER2 antibody prolongs tumor-free survival



- During the efficacy study, mouse survival was monitored.
- Mice were sacrificed when any dimension of tumor mass reached 20mm.
- Kaplan Meier survival analysis was done using Prism GraphPad software 9.

Summary

- Our data indicate that our synthetic mammalian riboswitch works efficiently both *in vitro* and *in vivo* in precisely regulating therapeutic antibody expression via a riboswitch small molecule inducer.
- Therapeutic antibody expression was induced in a dose dependent manner via orally available small molecule inducer, enabling precise control of therapeutic antibody levels *in vivo.*
- The induced anti-HER2 antibody is efficacious in suppressing HER2⁺ tumor growth and prolonging tumor-free survival in a dose responsive fashion to the oral small molecule inducer.
- Our data demonstrate the therapeutic potential of optimized vectorized antibody constructs regulated precisely by the dose of oral small molecules using our synthetic mammalian riboswitch technology.