

Riboswitch-controlled delivery of therapeutic antibodies for gene therapy

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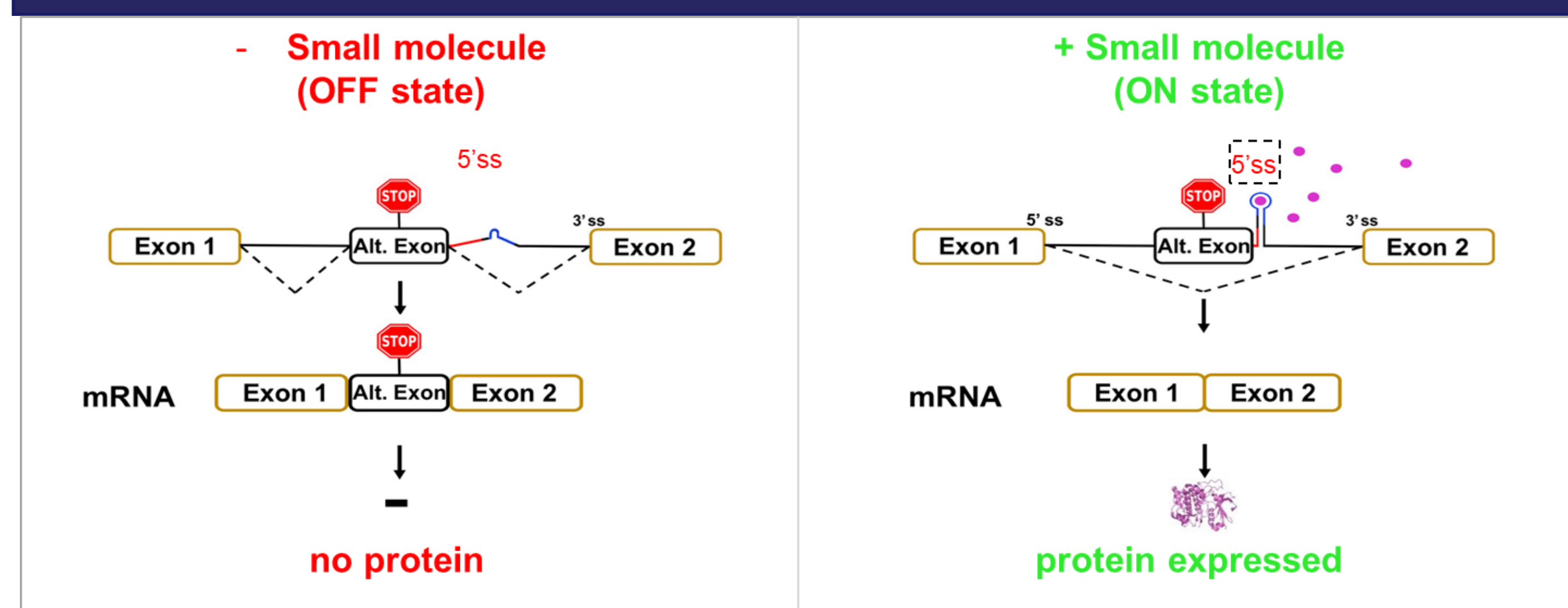
Gene Regulation, MeiraGTx, New York



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 success of gene therapy. A genetic switch or gene control mechanism would provide a gene therapy approach that can be safely controlled and is applicable to a broader range of disease areas outside of inherited diseases. Here, we present the development of regulated antibody genes, whose expression is controlled by riboswitch via small molecule inducer. In contrast to previously reported gene regulation systems that involve the use of exogenous protein components, our gene expression platform utilizes a 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. When antibody gene with riboswitch was delivered in AAV to mice, orally dosed small molecule induced antibody expression, which subsequently diminished and returned to baseline level following withdrawal of the small molecule inducer. 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

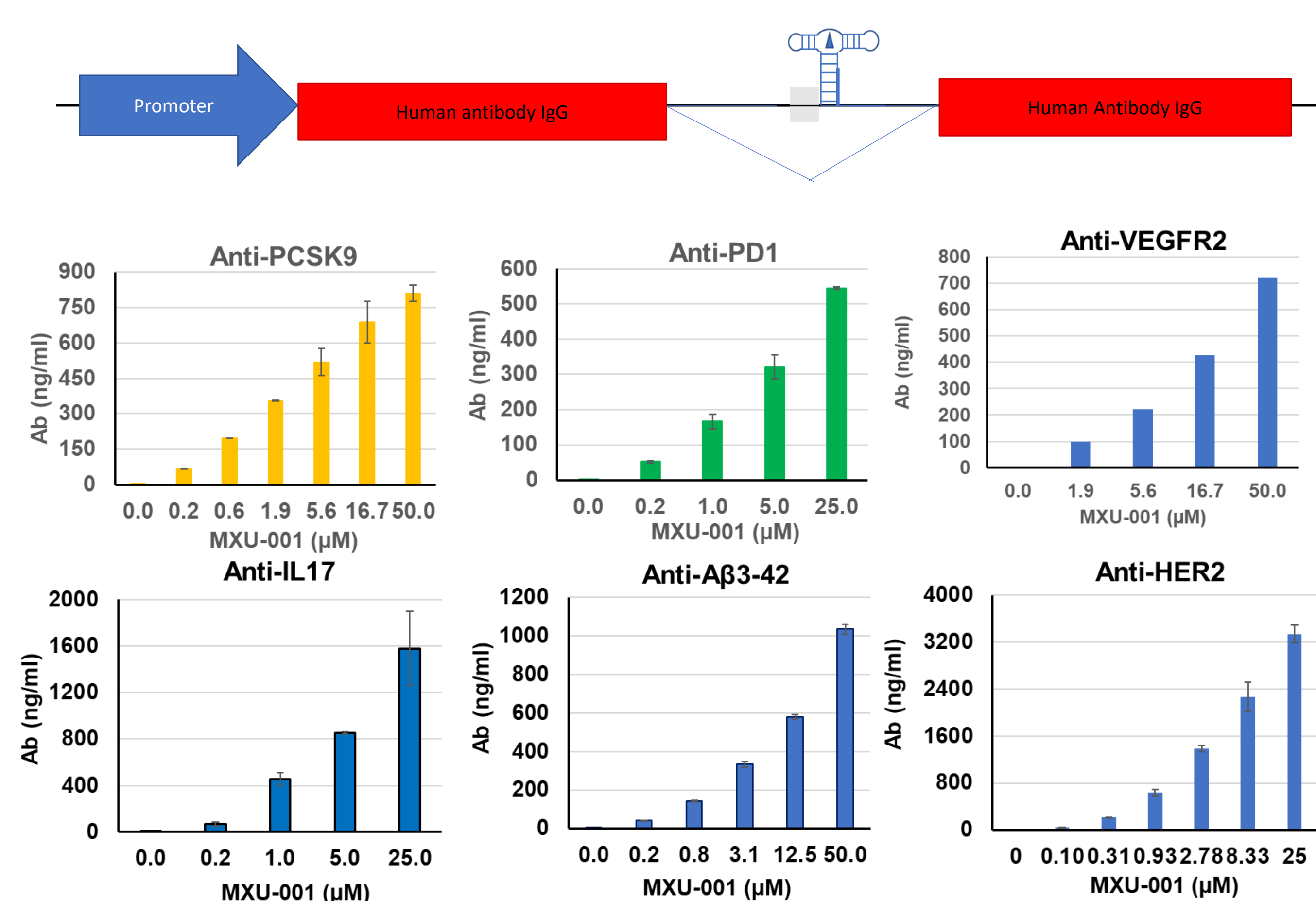
In the absence of small molecule (Left panel):

- Alt. exon 5' ss is accessible
- Alt. exon is included
- No protein expressed

In the presence of small molecule (Right panel):

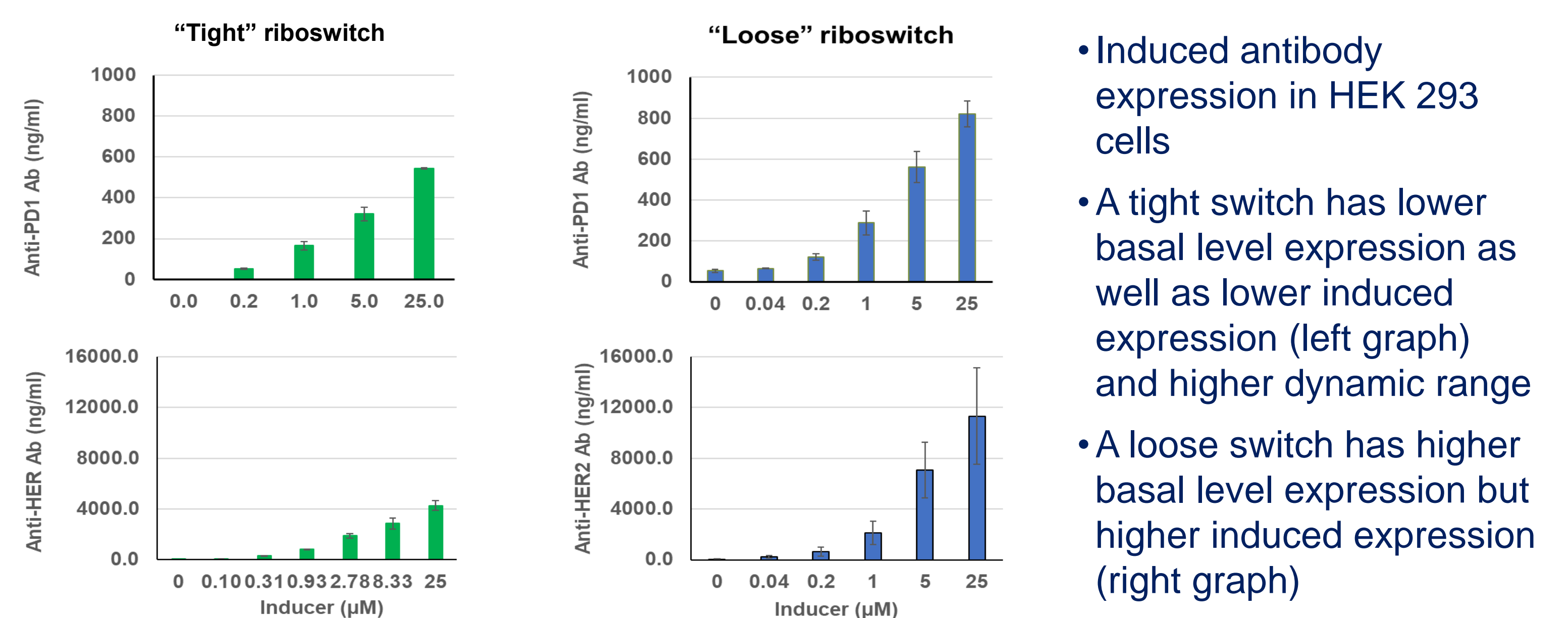
- Alt. exon 5' ss is sequestered
- Alt. exon is skipped
- Protein expressed

Riboswitch regulated therapeutic antibody expression *in vitro*



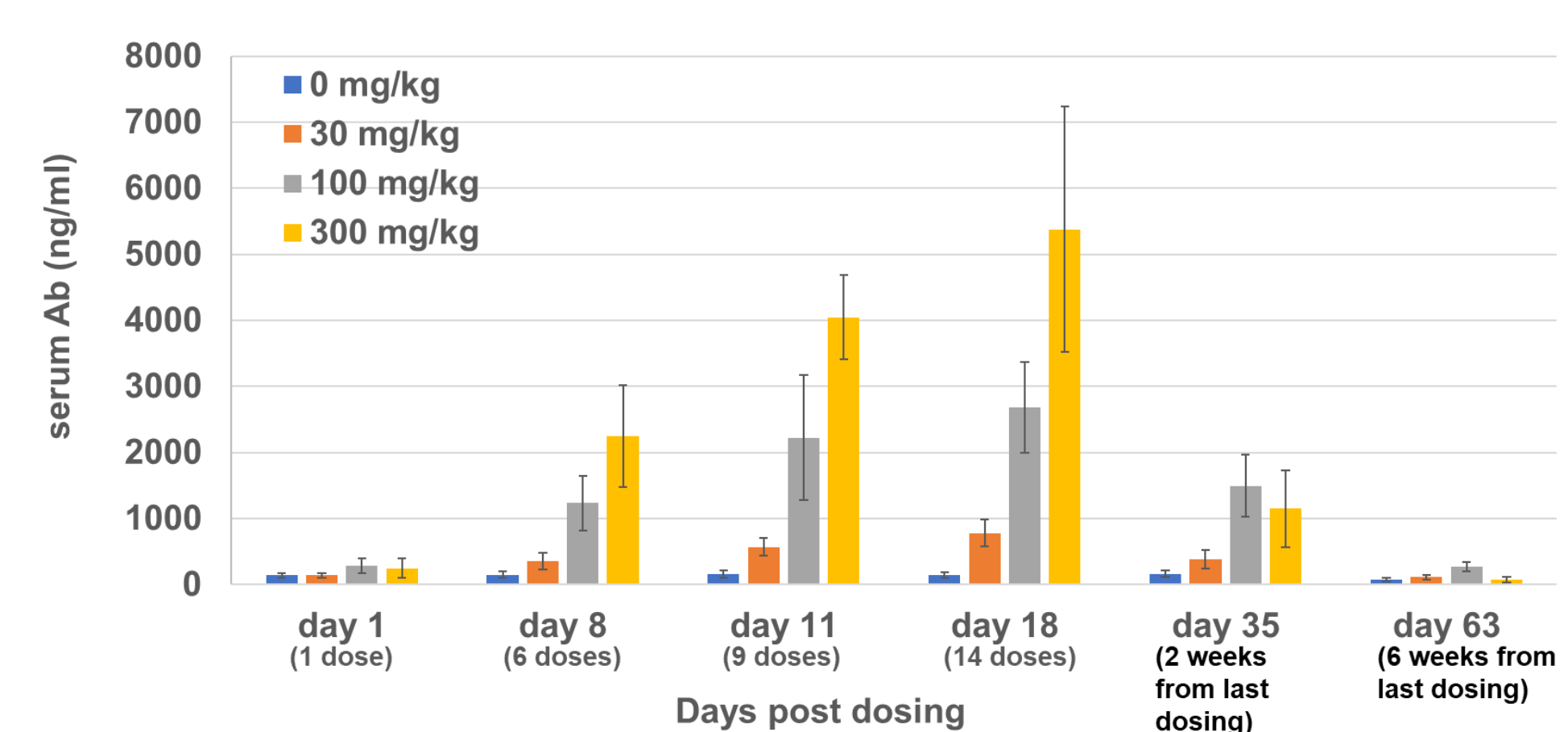
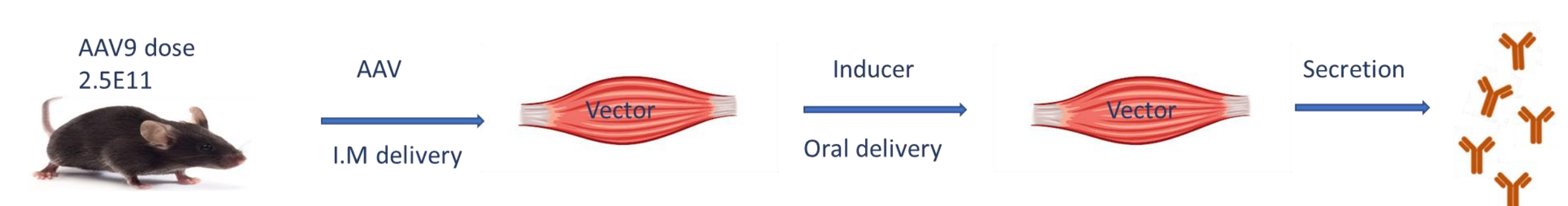
- Schematics of therapeutic antibody expression constructs with riboswitch cassette being inserted in the antibody coding sequence
- Antibody constructs were transfected into HEK 293 cells.
- Transfected HEK 293 cells were treated with small molecule inducer MXU-001 at different concentrations
- In the absence of riboswitch inducer, no or minimal antibody expression was detected
- In the presence of riboswitch inducer, antibody expression was increased.
- Antibody expression was induced in response to riboswitch inducer in dose dependent manner

Tight vs. loose riboswitch in controlling the dynamic range of induced antibody expression



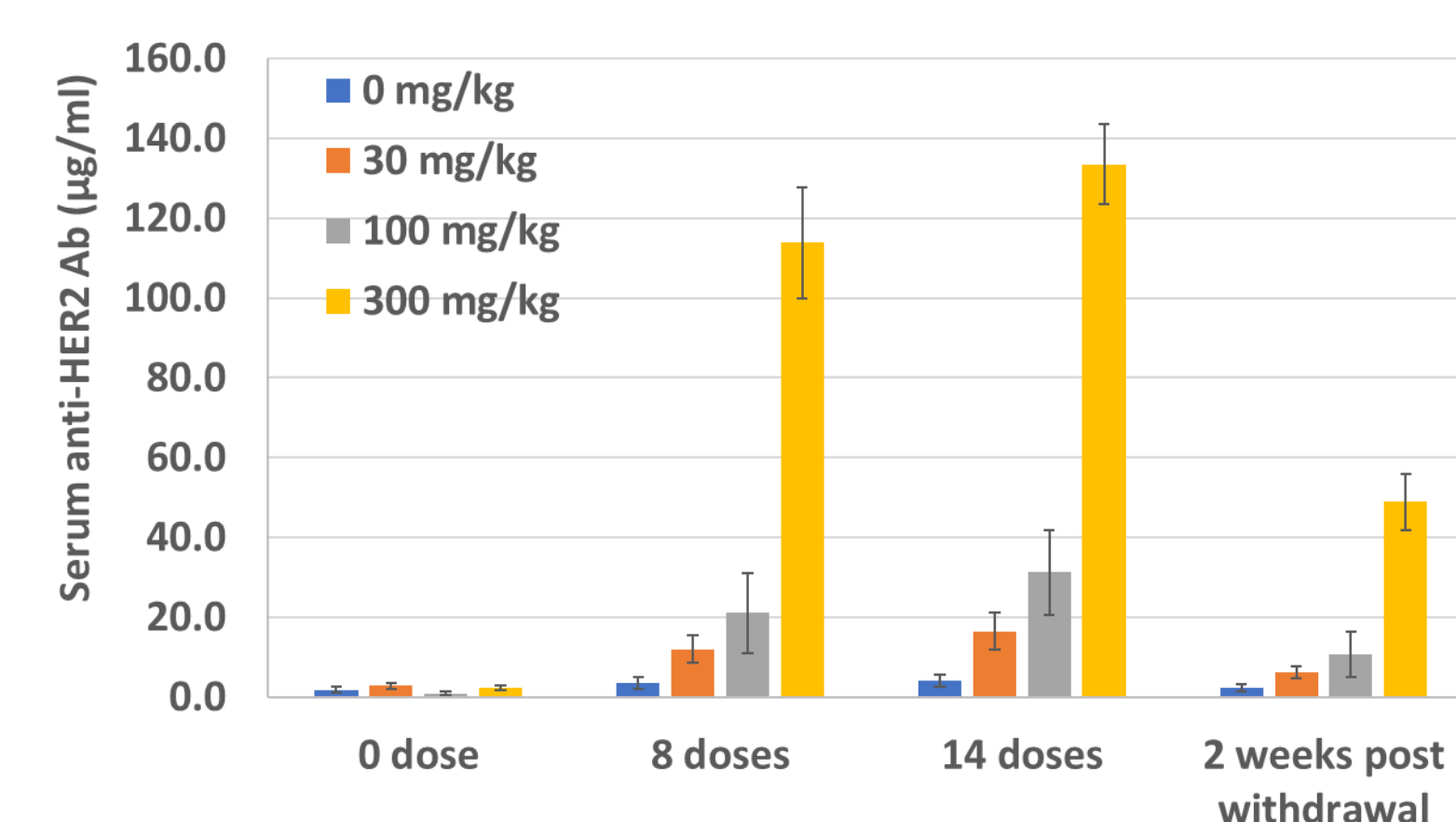
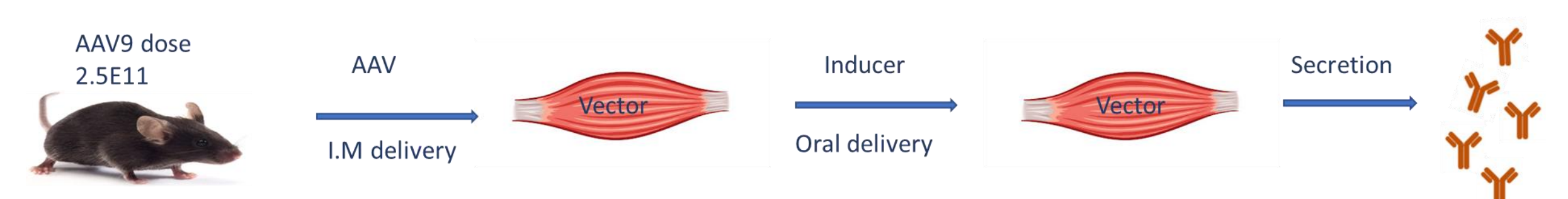
- Induced antibody expression in HEK 293 cells
- A tight switch has lower basal level expression as well as lower induced expression (left graph) and higher dynamic range
- A loose switch has higher basal level expression but higher induced expression (right graph)

Riboswitch regulated expression of anti-PD1 antibody via orally dosed inducer *in vivo*



- AAV9-anti-PD1 vector was injected to the muscle of mice. 4 weeks post AAV delivery, MXU-001 was administered orally once daily for the indicated number of days and doses. Antibody expression in serum post dosing was measured by ELISA.
- Antibody expression was induced by MXU-001 in a dose dependent manner at multiple dose levels, and the level of antibody decreased to baseline level after withdrawal of inducer.
- This results demonstrate the on/off state of the therapeutic gene controlled by small molecule inducer in animal.

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



- AAV9-anti-Her2 vector was injected to the muscle of mice. 4 weeks post AAV delivery, MXU-001 was administered orally once daily for the indicated number of days and doses. Antibody expression in serum post dosing was measured by ELISA.
- Antibody expression was induced by MXU-001 in a dose dependent manner at multiple dose levels, and the level of antibody decreased after withdrawal of inducer.
- This results demonstrate the on/off state of the therapeutic gene controlled by small molecule inducer in animals.

Summary

- Riboswitch regulates gene expression post transcriptionally
- Riboswitch comprises no exogenous protein components
- Riboswitch controls gene expression by small molecule inducer
- Therapeutic antibody expression was tightly controlled by riboswitch *in vitro* and *in vivo*
- Therapeutic antibody expression was induced by small molecule inducer in dose dependent manner via orally available small molecule inducer
- Riboswitch enables precise control of therapeutic antibody expression by the dose of inducer