circVec: A powerful circular RNA expression platform that enhances AAV transgene output and enables significant dose reduction

Number : 1528

Type: Oral and/or Poster

Topic: A: Gene Addition: AAV

Eoghan O'leary[1], Juan Zhang[1], Summer Stefanelli[1], Mohamed Warsame[1], Erik Wiklund[1], Victor Levitsky[1], Thomas Hansen[1]

[1] Circio AB, Huddinge, Sweden

Introduction

The therapeutic efficacy of AAV gene therapy is frequently limited by inefficient and variable transgene expression, necessitating high vector doses that constrain the therapeutic window and increase toxicity risks. Improving transgene output per viral genome is therefore a key requirement for the development of next-generation safe and effective AAV platforms. Here, we describe circVec, a novel and versatile expression platform designed to substantially enhance AAV-mediated transgene expression via a long-lived circular RNA (circRNA) intermediate, enabling significantly increased protein production at reduced vector doses.

circVec is a modular genetic construct that drives spliceosome-mediated production of protein-coding circRNA from viral and non-viral DNA vectors. circVec-based expression leverages the intrinsic resistance of circRNAs to exonucleolytic degradation, resulting in prolonged transcript half-life and elevated steady-state levels of transgene-encoding RNA, thereby supporting sustained and enhanced transgene expression.

Methods

The circVec platform was systematically optimized through the incorporation of novel regulatory elements. Several next-generation circVec designs were screened to identify key determinants that maximise transgene output. Optimized circVec constructs were then evaluated *in vivo* across multiple AAV serotypes, promoters, tissues, and routes of administration, with firefly luciferase transgene expression directly compared to conventional mRNA-based AAV vectors (mVec-AAV).

Results

Optimization of circVec constructs identified regulatory features that significantly enhanced expression, with the latest iteration, circVec4.0, achieving a 37-fold improvement in protein expression compared to early circVec designs and outperforming conventional mRNA-based vectors. These optimised circVec constructs were next evaluated in vivo using AAV vectors to assess transgene expression in disease relevant tissues.

In the eye, intravitreal delivery of circVec-AAV vectors has demonstrated significantly increased transgene expression across dose levels compared to conventional mVec-AAVs. For an early generation circVec 2.0 under the CMV promoter in AAV8, ocular firefly luciferase expression increased 7-fold relative to mVec-AAV 10 weeks post-injection. In a subsequent study, circVec 2.1 under the CMV promoter in AAV2, outperformed mVec-AAV by 7-fold and 10-fold at high (5×10^9 vg) and low (5×10^8 vg) doses, respectively, six weeks post-injection. The latest circVec4.0-AAV construct design further elevates gene expression vs mVec-AAVs by over 60-fold at both high and low dose levels. Notably, circVec4.0-AAV exceeds mVec-AAV transgene expression by 22-fold at a 10x lower dose, thereby directly demonstrating the substantial dose-sparing potential of the circVec platform.

Consistent with these findings, circVec has also been shown to enhances AAV-mediated transgene expression in other clinically relevant tissues. Intracerebroventricular delivery of circVec 2.1-AAV9 vectors under a neuronal-specific promoter resulted in a 4-fold increase in CNS expression. Similarly, systemic delivery of 5e12 vg/kg circVec3.2-AAV9 vectors under a cardiac-specific promoter produced a 40-fold increase, and highly localized cardiac transgene expression compared to mVec-AAV at the same dose level.

Conclusion

Circular RNA-based expression represents a powerful new strategy to substantially enhance AAV-mediated transgene output. The circVec platform enables consistent and robust increases in gene expression in vivo across the eye, heart, and CNS, demonstrating broad applicability across serotypes, promoters, and delivery routes. By enabling therapeutic expression at significantly reduced vector doses, circVec has the potential to expand the therapeutic window, reduce dose-limiting toxicities, and enhance the translational and clinical feasibility of next-generation AAV gene therapies.