Biodistribution and safety of a novel AAV9 gene therapy for treatment of temporal lobe epilepsy shown in non-human primates

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ABSTRACT

Temporal lobe epilepsy (TLE) is a chronic disorder of the nervous system characterized by recurrent spontaneous seizures. Many TLE patients are drug-resistant to available anti-seizure medications resulting in a high unmet medical need for new therapeutic options. In a pilocarpine mouse model, we observed anti-seizure activity with a novel AAV9 vector containing an engineered miRNA against the mRNA of GRIK2 subunit of kainate receptors. The work presented here reveals the biodistribution of the vector, transgene expression and target knock down in non-human primates together with minimal adverse findings.

INTRODUCTION

Aberrant kainate receptors are implicated in TLE[1] and previous work has demonstrated that the knock down of GRIK2 mRNA (kainate receptor subunit GluK2) has anti-seizure activity (Poster P128 ESGCT). Corlieve Therapeutics (now a subsidiary of uniQure N.V.) together with Regenxbio designed a series of miRNAs to be delivered by AAV9 for focal delivery of silencing miRNA to the hippocampus by convection-enhanced delivery (CED). Using the AAV9 capsid and a human neuronal specific promotor (human synapsin1), miRNA were able to target neurons to knock down aberrant GRIK2 (Gluk2 protein). The mature miRNA bind to specific sites on the GRIK2 mRNA promoting translation repression and mRNA degradation.

In order to understand the basic biodistribution of our miRNA after MRI guided CED, we constructed an AAV9-hsyn1-eGFP vector as a surrogate. The results of this work allowed the further development of a dual miRNA cassette vector for clinical development.

METHODS

Non-human primates (NHP) were administered vectors by MRI-guided convection-enhanced delivery to the hippocampus. The animals received bilateral 60 µL injections per hippocampus.

A pilot study investigating the basic vector construct was designed to confirm local transduction of the vector in the hippocampus and cell specific expression of GFP in the neuronal tissues. The AAV9.hSyn1.eGFP had already been confirmed to have the desired expression pattern in mice (see poster P128 at ESGCT).

The final optimized vector design shown below with AAV9 containing a dual miRNA cassette under the control of hSyn1 was then tested in a dose range finding study in NHP with low, medium and high doses.

Biofluids (serum, CSF) were sampled in life and after 4 weeks at necropsy, the peripheral organs were sampled for DNA and RNA analyses. The brain was removed and sliced coronally into 3-4 mm thick slices. Alternate brain slices were used for either histological examinations or bioanalysis of vector, miRNA and mRNA. Brain punch (BP) samples (approx. 4 mm) were taken from the odd numbered brain slices (plus the injection site and adjacent slices) to cover the different brain regions.

Vector DNA distribution, miRNA expression and GRIK2 mRNA quantification was performed in qualified assays.

Table 1. AMT-260 vector construct

AMT-260 AAV9 vector with hSyn1 promotor and miR38R and miR3bR

RESULTS

Confirmed neuronal expression of transgene

For the AAV9.hSyn1.eGFP vector administered by local CED, GFP expression was shown to be localised to the hippocampus with staining prominent in the neuronal cell bodies (**Figure 2**). The vector was also detected in the entorhinal cortex but little or no distribution to other brain regions and levels were at least 100-1000x lower than the hippocampus (data not shown).

Figure 2. eGFP immunostaining in brain tissue of NHP at low (left) and high (right) magnification

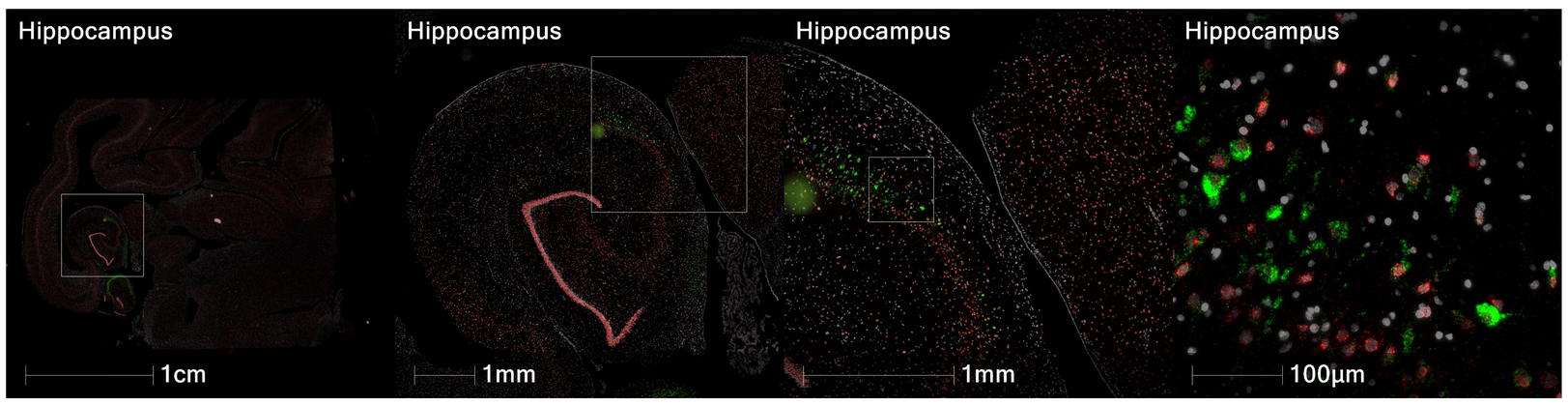
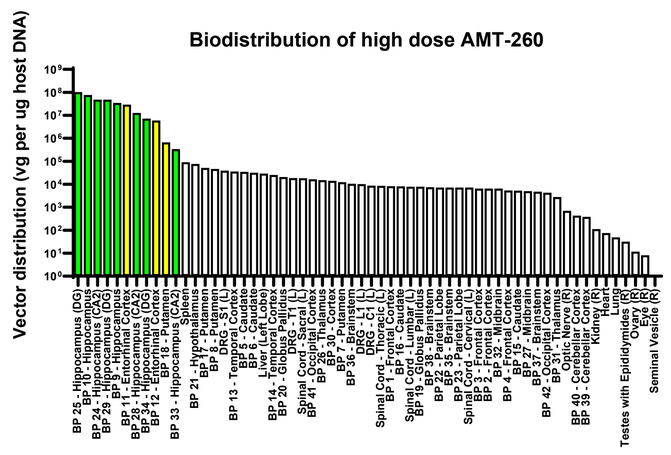


Figure 4. RNAscope of miR38 specifically localized to the hippocampal tissue of NHP (red =GRIK2 mRNA, green = miR38).

Local vector distribution to the hippocampus

The AMT-260 vector showed a similar vector distribution to the earlier GFP vector construct with high vector genomes in the hippocampal tissue and in the adjacent entorhinal cortex but orders of magnitude less in other brain tissues and negligible amounts in the peripheral organs. **(Figure 3**).

Figure 3. Biodistribution (qPCR) of AMT-260 in brain tissues of NHP

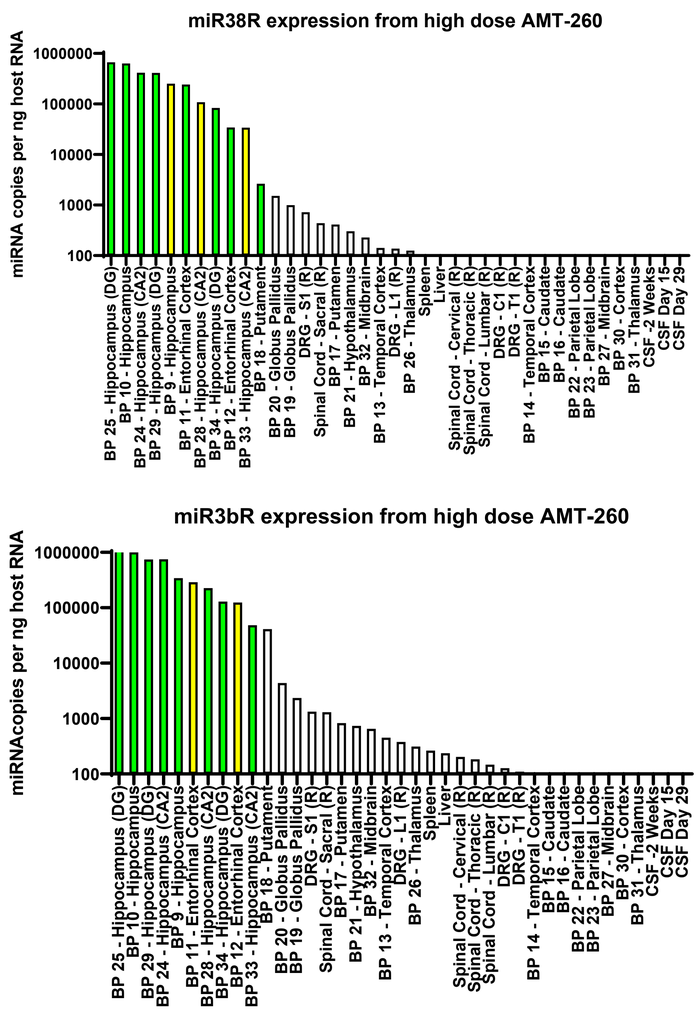


Bars represent tissue punches from the hippocampus (green), adjacent tissue (yellow) and other brain regions and peripheral organs (white).

Tissue specific miRNA expression

Both miRNA were expressed equally efficiently and show neuronal specific tissue expression (**Figure 4**). The highest miRNA levels (shown for miR3bR in **Figure 5**) were within the hippocampus near the injection site reaching 1E+6 copies per ng host RNA. Less was detected in the hippocampal punches further from the injection site and less still in the adjacent entorhinal cortex. Other brain tissues had <1000 copies per ng host RNA and were considered to be inactive.

Figure 5. miRNA expression for miR38R and miR3bR in NHP brain tissues (stem-loop RT-qPCR)

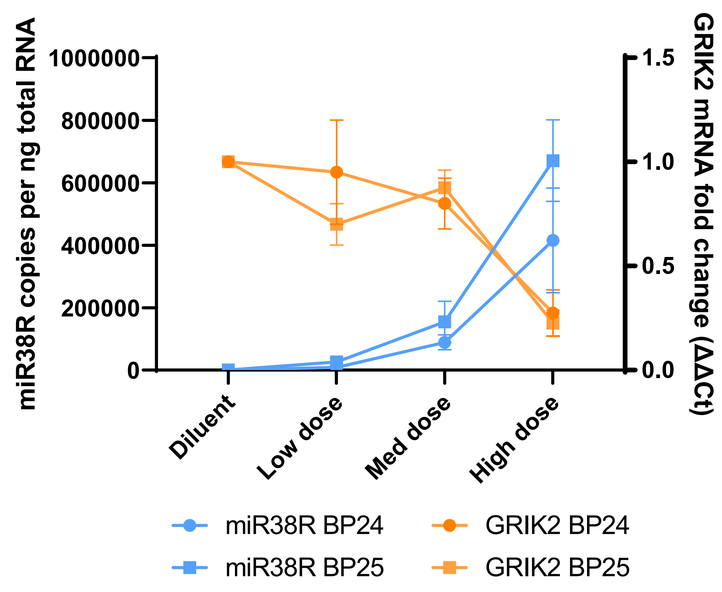


Bars represent tissue punches from the hippocampus (green), entorhinal cortex (yellow) and other brain regions (white).

Successful knock down of GRIK2

GRIK2 lowering was observed in the hippocampus when significant quantities of miRNA were expressed. In **Figure 6**, the relationship between miRNA expression and GRIK2 knock down was dose dependent and reached up to 90% reduction in individual animals.

Figure 6. Knock down of GRIK2 mRNA in the hippocampus and its relationship to miRNA levels.



BP24 corresponds to brain punch for the hippocampus CA2 region and BP25 to the DG region. Results for only one of the miRNA shown.

AMT-260 was well tolerated

AMT-260 was safe and well tolerated. There were no clinical signs, body weight changes or clinical pathology changes related to the test article. Minor microscopic findings were restricted to the hippocampal tissues of the the injection site and in high dose animals only.

CONCLUSION

- We confirm the feasibility of MRI-guided intrahippocampal convection-enhanced delivery in NHP
- AAV9 vector constructs using the hSyn1 promoter show neuron specific expression in NHP
- Peripheral vector distribution and miRNA expression is absent or negligible.
- The dual miRNA cassette successfully knocked down GRIK2 mRNA in the hippocampus by up to 90% in NHP
- Excellent safety profile of our clinical candidate AMT-260 with only minor findings at the injection site of the high dose group only.

These non-human primate data show that local MRI-guided administration is a viable strategy for delivering AAV9 vectors to the brain and represents a promising novel approach for treatment of refractory TLE.

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