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Concept Paper

# Microglial Replacement and COURIER or SPIT for Neuronal Gene Editing

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**Abstract:** Adeno-associated viral (AAV) vectors can be used for gene delivery. Recently, AAV.CAP-Mac was developed; it can cross the blood-brain barrier and transduce cells throughout the brain. However, while parts of the cortex and thalamus in 17-year-old rhesus monkeys were transduced, other brain regions were not. Additionally, AAV vectors may also be genotoxic and cytotoxic, especially at higher doses. Finally, AAV vectors are very expensive to produce at sufficiently high titers for treatment. In contrast, an off-the-shelf, cell-based delivery system may be ideal in terms of safety, effectiveness, and cost. Induced pluripotent stem cell-derived macrophages (iPSC-Macs) can engraft in the brain and take on a microglial phenotype after microglial depletion. The iPSC-Macs could then inducibly become hyper-motile and continuously export mRNA packaged in protein nanocages, which would potentially be less immunogenic than virus-like particle capsid proteins.

**Keywords:** microglial replacement; adeno-associated viral (AAV) vectors; COURIER; SPIT; base editing; and prime editing

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## Introduction

The golden era of biotechnology ushered in a plethora of new tools for our toolkits, including precision gene editing via homologous recombination [1], base editing[2], prime editing[3–5], large serine recombinase-mediated gene insertion[6], and CRISPR transposases[7]. These have the potential to cure any genetic illness. With adeno-associated viral (AAV) vectors, gene therapy has been brought to bear in the human body, sometimes greatly improving function in patients[8]. However, full cures are generally not possible, and many genetic diseases remain untreatable currently. In the cases wherein effective treatments or cures are not attainable, it is not for a lack of in vitro tools, but rather our ability to deliver them to the cells around the body that need to be repaired.

With regard to central nervous system (CNS) disorders, AAV.CAP-Mac was recently described, which can cross the blood-brain barrier and reach cells throughout the brain[9]. However, although parts of the cortex and thalamus were effectively transduced in 17-year-old adult rhesus monkeys, other regions of the brain were not[9]. It is also possible that AAV.CAP-Mac may not work in humans as well as it did in non-human primates. Additionally, the cost of producing sufficiently high titers of AAV vectors and other viral vectors for therapeutic purposes is at least currently very steep[10–12]. Moreover, AAV therapies are possibly genotoxic or cytotoxic, especially at high doses[13]. In contrast to AAV vectors, a transient, off-the-shelf, cell-based treatment enabled by inducible mRNA delivery would likely be much cheaper and safer.

## Microglial Replacement

The small molecule CSF1R inhibitor, PLX5622, potently depletes microglia[14]. PLX3397 is another, FDA-approved small molecule CSF1R inhibitor, and it also potently depletes microglia in mice and non-human primates[15]. Microglial depletion in both model organisms appears safe, and can be repeated multiple times in mice, given sufficient time between depletion-repopulation cycles[16,17].

PLX3397 may also potently deplete microglia in humans as well, although the clinical trial that measured microglial depletion may not have administered PLX3397 for long enough to thoroughly

test its efficacy in this regard[18]. It might be possible to edit induced pluripotent stem cell-derived macrophages (iPSC-Macs) *ex vivo*, and then infuse them intrathecally or intravenously to replace a patient's microglia with ones that can employ a system called COURIER (controlled output and uptake of RNA for interrogation, expression, and regulation)[19,20].

Normally, the remaining population of microglia or peripheral monocytes might outcompete iPSC-Macs that are infused into the bloodstream[21,22], but an inhibitor-resistant CSF1R variant has been developed[23]. Thus, constant selection is possible - which should enable non-invasive microglial replacement.

For gene editing purposes, the iPSC-Macs could at least eventually be "off-the-shelf"[24,25]. This would substantially decrease the cost of the therapy. After treatment, a small molecule like a rapamycin analog that can penetrate the blood-brain barrier (BBB) can be administered to eliminate them via caspase-9 activation[26], and the patient's hematopoietic stem cells (HSCs) would repopulate the microglia.

Non-genotoxic HSC transplant is also a possibility if the direct intravenous or intrathecal infusion of edited cells is ineffective[27].

Also, while microglia sample their surrounding microenvironment constantly with protrusions that extend and retract, they may not move around much from place to place[28,29]. To address this potential issue, random migration of the iPSC-Mac-derived microglia in the CNS could be induced or enhanced by inhibiting LRRK2[30]. Other methods of inducing hyper-motility are also possible[31,32].

### Neuronal Gene Editing

Base editing of the SMN2 gene in neurons is a viable strategy for spinal muscular atrophy[33,34]. In this case, the COURIER cargo could be an mRNA molecule encoding a zinc finger base editor. However, they currently have more off-target activity than CRISPR base editors[35]. TALE base editors exist, as well[36]. Alternatively, the cargo could be a self-amplifying RNA (saRNA) vector[37]. An saRNA vector could enable the use of a CRISPR base editor, wherein a subgenomic promoter effectively replicates an sgRNA module but weakly replicates the proteinaceous component of the base editor[38,39]. As larger RNA molecules were packaged less efficiently in COURIER, but dual delivery was possible, a trans-amplifying RNA (taRNA) vector could be employed[40]. In either case, the RNA-dependent RNA polymerase could be inhibited in the edited microglia via an orthogonal degree, perhaps.

With saRNA and taRNA vectors, there may be some considerations with superinfection exclusion, copy number restriction, and possibly gene dosage compensation[41–43].

As opposed to an AAV vector, microglial replacement and cell-based treatment might be too slow for SMA1 patients. However, as described in[33], clinicians could co-administer the antisense oligonucleotide drug nusinersen to extend the therapeutic window.

Two other CNS genetic illnesses that microglial replacement could potentially help with are Tay-Sachs and Huntington's disease. For Tay-Sachs, prime editing could remove a causative four-base duplication[3]. Base editing could diminish the disease-causing mutation in Huntington's[44]. Twin prime editing combined with a site-specific recombinase could also enable the targeted excision of large trinucleotide repeat regions[4,45].

Furthermore, the APOE4 allele, which is a genetic risk factor for Alzheimer's disease, could be edited to APOE3[46].

Secreted Particle Information Transfer (SPIT) could also be exported for gene editing[47]. However, mRNA export may be more efficacious, as opposed to RNP secretion. Also, SPIT utilizes viral capsid proteins, which would probably be potently immunogenic.

### COURIER Immunogenicity

As Dr. Horns et al. mentioned[20], COURIER protein nanocages may not stimulate an excessive immune response. Low-dose dexamethasone could possibly be sufficient to prevent undue

inflammation from all of the COURIER components. Dexamethasone has been used to counter excessive immune responses in patients with SARS-CoV-2[48].

If not, there are other strategies. The innate immune response to dsRNA generated from vector replication could possibly be attenuated by the expression of the MERS-CoV ORFa protein[49]. If cyclic induction is required over a long period of time, the adaptive immune response to the gene editing components may need to be attenuated as well. First, the vector could encode a deimmunized dCas9 protein[50]. Second, it may help if the vector were to incorporate multiple, tandem miR-142-3p binding sites in the dCas9 mRNA 3'UTR[51]. Third, the vector could express the SARS-CoV-2 ORF6 protein, which inhibits the MHC class I pathway[52].

Of course, adding more elements requires a more packaging space. If necessary, one could theoretically minimize the size of the required elements. Or, as was also mentioned in the COURIER article, alternative nanocage architectures could tune cargo capacity.

### Other Uses for Microglial Replacement

#### CNS Senescent Cell Elimination

Senescent cells in the CNS could be eliminated by a system involving a replicating RNA COURIER cargo molecule and ADAR-mediated detection of the *p16<sup>Ink4a</sup>* transcript[53,54]. Detection would lead to the triggering of caspase 8 production.

The caspase's activity could be attenuated via mutation if necessary to ensure a proper therapeutic window.

#### Mitochondrial Transplantation

Instead of COURIER, arrestin domain containing protein 1 [ARRDC1]-mediated microvesicles (ARMMs) could be employed to transfer mitochondria to aged cells with mutated or damaged mitochondria[55,56].

PGC-1 $\alpha$  could be overexpressed by the edited microglia to increase their intracellular stores of mitochondria[57].

TFAM could also be directly overexpressed in order to maximally increase the mitochondrial DNA (mtDNA) copy number of the pristine mtDNA in the edited microglia. However, PGC-1 $\alpha$  overexpression would accomplish this to some extent at least.

Miro1 overexpression might help to improve donation efficiency[58].

These mitochondria could be imbued with SNPs in non-coding regions - or synonymous or non-synonymous SNPs - that allow for targeting of aged mtDNA with pre-imported nucleases[59]. Alternatively, the ARMMs could be loaded with mRNA encoding nucleases. This might work better than pre-imported nucleases because it would not rely on target cell mitochondrial fusion-fission dynamics. COURIER can be used instead of loading the mRNA into ARMMs, but would not be directional as currently formulated.

Some SNPs are actually associated with endurance-enhancing or pro-longevity effects [60,61].

Perhaps an ideal approach would be to load saRNA or taRNA into the ARMMs that encode TFAM and the nucleases - to destroy old mtDNA, while simultaneously promoting the replication of the new mtDNA.

### Conclusion

Microglial replacement in combination with COURIER or SPIT could enable cheap and effective CNS gene editing.

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