Lowering alpha-synuclein as a Therapeutic Strategy for Parkinson’s disease: Challenges & Possibilities

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Overview

Why is alpha-synuclein a therapeutic target for Parkinson’s disease?

What are the different therapeutic concepts for targeting alpha-synuclein?

What are the challenges?

Where are the next breakthroughs?
WANTED!

Name: Alpha-synuclein, alias: NACP, PARK1/PARK4
Address: Chromosome 4q22.1
Height/weight: 140 amino acids, 14 kDa protein
Appearance: monomer, tetrameric α-helical oligomer, associates with biological membranes
Crime: aggregates can cause Parkinson’s
Alpha-synuclein genetics, part 1: point mutations

• First point mutation discovered in 1997
• Major component of Lewy bodies
• Frequency 0.5% in familial and sporadic cases
• Only a few point mutations
• Mutations cause PD by increasing aggregation propensity
Alpha-synuclein genetics, part 2: copy number mutations

- Larger genomic regions multiplied (duplication or triplication) = copy number (mutation) variant
- Discovered in 2003
- Regions may include many genes
- Alpha-synuclein is common ‘denominator’
- Too much normal alpha-synuclein causes PD

Alpha-synuclein genetics, part 3: non-coding variants

- Genetic changes outside the gene
- Increase risk for PD
- Might alter alpha-synuclein expression

Mechanisms of alpha-synuclein pathology in Parkinson’s disease

Papadopoulos 2018, Irwin, Lee & Trojanowski 2013
How much reduction of alpha-synuclein is beneficial?
Clinical spectrum of a-syn gene dosage from autism to Lewy body dementia

- Cautious when design of a-syn downregulation

*Torres et al. 2020, Int. J. Molecular Sciences*
Therapeutic concepts of lowering alpha-synuclein

1. ↓ α-Synuclein Production
   - DNA, RNA Transcription
   - Translation

2. ↓ α-Synuclein Aggregation
   - Monomer, Misfolded seed, Aggregated

3. ↑ α-Synuclein Degradation
   - Lysosome

4. ↓ Extracellular α-Synuclein

5. ↓ α-Synuclein Cellular Uptake

6. ↑ GCase Activity
   - Lysosome

Stanford Medicine | Department of Pathology
1. Reducing production of alpha-synuclein

Rationale: if production of a-syn can be prevented then less protein is produced and toxicity of protein misfolding can be avoided.
Therapeutic strategies follow principles of the central dogma of biology.

DNA = alpha-synuclein gene

(m)RNA = alpha-synuclein mRNA

Alpha-synuclein Protein
Therapeutic strategies follow principles of the central dogma of biology

Gene editing

DNA = alpha-synuclein gene

Antisense oligonucleotides

(m)RNA = alpha-synuclein mRNA

Small molecules

Alpha-synuclein Protein

Antisense oligonucleotides

Lattice α-synuclein

Oligomers

Fibrils

Antibody therapies

Lewy body

Transmission

Nature Reviews | Drug Discovery
Gene editing targets the DNA

DNA = alpha-synuclein gene

(m)RNA = alpha-synuclein mRNA

Alpha-synuclein Protein

Native α-synuclein → Oligomers → Fibrils → Lewy body

Nature Reviews | Drug Discovery
CRISPR regulation of a-syn gene expression

• “Molecular scissors” for gene engineering
• SLS-004 is lentiviral vector
• Targets epigenome of alpha-synuclein
• *In vitro*: reduces alpha-synuclein expression (by ~30%) & rescued function in dopaminergic neurons
• *In vivo*: single dose leads to 40% alpha-synuclein reduction
• Seelos Therapeutics, Inc. announced the exclusive worldwide licensing of SLS-004 from Dr. Chiba-Falek, Duke University in Summer 2019
Antisense oligonucleotides = ASO
“shooting the messenger”

DNA = alpha-synuclein gene

(m)RNA = alpha-synuclein messenger RNA

Alpha-synuclein Protein
Antisense oligonucleotides = ASO “shooting the messenger”

DNA = alpha-synuclein gene
(m)RNA = alpha-synuclein mRNA

Alpha-synuclein Protein
Antisense oligonucleotides downregulate alpha-synuclein mRNA

- Synthetic strands of nucleic acid that modulate gene expression
- ASO therapeutics recently FDA-approved for muscular atrophy
- Selective reduction of α-syn production in midbrain monoaminergic regions

- **Clinical trial in MSA via intrathecal injection (HORIZON), NCT04165486**

*Primate brain and CSF, Cole et al. 2021*
2. Reducing aggregation of alpha-synuclein

**Rationale:** if aggregation of α-syn can be prevented then its normal function can be sustained and toxicity of protein misfolding can be avoided.
Small molecule binds to alpha-synuclein oligomers

DNA = alpha-synuclein gene
(m)RNA = alpha-synuclein mRNA
Alpha-synuclein Protein
Prevention of alpha-synuclein fibril formation

DNA = alpha-synuclein gene
(m)RNA = alpha-synuclein mRNA
Alpha-synuclein Protein
Anle138b modulates alpha-synuclein oligomers

- Binding to oligomers alters structure & inhibits neurotoxic properties
- Modulation towards non-toxic oligomers and monomers
- normal alpha-synuclein function regained
- depletes pre-amyloid oligomers and impairs fibril growth, but not a “fibril breaker”

- *First-in-Human Study of Single and Multiple Doses of anle138b in Healthy Subjects shows good safety profile (ClinicalTrials.gov Identifier: NCT04208152)*

Teva and MODAG Announce Licensing Collaboration for Neurodegenerative Disease Drug Candidate

Small molecule candidate Anle138b targets disease modification for multiple system atrophy and other neurological disorders
3. Increasing degradation of alpha-synuclein

Rationale: Autophagy plays major role in degradation of a-syn aggregates
Enhancing autophagy/lysosomal function

• Enhancement of autophagic processes leads to increased degradation of pathological a-syn

• Examples:
  • Rapamycin (natural anti-fungal antibiotic)
  • Trehalose (natural sugar)
  • Modulator of the mitochondrial pyruvate carrier (MSDC-160)

Challenges:

• Lack of specificity for a-syn (effects on other essential pathways)
• Side-effects (e.g. immunosuppression)
GBA encodes the GCase enzyme & mutations increase risk for PD

- GBA mutations account for 7-10% of PD
- GBA carriers have 20-fold increased risk of developing PD
- GBA encodes lysosomal enzyme, beta-glucocerebrosidase (GCase),
- Required for degradation & recycling of glycolipids
- GBA mutation affects:
  - severity
  - age of onset
  - rate of progression
  - likelihood of dementia

GBA = gene, GCase = gene product/enzyme
Restauration of GCase enzyme function

*Enzyme deficiency in GBA leads to build-up of GlcCer

Sanofi trail failed

Schneider & Alcalay 2020
GBA gene therapy

- Study PRV-PD101 is a Phase 1/2a first in-human study
- Evaluate safety of intracisternal PR001A administration
- Patients with at least 1 pathogenic GBA1 mutation
- PR001 utilizes an AAV9 viral vector to deliver the GBA1 gene
- Goal: correct lysosomal enzyme deficiency
- Open-label, 12 participants, multi-center

ClinicalTrials: Phase 1/2a Clinical Trial of PR001 in Patients With Parkinson's Disease With at Least One GBA1 Mutation (PROPEL) NCT04127578
Ambroxol “shuttles” GCase through the cell

- Small-molecule chaperones drive correct folding of mutant GCase molecules in the cell
- Efficient transport to lysosomes & increased GCase activity, rescue neurodegeneration
- Ambroxol, an FDA-approved over-the-counter cough medication

- *Four clinical trials ongoing or completed testing efficacy of Ambroxol in PD and Dementia with Lewy bodies*
4. Reducing extracellular alpha-synuclein

Immunotherapies
Antibodies target extracellular alpha-synuclein

DNA = alpha-synuclein gene

(m)RNA = alpha-synuclein messenger RNA

Alpha-synuclein Protein
Antibodies target extracellular alpha-synuclein

DNA = alpha-synuclein gene

(m)RNA = alpha-synuclein messenger RNA

Alpha-synuclein Protein
Immunotherapies target extra-cellular a-syn

- Antibodies are unable to enter cells
- Immunotherapy reduce a-syn aggregation in transgenic mice overexpressing a-syn and prevent rescue phenotypes
- Anti-inflammatory effect in neurodegenerative models

Challenges:
- potential to trigger off-target responses
- non-specific inflammatory reactions
- need for repetitive administration
- limited penetration of antibodies into the CNS (0.1-1%)
Completed phase I trials – low BBB penetration

Table 1. Completed phase I trials of α-synuclein-targeted immunotherapy

<table>
<thead>
<tr>
<th>Name</th>
<th>Type</th>
<th>Target of the antibody</th>
<th>Affinity (oligomeric/monomeric)</th>
<th>Trials</th>
<th>Dose</th>
<th>Half-life (d)</th>
<th>Change in serum α-syn level</th>
<th>Change in CSF α-syn level</th>
<th>BBB penetration rate</th>
<th>Safety</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRX002</td>
<td>Passive</td>
<td>C-terminus</td>
<td>&gt; 400 fold</td>
<td>Phase Iα</td>
<td>Single ascending dose</td>
<td>18.2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>No severe or serious TEAEs</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Phase Ib*</td>
<td>Multiple ascending doses</td>
<td>10.2</td>
<td>Up to 97% reduction</td>
<td>No</td>
<td>0.3%</td>
<td>No severe or serious TEAEs</td>
</tr>
<tr>
<td>BIIB054</td>
<td>Passive</td>
<td>N-terminus</td>
<td>&gt; 800 fold</td>
<td>Phase I†</td>
<td>Single ascending dose</td>
<td>28–35</td>
<td>-</td>
<td>-</td>
<td>0.3–0.5%</td>
<td>No severe or serious TEAEs</td>
</tr>
<tr>
<td>PD01A</td>
<td>Active</td>
<td>C-terminus</td>
<td>-</td>
<td>Phase I</td>
<td>Single dose</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>No serious adverse events</td>
</tr>
</tbody>
</table>

*adapted from Jankovic et al.64; †adapted from Brys et al.65; d: day, syn: synuclein, CSF: cerebrospinal fluid, BBB: blood-brain barrier, TEAEs: treatment-emergent adverse events.

Shin et al. 2020, J. Mov. Disorders
Biogen alpha-synuclein antibody therapy failed

Biogen tosses out Parkinson's hopeful cinpanemab, pays $75M for its syn

by Ben Adams | Feb 3, 2021 7:55am

No results were found for cinpanemab.
Getting through ---- the blood brain barrier

using TVs = transport vehicles

carrying a drug across the blood-brain barrier = receptor-mediated transcytosis
5. Reducing cellular uptake of a-syn
Cell surface receptors bind misfolded alpha-synuclein

- Extracellular alpha-synuclein propagates between cells
- Oligomeric alpha-synuclein binds to cell surface receptors
- induces transmission of signals into cells

New therapeutic avenues: modulate receptors to inhibit alpha-synuclein update

Surguchev et al. 2019 Molecules
Summary

- Alpha-synuclein is well-established therapeutic target for alpha-synucleinopathies
- Different strategies of alpha-synuclein lowering strategies
- Some are already in clinical trials
- Challenges: route of administration, passing BBB, frequency of treatment
Thanks so much for your attention!

Any questions? Contact: bschuele@stanford.edu
Bibliography and References cited