Novel α-Synuclein positron emission tomography (PET) tracers

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ATT-AD/PD, Torino
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Disclosures

Jan Stöhr is an employee of AC Immune
No off-label nor investigational use of therapeutic products will be presented
Clinical need for an α-Synuclein PET tracer
Biomarker for early diagnosis and disease progression

- α-Synuclein misfolding and inclusion are believed to be the molecular basis for disease progression
- >90% of PD cases are sporadic - diagnosis can not rely on genetic testing
- Imaging α-Synuclein has the potential to detect PD in its earliest stages
- Imaging marker for the recruitment of patients and longitudinal efficacy measurements in clinical trials targeting pathological α-Synuclein
- Detection/quantification of α-Synuclein copathologies in other neurodegenerative diseases

(1) Shah et al., 2014 Journal of Nuclear Medicine
**α-Synuclein radiotracer**

Specific challenges for PET ligand development

- Pathological α-Synuclein detected in multiple forms: Lewy bodies/neurites, fibrils, oligomers and pore-like assemblies\(^{(1)}\)

- α-Synuclein deposits can contain other proteins: parkin, tau, amyloid beta, etc\(^{(2)}\)

- Pathological α-Synuclein can be extensively modified posttranslationally\(^{(3,4)}\)

- Pathological aggregates of α-Synuclein are not as abundant ("low B\(_{\text{max}}\)”) as amyloid beta, thus low K\(_i\) & high selectivity for α-Synuclein over amyloid beta and tau is required \(^{(5)}\)

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\(^{(1)}\) Paleologou et al., 2009 Brain

\(^{(2)}\) Kotzbauer et al., 2012 Arch Neurol.

\(^{(3)}\) Schildknecht et al., 2013 J Neurochem.

\(^{(4)}\) Anderson et al., 2006 J Biol Chem.

\(^{(5)}\) Eberling et al., 2013 J Parkinsons Dis
Compound characterization cascade

- Qualitative/semi-quantitative
  - Direct, fluorescence staining to define distinct chemical series from the Morphomer™ platform

- Quantitative, used for SAR
  - Radiobinding assay with recombinant α-Syn fibers and AD-brain homogenates
  - Radiobinding assay: PD-brain homogenates
  - ARG (PD-brain tissue)
  - 18F mouse PK
  - 18F NHP PK

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Target engagement on Braak stage V-VI PD tissue
Representative results of staining amygdala tissue sections

Cpd-F’ shows good staining of α-Synuclein aggregates. Fluorescence staining of α-Synuclein aggregates is detected as low as 500 nM of Cpd-F’
Screening for tool compounds by immunohistochemistry

Target engagement on α-Synuclein in early PD Braak stages, DLB and MSA

Selected compounds showed target engagement on early Braak stage PD amygdala sections as well as in human brain sections from different synucleinopathies

Ref.: Adapted from Capotosti et al. AD PD 2017
Selectivity over Amyloid-β in Parkinson’s Disease

Representative results of staining amygdala tissue sections

Cpd-F’ selectively stains α-Synuclein aggregates decorating Aβ plaques in PD tissue with mixed pathology

Ref.: AC Immune unpublished data
Selectivity over Amyloid-β in Alzheimer’s Disease

Representative results of staining amygdala tissue sections

Cpd-F’ does not stain Aβ plaques when devoid of synuclein co-pathology

Ref.: AC Immune unpublished data
Selectivity over Amyloid-β in Alzheimer’s Disease

Autoradiography on amygdala tissue with an Amyloid-β ligand

Both compounds do not compete with an Aβ ligand on AD amygdala sections

Ref.: AC Immune, unpublished data
Resolution limit of ARG: Quantitative μARG

- AD patient-derived tissue was chosen for this comparison to appreciate the labeling of amyloid plaques with compound $^3$H-ACI-Cpd-N in classical ARG (upper panel).
- ARG needed to be adopted to subcellular resolution to be applicable to PD tissue because of size and density of the underlying pathology.
- Comparison of classical ARG and the micro-ARG on AD patient-derived tissue illustrates the substantial gain in resolution.

Ref.: AC Immune, unpublished data.
**μ-ARG: Quantification**

Dose-response curves using $^3$H-ACI-Cpd-B’ on PD brain sections

- Increasing concentration of unlabeled compounds leads to competition of the radiolabelled compound binding and consequently the decrease of tritium induced Ag grains colocalizing with aSyn mAb (Syn211)
- Micro-ARG and immunohistochemical signal can be automatically quantified to obtain IC$_{50}$ values in PD and MSA cases

Ref.: AC Immune, unpublished data
High resolution, quantitative autoradiography
Target engagement with ACI-Cpd-AA on human PD brain sections

μ-autoradiography (ARG) for ACI-Cpd-AA

ACI-Cpd-AA displaced the μARG signal from both Lewy bodies and neurites with single-digit to sub-nM affinity in PD brain sections

ACI-Cpd-AA: 0.1 nM
ACI-Cpd-AA: 5 μM

Ref.: AC Immune unpublished data
Radiobinding assay for ligand characterization
Biochemical, radiobinding assay for pathological \( \alpha \)-synuclein

- Two radiobinding assays were implemented to complement the quantitative, autoradiography assay

- Recombinant \( \alpha \)-Synuclein fibrils

- PD-patient derived \( \alpha \)-Synuclein

In addition to micro-autoradiography we routinely use radiobinding assays with recombinant \( \alpha \)-Synuclein fibrils, but more importantly with PD-patient derived \( \alpha \)-Synuclein.
## Affinity measurements on PD-derived α-Syn

**Compound affinity measurement using micro-ARG and radiobinding assays**

<table>
<thead>
<tr>
<th>ACI Compound ID</th>
<th>Radiobinding</th>
<th>μARG</th>
<th>Radiobinding</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ki on α-Syn from PD brain homogenates (nM)</td>
<td>IC50 α-Syn aggregates on PD brain sections (nM)</td>
<td>Ki on rec. α-Syn fibers (nM)</td>
</tr>
<tr>
<td>ACI-Cpd-B’</td>
<td>1.6</td>
<td>7.3</td>
<td>44.9</td>
</tr>
<tr>
<td>ACI-Cpd-T’</td>
<td>2.6</td>
<td>2.3</td>
<td>26.6</td>
</tr>
<tr>
<td>ACI-Cpd-W’</td>
<td>1</td>
<td>10.8</td>
<td>9.5</td>
</tr>
<tr>
<td>ACI-Cpd-O’</td>
<td>5.6</td>
<td>6.2</td>
<td>6</td>
</tr>
<tr>
<td>ACI-Cpd-AA</td>
<td>1.7</td>
<td>0.6</td>
<td>13.3</td>
</tr>
<tr>
<td>ACI-Cpd-X’</td>
<td>14</td>
<td>&gt;100</td>
<td>35</td>
</tr>
<tr>
<td>ACI-Cpd-G’</td>
<td>17.5</td>
<td>725</td>
<td>76.9</td>
</tr>
<tr>
<td>ACI-Cpd-Y’</td>
<td>5.3</td>
<td>&gt;100</td>
<td>24.4</td>
</tr>
</tbody>
</table>

Ref.: AC Immune unpublished data

Our screening approach enabled us to optimize compounds leading to low nM affinity for pathological α-Synuclein and preservation of selectivity over AD-derived Aβ.
Pharmacokinetics of $^{18}$F-labeled compounds

Mouse & non-human primate pharmacokinetics (NHP-PK)

Mouse i.v. PK with $^{18}$F-labeled ACI-compounds

![Graph showing pharmacokinetic profiles of ACI-Cpd-S', ACI-Cpd-J', and ACI-Cpd-AA](image)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Brain uptake Time to Cmax</th>
<th>Brain uptake % ID/g</th>
<th>Washout Time to Cmin</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACI-Cpd-S'</td>
<td>0.9 min</td>
<td>3.7</td>
<td>&gt; 82 min</td>
</tr>
<tr>
<td>ACI-Cpd-J'</td>
<td>0.5 min</td>
<td>5.6</td>
<td>110 min</td>
</tr>
<tr>
<td>ACI-Cpd-AA</td>
<td>1 min</td>
<td>6.9</td>
<td>45 min</td>
</tr>
</tbody>
</table>

NHP i.v. PK with $^{18}$F-labeled ACI-Cpd-AA

![Graph showing SUV (g/ml) over time for ACI-Cpd-AA](image)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Brain uptake Time to Cmax</th>
<th>Brain uptake % ID/g</th>
<th>Washout Peak/half peak</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACI-Cpd-AA</td>
<td>5 min</td>
<td>4.0</td>
<td>20 min</td>
</tr>
</tbody>
</table>

Selected compounds from our lead series showed a $^{18}$F PK profile in rodents and NHP suitable for further development as PET imaging ligands.
Novel PET radioligands for α-Synuclein

Conclusions

- Misfolded α-Synuclein can be specifically targeted for PET radiotracer development in synucleinopathies

- Synthetic fibrils are a reasonable structural surrogate for PD derived aggregates, but selection of compounds should be guided by binding to PD-patient derived α-Synuclein

- Cpd-AA has been identified as lead compound:
  - Low nM affinity to recombinant α-Synuclein fibrils
  - Low nM affinity on patient-derived α-Synuclein aggregates
  - Up to 500-fold selectivity over Aβ (AD)

- Cpd-AA has a good pharmacokinetic profile in rodents and non-human primates

- Clinical development process is initiated and first in human study is scheduled for the second half of 2018
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