Bench to Bedside at Georgetown: Preclinical Science to Clinical Trials with Nilotinib

Charbel Moussa, MB, PhD
Laboratory for Dementia and Parkinsonism
Department of Neuroscience
Georgetown University Medical Center
Overview – experiments and regulations

What we did

How we got to clinical trials

The role of basic research in elucidating disease mechanisms

The role of pre-clinical science in modifying disease mechanisms

The importance of post-mortem human tissues to validate/correlate mechanisms

Our hypothesis to re-position anti-cancer drugs to treat neurodegenerative diseases

Nilotinib (Bosutinib) clinical trials - hypothesis, design and preparation
FACES WE KNOW

President Ronald Reagan - Alzheimer
Michael J. Fox - Parkinson
Lou Gehrig -ALS

Many other faces of beloved family and friends we also know?

Alzheimer’s disease (AD): 5.3 million people in the U.S. The number of AD and other dementias will rise to 16 million by 2050. The direct and indirect costs for the dementias was more than $148 billion in 2005. In 2008, there were 9.9 million family and other unpaid who provided 8.5 billion hours of care, valued at $94 billion (NINDS).

By 2060: The aging population is projected to increase form 1.96% to 4.33% > 85 years of age and 14.84% to 21.90% > 65 years – US Census Bureau
Life expectancy: male 77.1 (2005) to 81.8 (2050). Females 81.8 (2005) to 85.9 (2050). US Census Bureau

Can we eradicate these diseases: Average age for PD is 65 and AD is 85 years?
Parkinson’s disease (PD) is predominantly sporadic, but some disease-causing mutations suggest a genetic component in the pathogenesis of this disorder.

<table>
<thead>
<tr>
<th>Loci</th>
<th>Chromosome</th>
<th>Genetic variants (penetrance)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SNCA (PARK1/4)</td>
<td>4q21</td>
<td>A53T, A30P, E46K duplication, triplication</td>
</tr>
<tr>
<td></td>
<td></td>
<td>REP1, rs2736990, rs11931074</td>
</tr>
<tr>
<td>LRRK2 (PARK8)</td>
<td>12p12</td>
<td>R1441C/G/H, I2020T, Y1699C, G2019S</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R1628P, G2385R, rs1994090</td>
</tr>
<tr>
<td>GBA</td>
<td>1q21</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td>N370S, L444P, others</td>
</tr>
<tr>
<td>MAPT</td>
<td>17q21</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td>H1 haplotype, rs393152</td>
</tr>
<tr>
<td>BST1</td>
<td>4p15</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs4538475</td>
</tr>
<tr>
<td>PARK16c</td>
<td>1q32</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs947211, rs823128</td>
</tr>
</tbody>
</table>

Autosomal dominant mutations in α-SNCA cause late onset (age-related>65 years) PD.
Mutations in the autosomal recessive gene *Parkin* are the most common causes (50%) of familial autosomal recessive early onset (Juvenile 14+ years old) PD.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Chromosome</th>
<th>Protein function</th>
<th>Clinical phenotype</th>
<th>Pathology</th>
</tr>
</thead>
<tbody>
<tr>
<td>parkin (PARK2)</td>
<td>6q25</td>
<td>E3 ubiquitin protein ligase</td>
<td>AR-JP, often indistinguishable from PD, but also with dystonia, reflex changes</td>
<td>Nigrostriatal degeneration, no inclusions</td>
</tr>
<tr>
<td>PINK1 (PARK6)</td>
<td>1p36</td>
<td>Mitochondrial serine-threonine kinase</td>
<td>AR-JP, similar to parkin</td>
<td>Unknown</td>
</tr>
<tr>
<td>DJ-1 (PARK7)</td>
<td>1p36</td>
<td>Unknown, possible role in stress response</td>
<td>AR-JP, similar to parkin</td>
<td>Unknown</td>
</tr>
<tr>
<td>ATP13A2 (PARK9)</td>
<td>22q13</td>
<td>Lysosomal cation transporter ATPase</td>
<td>Kufor-Rakeb disease (AR), parkinsonism and dementia</td>
<td>Unknown</td>
</tr>
<tr>
<td>FBXO7 (PARK15)</td>
<td>22q12</td>
<td>E3 ubiquitin protein ligase</td>
<td>Pallido-pyramidal syndrome (AR), dystonia and parkinsonism</td>
<td>Unknown</td>
</tr>
<tr>
<td>PLA2G6 (PARK14)</td>
<td>22q13</td>
<td>Phospholipase A2</td>
<td>NBIA-2 (AR), dystonia, parkinsonism, and dementia</td>
<td>Neuroaxonal dystrophy, iron accumulation</td>
</tr>
<tr>
<td>PANK2</td>
<td>20p13</td>
<td>Pantothenate kinase</td>
<td>NBIA-1 (AR), dystonia, parkinsonism, and dementia</td>
<td>Neuroaxonal dystrophy, iron accumulation</td>
</tr>
<tr>
<td>ATP7B</td>
<td>13q14</td>
<td>Copper transporter ATPase</td>
<td>Wilson's disease (AR), parkinsonism, liver failure, neuropsychiatric symptoms</td>
<td>Basal ganglia copper accumulation and degeneration</td>
</tr>
<tr>
<td>GRN</td>
<td>17q21</td>
<td>Progranulin, growth factor</td>
<td>Frontotemporal dementia (AD), dementia with or without parkinsonism</td>
<td>Ubiquitin/TDP-43 inclusions</td>
</tr>
<tr>
<td>ATXN2 (SCA2)</td>
<td>12q24</td>
<td>Unknown, enriched in Golgi apparatus</td>
<td>Spinocerebellar ataxia (AD), with or without parkinsonism</td>
<td>Polyglutamine-repeat nuclear inclusions</td>
</tr>
<tr>
<td>GCH1 (DYT5)</td>
<td>14q22</td>
<td>GTP cyclohydrolase I, dopamine biosynthesis</td>
<td>Dopa-responsive dystonia (AD), occasionally phenocopied by parkin</td>
<td>No nigral degeneration, no inclusions</td>
</tr>
</tbody>
</table>
Familial and sporadic PD are both characterized by death of dopaminergic neurons in the Substantia Nigra pars compacta (SN) AND cytosolic \( \alpha \)-Synuclein inclusions known as Lewy bodies (LBs)

PD (parkinsonism) and AD (dementias) share common pathological, neuroanatomical and clinical characteristics

FTD (TAU)/FTLD (TDP-43)
Can we halt neurodegenerative pathologies, including misfolded protein accumulation and pathogenic propagation? What are the possible quality control mechanisms for protein degradation or clearance!!

- **Route A**: Proteasome
- **Route B**: Golgi/ER
- **Route C**: Exosomal/exocytosis

**Amyloids**: Tau, Aβ, α-Syn, Prions Htg, TDP-43, etc.

**Cell-to-cell propagation**

**Glial cells**
What is Parkin?

- It is a cytosolic protein
- An E3 ubiquitin ligase
- Involved in proteasomal and autophagic degradation and aggresome formation
- Loses function in early onset autosomal recessive PD

ALSO
- Dysfunctional in neurodegeneration, independent of disease-causing mutations
Pre-clinical science is the place to start - 1

1- Understanding disease mechanisms
Pre-clinical science is the place to start - 2

2- Testing starts on the bench using post-mortem human brains

Human α-Synuclein ELISA in autophagic vacuoles form human samples (% control)

Human Aβ1-42 ELISA in autophagic vacuoles
Pre-clinical science is the place to start - 3

Th+DAB
Parkin+
GFAP+
DAPI

PLA: Parkin+beclin-1+
DAPI

Midbrain Ctl
Case # 2201

Midbrain PD
Case # 2315

Amyloids
Phagophore-AV10
Autophagosome-AV20
Lysosome: degradation

Interacts
Parkin
Beclin-1

1. Incubate with target primary antibodies
2. Add PLA probes PLUS and MINUS
3. Hybridize connector oligos
4. Ligase to form a complete DNA circle
5. Rolling circle amplification
6. Add fluorescent probe to reveal interaction

Control-cortex
Case# 1855

62μm
AD-cortex
Case # 1833

55μm

Control-Hipp
Case # 1352

48μm
AD-Hipp
Case # 1861

55μm

Control-caudate
Case # 1683

53μm
AD/PD-caudate
Case # 2352

52μm

Interacts
Pre-clinical science is the place to start
How is autophagy clinically exploited via tyrosine kinase inhibition?
Nilotinib and Bosutinib increase Parkin activity via self-ubiquitination

**Ubiquitination Increases Parkin Activity to Promote Autophagic α-Synuclein Clearance.**

Lonskaya I, Desforges NM, Hebron ML, Moussa CE.

**Nilotinib-induced autophagic changes increase endogenous parkin level and ubiquitination, leading to amyloid clearance.**

Lonskaya I, Hebron ML, Desforges NM, Schachter JB, Moussa CE.
Nilotinib reverses loss of dopamine neurons and improves motor behavior via autophagic degradation of α-synuclein in Parkinson's disease models.
Hebron ML, Lonskaya I, Moussa CE.

Tyrosine kinase inhibition increases functional parkin-Beclin-1 interaction and enhances amyloid clearance and cognitive performance.
Lonskaya I, Hebron ML, Desforges NM, Franjie A, Moussa CE.

Tyrosine kinase inhibition facilitates autophagic SNCA/α-synuclein clearance.
Hebron ML, Lonskaya I, Moussa CE.
TKIs reverse the failure of Parkin-Beclin-1 interaction in transgenic models
More regulatory approval: EH&S and GUACUC

1- Lentiviral packaging and preparation (safety)

2- Stereotaxic animal surgery to transfer genes – what kind of animals

3- Drug treatment
Nilotinib and Bosutinib fail to clear α-Synuclein in Parkin−/− mice.
TKIs reduce extracellular plaques and intracellular Aβ in Tg-APP and WT, but not in parkin⁻/⁻ mice.
Possible mechanisms of Parkin activation and amyloid clearance

**Normal conditions**

- **Inactive Parkin**
  - Ubiquitin
  - Ubiquitination
  - Recycling
  - Clearance
  - Amino acids
  - Amyloids
  - Parkin interacts with Beclin-1
  - Phagophore-AV10
  - Maturation
  - Autophagosome-AV20
  - Fusion
  - Lysosome: degradation

**TKIs**

- **Parkin active**
  - Ubiquitin
  - Ubiquitination
  - Recycling
  - Clearance
  - Amino acids
  - Amyloids
  - Parkin interacts with Beclin-1
  - Phagophore-AV10
  - Maturation
  - Autophagosome-AV20
  - Fusion
  - Lysosome: degradation

**Aging/Pathology**

- **Inactive Parkin**
  - Ubiquitin
  - De-ubiquitination?
  - Parkin unstable
  - Degradation
  - Decreased solubility/inactivity

- **Proteasome**

**TKIs**

- **Proteasome**
TKIs mediate TDP-43 localization in transgenic TDP-43, but not Parkin−/−, mice.
TKI improves memory in transgenic TDP-43 but not Parkin-/- mice.

Before treatment:
- C57BL/6
- TDP-43

After treatment:
- TDP-43+DMSO
- TDP-43+Nilo
- TDP-43+Bos
TKI improves motor and cognitive behavior in transgenic TDP-43 but not Parkin-/- mice.
Pulsatile autophagy: an ON/OFF clearance strategy that exploits post-mitotic (non-dividing) neuronal biology to induce degradation of toxic intraneuronal debris, without causing the self-cannibalization seen in rapidly dividing tumor cells.

**CLINICAL TRIALS !!**

Debris accumulation: Tau, β-amyloid, α-Synuclein, TDP-43, Damaged organelles

- **Disease onset**
  - Time in years
  - Debris accumulation
  - Neurodegeneration

- **AUTOPHAGY ON**
  - Time in days
  - Nilotinib 4-8hrs
  - Bosutinib 6-12hrs
  - Parkin
  - Beclin-1
  - Autophagic clearance

- **AUTOPHAGY OFF**
  - Time in days
  - Nilotinib 4-8hrs
  - Bosutinib 6-12hrs
  - Parkin
  - Beclin-1
  - Re-accumulation

- **Halting neurodegeneration**

Lewy Body Dementia (LBD) is at the interface of PD and AD pathologically and clinically:
- No known cure
- Rapidly progressing, debilitating and extremely frustrating for caregivers
- These trials can also be applied to similar disorders like MSA, PSP and CBD
A new therapeutic concept

- This is a NOVEL life transforming technology because:

  1- It is a successful pre-clinical strategy to decrease Parkinsonism-linked α-Synuclein and Alzheimer-associated Tau and β-amyloid proteins

  2- This technology is a stark contrast with failed anti-AD vaccination therapies that target extracellular β-amyloid and leave the neuron vulnerable to the detrimental effects of intraneuronal accumulation of a variety of proteins, including α-Synuclein, TDP-43, β-amyloid and Tau
Design, preparation, and execution of clinical trials

- Designing clinical trials (types and disease indication)
- Patient population (Safety, inclusion criteria, primary, secondary and tertiary outcomes)
- Therapeutics (drugs, dosing and safety)
- Trials duration and recruitment
- FUNDING
- IRB approval
- FDA approval – safety, safety, safety (IND)
- Treatment (CRU) and data collection
- DSMB and data analysis
- Recommendations (safety, efficacy, continuation etc)
- More FDA to licensing
A Georgetown University journey from bench to bedside

• This invention is owned by Georgetown

• It has the potential to eradicate neurodegenerative diseases and decrease the national economic and social burdens (invest in a cure/nursing home?)

• We are working with pharmaceutical companies (Merck and Janssen collaboration) to develop more TKIs

• We are talking to Novartis (Nilotinib) and Pfizer (Bosutinib) to provide the drugs for clinical trials
**Laboratory members:**
Charbel Moussa, MD. PhD

**Post-Docs**
Irina Lonskaya, PhD
Preety Khandelwal, PhD
Ashot Shekonyan, MD, PhD
Wenqiang Chen, MD

**Lab Manager/ Research Assistant**
Michaeline Hebron, MS
Sandra Selby, RN
Alexander Herman, MS

**Internship /Graduate Students**
Norah Algarzae
Nicole Desforges
Odalys Amador
Kayde Sharpe
Kithmini Weersinghe
Alexander Franjie
Catherine Domingo
Irin Nizam
Michael Colon
Shay Seager
Lanier Heyburn
Yue Feng
Zainab Ibrahim
Badryah Omar

**Funding:**
- NIH-National Institute on Aging
- Merck & Co
- Alzheimer’s Association
- Cure Foundation-Charleston Conference on Alzheimer’s Disease
- Michael J. Fox. Foundation
- National Parkinson’s Disease Foundation
- Georgetown University
- Georgetown University- Music for the Mind
- Georgetown University – Regents Board
- Langer Family Charitable Association

**THANK YOU!**

**Collaborations:**
- Jim Driver, PhD (Electron Microscope Facility- Montana)
- Joel Schachter, PhD (Merck Neuroscience Laboratories)
- Milton Brown, MD, PhD (Drug Discovery Laboratory- Georgetown)
- Bradoslav Goldman, PhD (Proteomics and Mass Spect- Georgetown)
- Fernando Pagan, MD (Movement Disorders Clinic, GUH)
- Brent Harris, MD, PhD
- Mark Burns, PhD
- Joseph Neale, PhD
- Ben Wolozin, PhD
- Italo Mocchetti, PhD
- Scott Turner, MD. PhD (Memory Disorders Program, GUMC)