LRRK2 AS THERAPEUTIC TAR

Jan Egebjerg

Scientific and Medical Rationale(s)

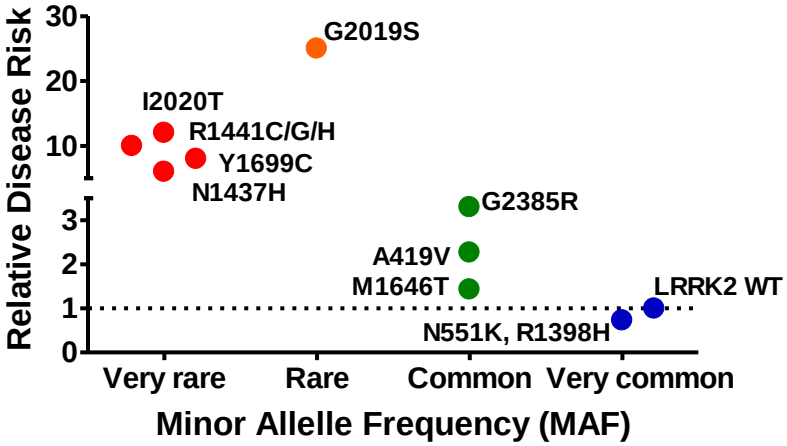
- **Scientific**
  - Strong genetic evidence causally associates LRRK2 to familial PD.
  - Combined genetic and biochemical evidence supports a hypothesis in where the LRRK2 kinase function correlates with disease risk and that LRRK2 kinase inhibitors would be a new treatment paradigm for PD
  - Expression of LRRK2 is highly enriched in brain, lung, kidney and blood.
  - Successful LRRK2 inhibition would successfully dampen LRRK2 kinase activity in the brain with a sufficient TI
- **Medical Rationale**
  - Current PD treatments treat symptoms and have no effect on disease progression and limited of effect at late stages of disease
  - Large unmet need for effective treatments and in particular treatments that may alter the progression of the disease or even modifying the disease.

How difficult can it be?

- Identify a selective LRRK2 kinase inhibitor
- Target engagement: Tools to determine target engagement in brain
- Pharmacological relevance: Disease relevant model for dose finding and time for intervention
- Patient selection and outcome measures for clinical trials

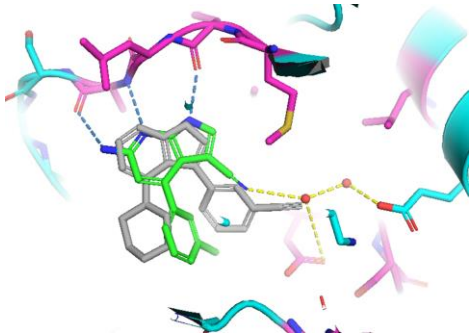
LRRK2 as a target for some PD patients ?

- Disease hypothesis: Increased LRRK2 kinase activity causal for Parkinsons Disease
  - Patient population?
    - Target only G2019S carriers: develop a LRRK2;G2019 S selective compound
    - Develop a selective ”pan-LRRK2” inhibitor and identify patients with increased risk or LRRK driven pathology



Highly potent and ”selective” LRRK2 kinase inhibitors have been identified.

- Challenging target (no approved CNS active kinase inhibitors) – transition peripheral target properties to CNS drug properties
- High quality compounds have been shared with the community



Patenting Activity 2013-2015

Merck	Sanofi
Genentech	Southern Research Institute
Pfizer	Arrien Pharmaceuticals
GSK	Elan
Lundbeck	Zenobia
Origenisis	Cellzome
Ipsen	

- Issue: PK properties for appropriate therapeutic index (TI)
- Pharmacodynamics: Dose finding based on a pathophysiological relevant readout

Tg rodent models

Impaired dopaminergic neurotransmission and microtubule-associated protein tau alterations in human *LRRK2* transgenic mice

H.L. Melrose [a,*], J.C. Dächsel [a], B. Behrouz [a], S.J. Lincoln [a], M. Yue [a], K.M. Hinkle [a], C.B. Kent [a], E. Korvatska [b], J.P. Taylor [a], L. Witten [d], Y.-Q. Liang [a], J.E. Beevers [a], M. Boules [a], B.N. Dugger [a], V.A. Serna [a], A. Gaukhman [a], X. Yu [a], M. Castanedes-Casey [a], A.T. Braithwaite [a], S. Ogholikhan [a], N. Yu [a], D. Bass [a], G. Tyndall [a], G.D. Schellenberg [c], D.W. Dickson [a], C. Janus [a], M.J. Farrer [a,*]

Leucine-Rich Repeat Kinase 2 Regulates the Progression of Neuropathology Induced by Parkinson's-Disease-Related Mutant α-synuclein

Xian Lin,[1,9] Loukia Parisiadou,[1,9] Xing-Long Gu,[1,9] Lizhen Wang,[1,9] Hoon Shim,[1,10] Lixin Sun,[1] Chengsong Xie,[1] Cai-Xia Long,[1] Wan-Jou Yang,[1] Jinhui Ding,[2] Zsu Zsu Chen,[7] Paul E. Gallant,[3] Jung-Hwa Tao-Cheng,[4] Gay Rudow,[5] Juan C. Troncoso,[5] Zhihua Liu,[5] Zheng Li,[6] and Huaibin Cai[1,*]

Conditional expression of Parkinson's disease-related R1441C LRRK2 in midbrain dopaminergic neurons of mice causes nuclear abnormalities without neurodegeneration

Elpida Tsika [a], Meghna Kannan [a], Caroline Shi-Yan Foo [a], Dustin Dikeman [b,c], Liliane Glauser [a], Sandra Gellhaar [d], Dagmar Galter [d], Graham W. Knott [e], Ted M. Dawson [b,c,f,g,h,i], Valina L. Dawson [b,c,f,g,h,i], Darren J. Moore [a,i,*]

Enhanced Striatal Dopamine Transmission and Motor Performance with LRRK2 Overexpression in Mice Is Eliminated by Familial Parkinson's Disease Mutation G2019S

Xianting Li,[1] Jyoti C. Patel,[1] Jing Wang,[1] Marat V. Avshalumov,[1,3] Charles Nicholson,[2] Joseph D. Buxbaum,[2,4] Gregory A. Elder,[4,7] Margaret E. Rice,[1,6] and Zhenyu Yue[1,2]

Progressive dopaminergic alterations and mitochondrial abnormalities in LRRK2 G2019S knock-in mice

M. Yue [a], K.M. Hinkle [a], P. Davies [c], E. Trushina [d], F.C. Fiesel [a], T.A. Christenson [f], A.S. Schroeder [d], L. Zhang [d], E. Bowles [a], B. Behrouz [a], S.J. Lincoln [a], J.E. Beevers [a], A.J. Milnerwood [e], A. Kurti [a], P.J. McLean [a,b], J.D. [a,b], W. Springer [a,b], D.W. Dickson [a,b], M.J. Farrer [e], H.L. Melrose [a,b,*]

The I2020T Leucine-rich repeat kinase 2 transgenic mouse exhibits impaired locomotive ability accompanied by dopaminergic neuron abnormalities

Tatsunori Maekawa[1], Sayuri Mori[2], Yui Sasaki[3], Takashi Miyajima[4], Sadahiro Azuma[5], Etsuro Ohta[6] and [7*]

R1441C mutation in LRRK2 impairs dopaminergic neurotransmission in mice

[b], Emmanuel N. Pothos[c],

Inhibitors of leucine-rich repeat kinase-2 protect against models of Parkinson's disease

Byoung Dae Lee[1,2], Joo-Ho Shin[1,2], Jackalina VanKampen[3], Leonard Petrucelli[3], Andrew B West[1,2,10], Han Seok Ko[1,2], Yun-Il Lee[1,2], Kathleen A Maguire-Zeiss[4], William J Bowers[5], Howard J Federoff[6,7], Valina L Dawson[1,2,8,9,11] & Ted M Dawson[1,2,8,11]

Mutant *LRRK2*^{R1441G} BAC transgenic mice recapitulate cardinal features of Parkinson's disease

Yanping Li[1,5], Wencheng Liu[1,5], Tinmarla F Oo[2], Lei Wang[1,3], Yi Tang[1,4], Vernice Jackson-Lewis[2], Chun Zhou[2], Kindiya Geghman[1], Mikhail Bogdanov[1,3], Serge Przedborski[2], M Flint Beal[1], Robert E Burke[2] & Chenjian Li[1]

Dopaminergic Neuronal Loss, Reduced Neurite Complexity and Autophagic Abnormalities in Transgenic Mice Expressing G2019S Mutant LRRK2

David Ramonet[1¶], João Paulo L. Daher[2,3,4¶], Brian M. Lin[2,3], Klodjan Stafa[1], Jaekwang Kim[5,6], Rebecca Banerjee[7], Marie Westerlund[8], Olga Pletnikova[5], Liliane Glauser[1], Lichuan Yang[7], Ying Liu[5], Deborah A. Swing[10], M. Flint Beal[7], Juan C. Troncoso[5], J. Michael McCaffery[9], Nancy A. Jenkins[10¤], Neal G. Copeland[10¤], Dagmar Galter[8], Bobby Thomas[7], Michael K. Lee[5,6], Ted M. Dawson[2,3,11], Valina L. Dawson[2,3,11,12*], Darren J. Moore[1*]

LRRK2 overexpression alters glutamatergic presynaptic plasticity, striatal dopamine tone, postsynaptic signal transduction, motor activity and memory

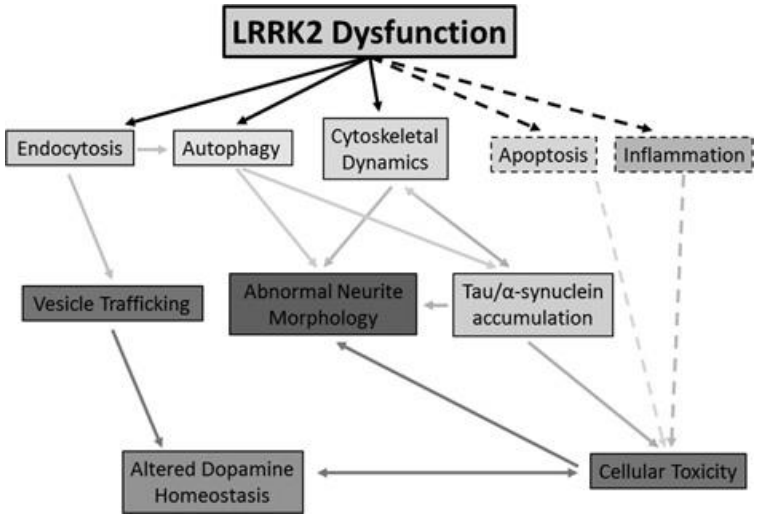
Dayne A. Beccano-Kelly[1,3,†], Mattia Volta[1,3,†], Lise N. Munsie[1,3], Sarah A. Paschall[1,3], Igor Tatarnikov[1,3], Kimberley Co[1,3], Patrick Chou[1,3], Li-Ping Cao[1,3], Sabrina Bergeron[1,3], Emma Mitchell[1,3], Heather Han[1,3], Heather L. Melrose[6], Lucia Tapia[1,3], Lynn A. Raymond[3,5], Matthew J. Farrer[1,3,4,†], and Austen J. Milnerwood[1,2,3,†,*]

(G2019S) LRRK2 activates MKK4-JNK pathway and causes degeneration of SN dopaminergic neurons in a transgenic mouse model of PD

C-Y Chen[1,5], Y-H Weng[2,5], K-Y Chien[3], K-J Lin[4], T-H Yeh[2], Y-P Cheng[1], C-S Lu[2] and H-L Wang[*,1]

Dose finding based on a pathophysiological relevant readout: PD

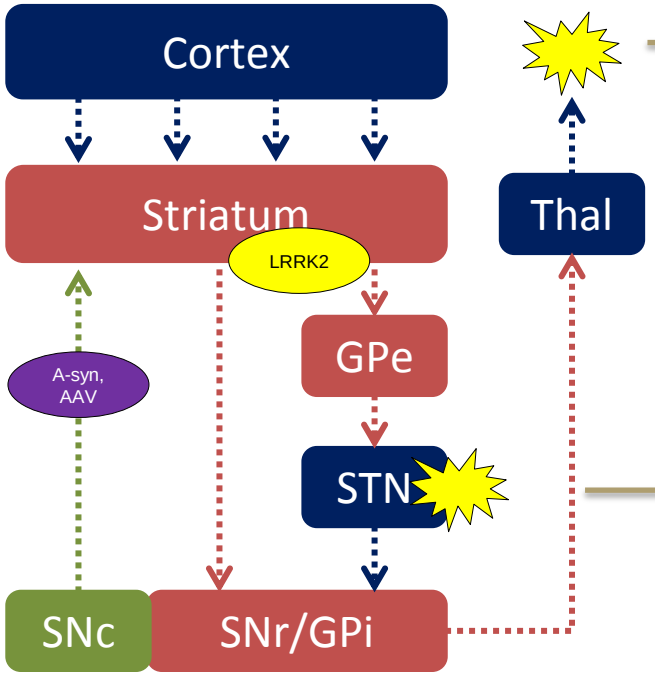
- There is no validated preclinical *in vivo* model for disease progression in Parkinson's Disease
- Rodent animals models that carry G2019S or other pathogenic variants do not present with Parkinson's Disease i.e. a-syn aggregates
- Several tg models exhibit changes in locomotor activity and striatal dopaminergic tone
- Robustness of models an issue for drug testing

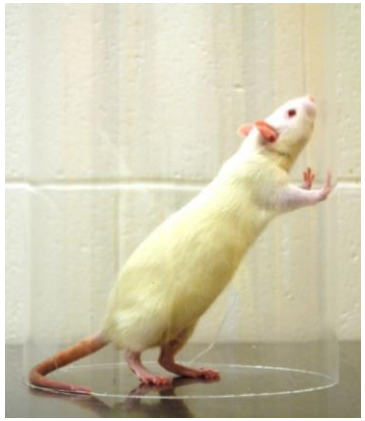


Sloan et al

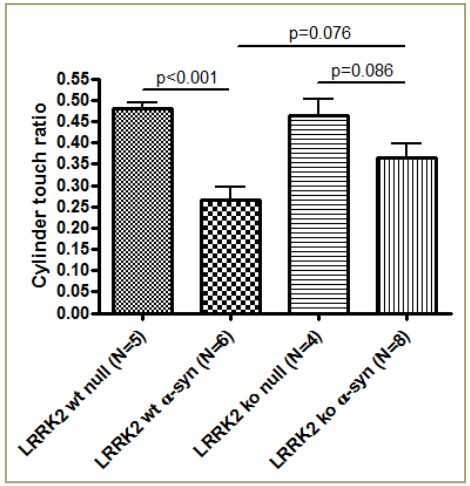
Basal ganglia circuitry in a PD-like state:
STN burst firing and behaviour

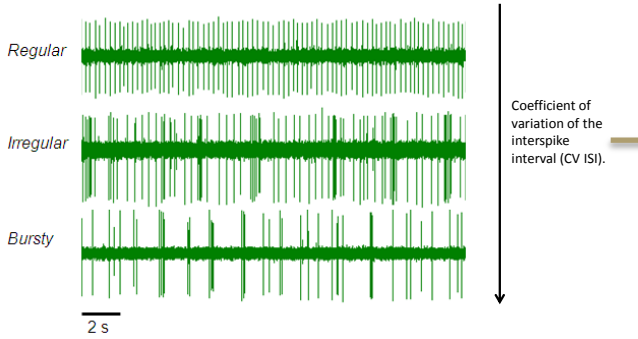
Rat aSyn AAV model



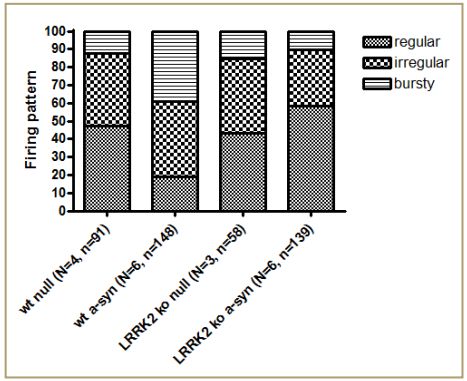


Cylinder test





Single unit recording



Findings – α-synuclein AAV rat model supportive of LRRK2 interaction- but still (several) inconsistencies

- LRRK2 KO studies – Long Evans rat
  - **LRRK2 KO modulates α-synuclein mediated burst firing**
  - **E**ffect on aSyn-pS129
  - **No significant effect on behavior** although a partial reversal has been observed

- LRRK2 inhibitor studies – Sprague Dawley rat
  - **Acute LRRK2 inhibition modulates α-synuclein mediated burst firing phenotype**
  - **Chronic LRRK2 inhibition modulates α-synuclein mediated behavioral phenotype**
  - **No significant chronic effect of LRRK2 inhibition on ephys**
  - **No significant effects on aSyn-pS129 after acute/chronic dosing**
  - **No significant effect on behavior after acute/chronic dosing**

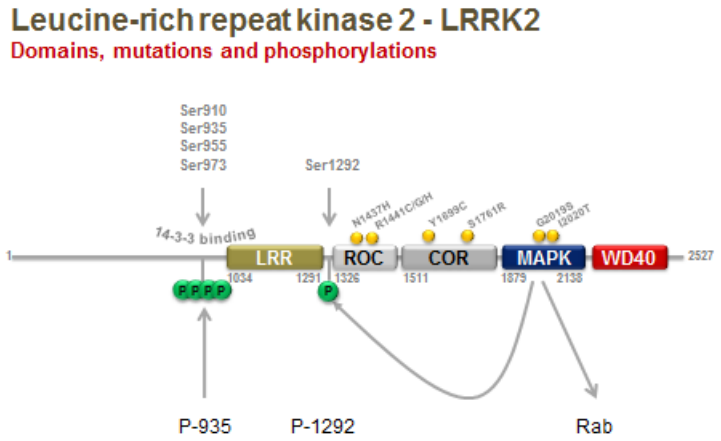
PK/PD modeling

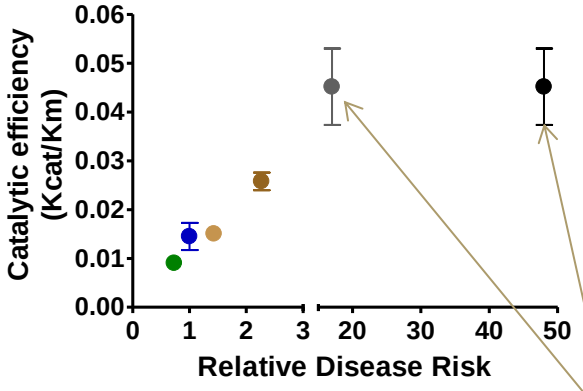
PK/PD based on mechanistic readout

- Auto-phosphorylation correlates with occupancy and disease risk
  - Direct P-1292 (not measurable in vivo)
  - Indirect P-935 (correlates with occupancy) – PD marker for PK/PD modeling?
- Rab phosphorylation as measure for pathophysiological pathway (link to α-synuclein)

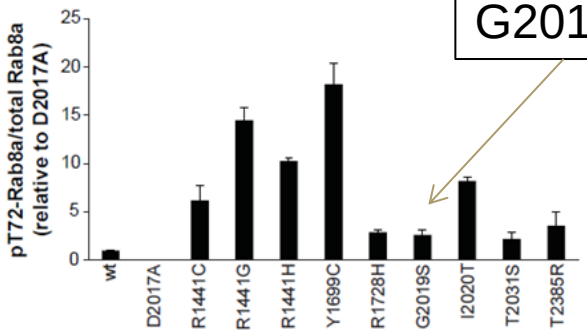
Dose qualification based on in vitro

- Hypothesis: Revert kinase activity of LRRK2 G2019S to the level of the protective form: IC80





G2019S



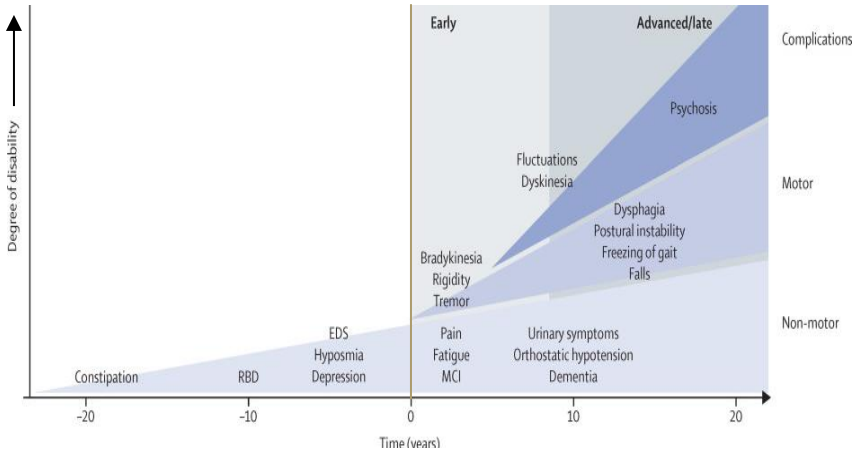
Target engagement: Translational tools to determine target engagement in human brain

- Markers for human studies – mandatory!
  - **PET ligands- very challenging target** – low abundance protein, lipophilicity of high affinity compound compromise signal/noise
  - P-LRRK2 levels in CSF exosomes (or brain specific exosomes isolated from blood)

PDBP Parkinson's Disease Biomarkers Program | National Institute of Neurological Disorders and Stroke

Clinical progression of Parkinson's Disease (PD)

FUNCTION



PATHOLOGY

Topics to resolve in preclinical models:

Time for intervention: Is LRRK2 dysfunction critical at particular stages of the disease?

What readout would be most sensitive to LRRK2 kinase inhibition?

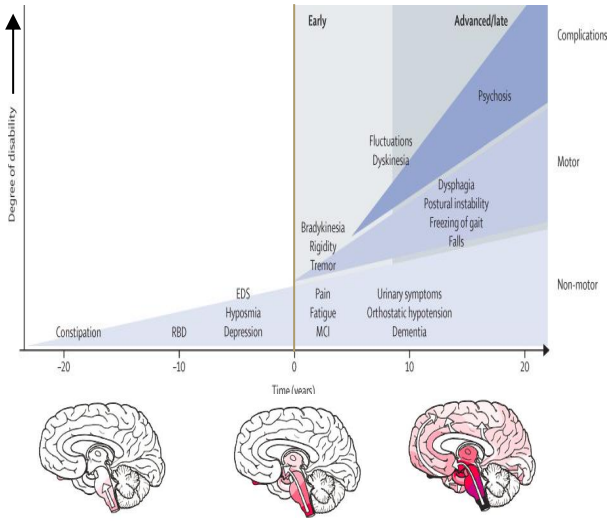
Model for clinical trial based on PPMI data (idiopathic PD patients)

PARKINSON'S PROGRESSION MARKERS INITIATIVE

Play a Part in Biomarker Research

- Early idiopathic PD (Hoehn & Yahr 2 or less)
- Positive DAT SPECT
- Time from diagnosis < 18mts
- +/- different concomitant treatments

- Δ-DAT – scan
- Δ-UDPRS



PARKINSON'S PROGRESSION MARKERS INITIATIVE
Play a Part in Biomarker Research

Modeling of change in disease progression

- Primary outcome at 1y: DAT imaging (change in striatum)
- Primary outcome at 2y: ΔUPDRS

DAT scan – 1y

Reduction (% mean striatum)	Mean change (SD)	Total sample size 80% power
30%	-0.17 -> -0.119 (0.20)	482
50%	*-0.17 -> -0.085* ***(0.18*)***	*144*

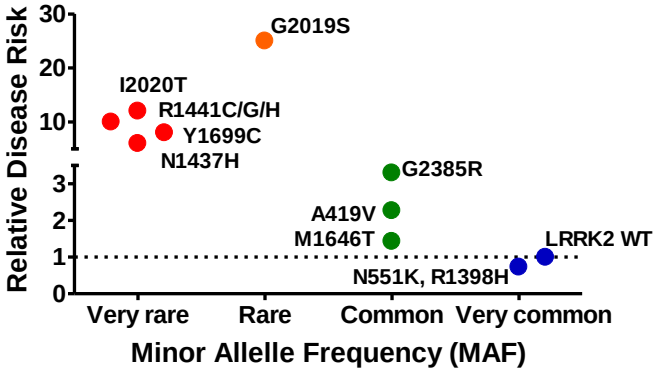
ΔUPDRS - 2y

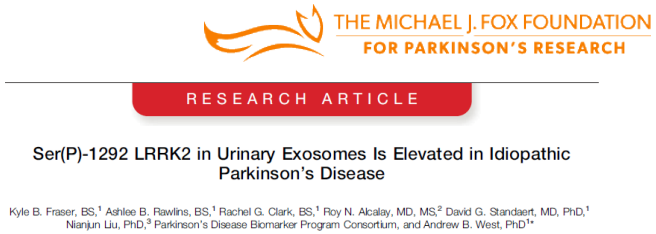
Difference (in Total Score)	Mean change (SD)	Total sample size 80% power
2 points (30%)	6.81 -> 4.81 (12.07)	1146
3.4 points (50%)	*6.81 -> 3.4* ***(10.86*)***	*322*

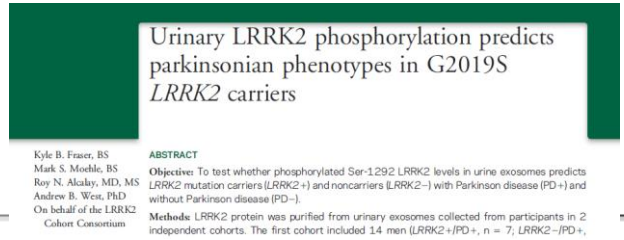
Lundbeck

Segmentation strategies

- Exonic variants stratification
  - **LRRK2 G2019S**
  - + LRRK2 risk variant
  - + PD minus LRRK protection carriers
  - All PD patients
- Biomarkers for elevated LRRK2 activity
  - PBMC – phosphorylation state
  - Exosomes in urine
  - Others
- Symptom differentiators







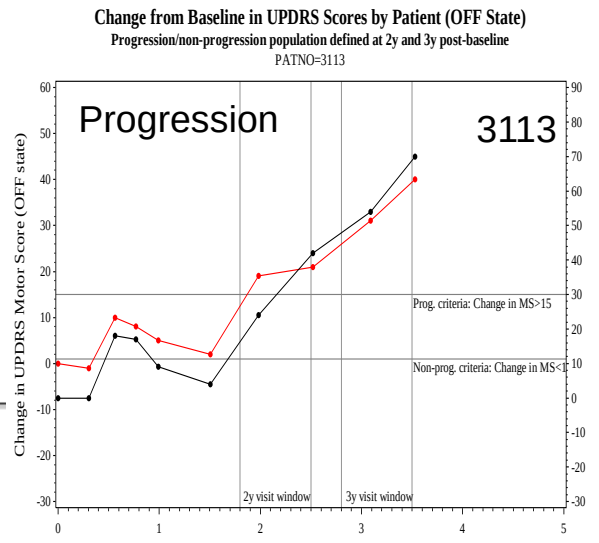
Focus area

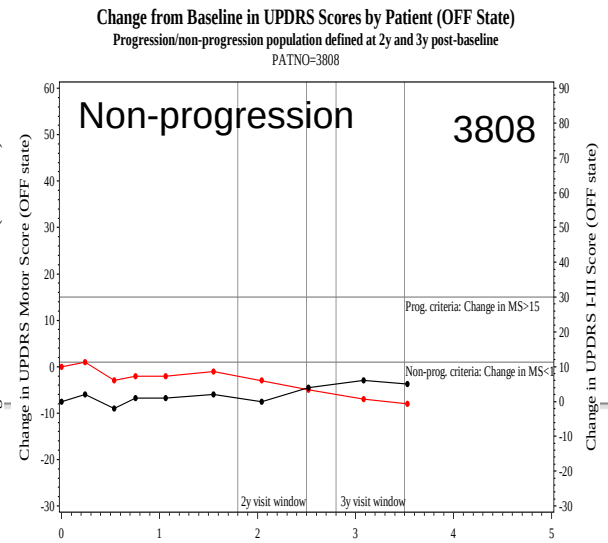
Increased focus on disease stratification

- Target/pathway specific markers for patient selection
- Biomarker approaches aiming at classifying patient heterogeneity

Use iPSCs as translational tool heterogeneity

- Patient disease pheno-/genotype defined iPSCs
  - Model disease heterogeneity
  - Confidence in target for dose estimation
  - Target identification





PARKINSON'S PROGRESSION MARKERS INITIATIVE

Play a Part in Biomarker Research

Lundbeck

How difficult is it ?

- Identify a selective LRRK2 kinase inhibitor
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