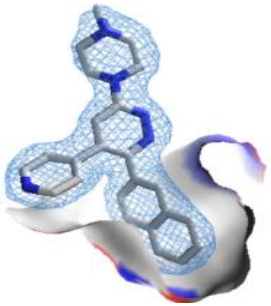
# Case Study From An Academic Consortium: Development of Novel Stress Kinase Inhibitor Drug Candidate

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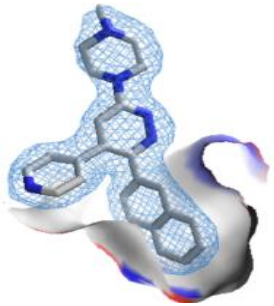
*Disclosures: DMW is funded by NIH and foundation awards to NU for the identification and validation of drug discovery targets and development of clinical candidates; NU and the NU-CU Consortium hold patents on work performed by DMW, JP, SMR and OA; Windisch, Pelletier, Arancio and Anderson constitute NKT, LLC, an academic spin out that has exclusive license from the NU-CU consortium for the clinical development of MW150.*

# Today's Presentation



- Overview of MW150
- Background on the general drug discovery platform
- Why p38aMAPK ?
- Key Concepts and Deliverables for MW150 project
- Structure Assisted, Fragment Expansion Approach to MW150
- Competitive Landscape for MW150 in CNS diseases
- Summary of Selected PK, PD and Efficacy End Points
- Commercial Scale GMP Clinical Drug
- First in Human Pharmacokinetics and Safety
- Key Aspects of the MW150 Study
- I wish I knew… and Project Learning Experiences

**Selected Relevant References to Data Presented:**

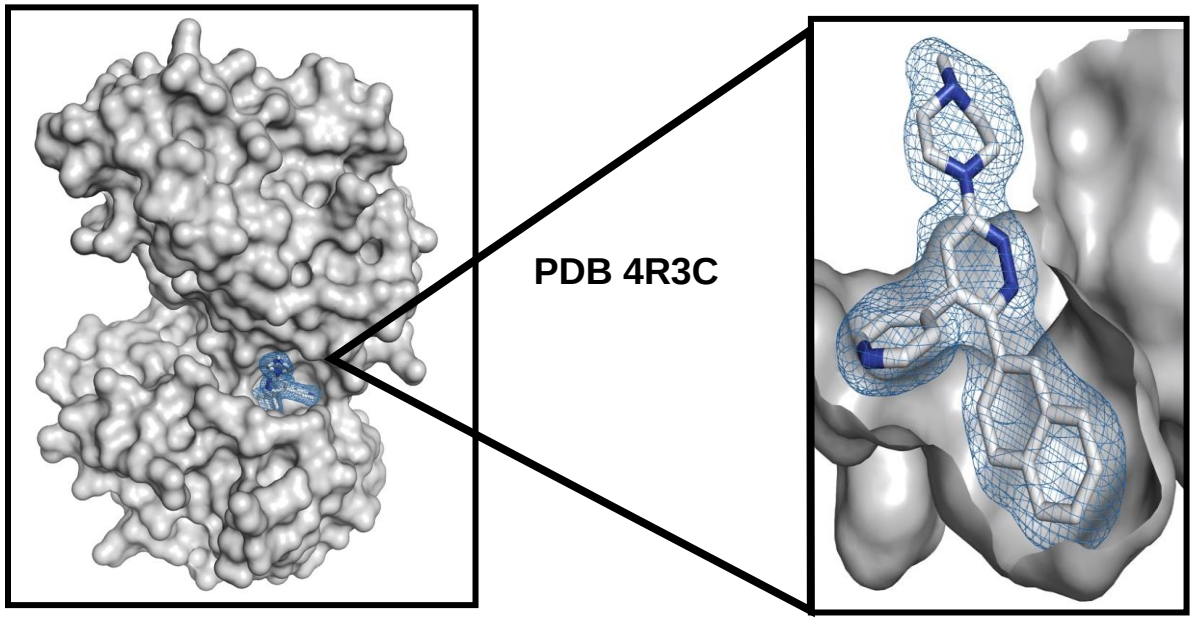

*Chico, L.K., et a. (2009) Nat Rev Drug Discovery 8:892-909; (2009) Drug Metab Disposition 37: 2204-2211.*
*Hu W, et al (2005) Curr Alzheimer Res 2: 197-205; (2007) Bioorg Med Chem Lett 17: 414-418.*
*Munoz et al., (2007) Journal of Neuroinflammation 4:21*
*Ralay Ranaivo et al. (2006) J Neurosci. 26(2): 662-70,*
*Robson MJ, et al (2018) Proc Natl Acad Sci U S A. 115:10245-10254.*
*Roy SM, et al (2015) ACS Chem Neurosci. 6:666-680; (2019) J Med Chem 62: 5298-5311.*
*Watterson DM, et al (2013) PLOS One. 8(6): e66226*

# MW150: A Phase 2 Clinical Asset

## *Key Features*

- **Efficacy/PD in diverse CNS disease models characterized by a neuroinflammation-synaptic dysfunction axis:**
  *Alzheimer's disease*
  *tauopathies*
  *neuropsychiatric disorders*
- **Unique, orally bioavailable, CNS exposure**
- **Phase 1 first-in-human clinical trials for safety and pharmacokinetics**
- **Extended GLP Toxicology (6 mo rat; 9 mo dog)**
- **No approved drugs or clinical trials with MW150's unique profile of molecular recognition, pharmacological selectivity and safety**
- **Potential to provide a new therapeutic intervention mechanism for neurologic disease modification**

## Atomic Level Knowledge of Target Recognition




**Summary of Discovery and Preclinical Development :**

S. M. Roy, G. Minasov, O. Arancio, L.W. Chico, L. J. Van Eldik, W. F. Anderson, J. C. Pelletier and D. M. Watterson (2019), ***Drug Annotation: A Selective and Brain Penetrant p38αMAPK Inhibitor Candidate for Neurologic and Neuropsychiatric Disorders That Attenuates Neuroinflammation and Cognitive Dysfunction***. J. Med Chem 62, 5298−5311

# Integrated, Recursive CNS Drug Discovery Platform

*Phenotypic or Single Molecular Target Approach (ICH/FDA driven)*

***I. Molecule Design stage***

**Design, selection of fragment or highly focused library, chemical diversification**

***All designs include pharmacoinformatics***

***II. Activity Screen Stage***

**Functional/ Pathway-Based Approach**

**OR**

**Single Molecular Target Approach**

**Concentration dependent, selective Inhibition of activity**

**"GO"**

***III. Pharmacology Assessment Stage***

**Campaign Driven**

***In vitro pharm. screens***

**Selected *in vivo* pharm. screens**

**"GO"**

***IV. In Vivo Efficacy/PD Stage***

***In vivo PD or* efficacy in relevant animal models**

**"NO GO"**

**Medicinal Chemistry Refinement**

**"GO"**

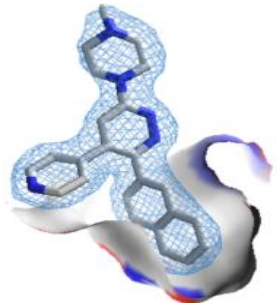
**Drug Development: GMP scheme, Preclinical GLP, FIH**

**Deliverables:**
**Phase 2 Ready, Brain Penetrant, Unique Small Molecules**

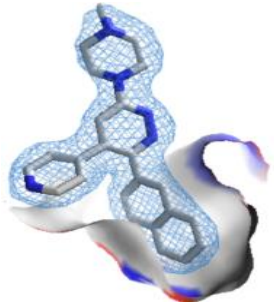
***THIS PRESENTATION***

***MW150:***

***case study from single molecular target approach***



# p38αMAPK: Biological Role Dependent on Physiological Context

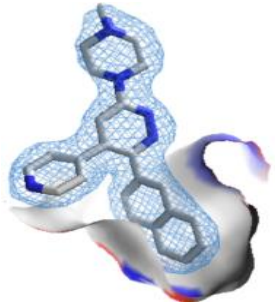


***A Stress Related Protein Kinase with Target Potential Dependent on Pathophysiology Progression & Disease Context***

**Prior art in the literature:**

- *Original identification of p38MAPK: molecular target for drugs that blocked increases in proinflammatory cytokine levels by infection related stressors (e.g, LPS)*
- *Roles independent of infections: "sterile inflammation" in injury, illness or aging.*
- *Pathophysiology biomarker in progressive neurodegenerative diseases, psychiatric disorders and drug resistance induced by chemotherapeutic exposure.*

# MW150: Isoform Selective p38αMAPKI



## *A Novel Single Target Pleiotropic Drug*

*exceptional single molecular target selectivity with ability to attenuate multiple disease processes and stress related pathways*

### *Approach and deliverables*

- *Structural genomics, pharmacoinformatics & secondary pharmacology*
- *Targeting pathophysiology progression mechanisms*
- *Disease agnostic approach* → *single target entity, multiple indications*
- *Early development focused on IND activities and early risk reduction*
- *New chemical entity candidates and CNS tissue exposure*

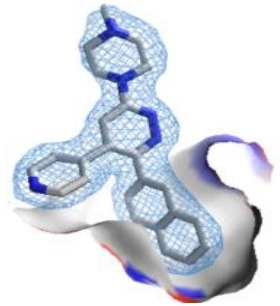
### *Secondary pharmacology screens drove refinement & optimization*

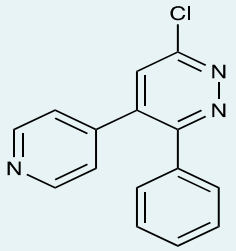
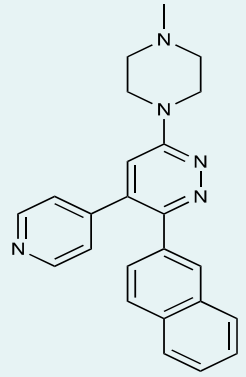
- Kinome-wide off-target screen (*>305 targets across all branches + key mutants*): **Negative**
- Functional GPCR agonist & antagonist screen (*>166 targets*): **Negative**
- Other target (ion channel, transporters, enzymes) screens *(>50 targets*): **Negative**
- p38αMAPK$^{T106M}$ KI mice: target recognition loss in active kinase **No PD**

# MW150 Case Study

## Design: *fragment expansion & pharmacoinformatics*

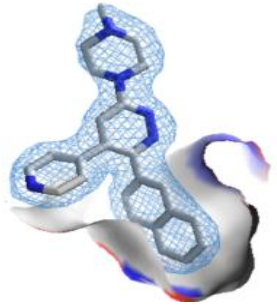
## Discovery: *structural genomics & secondary pharmacology filtering*



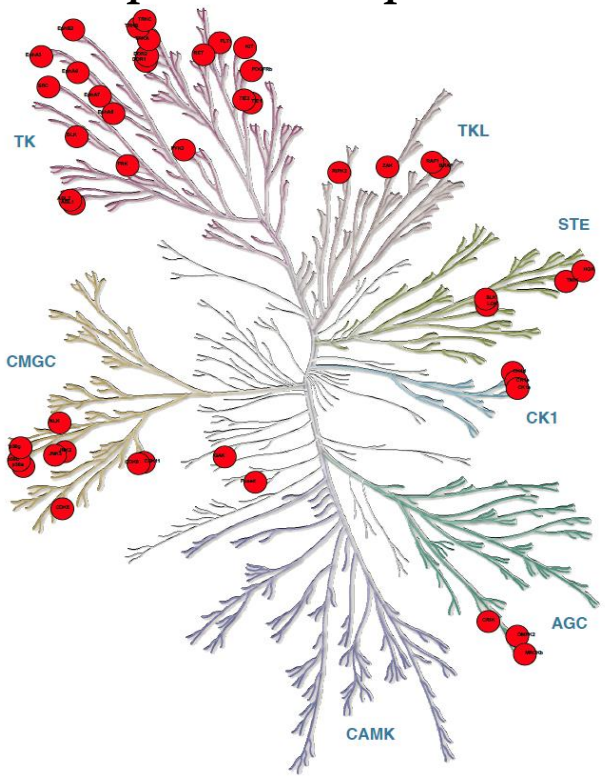
| MW01-6-180SRM<br>PDB 4ZTH | MW01-2-069aSRM<br>PDB 4EWQ | MW01-10-181SRM<br>PDB 4F9Y | MW01-11-108SRM<br>PDB 4F9W | MW01-18-150SRM<br>PDB 4R3C |
|---|---|---|---|---|
|  | *First synthesis* | *Lead compound with improved target selectivity & safety* | *Isoform selectivity* |  |
| *Inactive fragment bound in active site - defines key recognition* | *Hit compound with n vivo function in disease models* | *In vivo function retention* | *Safety & in vivo function retention* | *MW 150*<br>*ADMET optimized; Improved metabolic stability & exposure* |

# Competitive Landscape

*Contrast of MW150 kinome target selectivity vs widely used p38MAPK inhibitors*



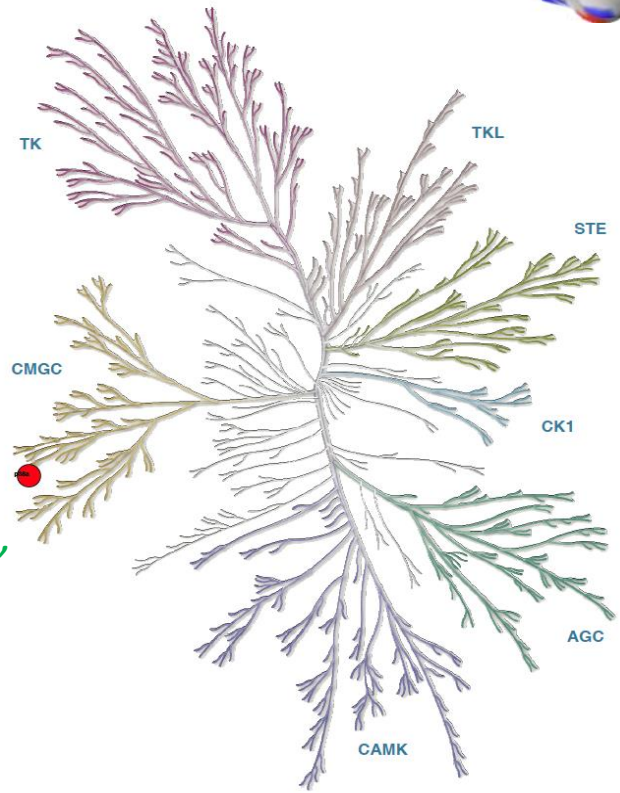
**A. Representative p38αMAPK inhibitors**




**Red circles denote kinases with inhibition $IC_{50}$ < 1 μM.**

*Off-target kinase & GPCR liabilities provide potential explanations for differences in drug effects & safety between prior art & MW150, including the only other brain penetrant candidate, V-745 (neflamapimod).*

**B. MW150**




**(A) Off-target kinases** ($IC_{50}$ <1μM) for widely used p38αMAPK inhibitor candidates:

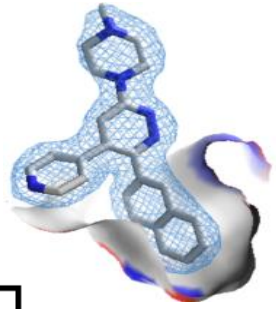
**VX-745 (**neflamapimod**)** include ABL1, ABL2, p38β, PDGFRβ, SRC;

**BIRB-796** include BLK, CDK5, CDK8, DDR1, DDR2, EPHA3, EPHA7, EPHA8, EPHB2, p38β p38γ, FLT1, FRK, NTRK1, JNK1, JNK2, JNK3, KIT, MAP4K4, MRCKβ, PTK2β, RET, SLK, STK10, TIE1, TIE2, TNIK, TRKB, TRKC, ZAK;

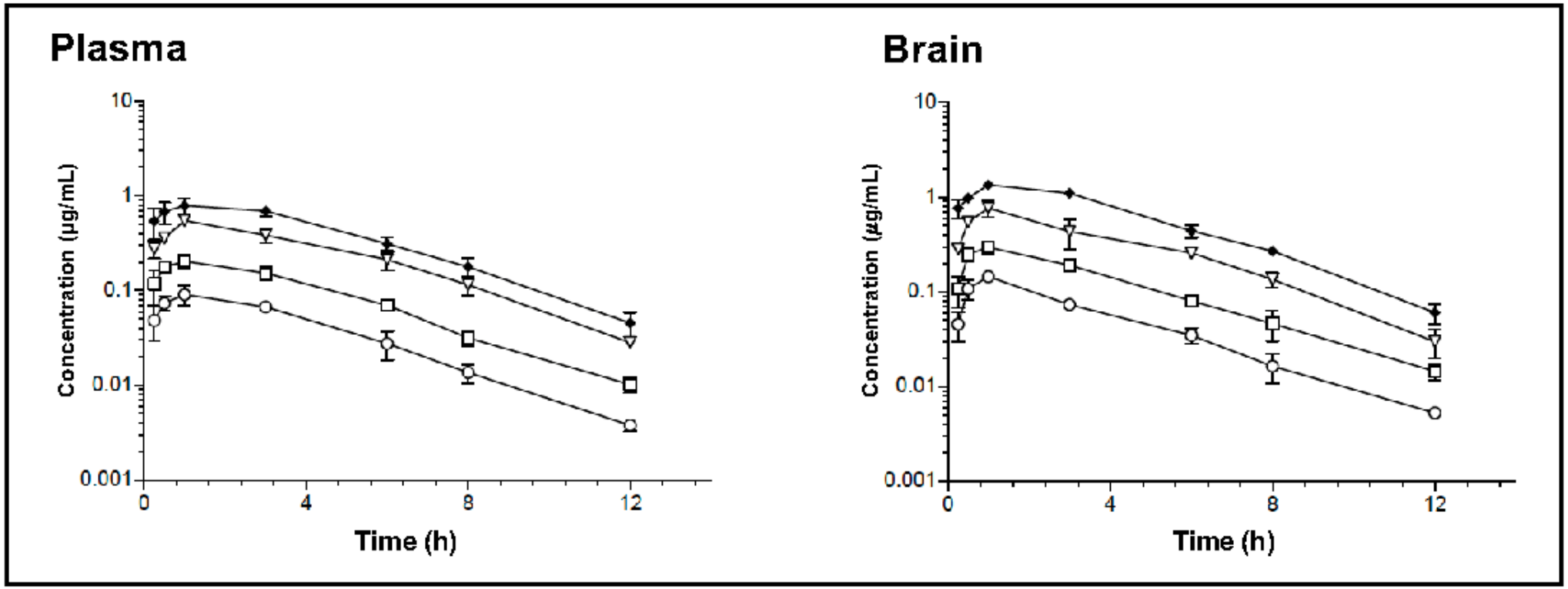
**SB203580** include BRAF, CIT, CK1δ, CK1ε, DMPK, GAK, JNK2, JNK3, NLK, p38β, RIPK2, STK36, TNIK.

**(B) MW150** has canonical $IC_{50}$ <1 μM for p38αMAPK.

# MW150: Orally Bioavailable with Brain Exposure



## Dose Dependent Oral Bioavailability



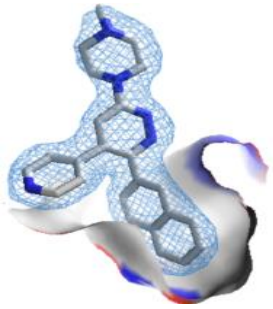

## Documents tissue exposure, including brain

***It is estimated that >95% of approved drugs lack sufficient blood brain barrier penetrance to engage their targets in the central nervous system (CNS)***

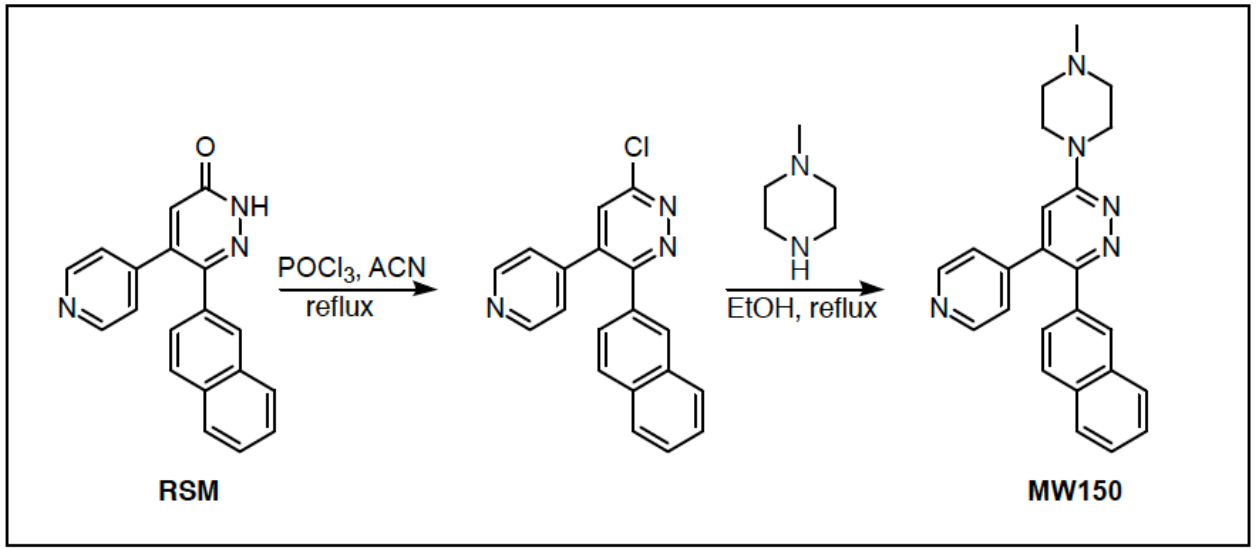
# MW150: Preclinical Efficacy & PD Consistent with MOA

| Property | Experimental System | End Point |
|---|---|---|
| Efficacy | Autism spectrum disorder (ASD) model: SERT Ala56 mouse | Repeat administration mitigates SERT Ala56-mediated 5-HT receptor hypersensitivities and altered social interaction; reverses SERT Ala56-mediated reductions in intestinal motility |
| | APP/PS1 Transgenic mouse | Improved performance in contextual fear memory and radial arm water maze (RAWM); no effect on sensory, motor and motivational mechanisms or in open field tasks |
| | $APP^{Nlh/Nlh}/PS1^{P264L/P264L}$ KI mouse | Improved performance in RAWM |
| | Tauopathy model: infusion of synthetic 4R/2N human tau into dorsal hippocampus | Attenuates defects in associative memory (contextual fear learning paradigm) and in short term spatial memory (RAWM); no effect on sensory, motor, motivational mechanisms or open field tasks |
| | Tauopathy model: rTg4510 (human tau, P301L mutation) mouse | Attenuates defects in associative memory (contextual fear learning paradigm) and in short term spatial memory (RAWM) |
| PD Endpoints | Autism spectrum disorder (ASD) model: SERT Ala56 mouse | Repeat administration normalizes 5-HT clearance in SERT Ala56 mice; no effects on SERT protein levels; no effects on brain 5-HT levels or turnover |
| | Anisomycin-treated CHO line | Decrease in anisomycin-induced 5-HT uptake |
| | LPS treated BV2 microglia line | Endogenous kinase inhibition measuring phosphorylated substrate pMK2: $IC_{50}$ = 332nM<br>Inhibition of IL-1β overproduction: $IC_{50}$ = 936nM |
| | $APP^{Nlh/Nlh}/PS1^{P264L/P264L}$ KI mouse | Cortex IL-1β and TNFα levels decreased in mice showing improved performance in RAWM |
| | APP/PS1 Transgenic | No effect on Aβ plaque burden |
| | $APP^{Nlh/Nlh}/PS1^{P264L/P264L}$ KI mouse | No effect on Aβ plaque levels or volume & no effect on PBS-soluble or formic acid-soluble Aβ40 or Aβ42 |
| | Drug Resistant p38α $MAPK^{T106M}$ KI mouse | IL-1β and TNFα levels do not change with MW150 treatment; no observed alternative PD |

# MW150: Commercial Scale GMP Production



## Clinical GMP Drug Production Optimized for Commercial Scale



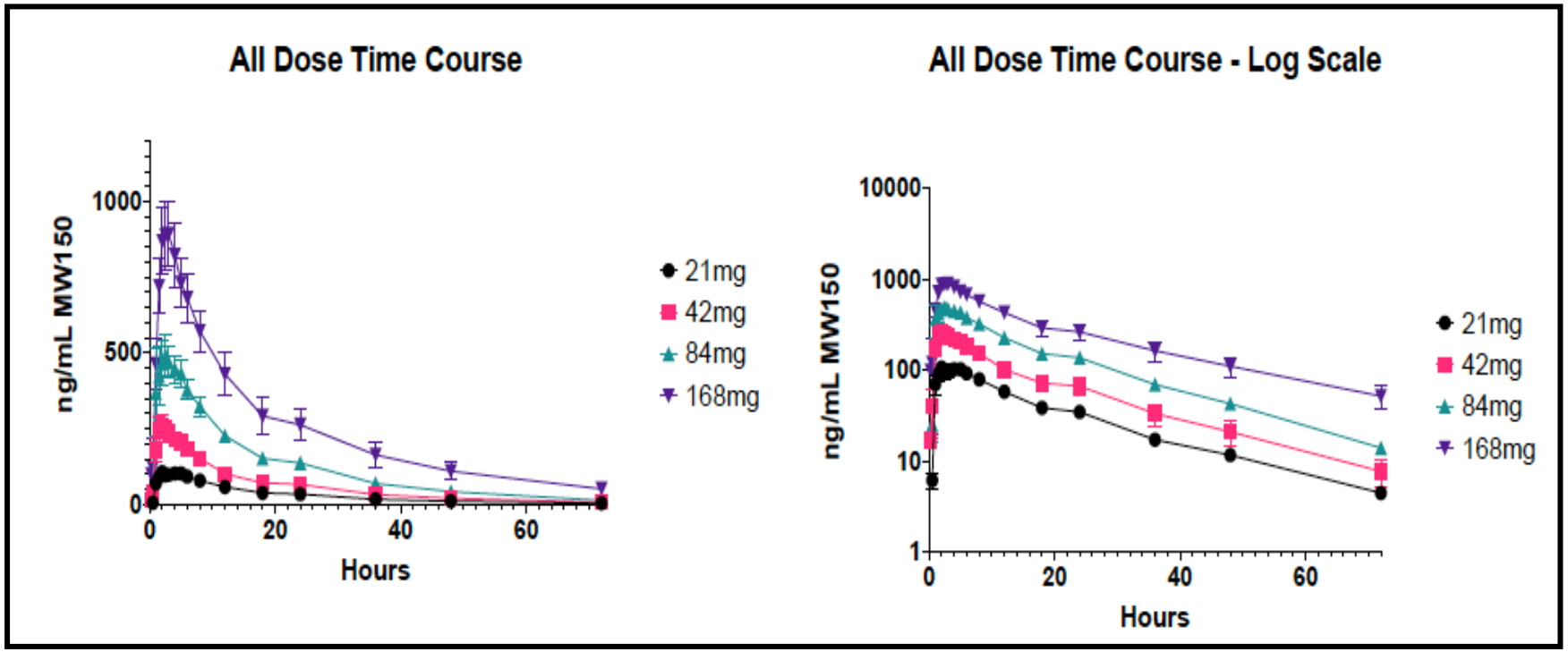

*Stress testing and storage stability analyses document long term stability of clinical drug, which is crystalline form.*

| Feature | Information |
|---|---|
| CAS Number | 1628502-91-9 |
| Molecular Weight | 381.47 |
| Physical Appearance | Light yellow, crystalline |
| logP | 2.80 ± 0.12 |
| PSA | 45.15 |
| Melting Point (DSC) | Single endotherm: Extrapolated Onset: 189.4°C<br>$\Delta H_f$ = 101.6 J/g |
| XRD | monoclinic, no polymorphs, no water or solvent |
| TGA | congruence of crystal & powder results |
| Crystal Structure | Space group: C2/c (no.15)<br>Unit cell: a = 28.090(11) Å, b = 9.296(4) Å, c = 17.273(6) Å, α 90°,<br>β = 116.996(8)°, γ 90°<br>Cell volume: 4019 (3) Å$^3$<br>T = 100K,<br>μ(CuKα) = 0.605 mm$^{-1}$<br>$D_{calc}$ = 1.261 g/mm$^3$<br>Z, Z': 8, 1 |
| pKa | Potentiometric: 3.83 ± 0.44; 7.27 ± 0.21<br>UV-metric: 3.62 ± 0.12; 7.27 ± 0.09 |
| Stability (24h, 37°C) | % remaining: 100% at pH 7.0 & pH 13.0; 87% at pH 1.0 |
| Purity | 99.0 wt % (anhydrous, solvent-free basis)<br>99.6 area% by HPLC |

# Human Pharmacokinetics: Phase 1a (SAD)

## Human PK: consistent with preclinical GLP investigations & once/day oral administration

*Exposure ($C_{max}$ & $AUC_{0\text{-}24h}$ ) increased close to dose proportionality. The log transform plot of the highest dose administered (168 mg, >> HED efficacy) reveals subject variation in $C_{max}$ within normal range and hints that one subject might have a clearance/metabolism distinct from others.*




- *$T_{max}$ = 2.1 - 3.1 h*
- *$T_{1/2}$ = 15.2 - 19.4 h*
- *$C_{max}$ = 114.2 - 915.6 ng/ml*
- *CL = 9.5 - 11.3 L/h; Vz = 206.9 - 261.8 L*
- *Last measurable sampling time was in general 72 h.*
- *Exposure, in terms of $C_{max}$ and $AUC_{0\text{-}24h}$ , increased proportionally with dose*

***Overall, findings are well below hepatic blood flow & exceed total body water (87 L/h & 42 L with 70 kg bw, respectively), consistent with low extra-hepatic elimination/metabolism & suggest good distribution into tissues.***

# Human Safety Biomarkers

## COAGULATION AND HEMATOPOIETIC

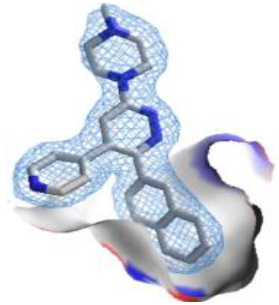
| Dose | | 0 mg (8 subjects) | 21 mg (8 subjects) | 42 mg (8 subjects) | 84 mg (8 subjects) | 168 mg (8 subjects) |
|---|---|---|---|---|---|---|
| Blood Coagulation | Thromboplastin time (TPZ) | All Normal | All Normal | All Normal | All Normal | All Normal |
| | APTT | All Normal | All Normal | All Normal | All Normal | All Normal |
| | INR | All Normal | All Normal | All Normal | All Normal | All Normal |
| Hematopoietic System | Erythrocytes | All Normal | All Normal | All Normal | All Normal | All Normal |
| | Neutrophils | All Normal except subject 10 at day 2 and 3, and subject 18 at day 1, 2, 3 and 4) | All Normal except subject 2 at day 2, subject 4 at day 1, 2 and 4) | All Normal except subject 13 at day 1, 2 and 4) | All Normal except subject 17 at day 2, subject 19 at day 1, subject 23 at day 3 and 4, subject 24 at day 2) | All Normal except subject 32 at day 2) |
| | Monocytes | All Normal | All Normal except subhect 2 at day 2 | All Normal except subject 12 at day 4, and subject 14 at day 1 | All Normal | All Normal |
| | MCV | All Normal | All Normal | All Normal | All Normal | All Normal |
| | Lymphocytes | All Normal except subject 10 at day 3 and 4, subject 18 at day 1, 2, 3 and 4, and subject 20 at day 2, 3 and 4 | All Normal except subject 2 at day2, subject 4 at day 1 and 2, and subject 8 at day 4) | All Normal excepot subjects 13 and 14 in day 1, 2, 3 and 4, and subject 15 in day 4) | All Normal except subject 17 at day 2, subject 19 at day 1, subject 23 at day 3 and 4, subject 24 at day 2, subject 23 at day 3 and 4, and subject 24 at day 2) | All Normal except subject 32 at day 2. |
| | Leukocytes | All Normal | All Normal | All Normal | All Normal | All Normal |
| | Haematocrit | All Normal | All Normal | All Normal | All Normal | All Normal |
| | Eosinophiles | All Normal exceot subject 10 at day 1 | All Normal except subject 4 at day 1 and 4, | All Normal | All Normal | All Normal |
| | Basophiles | All Normal except subject 10 at day 3 and 4 | All Normal except Subject 4 at day 2 | All Normal | All Normal | All Normal |
| | Thrombocytes | All Normal | All Normal | All Normal | All Normal | All Normal except subject 30 on days 1-4 |

# Human Safety Biomarkers

## ORGAN FUNCTION

| Dose | | 0 mg (placebo) (8 subjects) | 21 mg (8 subjects) | 42 mg (8 subjects) | 84 mg (8 subjects) | 168 mg (8 subjects) |
|---|---|---|---|---|---|---|
| Liver Function | Total protein | All Normal | All Normal | All Normal | All Normal | All Normal |
| | Total Bilirubin | All Normal | All Normal except subject 8 at day 4 | All Normal | All Normal | All Normal |
| | Albumin | All Normal | All Normal | All Normal | All Normal | All Normal |
| | GPT | All Normal | All Normal | All Normal | All Normal | All Normal except subject 27 at day 1, 2, 3 and 4 |
| | GOT | All Normal except subject 11 at day 1 | All Normal | All Normal | All Normal | All Normal |
| | γ-GT | All Normal | All Normal | All Normal | All Normal | All Normal |
| | Alkaline Phosphatase | All Normal | All Normal | All Normal | All Normal | All Normal |
| Renal Function | uric acid | All Normal except subj 11 on day 4 | All Normal | All Normal | All Normal | All Normal |
| | urea | All Normal | All Normal | All Normal except subject 12 at day 1 | All Normal | All Normal |
| GI/Renal Function | Mg2+ | All Normal | All Normal | All Normal | All Normal | All Normal |
| Cardiac Function | CK-MB, | All Normal except subject 11 at day 1, 2, 3 and 4) | All Normal except subject 4 at day 1 and 4, | All Normal except subject 12 at day 3 | All Normal | All Normal |
| | LDH | All Normal | All Normal | All Normal | All Normal | All Normal |
| Skeletal Muscle Function | CK | All Normal except subject 7 at day 3 and 4, and Subject 11 at day 1, 2, 3 and 4. | All Normal except subject 8 at day 4 | All Normal except subject 12 at day 4 | All Normal except subject 17 at day 1, 2, 3 and 4 | All Normal except subject 27 at day, 1, 2, 3, 4, and Subject 31 at day 4 |

# MW150 Safety Pharmacology & Toxicology



## *First-in-Human (FIH) Clinical Trial:*

## *No adverse events at human equivalent doses (HED) for efficacy & PD*

**Phase 1a (SAD) Clinical Trial Doses: 0.35, 0.7, 1.4 & 2.8 mg/kg**

***[Preclinical Efficacy Studies were done with doses ~ 0.2 mg/kg HED]***

***Preclinical Safety Pharmacology & Toxicology at Doses >> Efficacy & PD***

| Study | NOAEL | Human Equivalent Dose (HED)* |
|---|---|---|
| Aged Alzheimer knock-in mouse model (single administration) | >250 mg/kg (highest dose) | 20 mg/kg |
| Rat (DRF, 14 days, daily oral administration, no recovery, toxicokinetics) | 125 mg/kg | 20 mg/kg |
| Rat (28 day GLP toxicology, with recovery, toxicokinetics) | 75 mg/kg (highest dose) | 12 mg/kg |
| Rat (GLP respiratory safety) | 100 mg/kg (highest dose) | 16 mg/kg |
| Dog (GLP cardiovascular safety) | 30 mg/kg (highest dose) | 16 mg/kg |
| Dog (28 day GLP toxicology, with recovery, toxicokinetics) | 6 mg/kg (male)<br>10 mg/kg (female) | 3 mg/kg(male)<br>5 mg/kg(female) |

*HED of 10 mg/kg follows ICH Guidance: test & observe up to 50x efficacy dose or until clear toxicology biomarker change in majority of animals in the cohort. Regulatory guidance for first-in-human (FIH) is to start at 10% of NOAEL HED, which is MW150 efficacy dose in diverse disease models.

# MW150: Key Aspects of Approach

- *Structural genomics, pharmacoinformatics & secondary pharmacology*
- *Targeting pathophysiology progression mechanisms*
- *Disease agnostic approach* ➡ *single target entity, multiple PD*
- *Early development focused on IND activities and early risk reduction*
- *CNS tissue exposure and new chemical entity candidates*

# I Wish I Knew… and Project Learning Experiences

**I wish I knew….**

*…about options to avoid elapsed time gaps between technical completion & next stage funding.*

*…more about CYP substrate liability forecasting.*

*…more about when to terminate non-productive alternative approaches*

**Project Learning Experiences**:

1. how to stay focused on ICH/FDA goals and minimize fun science distractions.
2. new perspectives on risk reduction at preclinical development stage.
3. how to leverage infrastructure and knowledge base from previous campaigns for rapid POC.