CHARACTERIZATION OF HIGHLY POTENT, ORALLY BIOAVAILABLE, AND BRAIN PENETRANT SMALL MOLECULE TREM2 AGONISTS FOR THE POTENTIAL TREATMENT OF ALZHEIMER'S DISEASE

Christian Mirescu, PhD
Vice President of Neuroimmunology
Vigil Neuroscience is a clinical-stage microglia-focused therapeutics company.

Our purpose: to treat rare and common neurodegenerative diseases by restoring the vigilance of microglia, the brain’s sentinel immune cells.

<table>
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<th>Company</th>
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<th>Royalties/Patent</th>
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Regulation of TREM2 receptors, microglia function and neurodegenerative disease risk and resilience

Mechanisms of surface TREM2 regulation

- Shedding
- Receptor Clustering
- Internalization

Regulation of microglial function by TREM2 modulation

- Cell-to-cell function
  - Chemotaxis
  - Polarization state

- Cell autonomous
  - Microglia survival
  - Polarization state
  - Phagocytosis
  - Lipid metabolism

Tuning of microglia activation states in human neurodegeneration

- AD risk variants
- Nasu-Hakola disease variants
- Human genetic perturbogens
- TREM2 TUNING
- Drug discovery approaches

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Human TREM2 vs TREM1 selectivity
HEK engineered cells

Human microglia pSYK EC$_{50}$, WT TREM2: <5 nM
TREM2 KO microglia potency: >3,000 nM

Vigil Small Molecules Activate Human Microglia TREM2
TREM2 SM agonists retain activity across common & rare variants

**Note:** TREM2 signaling in transiently transfected HEKs.
Evidence of SM MOA as TREM2 molecular glues

High-order SM induced TREM2 receptor clustering in microglia

Vigil SM enhances the interaction of TREM2-DAP12

Vigil SM coordinate signaling via molecular glue MOA

Non-denatured states (Native PAGE) in iMGLs

TREM2 Complexes in iMGLs (kDa)

66 146 242 480

Neg CTL (DMSO) Vigil SM 10nM 100nM TREM2 KO

DMSO Vigil SM

TREM2-DAP12 interaction

Steady-state levels in HEK293 cells

TREM2 immunoprecipitation in HEK system

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TREM2 SM agonists enhance signaling responses to damage-associated ligands

**SUMMARY OF CO-TREATMENT RESPONSES**

- **TREM2 SM agonist shifts**
  - 4x improvement in potency
  - 4x increase in max response

- **TREM2 natural ligand shift**
  - 6x improvement max response
Engagement of human microglia TREM2 translates to reductions in surface and shed levels

<table>
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<tr>
<th>Time post-SM stim.</th>
<th>TREM2+ Microglia (% Baseline)</th>
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<tr>
<td>CTL</td>
<td>40</td>
</tr>
<tr>
<td>5 min</td>
<td>60</td>
</tr>
<tr>
<td>10 min</td>
<td>50</td>
</tr>
<tr>
<td>15 min</td>
<td>50</td>
</tr>
<tr>
<td>60 min</td>
<td>40</td>
</tr>
<tr>
<td>120 min</td>
<td>30</td>
</tr>
<tr>
<td>24 hr</td>
<td>20</td>
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**Surface TREM2 target engagement**

- Robust effect
- Rapid time-course

**Reduction of iMGL sTREM2 shedding**

- sTREM2 IC50 <5nM

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<tr>
<th>SM TREM2 agonist Log[nM]</th>
<th>iMGL sTREM2 Levels (% DMSO CTL)</th>
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<tbody>
<tr>
<td>-2</td>
<td>125</td>
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<tr>
<td>-1</td>
<td>100</td>
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<tr>
<td>0</td>
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</tr>
<tr>
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<td>25</td>
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<td>25</td>
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Oral dosing of SM TREM2 agonist selectively activate microglia gene signatures in hTREM2 mice

**Brain chemo-transcriptomic responses**
- mRNA level
-SM TREM2 Agonist Gene Signature
- log2(fold change vs vehicle)

**Brain pharmacodynamics**
- Protein level
- SM TREM2 Agonist (Oral)
- Brain IP-10 Levels (pg/g Tissue)

**Humanized TREM2 Mice**
- hTREM2 BAC transgenic
- Vs
- TREM2 Knockout (KO) Mice

**On-target TREM2 in vivo activity**

**SM TREM2-dependent signatures**
- hTREM2-CV^{TH} vs TREM2^{KO}

**Brain changes**
- Innate Immune Response
- Microglia Function
- Adaptive Immune Response

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SM agonist recapitulates TREM2 mAb effects in AD Mouse Models

Comparative chemo-transcriptomic profiling in 5xFAD amyloidosis model

Disease-associated microglia (DAM) signature enhanced by SM dosing

Pharmacodynamic responses in AD mouse model

Mouse Amyloidosis Model
5xFAD AD (mut APP/PS1) + hTREM2
Oral dosing in NHPs demonstrates CNS TREM2 engagement: Path to translational biomarker

Small molecule CSF biomarker enablement

Cynomolgus Macaque
Non-human primates (NHPs)

PK Timecourse
(hours)

CSF Drug Levels (nM)

Reduction in NHP CSF sTREM2 levels

Timecourse post-dosing (hours)
sTREM2 (%Baseline)

Low Dose, po
High Dose, po

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Acknowledgements

Vigil Neuroscience
Kell C. Larson
Borislav Dejanovic
Abbie Renoux
Matthew Figley
Daria Tchessalova
Beril Kiragasi
Frederick Gergits
David Gray

Jonathan Houze
Bhaumik Pandya
Evan Thackaberry
David Stiles
Liyue Huang

Washington University
Marco Colonna
Shoutang Wang
Susan Gilfillan