Microglia as a Therapeutic Target in Alzheimer’s Disease

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Microglia are the immune cell of the brain.

- Comprise ~12% of all cells in the brain.
- Function to protect from infections, and to clean up debris following damage and injury.
- Microglial dysfunction implicated in traumatic brain injury, aging, and neurodegeneration.
Activation of microglia

- Infection or damage causes microglia to become “activated”
- Primed to fight pathogens.
- After the infection/damage is contained microglia revert to resting state.
AD Neuropathology
AD Neuropathology

- **Plaques** composed of β-amyloid (Aβ) peptide
  - Initiates in cortical regions

- **Tangles** composed of hyperphosphorylated tau
  - Initiates in hippocampus (CA1)

- Synaptic/Neuronal dysfunction and death
Inflammation and Alzheimer’s disease

- The AD brain is characterised by the presence of plaques and tangles, extensive neuronal loss and an inflammatory response.

- Microglia surround plaques, in an attempt to phagocytose Aβ and remove it from the brain.
- They are unsuccessful, as the AD brain is still riddled with Aβ plaques.

- However, now there is also an increasing chronic inflammatory process...
CSF1R

- In the brain, microglia express the majority of the colony stimulating factor 1 receptor
  - expressed on monocytes, macrophages, dendritic cells and osteoclasts
- Has 2 ligands CSF1 and IL34
  - Cause dimerization and autophosphorylation
- Involved in cell proliferation, survival and migration.

Adapted from Hume and MacDonald, Blood 2012
Evaluation of CSF1R antagonists

- We use PLX3397 (Plexxikon Inc.) – entering Phase 3 clinical trials for Pigmented villonodular synovitis (PVNS), and Phase 2 for oncology indications.

- Specific for CSF1R and also related receptor c-Kit.

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Elmore and Najafi et al., Neuron 2014
Control

12-months PLX3397

21 days 14 3
represents a microglia

Elmore and Najafi et al., *Neuron* 2014
CSF1R inhibitors eliminate microglia

Elmore and Najafi et al., Neuron 2014
Microglial remnants seen throughout brain

Elmore and Najafi et al., Neuron 2014
Microglia are dependent on CSF1R signaling

- Administration of CSF1R inhibitors that cross the blood brain barrier lead to the rapid elimination of microglia throughout the CNS.

- Microglia undergo cell death.

- Peripheral macrophage/monocyte populations are not depleted.

- As microglia are the only cell type in the CNS to express CSF1R it provides a useful tool to study microglial function, as well as a potential therapeutic target.
Phenotype of microglia-depleted mice:

Elmore and Najafi et al., Neuron 2014
Tests of Anxiety

Elevated Plus Maze

Open Field
No Effect of Microglial Elimination on Anxiety

Elevated Plus Maze

- Frequency of Arm Entries
- Time Spent in Arms (s)

Open Field

- Frequency in Zone
- Time in Zone (s)

Elmore and Najafi et al. – Neuron (2014)
Tests of Motor Function

Open Field

Rotarod
No Effect of Microglial Elimination on Motor Function

Open Field

Rotarod

Elmore and Najafi et al. – Neuron (2014)
Tests of Cognitive Function

Barnes Maze

Fear Conditioning
No Effect of Microglial Elimination on Cognitive Function

**Barnes Maze**

![Barnes Maze Graph]

**Fear Conditioning**

![Fear Conditioning Graph]

Elmore and Najafi et al. – Neuron (2014)
Interim Conclusions

- Pharmacological inhibition of the CSF1R results in rapid microglia elimination from the CNS in 7-21 days.
- Microglia are not overtly necessary for cognition or behavior.
- We can now directly study the roles of microglia in the healthy and diseased/injured/aged brain.
- Can microglial-elimination be a therapeutic for brain disorders?
Effects of microglial-elimination in Alzheimer’s disease
Alzheimer’s disease:

- Genetically modified mice develop Alzheimer’s disease.
- They produce plaques in their brains, and become cognitively impaired.
CSF1R inhibition eliminates microglia in AD mice

- 3 months treatment in 23 month old 3xTg-AD mice.
- Removes >95% of all microglia.
- Is this beneficial?

A B

IBA1/6E10

C

represents a microglia

Control 3-months PLX3397

0 1000 2000 3000 4000 5000 6000 7000 8000

Control PLX3397

*
Elimination of microglia improves learning

![Graph showing latency to platform location over days of training. The graph compares control and PLX3397 groups. The control group shows a decrease in latency over time, while the PLX3397 group shows a more significant decrease, especially on the 6th day. The 24 Hour Probe Trial results show a similar trend, with PLX3397 having a lower latency to platform location and platform crosses compared to the control group.](image-url)
Elimination of microglia does not alter pathology
Elimination of microglia does not alter pathology
Elimination of microglia reduces inflammation

- Levels of IL-1β and TNFα are significantly reduced with microglial-elimination.
- Elevated levels of both of these are associated with memory impairments as well as synapto- and neurotoxicity.
Interim Summary

• Even microglia in the aged and diseased brain are fully dependent upon CSF1R signaling for their survival.

• We can achieve chronic microglial elimination in advanced AD mice.

• Elimination of microglia improves cognition, but has no impact on pathology.

• Treatment with CSF1R inhibitors may represent a useful therapy for AD and other disorders involving neuroinflammation.
AD models have plaques and tangles but not extensive neuronal loss.

Many drugs have progressed into human clinical trials after testing in AD models, and have then failed!

We also utilise a mouse model of extensive neuronal loss.

Yamasaki et al., J. Neurosci.
Experimental Design

A

CamKII tTa

- Dox

TRE DipA

Cell Loss

B

25-Day Lesion

- Dox

PLX3397

4 Weeks Recovery

5-8 Months of Age

Sacrifice

Cognition Testing

Control

PLX3397

Lesion

Lesion + PLX3397
D  Lesion + PLX3397
Activated microglia are dependent upon CSF1R signaling for survival.
Microglia elimination improves lesion-associated deficits on elevated plus maze
Microglia elimination improves performance on Morris water maze
Microglia elimination restores lesion-induced synaptic alterations

![Image of molecular layer showing effects of PLX3397 treatment on synaptophysin and PSD-95 puncta number and area.](image-url)
Conclusions

- Activated microglia are dependent upon signaling through CSF1R for survival

- Elimination of microglia following neuronal lesion facilitates:
  - Functional recovery
  - Synaptic alterations

- CSF1R inhibitors improve cognition in a model of AD and a model of robust neuronal loss. Therefore good rationale for developing further for neuroinflammatory disorders.
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Microglial elimination is fully reversible

- Once microglia are eliminated with CSF1R inhibitors then withdrawal of CSF1R inhibitors stimulates rapid repopulation with new microglia.

- New microglia arise from stem cells found throughout the CNS that divide and then differentiate into new microglia.

Elmore and Najafi et al., *Neuron* 2014
3 days recovery
7 days recovery
14 days recovery
Rapid repopulation of the microglia-depleted brain

IBA-1 Cells

Control 0 day 3 day 7 day 14 day 21 day

0 500 1000 1500 2000
CSF1R inhibitors eliminate microglia during brain injury
Microglia-depleted mice have no behavioral deficits

- Depletion of microglia for 2 months does not effect Elevated plus maze, or Open field analyses.

Elmore and Najafi et al., Neuron 2014
Mice depleted of microglia have significantly improved learning compared to intact animals.
Microglia-depleted mice have no learning deficits

- Mice depleted of microglia have significantly improved learning compared to intact animals.
Microglia-depleted mice have no motor deficits

- No deficits in Contextual Fear Conditioning
- No deficits in motor function in healthy mice

Elmore and Najafi et al., Neuron 2014