Conflicts of Interest

• None to declare
Amelioration of Ischemic Stroke Damage Through Inhibition of Interleukin-6 Signaling with Tocilizumab Requires Sex Specific Dosing

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Interleukin-6 and stroke

• Interleukin-6 (IL-6) has been extensively studied in inflammatory disorders both pre-clinically and clinically.

• Levels of IL-6 are increased in the plasma of stroke patients when compared to age-matched healthy controls.

• Between stroke patients, higher plasma IL-6 levels are correlated with larger infarct volume, increased stroke severity and poor long term prognosis.
IL-6 signaling can be mediated by membrane and soluble forms of the receptor
Classical Signaling

• IL-6 Signaling where IL-6 binds directly to the cell membrane (no intermediate)

• Limited to cells which have **IL-6R bound to the gp-130 complex expressed on the membrane** which includes:
  - Neutrophils, monocytes, some T-cells, microglia, astrocytes, neurons and hepatocytes
Trans-signaling

- Signaling of IL-6 using soluble IL-6R

- sIL-6R formed by *proteolytic cleavage* of membrane bound IL-6R (approx. 90%) and alternative splicing (approx. 10%)
**Tocilizumab (Actemra)**

* IgG1

- Antibody against the Interleukin-6 receptor (IL-6R)

- FDA approved treatment for:
  - *Rheumatoid Arthritis* (onset 40-60 years old)
  - *Giant Cell Arteritis* (onset 70-80 years old)
  - *Systemic Juvenile Idiopathic Arthritis* (onset 1-6 years old)

*** Women are more likely than men to develop both giant cell arteritis and rheumatoid arthritis***
Hypothesis:

Tocilizumab treatment will reduce stroke damage by blocking post-stroke interleukin-6 signaling.
35-day post-stroke survival study

*Using 18-month old males and females

**Day -3:**
- Baseline Behavior tests

**Day 0:**
- MCAO surgery or SHAM Surgery
  - Aged male and female mice, 60 minute MCAO

**Day 0:**
- IP injection of either Drug (20ug/1g mouse weight) or vehicle (non-specific IgG)

**Day 7:**
- Open-field
- Y-maze
- Corner Test

**Day 14:**
- Corner Test
- Forced Swim test

**Day 20:**
- NDS
- Open-field
- Y-maze
- Corner Test

**Day 21:**
- Digi gait
- Tail-suspension

**Day 27-30:**
- Train for Barnes maze

**Day 31:**
- Barnes Maze testing day

**Day 35:**
- Euthanasia and sample collection

**Male N:**
- Drug Stroke: 8
- Vehicle Stroke: 7
- Drug Sham: 5
- Vehicle Sham: 5

**Female N:**
- Drug Stroke: 9
- Vehicle Stroke: 9
- Drug Sham: 6
- Vehicle Sham: 6
Behavioral changes
Reduced corner test deficit in aged male mice with tocilizumab
Improved long term cognitive outcomes on the Barnes maze 31-days post-stroke for tocilizumab treated Aged male mice
Tocilizumab treatment reduced brain atrophy 35 days post-stroke in aged male mice

**N:**
Female Vehicle Stroke: 9
Female Drug Stroke: 9
Male Vehicle Stroke: 7
Male Drug Stroke: 8
Tocilizumab treatment reduced Post-Stroke Mortality in Aged mice
Why is tocilizumab effective in aged males but not females?
Females have a significantly higher level of soluble IL-6R post-stroke compared to males.
Hypothesis:

• Tocilizumab has been ineffective for aged females so far in the study do to an increase in sIL-6R post-stroke

• A higher dose of tocilizumab (100mg/kg) will lead to reduced infarct in aged female mice 72-hours post-stroke
A higher dose of tocilizumab (100mg/kg) led to significant reduction in infarct 72-hours post-stroke in aged females

High Drug Dose Female Stroke Test

**

% Hemisphere infarct 3 days

IgG Control | High drug dose

N:
Drug: 6
IgG control: 5
Why do females have a higher sIL-6R level post-stroke compared to males?
Previous research has shown that cell death pathways differ between males and females, with females undergoing primarily caspase dependent pathways.

Hypothesis:

• Caspase inhibition will lead to a reduction in sIL-6R release in females.

• Since neutrophils release much of the sIL-6R post injury, inhibiting caspase in an ex vivo bone marrow neutrophil assay will reduce the amount of sIL-6R released
Aged male and female ex vivo bone marrow assay

Neutrophils were then collected for mRNA and supernatant for ELISA analysis.
Interleukin-6 receptor shedding, not expression, is caspase dependent in aged female neutrophils but not aged male neutrophils

N:
Aged male: 10
Aged Female: 10
Clinical Relevance
Women also have a significantly higher level of soluble IL-6R post-stroke compared to men.
Conclusions Drawn from Study

- Inhibiting interleukin-6 signaling with tocilizumab ameliorates injury post-stroke in aged male mice and aged female mice at a higher dose.

- Sex difference seen in tocilizumab effectiveness likely caused by higher sIL-6R in females post-stroke a trend also seen in human stroke patient plasma.

- If translated into the clinic women would likely need a higher dose than men for therapeutic effect.
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