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Fingolimod as a potential cerebroprotectant

Results from the stroke preclinical assessment network

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SPAN Yale site





Dr. Sansing's lab



DISCLOSURES:

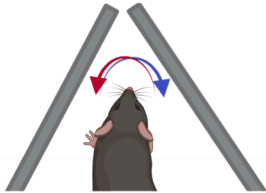
SPAN was funded by NINDS/NIH

Stroke preclinical assessment network (SPAN)

- First multi-site trial intended to identify a candidate treatment likely to succeed in human clinical trials
- State-of-the-art approach to minimize bias    
- Relevant comorbidities in stroke preclinical trials
- **Fingolimod** is an immunosuppressive drug that has been shown to improve functional recovery and reduce infarct size after stroke
(Hasegawa et al. 2010, Wei et al. 2011, Kraft et al. 2013, Campos et al. 2012)
 - Effects of Fingolimod examined in different comorbidity models

SPAN primary outcome (corner test)

Corner test



Sensorimotor test

10 trials/subject

baseline, d7 and d28

Hanging wire



Motor function deficit

Three trials of 120 sec

d7 and d28

Grid walk test



Locomotor function

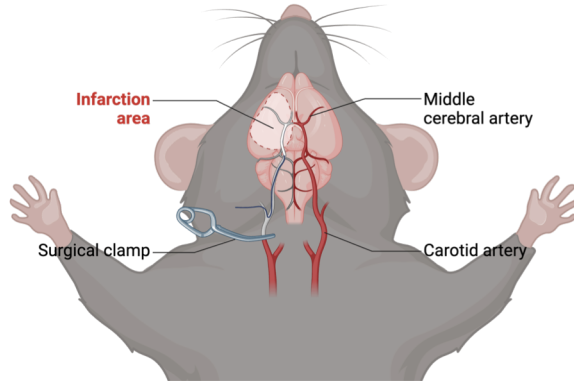
One trial - 5 min

d7 and d28

The tests were recorded, and the de-identified videos evaluated by SPAN-certified raters

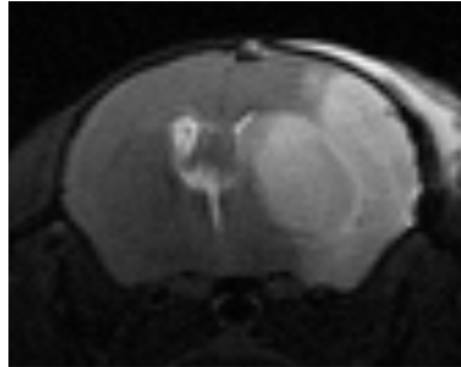
Secondary outcomes – MRI lesion fraction

Middle cerebral artery occlusion (tMCAO)

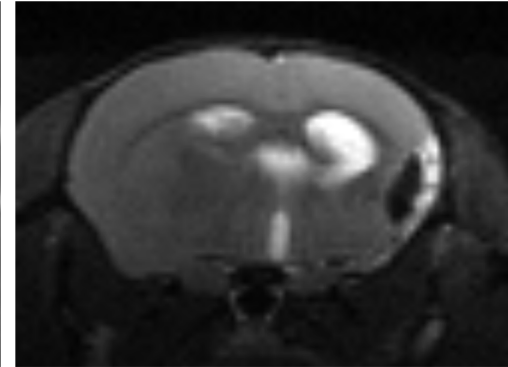


Reperfusion after 1h of occlusion

Day 2 post-tMCAO



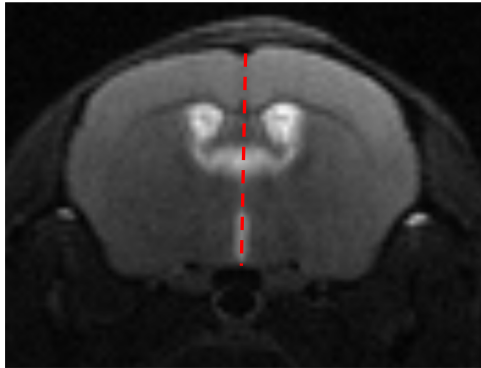
Day 28 post-tMCAO



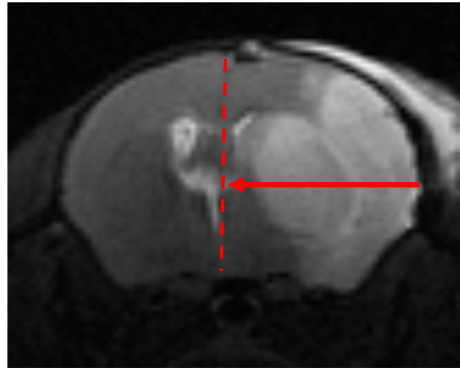
Cerebral morphometry was assessed using automated image analysis

Secondary outcomes – MRI midline shift

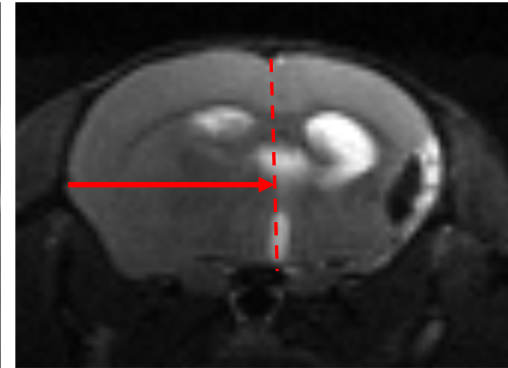
No lesion



Day 2 post-MCAO



Day 28 post-MCAO



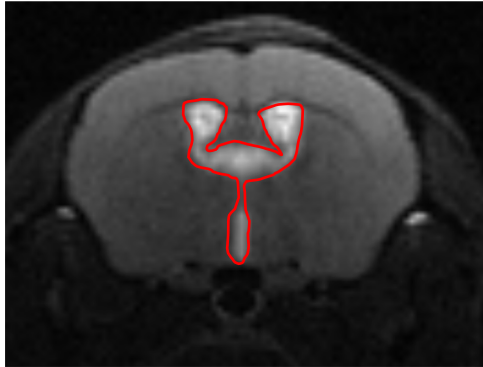
Shift to the left = brain edema

Shift to the right = brain atrophy

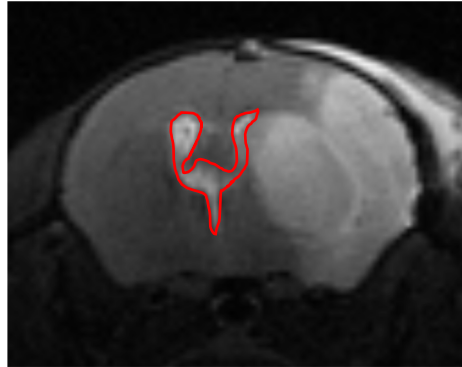
Cerebral morphometry was assessed using automated image analysis

Secondary outcomes MRI - cerebrospinal fluid (CSF) volume

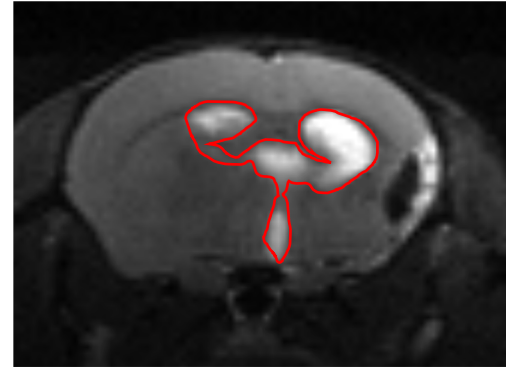
No lesion



Day 2 post-MCAO



Day 28 post-MCAO



CSF volume ↓ brain edema

CSF volume ↑ brain atrophy

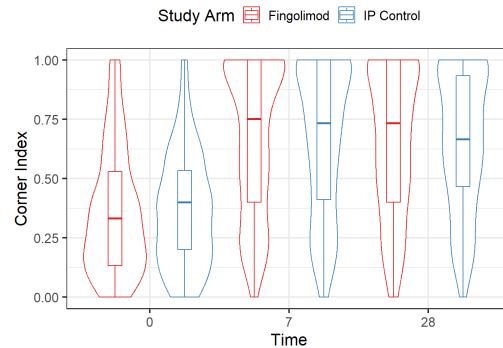
Cerebral morphometry was assessed using automated image analysis

Overall behavioral outcomes

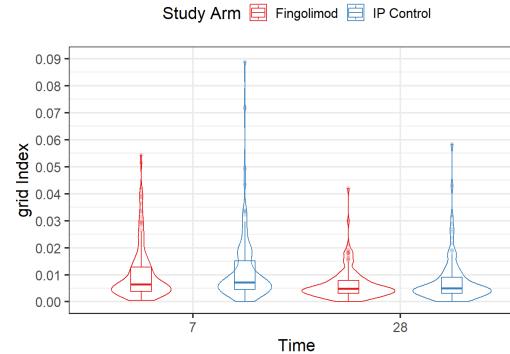
Overall cohort: Young, aging, obesity-induced hyperglycemic mice and spontaneous hypertensive rats

Enrolled: 410 total - 278 rodents at day 30

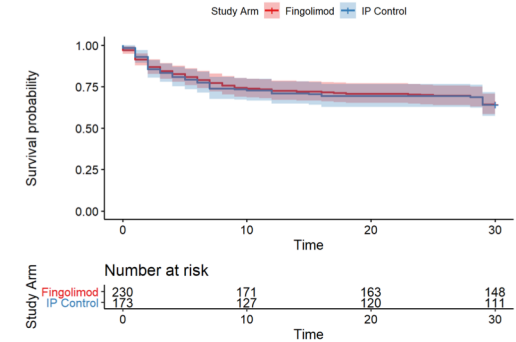
Alternative corner index



Grid walking index

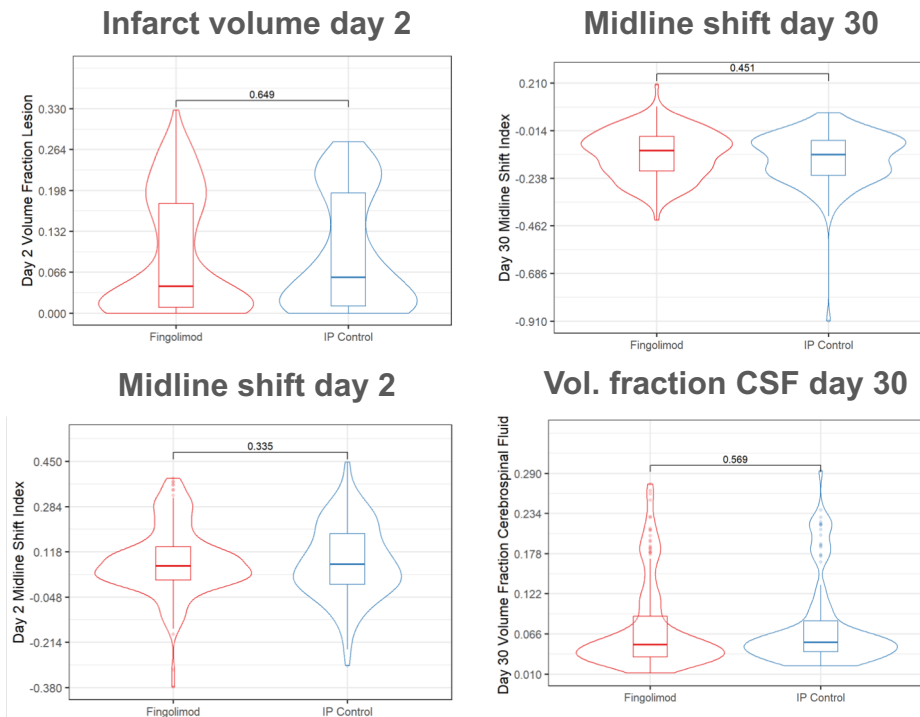


Mortality



Overall Fingolimod was not effective in comparison to control group

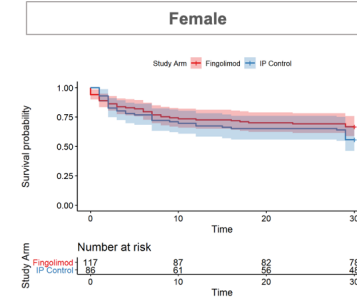
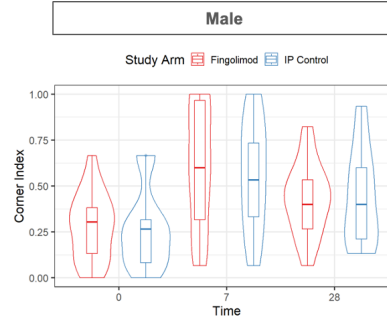
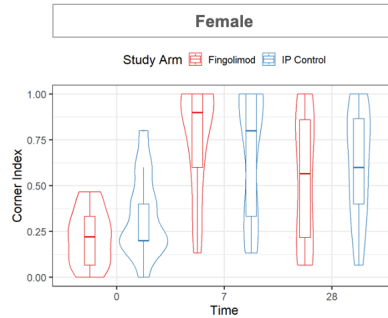
Fingolimod was not effective in comparison to control



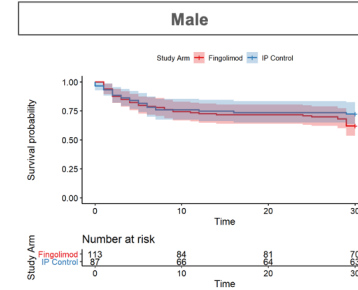
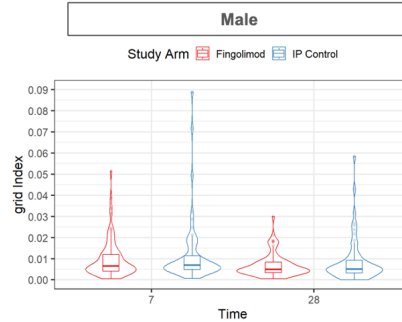
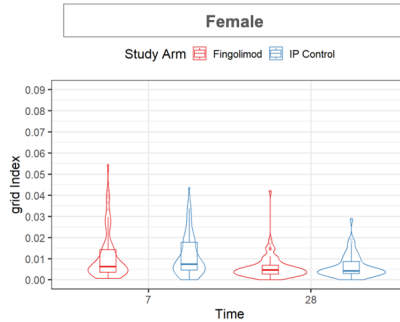
Overall Fingolimod was not effective to promote brain protection (morphometric evaluation – MRI)

Behavioral outcomes by sex

Alternative Corner index

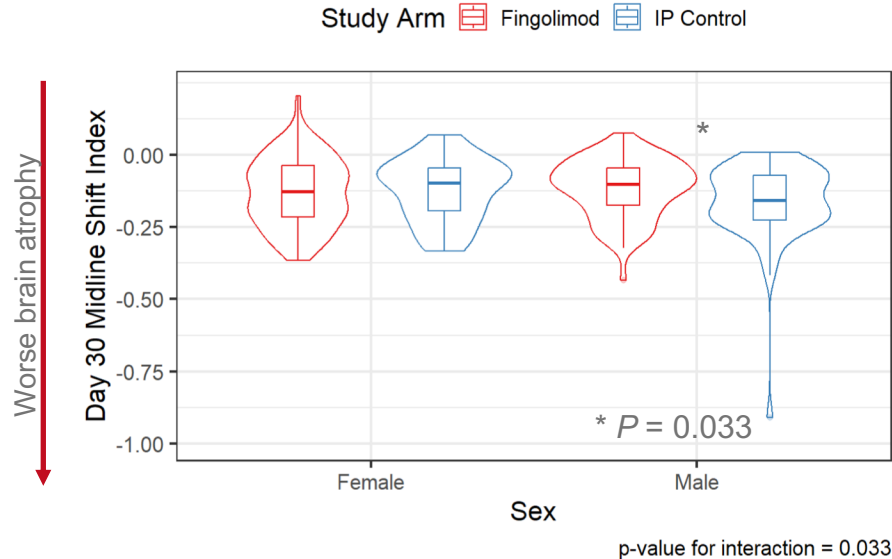


Grid walk index



MRI outcomes – treatment effect interacts with sex

Midline shift



- Treatment effect interacts with sex
- Control males presented a significant midline shift to the right (more negative) in comparison to Fingolimod group
- Fingolimod treatment was shown to be beneficial to males, but not females.

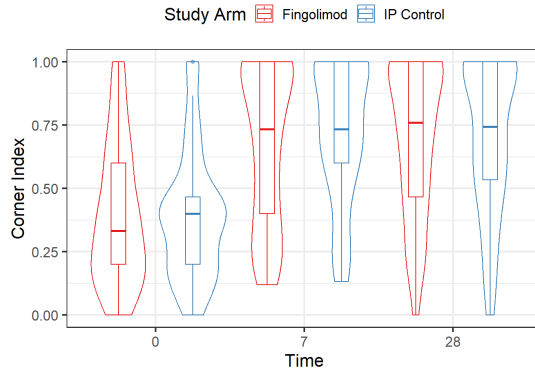
Fingolimod improved grid walk outcome in young healthy mice (YHM)

YHM model

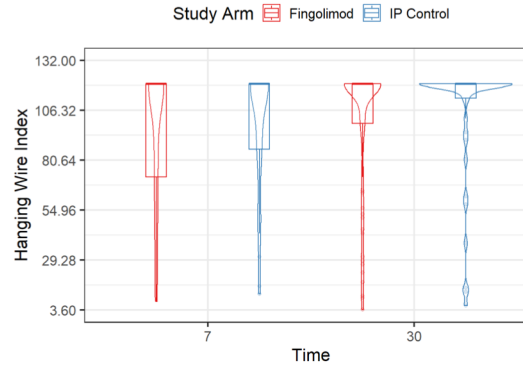


MCAO at 11 week-old (+/- 1 week) Total enrolled: 175 mice. 116 survived to day 30

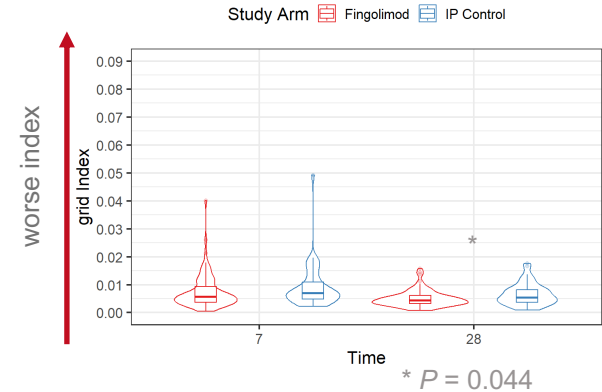
Corner test



Hanging wire test



Grid walk



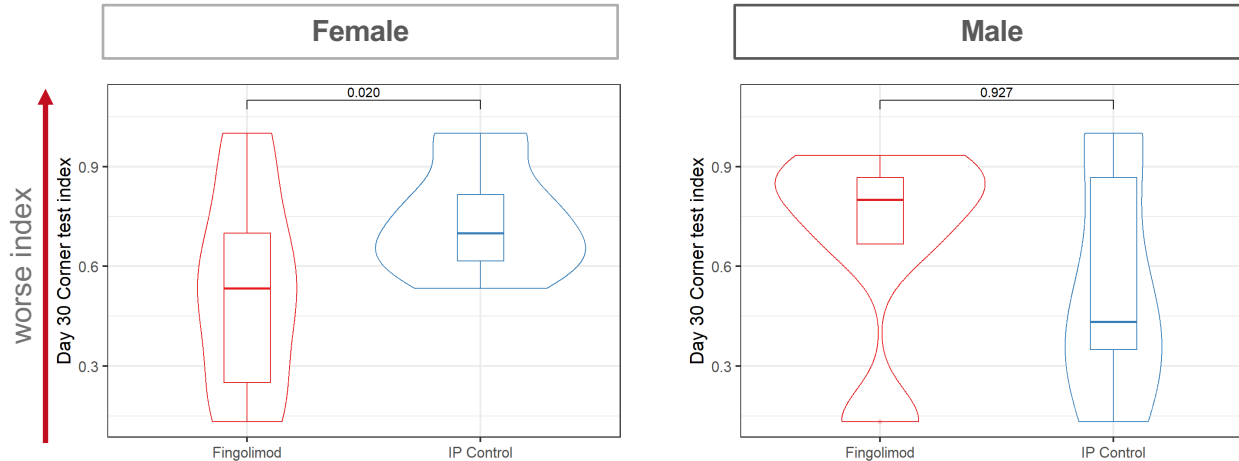
MRI outcomes: No differences were detected between control and fingolimod in the YHM group

Fingolimod improved primary outcome in aging females

Aging model

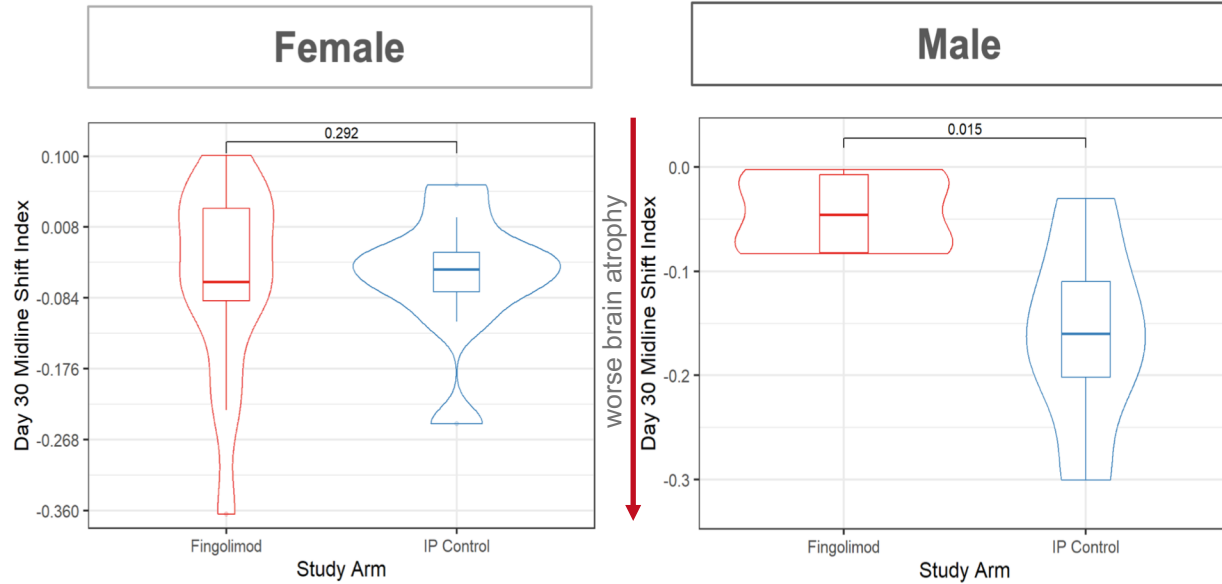


MCAO in 16 month-old (+/- 1 month). Total enrolled: 74. 35 mice survived to day 30



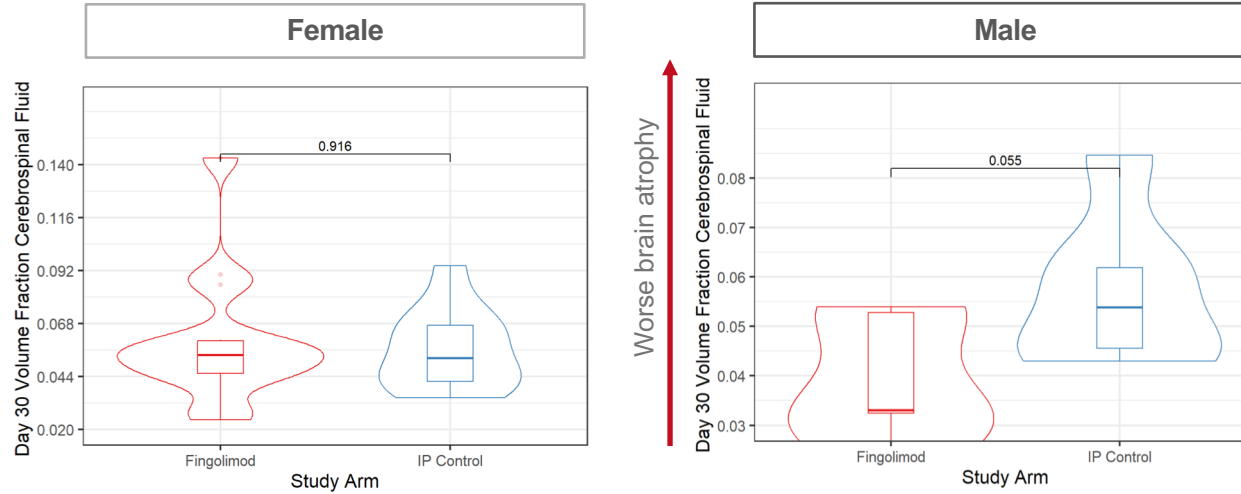
Fingolimod treatment was beneficial for aging females in the corner index

Fingolimod prevented brain atrophy in aging males



A greater ipsilateral midline shift was detected in the group control * $P = 0.015$

Fingolimod prevented brain atrophy in aging males



CSF volume was increased in the control group $P = 0.055$

Outcomes by model- Spontaneous hypertensive rats

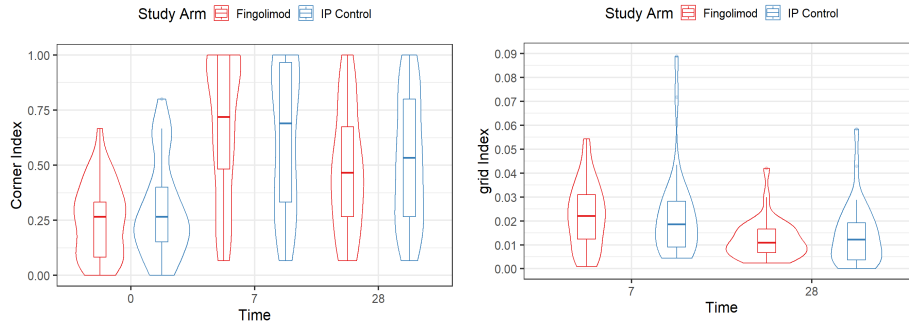
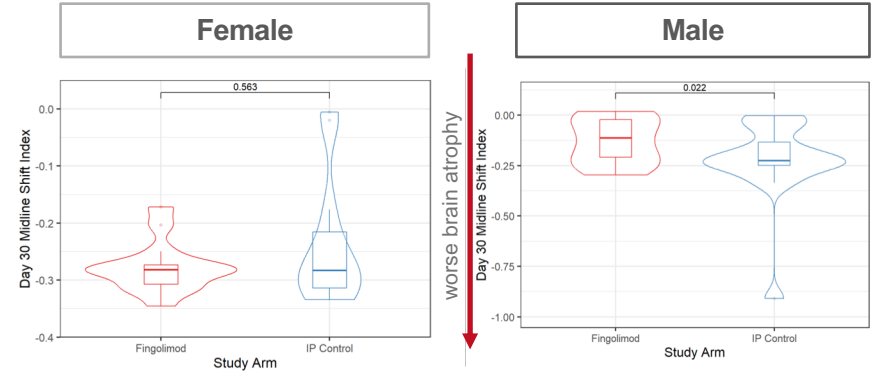
SHR model



MCAO at 15 week-old (+/- 1 week)

Stage 2 only

Animals included: 75 SHR rats. 55 survived at day 30



- Fingolimod prevented brain atrophy in SHR males

Fingolimod worsened outcome in obesity-induced hyperglycemic mice

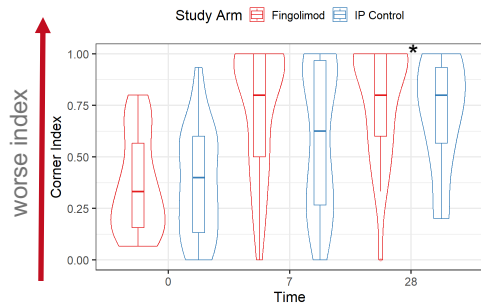
OIH model-



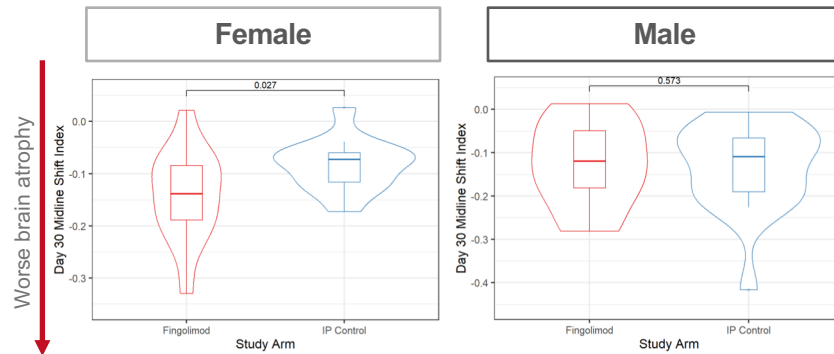
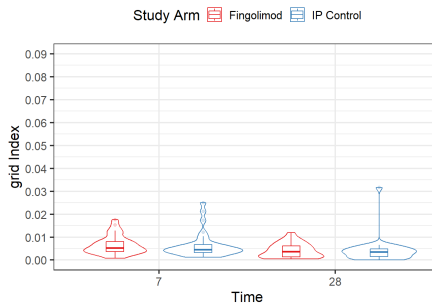
Enrollment at 4wk old – high fat diet started

MCAO at 16 week-old (+/- 1 week)

Animals included: 80 mice. 53 survived to day 30



* $P = 0.034$



- Fingolimod worsened outcome for corner index (at day 28)
- Fingolimod increased ipsilateral midline shift (brain atrophy) on OIH females

Results summary

Model	Functional outcomes			MRI outcomes				
	Corner index	Grid walk	Hanging wire	Lesion fraction d2	Midline shift day 2	Midline shift day 30	CSF volume day 2	CSF volume day 30
Overall	ns	ns	ns	ns	ns	✓ ♂	ns	ns
Young healthy mice	ns	✓ ♀♂	ns	ns	ns	ns	ns	ns
Aging	✓ ♀	ns	ns	ns	ns	✓ ♂	ns	P = 0.055
Spontaneous hypertensive rats	ns	ns	ns	ns	ns	✓ ♂	ns	ns
Obesity-induced hyperglycemia	✗ ♀♂	ns	ns	ns	ns	✗ ♀	ns	ns



favours Fingolimod



favours control

ns: not statistical significant P < 0.05

Conclusions

- Fingolimod failed to improve outcomes in the overall SPAN cohort
- Fingolimod showed benefits for specific models, but impaired stroke recovery in OIH
- Testing new drugs in different preclinical populations provides additional information on efficacy beyond young healthy male animals and may be an essential step to improving clinical translation

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