

Title	The impact of fingolimod on Treg function in brain ischaemia
Authors	Malone, Kyle;Shearer, Jennifer A.;Waeber, Christian;Moore, Anne C.
Publication date	2023
Original Citation	Malone, K., Shearer, J.A., Waeber, C. and Moore, A.C. (2023) 'The impact of fingolimod on Treg function in brain ischaemia', European Journal of Immunology, 53(9), 2350370 (13pp) . doi: 10.1002/eji.202350370
Type of publication	Article (peer-reviewed)
Link to publisher's version	10.1002/eji.202350370
Rights	© 2023 The Authors. European Journal of Immunology published by Wiley-VCH GmbH. This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License,which permits use, distribution and reproduction in any medium, provided the original work is properly citedand is not used for commercial purposes. - <a href="https://creativecommons.org/licenses/by-nc/4.0/">https://creativecommons.org/licenses/by-nc/4.0/</a>
Download date	2024-05-21 13:45:41
Item downloaded from	<a href="https://hdl.handle.net/10468/15605">https://hdl.handle.net/10468/15605</a>

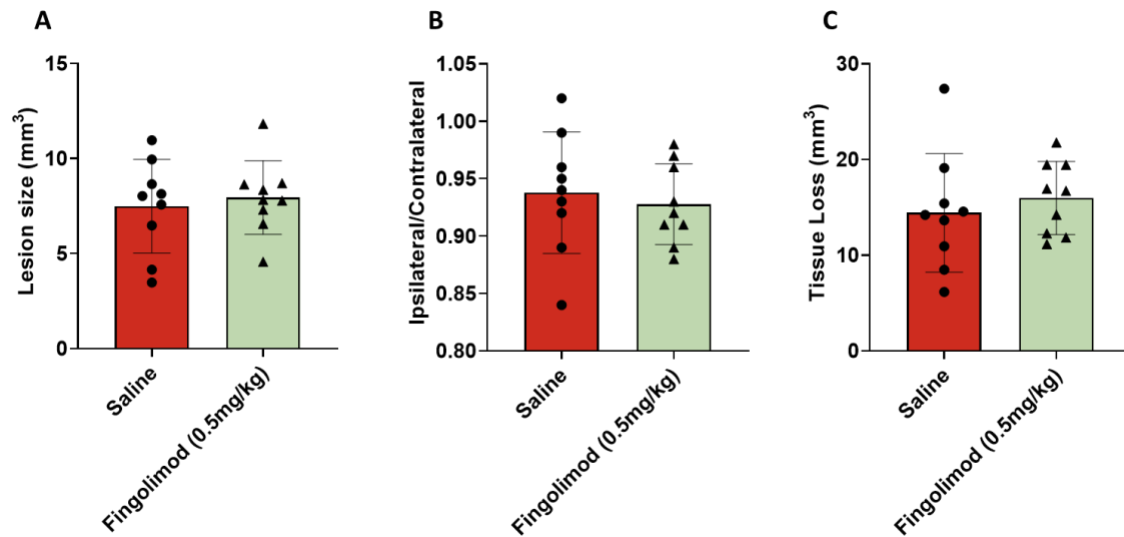


# UCC

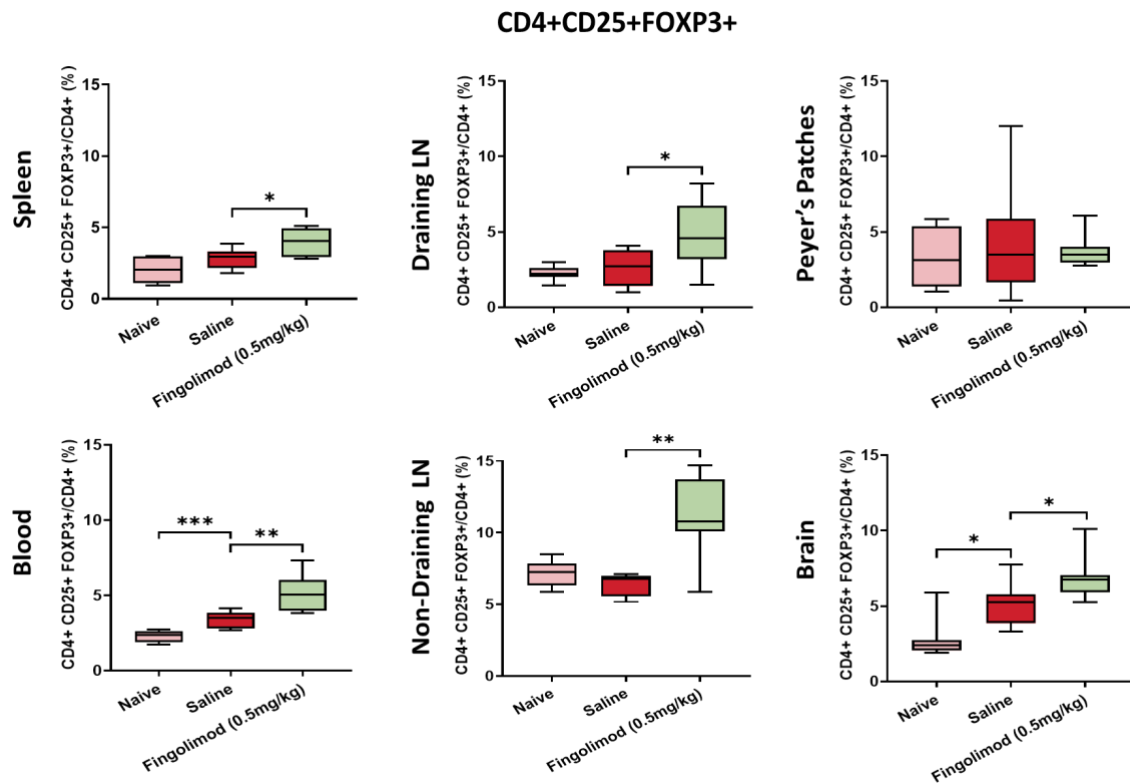
**University College Cork, Ireland**  
Coláiste na hOllscoile Corcaigh

**Supplemental Table 1:** Flow cytometry antibody reagents

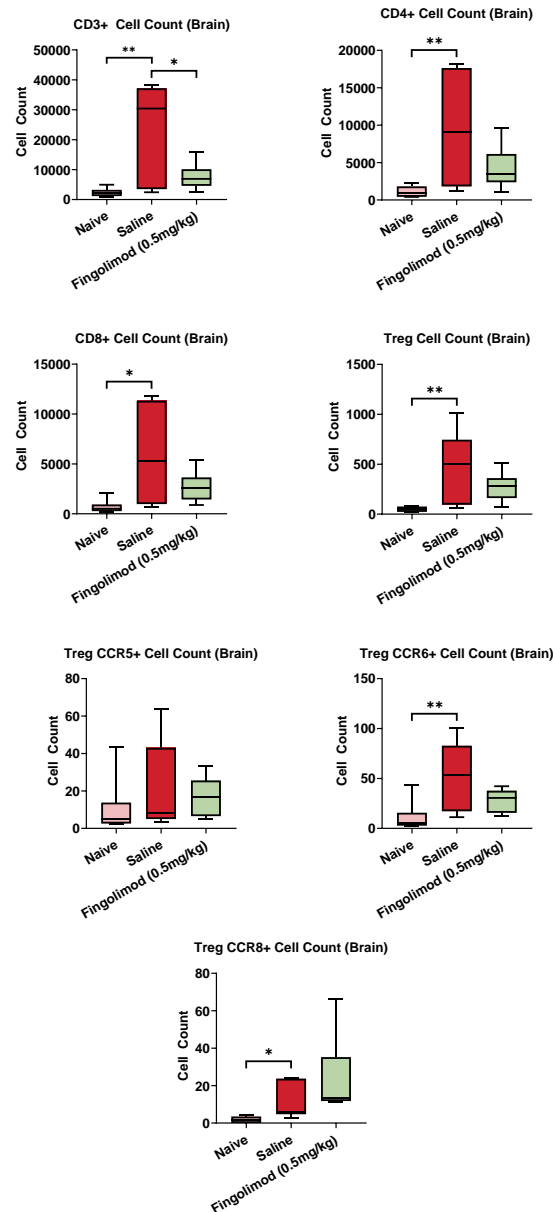
<b>Panel 1: Chemokine Expression (Target, fluorochrome, clone)</b>	<b>Cat #</b>	<b>Titration</b>	<b>Supplier</b>
Anti-Mouse CD45 PerCP-CY5.5 (30-F11)	#45045182	1:100	Thermo Fisher
Anti-Mouse CD3e PE-Cy7 (145-2C11)	#14003182	1:100	Thermo Fisher
Anti-Mouse CD4 FITC (RM4-5)	#11004282	1:800	Thermo Fisher
Anti-Mouse CD8a Pacific Blue (5H10)	#MCD0828	1:100	Thermo Fisher
Anti-Mouse CD25 APC (PC61.5)	#17025182	1:100	Thermo Fisher
CCR5: Anti-Mouse CD195 PerCP eFluor710 (HM-CCR5 (7A4))	#46195182	1:100	Thermo Fisher
CCR6: Anti-Mouse CD196 PE-Vio615 (REA277)	#130108396	1:20	Miltenyi Biotec
CCR8: Anti-Mouse CD198 PE-Vio615 (REA921)	#130119922	1:50	Miltenyi Biotec
Anti-mouse FoxP3 PE (FJK-16s)	#12577382	1:100	Thermo Fisher
Fixable Viability Dye eFluor 780	#65086514	1:10000	Thermo Fisher
<b>Panel 2: Cytokine Expression (Target, fluorochrome, clone)</b>	<b>Cat #</b>	<b>Titration</b>	<b>Supplier</b>
Anti-Mouse CD3e PE-Cy7 (145-2C11)	#14003182	1:100	Thermo Fisher
Anti-Mouse CD4 FITC (RM4-5)	#11004282	1:800	Thermo Fisher
Anti-Mouse CD8a Pacific Blue (5H10)	#MCD0828	1:100	Thermo Fisher
Anti-Mouse IFN- $\gamma$ eFluor610 (XMG1.2)	#61731182	1:100	Thermo Fisher
Anti-Mouse IL-17 PE (TC11-18H10)	#559502	1:100	BD
Anti-Mouse IL-10 PerCP-Cy5.5 (JES5-16E3)	#45710182	1:50	Thermo Fisher
Fixable Viability Dye eFluor 780	#65086514	1:10000	Thermo Fisher



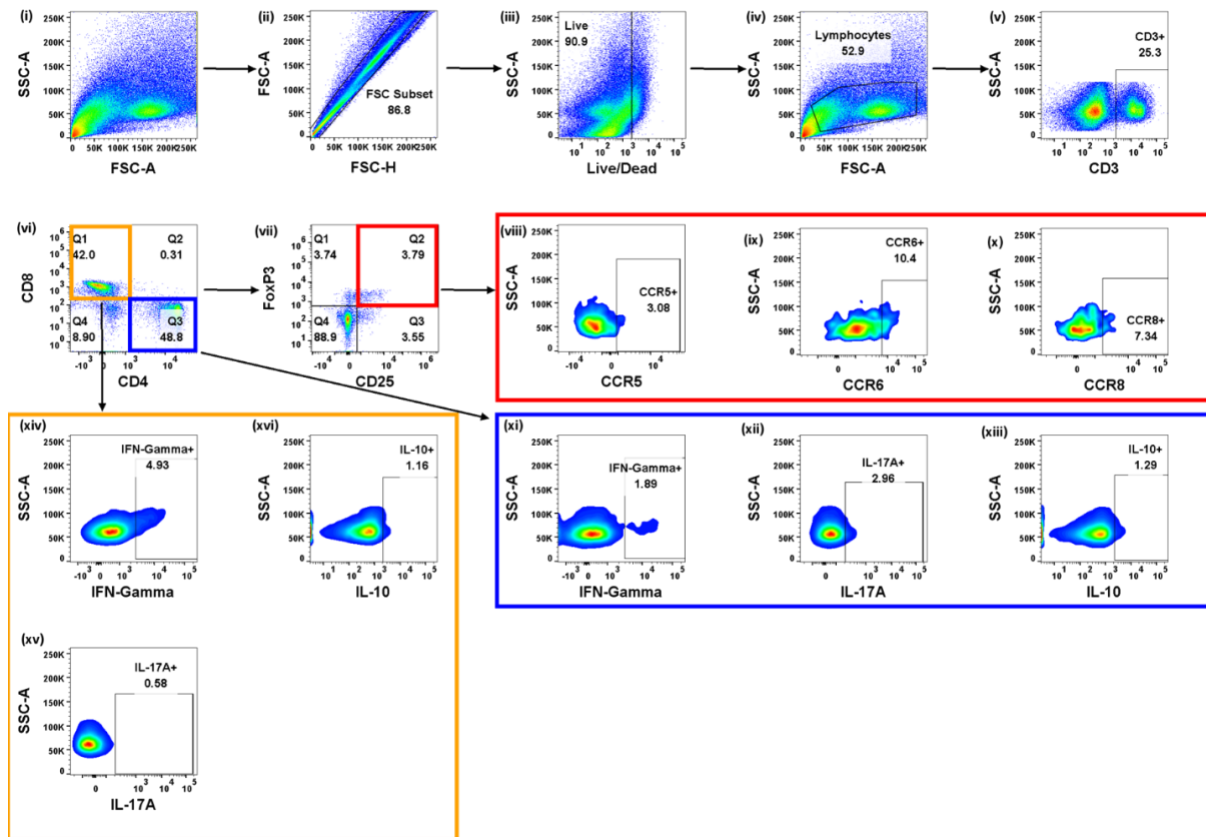
**Supplemental Figure 1:** Comparison of A) lesion size (mm<sup>3</sup>), B) ipsilateral/contralateral hemispheric volume ratio, and C) tissue loss (mm<sup>3</sup>) between saline (n = 9) and fingolimod-treated (n = 9) mice as quantified by H & E staining (t = 10 days). Two-sided, independent-samples t-tests was used to investigate differences between groups. Scatter plots depict mean  $\pm$  standard deviation.



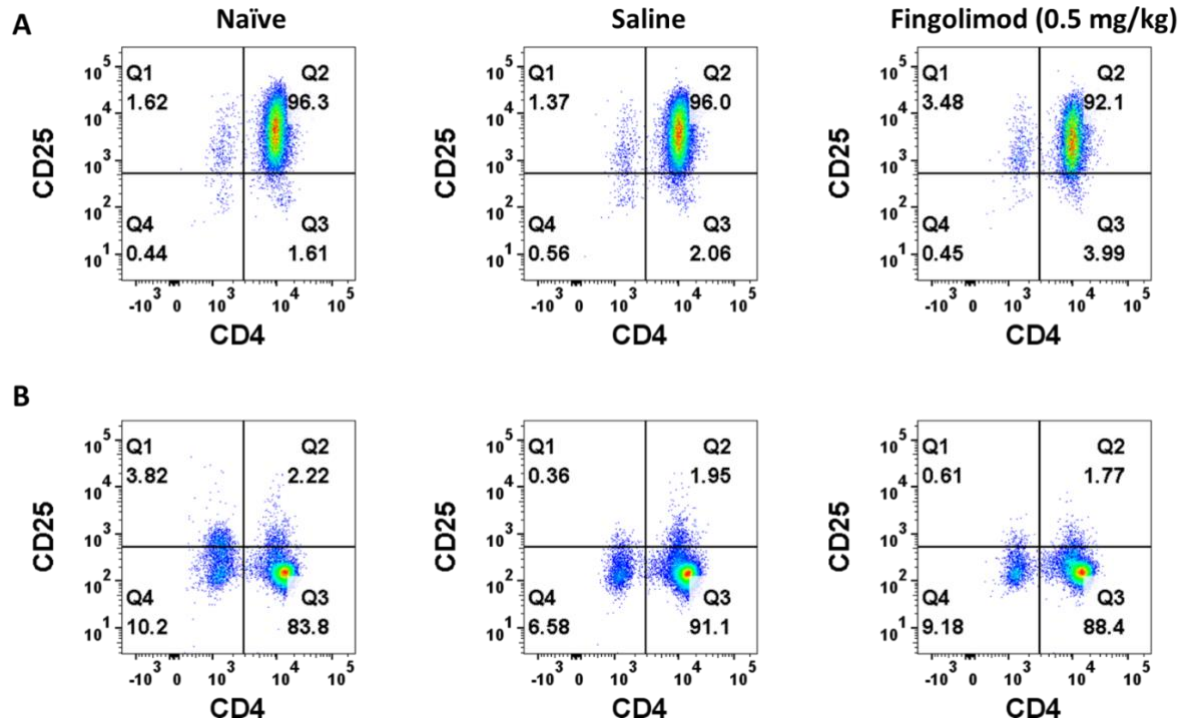
**Supplemental Figure 2:** Frequency of CD4<sup>+</sup> CD25<sup>+</sup> FoxP3<sup>+</sup> cells in blood, brain, and secondary lymphoid tissue in response to saline or fingolimod (0.5 mg/kg)) treatment post-brain ischaemia in young mice (t = 10 days). Two-sided, independent-samples T tests investigated differences between two groups (\* =  $p < 0.05$ , \*\* =  $p < 0.01$ , \*\*\* =  $p < 0.001$ ). Box and whisker plots display the 90/10 percentile at the whiskers, the 75/25 percentiles at the boxes, and the median in the centre line. Notably, once daily fingolimod 0.5 mg/kg treatment caused an increased Treg frequency in spleen, draining lymph nodes, non-draining lymph nodes, blood, and brain. N = 7 per group.



**Supplemental Figure 3:** Absolute cell counts of CD3+ T cells, CD4+ T cells, CD8+ T cells, Tregs, CCR5+ Tregs, CCR6+ Tregs, and CCR8+ Tregs in brain tissue in mice treated with saline or fingolimod (0.5 mg/kg) post-brain ischaemia in young mice (t = 10 days) or in untreated, naïve, mice (n = 7 mice per group). Two-sided, independent-samples t-tests investigated differences between two groups (\* =  $p < 0.05$ , \*\* =  $p < 0.01$ , \*\*\* =  $p < 0.001$  as compared to saline). Box and whisker plots display the 90/10 percentile at the whiskers, the 75/25 percentiles at the boxes, and the median in the centre line.



**Supplemental Figure 4:** Gating strategy for determination of chemokine expression among Tregs (red), cytokine expression among CD4+ cells (blue), and cytokine expression among CD8+ cells (yellow) in representative mouse splenocytes post-brain ischaemia (t = 10 days). (i) = Initial population, (ii) = singlets, (iii) = Live cells, (iv) = lymphocytes, (v) = CD3+ cells (T cells), (vi) = CD4+ vs. CD8+ cells, (vii) = CD4+ CD25+ FoxP3+ cells (quadrant in red) designated Tregs, (viii) = CCR5+ Tregs, (ix) = CCR6+ Tregs, (x) = CCR8+ Tregs, (xi) = CD4+ IFN $\gamma$ + cells, (xii) CD4+ IL-17A+ cells, (xiii) CD4+ IL-10+ cells, (xiv) CD8+ IFN $\gamma$ + cells, (xv) CD8+ IL-17A+ cells, (xvi) CD8+ IL-10+ cells. All gates were determined by both negative cells and fluorescence minus one controls.



**Supplemental Figure 5:** Treg and Tconv cells were isolated from spleens of naïve, saline-treated, and fingolimod-treated mice. Pseudocolour plots depict representative image from 1 replicate (n = 3 replicates total). A) Frequency of CD4 and CD25 +/- cells among enriched CD4<sup>+</sup> CD25<sup>+</sup> “Treg” cells. B) Frequency of CD4 and CD25 +/- cells among remaining cells in the CD25<sup>-</sup> fraction, termed “Tconv” cells.