Title: CCR5 antagonists as cerebroprotective and stroke recovery enhancing agents: a preclinical systematic review and meta-analysis

Abbreviated Title: CCR5, cerebroprotection, and stroke recovery

Authors: Ayni Sharif[1,2,*], Matthew S. Jeffers[1,2,*], Dean A. Fergusson[1,2,3], Raj Bapuji[1], Stuart G. Nicholls[4], John Humphrey[5], Warren Johnston[5], Ed Mitchell[5], Mary-Ann Speirs[5], Laura Stronghill[5], Michele Vuckovic[5], Susan Wulf[5], Risa Shorr[6], Dar Dowlatshahi[2,7,8], Dale Corbett[8], Manoj M. Lalu[1,2,8,9,#]

Affiliations: [1]Clinical Epidemiology Program, Blueprint Translational Research Group, Ottawa Hospital Research Institute, Ottawa, Canada
[2]School of Epidemiology and Public Health, University of Ottawa, Ottawa, Canada
[3]Department of Medicine, University of Ottawa, Ottawa, Canada
[4]Office for Patient Engagement in Research Activity (OPERA), Ottawa Hospital Research Institute, Ottawa, Canada
[5]Patient Partner, Blueprint Translational Research Group, Ottawa Hospital Research Institute, Ottawa, Canada
[6]Ottawa Hospital Library Services, The Ottawa Hospital, Ottawa, Canada
[7]Division of Neurology, Department of Medicine, The Ottawa Hospital, Ottawa, Canada
[8]Department of Cellular and Molecular Medicine, Faculty of Medicine, University of Ottawa, Ottawa, Canada
[9]Department of Anaesthesiology and Pain Medicine, The Ottawa Hospital, Ottawa, Canada
*These authors contributed equally

Corresponding Author:
[#]Manoj M. Lalu, mlalu@toh.ca
Department of Cellular and Molecular Medicine, University of Ottawa
Clinical Epidemiology and Regenerative Medicine Programs, Ottawa Hospital Research Institute
Ottawa, Ontario, Canada, K1Y 4E9

X: @ManojLalu

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ABSTRACT

Background: C-C chemokine receptor type 5 (CCR5) antagonists may improve both acute stroke outcome and long-term recovery. Despite their evaluation in ongoing clinical trials, gaps remain in the evidence supporting their use.

Methods: With a panel of patients with lived experiences of stroke, we performed a systematic review of animal models of stroke that administered a CCR5 antagonist and assessed infarct size or behavioural outcomes. MEDLINE, Web of Science, and Embase were searched. Article screening and data extraction were completed in duplicate. We pooled outcomes using random effects meta-analyses. We assessed risk of bias using the Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE) tool and alignment with the Stroke Treatment Academic Industry Roundtable (STAIR) and Stroke Recovery and Rehabilitation Roundtable (SRRR) recommendations.

Results: Five studies representing 10 experiments were included. CCR5 antagonists reduced infarct volume (standard mean difference −1.02; 95% confidence interval −1.58 to −0.46) when compared to stroke-only controls. Varied timing of CCR5 administration (pre- or post-stroke induction) produced similar benefit. CCR5 antagonists significantly improved 11 of 16 behavioural outcomes reported. High risk of bias was present in all studies and critical knowledge gaps in the preclinical evidence were identified using STAIR/SRRR.

Conclusions: CCR5 antagonists demonstrate promise; however, rigorously designed preclinical studies that better align with STAIR/SRRR recommendations and downstream clinical trials are warranted.

Registration: Prospective Register of Systematic Reviews (PROSPERO CRD42023393438)

Key Words: stroke; neuroprotection; impairment; recovery; preclinical; CCR5 antagonists; maraviroc; patient engagement in research; CAMAROS; cerebroprotection

Non-standard Abbreviations and Acronyms:

CAMAROS	Canadian Maraviroc RCT to Augment Rehabilitation Outcomes After Stroke
CCR5	C-C chemokine receptor type 5
DAPTA	D-Ala-Peptide T-Amide
SRRR	Stroke Recovery and Rehabilitation Roundtable
STAIR	Stroke Treatment Academic Industry Roundtable
SYRCLE	Systematic Review Centre for Laboratory Animal Experimentation
TAK-779	Takeda 779

Introduction

C-C chemokine receptor type 5 (CCR5) is expressed across a variety of leukocyte subtypes, endothelial cells, and cell types in the brain (e.g., neurons, microglia, astrocytes), and is thought to play crucial roles in post-stroke neuroinflammation, blood-brain barrier repair, and neuronal survival / repair processes.[1] CCR5 antagonists have emerged as potential therapeutic candidates for stroke, demonstrating both cerebroprotection and improved neural repair/recovery in preclinical animal models.[2–6] However, no CCR5 antagonist drug has an approved indication in the stroke context, necessitating studies to establish safety and efficacy this population. This has led to an ongoing clinical trial to investigate efficacy of CCR5 antagonists in combination with post-stroke rehabilitation.[7] Assessment of the preclinical evidence supporting CCR5's role in stroke is needed to identify areas of potential benefit, and knowledge gaps, that should be addressed by future preclinical research.[8]

The stroke cerebroprotection and recovery communities have advocated for alignment of preclinical and clinical study parameters through publication of consensus recommendations for preclinical research, in an effort to enhance the translation of new stroke therapies.[9–11] Examples include identification of more sensitive and clinically relevant preclinical outcome measures and incorporation of potentially important effect modifiers of treatment efficacy, such as age, sex, and stroke-related comorbidities (hypertension, diabetes, etc.).[9–11] These recommendations aim to improve the translation of novel stroke therapeutics from preclinical to clinical populations, but the degree to which preclinical evidence for CCR5 antagonists satisfy these recommendations is unknown.

We sought to comprehensively evaluate the preclinical evidence for CCR5 antagonist drugs as both cerebroprotective and stroke recovery-promoting agents.[9,11] Both perspectives are necessary to fully understand the therapeutic potential of stroke-related treatments, as distinct biological principles, time windows for treatment, and outcomes of interest underpin each of these treatment domains.[12,13] We conducted a systematic review and meta-analysis of the CCR5 literature in conjunction with a panel of individuals with lived experiences of stroke. This review sought to explore how the preclinical evidence for CCR5 antagonist drugs aligned with guidance for preclinical stroke research provided by previous expert committees and the parameters of an

ongoing clinical trial (The Canadian Maraviroc Randomized Controlled Trial To Augment Rehabilitation Outcomes After Stroke, CAMAROS, NCT04789616).[9–11]

Materials and Methods

We registered the review protocol on the International Prospective Register of Systematic Reviews (CRD42023393438).[14] The findings are reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (Table S1)[15] and Guidance for Reporting the Involvement of Patients and the Public (Table S2).[16] A panel of eight patients and caregivers with lived and living experience of stroke informed project development and were actively involved throughout research conduct (see Table S2 for more information).

Eligibility criteria

- *Animals:* Any preclinical *in vivo* animal models of adult stroke were included. Human, invertebrate, *in vitro*, *ex vivo*, and neonatal animal studies were excluded.
- *Model:* Focal ischaemic or intracerebral haemorrhagic stroke models were included, while animal models of global ischaemia were excluded.
- *Intervention:* Studies administering a CCR5 antagonist drug (e.g., maraviroc, D-Ala-Peptide T-Amide (DAPTA), Takeda 779 (TAK-779)). Study arms in which CCR5 was genetically manipulated (e.g., CCR5 knockout strain) were excluded.
- *Comparator:* Vehicle-treated control groups where stroke was induced. CCR5 antagonist control groups without stroke were excluded.
- *Outcome:* Studies reporting at least one of the following: infarct size, behavioural tests, mortality, adverse events, and spasticity were included.
- *Study design and publication characteristics:* Controlled interventional studies (randomized, pseudo-randomized, or non-randomized) published as full journal articles in any language or year were included. Abstracts, review articles, opinion-based letters/editorials, and unpublished grey literature were excluded.

Information sources and search strategy

An information specialist with experience in systematic searches of the preclinical literature developed a comprehensive search strategy based on a previously published strategy for

identifying animal experimentation studies.[17] The search strategy underwent peer-review using the Peer Review of Electronic Search Strategies (PRESS) checklist.[18] We searched MEDLINE (OVID interface, including In-Process and Epub Ahead of Print), Web of Science, and Embase (OVID interface). The search was conducted October 25, 2022, to align with the listed launch date of the CAMAROS trial (September 15, 2022). Our intention in doing so was to collate and assess all preclinical evidence that could have feasibly informed the clinical trial. We sought to assess the comprehensiveness of evidence and readiness for translation of CCR5 antagonist drugs at the time of their actual translation into human clinical trials, as well as the alignment of the CAMAROS trial design to the existing preclinical evidence base. See the Supplemental Methods for the full search strategy and exact search dates for each database.

Selection process and data collection

We deduplicated citations and uploaded them into DistillerSR® (Evidence Partners, Ottawa, Canada). Two reviewers independently screened citations by title and abstract using an accelerated method (one reviewer required to include, two reviewers required to exclude). We then screened and extracted data from full-text articles in duplicate. Graphical data was extracted using Engauge Digitizer.[19] A third reviewer with content expertise in preclinical stroke studies audited all data extraction. Conflicts between reviewers were resolved by consensus discussion. See Supplemental Methods for the complete list of data extraction elements.

Effect measures and data synthesis

We performed quantitative analyses using the R (version 4.1.2) “metafor” package (version 4.0.0)[20] with inverse variance random effects modelling. We expressed continuous outcome measures as standardized mean differences (SMDs) with 95% confidence intervals (CIs) and assessed statistical heterogeneity of effect sizes using the Cochrane I^2 statistic.[21] This was necessary due to the variety of outcome measures and measurement scales used across studies, which is a common feature of preclinical systematic reviews. Sensitivity analyses were performed using original measurement scales where possible. From patient partner input, subgroups were analyzed based on timing / dose / route of intervention, stroke model, stroke type, species, type of behavioural outcome (i.e., motor, cognitive), and comorbidities.[22] Our planned subgroup analyses on study quality, specific regions/areas of the brain, and post-stroke

rehabilitation paradigms were not performed due to insufficient number of studies. We did not assess publication bias using Egger plots due to fewer than 10 studies being included in the analysis, as per Cochrane recommendations.[23]

Risk of bias assessment

Two independent reviewers used the Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE) risk of bias tool to assess each study as having a "Low Risk", "Unclear Risk", "Some Concerns of Risk", or "High Risk" across domains such as randomization, blinding and outcome reporting.[24] "Some Concerns of Risk" indicated reporting of a domain (i.e., randomization), but lacking methodological details. This differed from "Unclear Risk" where there was no mention of the domain in the study.

Comprehensiveness of preclinical evidence and alignment with clinical trials

We assessed comprehensiveness of the *overall body* of preclinical evidence for CCR5 antagonists as a cerebroprotective treatment using The Stroke Treatment Academic Industry Roundtable (STAIR) I, VI, and XI Consolidated Recommendations.[11,25,26] These recommendations encompass "candidate treatment qualification" (e.g., dose, timing of dose, outcomes) and "preclinical assessment and validation" (e.g., age, sex, sample size, animal type).[11] We excluded domains that were redundant with risk of bias (e.g., randomization, blinding, etc.) and included additional items relevant to stroke recovery studies from the Stroke Recovery and Rehabilitation Roundtable (SRRR) Translational Working Group consensus recommendations.[9] Two reviewers extracted data to determine if the overall evidence across studies satisfied each of the STAIR and SRRR recommendations. A third reviewer audited this analysis. We then assessed alignment of existing preclinical evidence with an ongoing clinical trial of CCR5 antagonists for stroke (CAMAROS, NCT04789616).[7]

Protocol deviations

We incorporated an additional assessment using the PRIMED[2] tool for assessing the readiness of stroke cerebroprotection therapies to be translated to clinical trials based on the body of preclinical evidence.[27] Additionally, a list of outcomes used to determine CCR5's potential

mechanisms of action were extracted (Figure S1, Table S3) based on feedback from individuals that reviewed the initial manuscript drafts.

Results

Study selection

Our search identified 263 citations, which was reduced to 166 unique studies after deduplication. Five studies representing 10 experiments met the eligibility criteria (Figure 1).[2–6] Herein, "studies" refer to the published articles as a unit, while "experiments" refer to distinct investigations within each published article used to test various hypotheses (i.e., a subunit of "studies" comprised of a select cohort of animals).

Study and animal model characteristics

Most studies used ischaemic stroke (n=4/5). This was induced via intraluminal suture (n=2), cauterization (n=1), or photothrombosis techniques (n=1; Table 1). Haemorrhagic stroke was induced in one study via autologous whole blood injection. All studies used mouse (n=4) or rat (n=1) models, comprised of exclusively male animals (n=5). Relevant stroke comorbidities highlighted by patient partners and STAIR/SRRR recommendations (e.g., hypertension, diabetes) were not used in any study.

Intervention characteristics

Maraviroc was used in six of the experiments (n=6/10), TAK-779 in three of the experiments (n=3), and D-Ala-Peptide T-Amide (DAPTA) in one (n=1; Table 2). These CCR5 antagonists were delivered intraperitoneally (n=4), intranasally (n=2), intracerebroventricularly (n=2), subcutaneously (n=1), and intravenously (n=1) at a dose range from 0.01 to 100 mg/kg. Most studies delivered a single dose of the drug (n=6); experiments with multiple administrations (n=4) ranged from 3–63 doses. Time of initial treatment administration varied widely. Two studies (3 experiments) administered treatment pre-stroke (10-15 minutes),[2,3] and 4 studies (6 experiments) in the acute, potentially cerebroprotective, post-stroke period (50 minutes – 24 hours post-stroke).[3–6] One study (1 experiment) was conducted in the late sub-acute/early chronic period beginning at three to four weeks post stroke, which would be oriented towards recovery, rather than cerebroprotective, effects.[6] Patient partners had identified several *a priori* interests

(physical therapy alongside CCR5 administration, spasticity), which were also aligned with SRRR recommended considerations for preclinical stroke recovery studies (see Supplemental Methods).[9] These were not reported in any included studies.

Meta-analysis of infarct volume

Infarct volume was reported in six experiments (n=6/10) from four different studies with an overall pooled analysis demonstrating marked cerebroprotection with CCR5 antagonists (SMD -1.02, 95% CI -1.58 to -0.46, $p < 0.0001$, I^2=34%; Figure 2). Five of these experiments measured infarct volume at 1-3 days post-stroke, and one experiment measured infarct volume at a delayed time point of 63 days post-stroke. No significant differences between pre- or post-stroke administration were observed (P=0.47). Post-hoc sensitivity analysis removing one experiment with extreme values[2] demonstrated that cerebroprotection in the remaining two experiments remained statistically significant while reducing heterogeneity (SMD -0.81, 95% CI -1.25 to -0.37, $p < 0.001$, I^2=0%). A second sensitivity analysis excluded one study that measured infarct volume in mm^3 so that all other studies could be meta-analyzed using mean differences on the percentage scale (Figure S2; MD -9.1%, 95% CI -11.6 to -6.7%, $p < 0.001$, I^2=0%). This demonstrated a similar cerebroprotective effect as the other analyses. Further sub-group analyses by route of administration, time of administration, stroke model, species, CCR5 antagonist, dose, and whether behaviour tests were assessed are described in Figure S3-S5.

Synthesis of behavioural outcomes without meta-analysis

Motor behavioural outcomes were reported in six experiments from three studies and represented seven different behavioural tasks. An additional study reported motor behavioural outcomes without standard deviations or standard errors, and thus could not be included (authors did not respond to email requests for data).[4] A cognitive outcome (Morris Water Maze) was measured in one study. Overall, CCR5 inhibition was effective in 11 of 16 behavioural outcomes tested (Figure 3). Meta-analysis and planned subgroup analysis were contra-indicated due to an inadequate number of studies for each given outcome measure, which necessitated the synthesis without meta-analysis presented below.[28]

Behavioural outcomes are presented by time of CCR5 antagonist administration, as discussed in the Intervention Characteristics section above, as administration time directly relates to the treatment context and patient population to which the results apply (i.e., acute cerebroprotection vs. late sub-acute/early chronic neural repair). Li et al. reported that pre-stroke administration (relevant to surgical contexts with high risk of thrombosis) of DAPTA did not result in significantly greater performance on the neurological deficit score.[2]

Regarding acute post-stroke administration times with the potential for *cerebroprotective* effects (up to 24 hours post-stroke based on observed infarct reductions in Figure 2), Yan et al. observed that CCR5 antagonist (maraviroc) administration one-hour following stroke resulted in greater motor performance on the corner test, limb placement, modified Garcia, foot fault, and rotarod tasks compared to vehicle-treated controls. Cognitive outcomes in the Morris Water Maze task (proportion of time spent in the probe quadrant) were also improved by this one-hour post-stroke administration. Significantly improved motor performance was not observed if CCR5 antagonist was administered 24 hours post-stroke, using this model of *intracerebral haemorrhage* (Figure 3).[5] In contrast, using a *focal ischaemia* model, Joy et al. observed that CCR5 antagonist (maraviroc) administration initiated 24 hours post-stroke and continued daily for 3 weeks (which could potentially represent a mix of cerebroprotective and recovery-induced effects) resulted in greater performance on the cylinder and grid walk tasks compared to vehicle-treated controls.

Joy et al., also performed one experiment with *late sub-acute/early chronic* administration (a potentially critical time for neural repair) initiated at three to four weeks post-stroke and continued daily for 11 weeks, that demonstrated significantly improved performance for the grid walk, but not cylinder, task.[6] This experiment was initiated outside the plausible range for a cerebroprotective effect, implying behavioural improvement involved *recovery-promoting mechanisms*. However, equivalent infarct volumes were not demonstrated between the treated and control groups in this cohort, which could potentially lead to confounding effects.

Risk of bias

All articles had a ‘high’ risk of bias in at least one domain of the SYRCLE tool.[24] Most domains within each study demonstrated an ‘unclear’ risk of bias. All studies reported randomizing

animals; however, as commonly observed in the preclinical literature,[29–31] only one of these studies provided sufficient detail to ensure that the randomization method had a low risk of bias. The SYRCLE domains with the highest risk of bias were incomplete outcome data, with 80% of studies (n=4/5) failing to provide complete data for all animals initially included in the study, as well as selective outcome reporting, with 60% (n=3/5; all studies of maraviroc) not providing complete data for all expected outcomes discussed in the methods (Figure S6).

Comprehensiveness of preclinical evidence and alignment with clinical trials

We assessed comprehensiveness of the preclinical evidence using the STAIR and SRRR recommendations[9,11,25,26] as well as alignment with study parameters of the CAMAROS trial.[7] A summary of assessment items from STAIR XI is provided in Table 3, with additional items from STAIR I, VI, and SRRR recommendations included in Table S4.

For CCR5 antagonists as a post-stroke cerebroprotectant, the overall body of evidence satisfied all five STAIR XI domains assessing ‘candidate treatment qualification’ (Table 3). Overall, a range of doses and clinically relevant administration times for cerebroprotection were evaluated across a variety of motor and cognitive behavioural domains. All studies tested both behavioural and histological outcomes and demonstrated cerebroprotective effects, but most studies failed to measure and control post-stroke temperature, which could potentially confound the observed cerebroprotection (Table S4).[32] Most histological measurements were also assessed at <72 hours, which could confound the observed cerebroprotective effects if cell death was merely delayed.[32] For CCR5 antagonists as a post-stroke recovery-inducing treatment, one experiment assessed the effects of initiating CCR5 administration in a similar post-stroke phase as the CAMAROS trial. This experiment (Joy et al.)[6] did not demonstrate that each treatment group had equivalent baseline stroke volumes, which may potentially confound observed behavioural effects. Furthermore, the maximum dose used in mice (100 mg/kg) was not sufficient to attain cerebrospinal fluid levels of maraviroc observed in humans using the CAMAROS dosing regime (mice: 13.8±5.4 ng/mL; humans 33.6–60 ng/mL).[6]

Areas of concern were identified in all STAIR XI domains assessing ‘preclinical assessment and validation’ (Table 3). Although adult animals were used in most of the preclinical studies, the

reported weights and ages of these animals corresponded to young adults, rather than the aged adults that better represent the stroke population. All experiments used only male rodents that were free of common stroke comorbidities. It was also unclear if behavioural testing was performed during the inactive circadian phase or active (dark) phase, which could result in confounding if CCR5 antagonists affect arousal of animals during their inactive period.[33] Furthermore, none of the studies had their protocols or results directly replicated by an independent laboratory, or across multiple sites in a multilaboratory study.[9,34] Regarding stroke recovery, no studies assessed behavioural effects on upper extremity skilled reaching / grasping or potential interactions of CCR5 antagonists with rehabilitative therapies or established recanalization procedures (Table S4).[35–38] These elements are highly relevant to the CAMAROS trial, as one of the primary outcomes of this trial is upper extremity performance on the Fugl-Meyer and maraviroc administration will be paired with an 8-week exercise program. These findings were supported by the PRIMED[2] tool, which resulted in a Readiness for Translation Score of “medium” (on a scale of “low”, “medium”, “high”). This tool highlighted similar promising elements, as well as weaknesses, as our analysis above (Table 3, Table S4), identifying the limited preclinical evidence for the effects of CCR5 antagonists in clinically relevant sexes, ages, species, and disease comorbidities, without sufficient dose-response information to inform trials (Table S5). Overall, our assessments highlight a variety of knowledge gaps, potential confounding factors, and areas of misalignment between the preclinical evidence and clinical trial parameters that could be improved with further preclinical experimentation.

Discussion

The overall body of preclinical evidence for CCR5 antagonists in stroke demonstrates potential acute cerebroprotection with corresponding impairment reduction, as well as improved functional recovery in the sub-acute/early chronic phase. Our systematic review also highlights evidence gaps that could impact successful clinical translation of CCR5 antagonists. Our analysis of 10 independent experiments, identified that acute administration of CCR5 antagonists within the first 24 hours post-stroke was associated with a marked reduction in infarct volume. This cerebroprotective reduction of infarct volume did not significantly vary based on treatment dose

or any other experimental characteristics.[3,4,6] Overall, the majority of behavioural effects appeared to be in a positive direction, but the low number of included studies precluded meta-analysis of these results. Indeed, no individual behavioural experiment included more than ten CCR5-treated animals, and given the wide range of dosages, timings, routes, stroke models, and rodent strains involved, the certainty of these findings is limited and should be interpreted cautiously. Pooling data across heterogenous experimental designs, animal/stroke models, and treatment parameters, as we have done with the infarct volume analysis in the present study, can introduce variability that increases the risk of overestimating or underestimating the true effect of the intervention.[39] Treatment effects observed across model systems and therapeutic compounds may represent different biological mechanisms. Despite this potential limitation, meta-analysis can provide valuable insights, especially in preclinical settings where the sample sizes of individual studies may be too small to detect significant effects on their own. In these cases, pooling data across studies can help identify overarching estimates of benefits and harm, highlight subgroups of interest, and help guide areas of future research. As described in the results above, we attempted to mitigate the risks of inappropriate data pooling through careful investigation of heterogeneity, subgroup analyses, and differentiation between outcomes where we felt that meta-analytic pooling was (infarct volume) and was not (behavioural outcomes) appropriate. Overall, we believe that our results indicate that further investigation is warranted to determine the optimal timing of administration and behavioural domains under which CCR5 antagonists exhibit the strongest post-stroke cerebroprotective and recovery-inducing effects.

Despite the positive direction of treatment effects across all studies of CCR5 antagonists, we found a substantial risk of bias in the underlying studies.[24] As is commonly observed in the preclinical literature, all studies either did not adequately report their randomization / blinding methods and exhibited evidence of selective / incomplete reporting.[29–31] Such features are associated with biased overestimations of preclinical treatment efficacy, which raises further concerns about the reliability and validity of the present findings.[40–42] Future studies should carefully incorporate all elements of the ARRIVE 2.0 guidelines to help ensure that results are transparently reported and improve confidence in the findings.[43]

Comprehensiveness of the preclinical evidence for CCR5 antagonists was assessed in relation to STAIR and SRRR consensus recommendations.[9,11,25,26] These recommendations aim to provide investigators and regulators with "assurance that the candidate treatment shows signals of efficacy and safety, before embarking on an expensive clinical development program".[11] The included studies provide good initial evidence for acute cerebroprotection, as well as mechanistic and behavioural evidence for enhanced recovery in the late sub-acute/early chronic post-stroke phase. However, demonstration of efficacy under a wider range of conditions, such as in aged animals, females, animals with stroke-related comorbidities, more clinically relevant timing of dose administrations, or in conjunction with rehabilitative therapies are necessary to provide further confidence in these findings. In addition, all studies used unique doses, timings, and outcomes, so independent replication of the most promising study parameters would further increase certainty in the evidence.[9,11] Future preclinical research should aim to address these evidence gaps to further increase the clinical relevance and comprehensiveness of evidence for CCR5 antagonists in stroke.

In relation to the ongoing CAMAROS trial assessing maraviroc in the subacute post-stroke phase,[7] the most relevant preclinical evidence comes from one experiment within the Joy et al. study[6] where maraviroc was initially administered at three to four weeks post-stroke and continued daily for 11 weeks. This experiment demonstrated that administration of maraviroc in the late sub-acute/early chronic post-stroke phase improved functional recovery on the grid walk, but not cylinder, task. These experimental conditions could potentially align with the putative therapeutic window and outcomes of interest being assessed in CAMAROS (e.g. 10-minute walk test co-primary outcome).[7] However, caution is warranted as this pivotal supporting preclinical evidence is based on a low sample size (n=9). Moreover, potential differences in dosing, severity of infarct, concomitant rehabilitative therapy, and other factors discussed above could influence the degree to which these results successfully translate to the clinical environment. Finally, clinically relevant secondary outcomes in the CAMAROS trial, such as cognitive and emotional domains as measured by the Montreal Cognitive Assessment (MoCA) and Stroke Aphasia Depression Questionnaire (SADQ) were not modelled in the preclinical literature. Although one study included a cognitive outcome, the other treatment parameters of this study were not aligned to the CAMAROS trial.[5] Future preclinical studies should assess a more diverse and

comprehensive battery of clinically relevant behavioural tasks, which could be based on the range of outcomes employed in the CAMAROS trial, or those found in the SRRR recommendations.[9] Nevertheless, the Joy et al., study provides a plausible biological mechanism and "proof of concept" for how CCR5 antagonism might enhance neuroplasticity that improves functional recovery after stroke.

Our present synthesis of the preclinical evidence for CCR5 antagonists used novel approaches to increase its utility for assessing certainty of the findings and identification of knowledge gaps. First, we engaged patients with lived experiences of stroke throughout the review process to ensure that our research questions, outcomes, and interpretations aligned with the priorities of the ultimate end-user of stroke research. Second, we incorporated consensus recommendations for both preclinical cerebroprotection and recovery research as an evidence evaluation tool, which we found often aligned with the priorities of our patient panel.[9,11,25,26] This guided assessment of the alignment of preclinical evidence with parameters of ongoing clinical trials, as well as appraisal of comprehensiveness of the preclinical evidence with a focus on translational validity[44] rather than only internal validity and risk of bias.[24] We also used this method to provide concrete avenues for future preclinical studies to close knowledge gaps and improve certainty in the effects of CCR5 antagonists under clinically relevant experimental conditions. Similar approaches should be considered by future preclinical systematic reviews to improve interpretation of the preclinical evidence from a translational perspective.

In conclusion, CCR5 antagonists show promise in preclinical studies for stroke cerebroprotection, corresponding reduction in impairment, as well as improved functional recovery related to neural repair in the late sub-acute/early chronic phase. However, high risk of bias and the limited (or no) evidence in clinically relevant domains underscore the need for more rigorous and transparent preclinical research to further strengthen the current preliminary evidence available in the literature. Addressing these concerns will not only enhance the reliability of preclinical evidence but also better inform the design and execution of clinical trials of CCR5 antagonists, such as the ongoing CAMAROS trial.[7] The integration of expert recommendations, such as STAIR and SRRR, should guide future preclinical investigations and synthesis of the body of preclinical evidence in stroke recovery research.[9,11] Our present

approach serves as a template by which the preclinical evidence supporting translation to clinical trials can be weighed when justifying early clinical trials of novel interventions for stroke cerebroprotection and recovery.

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Supplemental Material:

Supplemental Methods
Figures S1-S6
Tables S1-S5

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Figure

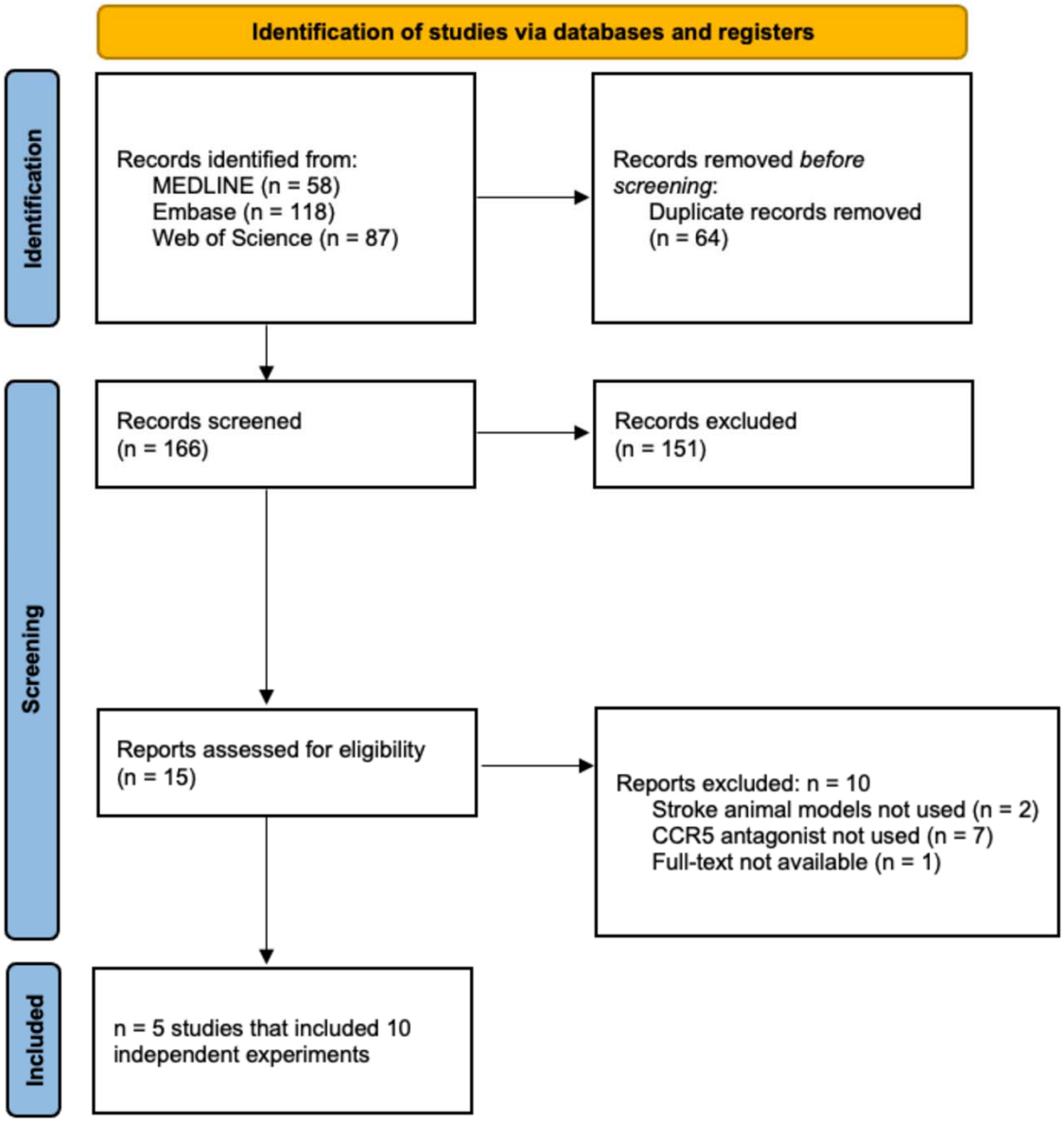

Figure 1. Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) flow diagram.

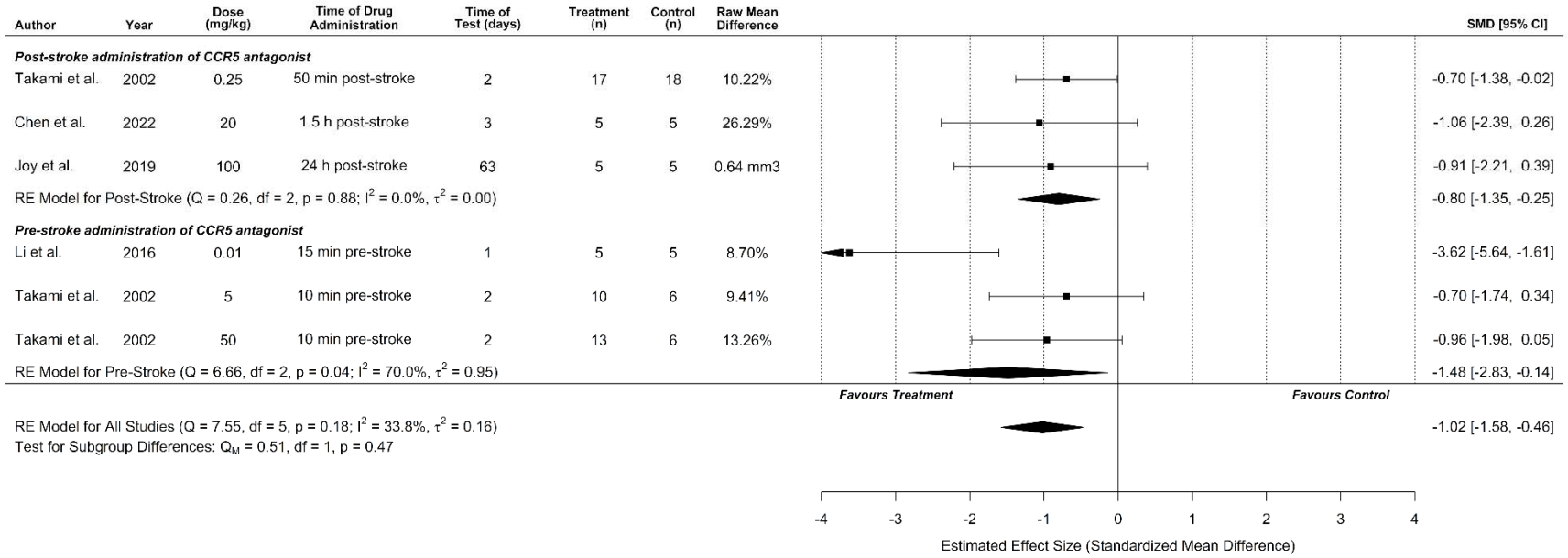

Figure 2. CCR5 antagonists reduce infarct volume. Data is presented as a forest plot with standardized mean differences and 95% confidence intervals. Effect sizes <0 favours drug treatment and >0 favours control. Data is stratified by timing of CCR5 antagonist administration (pre- or post-stroke induction). The 'RE Model for All Studies' represents a pooled estimate of the CCR5 antagonist drug effect on infarct volume from all studies combined. Separate pooled estimates are also reported for post-stroke and pre-stroke CCR5.

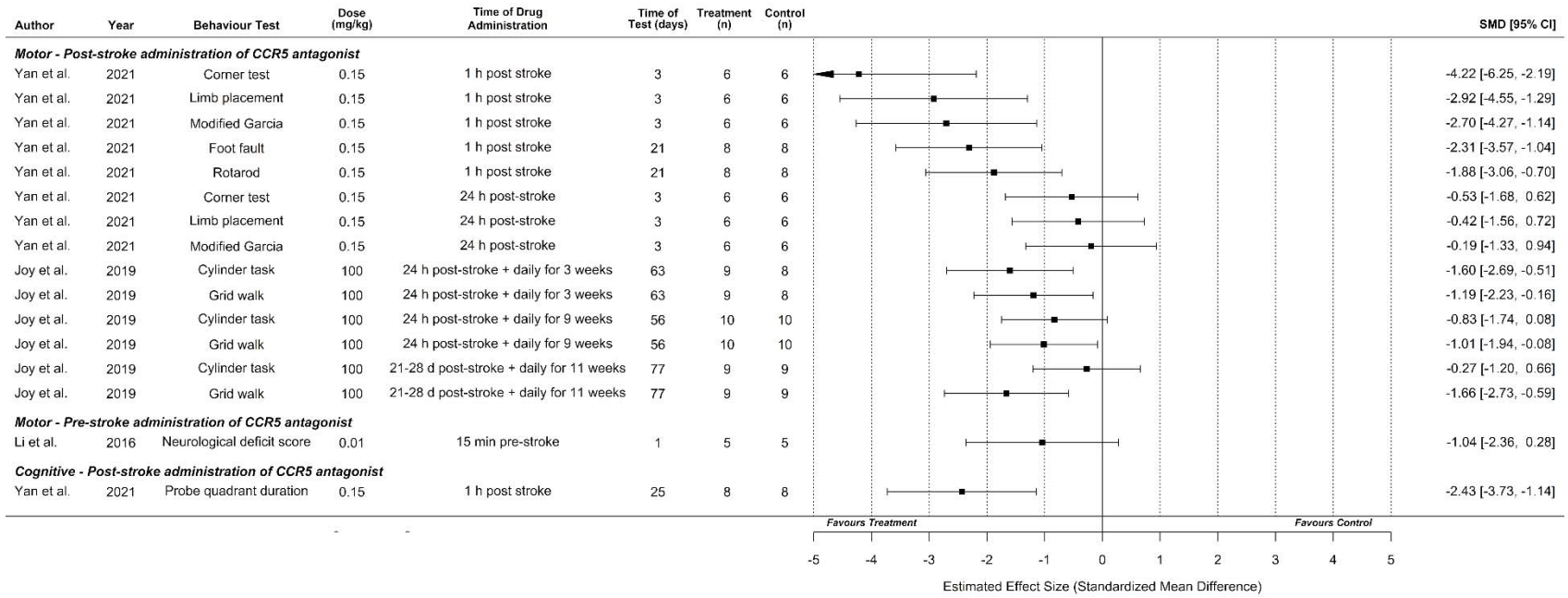

Figure 3. Synthesis without meta-analysis for all included preclinical CCR5 antagonist studies that reported motor and/or cognitive behavioural outcomes. Data is presented as a forest plot with a standardized mean difference and 95% confidence intervals. Effect sizes <0 favours drug treatment and >0 favours control.

Tables

Table 1. Summary of study and animal model characteristics of included articles.

Author	Year	Country	Species	Strain	Stroke Type	Stroke Model	Sex	Weight	Age
Li et al.[2]	2016	China	Rat	Wistar	Ischaemic	Intraluminal suture	Male	260–300 g	N/A
Takami et al.[3]	2002	Japan	Mice	ddY	Ischaemic	Intraluminal suture	Male	N/A	4 weeks
Chen et al.[4]	2022	China	Mice	C57/BL6	Ischaemic	Permanent middle cerebral artery occlusion	Male	25–30 g	8–10 weeks
Yan et al.[5]	2021	China	Mice	CD1	Haemorrhagic	Intracerebral haemorrhagic	Male	30–40 g	N/A
Joy et al.[6]	2019	USA	Mice	C57/BL6	Ischaemic	Photothrombotic	Male	25–30 g	8–20 weeks

Table 2. Summary of intervention characteristics.

Author	Drug	Dose (mg/kg)	Route	Timing	Doses (total #)	Outcomes Measured (Treatment n/Control n)	Outcome Window (post-stroke))
Li et al.[2]	DAPTA (D-Ala-Peptide T-Amide)	0.01	SC	15 min pre-stroke	1	- Infarct volume (5/5) - Neurological deficit score (5/5)	24 h
Takami et al.[3]	TAK-779	5	ICV	10 min pre-stroke	1	- Infarct volume (10/6)	48 h
		50	ICV	10 min pre-stroke		- Infarct volume (13/6)	
		0.25	IV	50 min post-stroke		- Infarct volume (17/18)	
Chen et al.[4]	Maraviroc	20	IP	1.5 h, 24 h, and 48 h post-stroke	3	- Infarct volume (5/5) - Longa score (5/5) - Neurological deficit score (5/5)	72 h
Yan et al.[5]	Maraviroc	0.15	IN	1 h post-stroke	1	- Garcia test (6/6) - Limb placement (6/6) - Corner turn test (6/6)	72 h
						- Foot fault (8/8) - Rotarod (8/8)	3 weeks
						- Probe quadrant duration (8/8)	25 days
				24 h post-stroke		- Garcia test (6/6) - Limb placement (6/6) - Corner turn test (6/6)	72 h
Joy et al.[6]	Maraviroc	100	IP	24 h post-stroke through daily injections for 9 weeks	63	- Infarct volume (5/5)	9 weeks
						- Grid walk (10/10) - Forelimb (10/10)	8 weeks
				24 h post-stroke then daily for 3 weeks	21	- Grid walk (10/10) - Cylinder test (9/8)	9 weeks
				3 to 4 weeks post-stroke then daily for 11 weeks	56	- Grid walk (9/9) - Cylinder test (9/9)	11 weeks

Abbreviations: ICV = intracerebroventricular; IN = intranasal; IP = intraperitoneal; IV = intravenous; SC = subcutaneous

Table 3. Alignment of included preclinical studies with STAIR recommendations and CAMAROS trial parameters.

Recommendation	Overall preclinical evidence	CAMAROS trial alignment	Notes
Candidate treatment qualification			
1) Establish treatment dose-response	Yes	No	• **Preclinical** – doses across all studies from 0.01 to 100 mg/kg • 6 doses tested for infarct volume, 3 doses for behavioural effects • Joy et al. demonstrated that 100 mg/kg of maraviroc in mice results in cerebrospinal fluid levels lower than in humans (13.8±5.4 vs. 33.6–60 ng/mL) • **CAMAROS** – participants take 300 mg doses twice daily for 8 weeks • **Interpretation** – maximum preclinical dose may not represent clinical levels
2) Treatment given after clinically relevant delayed times (one to 4.5 hours post-stroke)	Yes	Yes	• **Preclinical** – 3/5 studies (all maraviroc) administered drug from 1 to 24 hours post-stroke (acute phase) • Joy et al. initiated maraviroc administration at 20 days post-stroke for 11 weeks (subacute phase) • **CAMAROS** – participants recruited between 5 days and 6 weeks post-stroke (acute / early subacute) • **Interpretation** – one experiment in a time window relevant to clinical trial
3) Both histologic and behavioural outcomes at acute (1 to 3 days) and chronic (7 to 30 days) time points	Yes	Yes	• **Preclinical** – 2/5 studies included both histologic and behavioural outcomes • Mainly tasks of spontaneous movement (e.g., cylinder) or motor coordination (e.g., grid walk) • Tests ranged from 3 days (acute) to 11 weeks (chronic) post-stroke for all included studies, but short term assessment may only delay cell death[32] • **CAMAROS** - motor learning (Fugl-Meyer Upper Extremity Assessment Score and 10-Meter Walk Test Score) measured as the primary outcome at baseline, 4-week (late subacute), 8-week (late subacute), and 6-month (chronic) • **Interpretation** – challenging to directly compare preclinical and clinical motor tasks, but testing range is comparable. Most relevant Joy et al. experiment (subacute administration) did not measure histologic outcomes
4) Treatment reaches presumed target and causes expected physiological effects that can be assessed with a clinically relevant biomarker	Yes	NA	• **Preclinical –** 5/5 studies demonstrated trends for CCR5 antagonism to reduce infarct volume. Plausible that CCR5 influences stroke-relevant mechanisms • **CAMAROS** – does not include outcomes to assess treatment mechanisms
5) Treatment able to pass the blood-brain barrier	Yes	NA	• **Preclinical** – 1/5 studies used mass spectrometry to demonstrate that maraviroc is present in the brain and cerebrospinal fluid • **CAMAROS** – does not include outcomes to assess presence of drug in the brain

Table 3 (continued). Alignment of included preclinical studies with STAIR recommendations and CAMAROS trial parameters.

Recommendation	Overall preclinical evidence	CAMAROS trial alignment	Notes
Preclinical assessment / validation			
1) Aging/adult age	No	No	• **Preclinical** – 4/5 studies used rodents with weights/ages consistent with young adulthood; 0/5 studies used rodents ≥10 months old. • **CAMAROS** – Age ≥18 year old adult participants eligible for recruitment • **Interpretation** – Preclinical studies consistent with CAMAROS eligibility criteria; however, no preclinical study examined middle-aged or elderly animals that would be more representative of the trial and overall stroke populations
2) Male and female animals	No	No	• **Preclinical** – 0/5 studies included female animals • **CAMAROS** – both male and female sexes eligible for recruitment • **Interpretation** – effects of CCR5 antagonists for stroke recovery in female animals represents a critical knowledge gap
3) Animals with co-morbidities	No	No	• **Preclinical** – 0/5 studies included animals with common stroke co-morbidities (e.g., diabetes, hypertension, etc.) • **CAMAROS** - participants with stroke co-morbidities eligible for recruitment • **Interpretation -** effects of CCR5 antagonists for stroke recovery in animals with common stroke comorbidities represents a critical knowledge gap
4) Evidence from two or more laboratories	Yes	No	• **Preclinical** – 5/5 studies demonstrated stroke-related benefits; however, no study had their results replicated by independent laboratories • **CAMAROS** – trial protocol will be replicated by multiple study sites • **Interpretation** – future preclinical experiments could consider assessing effects using a multilaboratory approach, or independently replicate the effects of existing preclinical studies
5) Gyrencephalic species	No	No	• **Preclinical** – 5/5 studies conducted in lissencephalic (rodent) species. • **CAMAROS** – human participants • **Interpretation** – effects of CCR5 antagonists for stroke recovery in gyrencephalic species represents a critical knowledge gap
6) Tests during the awake phase of animals	No	No	• **Preclinical** – 0/5 studies reported assessing outcomes at night (i.e. awake phase of rodents) • **CAMAROS** – follow-up occurs during daytime hours (i.e. awake phase of humans) • **Interpretation** – future preclinical studies should conduct behavioural testing during the awake phase to better align with human testing conditions