Novel Analytical and Methodological Approaches to Preclinical Knowledge Synthesis in Stroke

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Abstract

This thesis explores opportunities to bridge the gap between preclinical and clinical research in stroke through the conduct of three studies: a Network Meta-Analysis (NMA) and systematic review of preclinical stroke therapies, a systematic review and meta-analysis of C-C chemokine receptor type 5 (CCR5) antagonists co-designed with patient partners, and an assessment of patient engagement in the systematic review process. The first study identifies a number of potential therapies while also underlining the challenges in their translation to human trials. The second investigation highlights the promising efficacy of CCR5 antagonists in preclinical models and notes existing gaps towards demonstrating clinical effectiveness. The final study focusses on the integration of patient perspectives, revealing the potential to align research with patient-relevant outcomes. Collectively, the work highlights the importance of methodological rigour, transparent reporting, and patient engagement in enhancing the relevance and translatability of preclinical findings to clinical practice in stroke research.

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Chapter 1: Introduction

STROKE AND PRECLINICAL STUDIES

Stroke is the leading cause of adult disability, and the second leading cause of death worldwide with 143 million people facing new permanent disabilities annually and more than six and a half million experiencing deaths.[1,2] Specifically in Canada, as of 2022, 878,000 Canadians have had a stroke or are living with stroke, and more than 89,000 strokes occur each year.[3] There are two main types of strokes: ischemic and hemorrhagic. Ischemic strokes account for about 87% of all strokes and occur when a blood vessel in the brain is obstructed (e.g. by a blood clot) .[4]

Hemorrhagic strokes occur when weakened blood vessels rupture and bleed into the surrounding brain, which accumulates and compresses the surrounding brain tissue.[5]

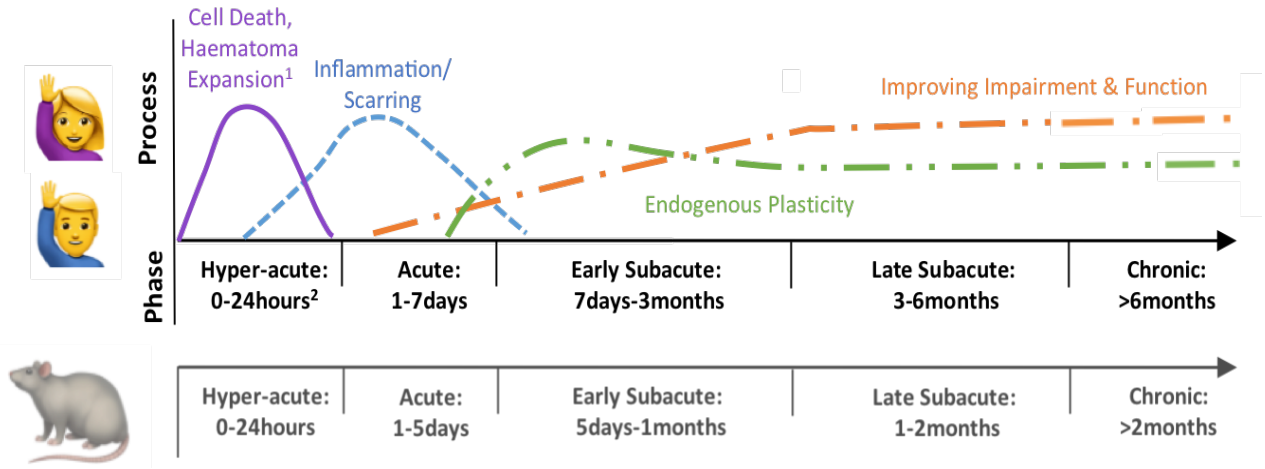

Figure 1. This figure shows the comparison and progress from stroke onset being divided into five phases in humans and rodents: hyper-acute, acute, early subacute, late subacute, and chronic.[6] In the hyper-acute phase, blood, oxygen, and nutrient supply to the affected brain region has been disrupted. From acute to late subacute, spontaneous recovery and neural repair of damaged cells and connections begin. By the time the chronic phase is reached, most neuroplasticity (a process that involves adaptive structural and functional changes to the brain[7]) has taken place.

Unfortunately, treatment options for patients with acute stroke remain limited, with intravenous thrombolysis and endovascular thrombectomy being the only effective therapeutic intervention

options for **acute** ischemic stroke.[8] Intravenous thrombolysis refers to treatment, such as a drug (e.g. alteplase or tissue plasminogen activator, t-PA) to dissolve clots in blood vessels. Patients treated with alteplase are at an increased odds of 1.7 (1.0-2.8)[9] to have minimal or no impairment three months after stroke.[3] Whereas endovascular thrombectomy is the removal of a blood clot under image guidance has a reduced relative risk of death (0.85 [0.75-0.97])[10] and leads to better recovery.[3] Hemorrhagic stroke treatments are more understudied with only temporary treatment options and no known drug therapies. Depending on the severity of the hemorrhagic stroke, an individual may have a small amount of cerebrospinal fluid removed to reduce pressure and drain blood that collected in the brain or clamping/coil embolization can be performed for prevention (prevent bleeding before a stroke or to prevent rebleeding).

Both intravenous thrombolysis and thrombectomy have limitations associated with the degree of residual infarction and disability after receiving treatment.[11,12] Most people who experience stroke require ongoing recovery support. According to the Canadian Chronic Disease Surveillance System, the number of Canadians who survived a stroke grew by 40% in recent years.[13] The long-term impairments resulting from a stroke can be severe and debilitating. Canadians experiencing these impairments could be affected to a disabling degree, therefore continuing in their jobs, or even performing the necessary daily life tasks could be overwhelmingly challenging. Ninety percent of stroke victims suffer from a wide variety of mental, physical, psychological, and emotional conditions; and around 50% of stroke victims will be left severely impaired where they will be unable to rejoin the workforce.[1] It is important to note that the use of limited treatment options is further limited by a short window after stroke onset, excluding a majority of individuals who have had a stroke. Specifically, guidelines restrict treatment with alteplase to the first 4.5 hours following stroke onset due to its rapid decline in efficacy outside this window.[9,14] Considering a combination of timing, type and location of stroke, eligibility criteria, it is estimated only about 10-20% of patients with stroke are eligible for treatment.[15] This leaves most stroke survivors with no access to effective drug treatments. One potential solution is addressing these concerns in the earliest stages of drug development, the preclinical stage. This might be beneficial to address reoccurring issues and patient priorities at the very start of drug development when testing drug therapies in animals instead at the start of human clinical trials.

Preclinical studies are laboratory tests of a new drug done on animal subjects to provide reasonable evidence prior to testing in humans and human clinical trials.[16] Preclinical animal studies are important to help demonstrate that the novel drug is safe and effective.[17] One of the challenges with preclinical studies is the lack of translation of demonstrated efficacy from preclinical experiments to humans. Almost 95% of the drugs entering human trials fail, and according to the National Institutes of Health, 80-90% of research projects fail before they ever get tested in humans.[18] Thus, approval of new drug candidates from preclinical to clinical studies and then the final approved drug for market use is only 0.1%.[19] Currently, in preclinical stroke research, over 1000 potentially neuroprotective agents have been tested for adjunct treatments to alteplase, the gold standard treatment in stroke studies, in preclinical *in vivo* model settings and more than 114 potential therapies initially tested in animals failed in human trials.[20,21] Most studies failed because of a lack of effectiveness and poor safety profiles that were not predicted in preclinical and animal studies.[19,22] Reproducing the disease in animals requires reproducing the predisposing diseases that come with stroke (i.e. hypertension, obesity, older age) to make preclinical studies clinically relevant, which is a challenge in stroke animal models.[21] However, novel treatment options are mostly being tested preclinically at the hyper-acute and acute phases of care and rarely in the recovery phases (late subacute and chronic), where the time frame for these phases are different in animals compared to humans and can potentially raise the continued issue of eligibility for treatment to a vast proportion of stroke survivors. In addition, it is difficult to identify the most promising therapeutic candidates for further development partly because of the lack of comparison among all treatment options for efficacy.[23,24] By highlighting efficacy of all treatment options, any treatment showing preclinical efficacy could potentially move forward to early phase clinical trials.

To help address this issue, we can apply preclinical knowledge synthesis which collates and analyses all preclinical experiments for a given therapy rather than looking at experiments in isolation or in a potentially biased manner. Performing a comprehensive analysis of the available evidence helps to identity research gaps and draw reliable conclusions.[25] This allows for an intermediate step of systematic reviews with meta-analyses prior to considering translation to early-phase clinical trials. Since there are numerous stroke drugs being tested preclinically with no evidence of a robust comparison, a preclinical review testing competing stroke therapies would be beneficial in narrowing down the most promising therapy candidates for clinical use. In

addition, clinically relevant time frames are not being tested (or do not match) in preclinical experiments, which potentially leads to the lack of successful translation. The absence of clinical and patient priorities being considered at the early stages of drug development might bridge this gap of matching clinical endpoints in preclinical studies. There is little evidence of preclinical stroke research, and preclinical research in general, obtaining and integrating input from key stakeholders (i.e. patients) in their studies.[26,27] Identifying, incorporating, and obtaining patient-centred outcomes is potentially another beneficial approach to preclinical knowledge synthesis.

Box 1. Definitions of key terms used[7,16,28-30]

TERM	DEFINITION
PRECLINICAL	Research using animals to determine if an intervention is likely useful. Preclinical studies take place to inform human testing (modified from National Institutes of Health)
SYSTEMATIC REVIEW	A review that uses systematic methods to collate and synthesize findings of studies that address a clearly formulated question (modified from PRISMA 2020)
PATIENT	Individuals with personal experience of a health issue and their informal caregivers, including family and friends (Canadian Institutes of Health Research Strategy for Patient Oriented Research)
PATIENT ENGAGEMENT IN RESEARCH	Meaningful collaboration between researchers and 'patient partners' to co-create research (Canadian Institute of Health Research)
NEUROPLASTICITY	A process that involves adaptive structural and functional changes to the brain (StatPearls)

KNOWLEDGE SYNTHESIS

Systematic reviews and meta-analyses of randomized control trials form the backbone of knowledge synthesis, evidence-based medicine, and are integral to impactful healthcare and policy decisions.[31] Clinically, they have been used for decades to inform best practices.[32] Systematic reviews in laboratory-based studies (i.e. preclinical systematic reviews) that involve animal work have recently been gaining traction.[33] Preclinical has different meanings to different stakeholders within healthcare and research. However, for the purposes of this thesis, we will define preclinical as research using animals to determine if a drug, procedure, or treatment is likely to be useful; in addition, preclinical studies take place to inform human testing[16]. Preclinical systematic reviews can be used to plan future experiments and help researchers decide when it is time to pursue the evaluation of a novel treatment in human patients.[34]

I will use two novel approaches to preclinical systematic reviews to provide robust and current evidence: a network meta-analysis and a patient partnered co-design of a preclinical systematic review.

NETWORK META-ANALYSIS (NMA)

There are limitations to the traditional meta-analytic approach in systematic reviews,[35,36] which may be further exacerbated when analyzing preclinical data. For example, meta-analyses are limited to traditional pairwise comparisons directly comparing two treatment effects.[35] In many healthcare settings, decisions often involve numerous possible treatment options, many of which have never been directly compared head-to-head in a randomized control trial.[37] In the absence of robust large head-to-head trial evidence, this limitation can be addressed by using an NMA, which uses direct and indirect evidence to compare treatment efficacy.[38] However, when it comes to preclinical data, there is lack of standardization of reporting key methodological items and often different measurement landmarks. With the lack of standardized comparisons, it makes it difficult or unfavourable to conduct either direct or indirect analyses.

An NMA uses direct and indirect evidence to estimate comparisons among various treatments.[38,39] For example, treatment A has been compared to treatments B and C in separate studies. Treatments B and C have never been directly compared. An NMA uses evidence from the common comparison of treatment A (i.e. A vs. B and A vs. C) to compare the effects of B vs. C (See **Figure 1**). An NMA assumes that the B vs. C effect from the direct evidence is similar to the B vs. C effect from the indirect evidence (the consistency assumption).[39] Violations of the consistency assumption occur when there are differences in factors that interact with the treatment effects between studies making different comparisons. If there are loops of evidence, then the consistency assumption can be checked statistically.[39] NMAs produce consistent estimates of the relative effects of all interventions compared to all other interventions, and also, NMAs provide the ability to rank all interventions of interest.[38] The comprehensive summary of evidence produced by NMAs has made them increasingly popular. In fact, ten percent of Cochrane systematic reviews published since 2015 have used an NMA to analyze data.[40]

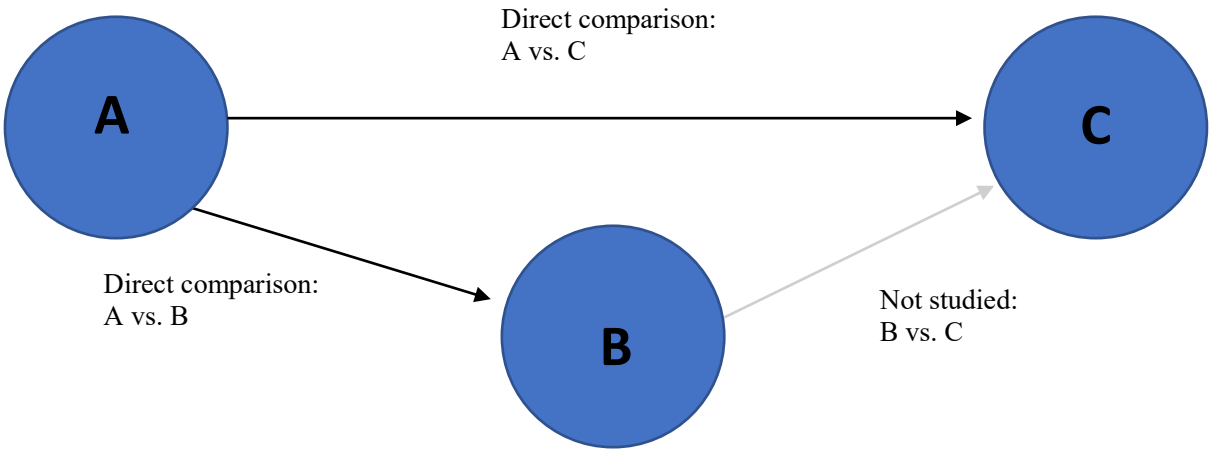

Figure 1: Network Meta-Analysis's use of both direct and indirect comparisons

Despite the increasing popularity of NMAs in clinical systematic reviews, they remain rarely used in preclinical systematic reviews. According to our search up to 2022, only four preclinical NMAs have been published to date.[41-44] Opposed to comparing only two therapies at a time (i.e. pairwise), systematic reviews with NMAs comparing multiple treatments provide a comprehensive overview of candidates with the potentially the most promising effective therapies for translation from the bench to the bedside. Therefore, using an NMA may provide evidence for potential future therapeutic targets in stroke research. Additionally, integrating key knowledge users, such as patients, can enhance the study by potentially strengthening its relevance, quality, and dissemination.

PATIENT ENGAGEMENT

When discussing key participants in the conduct of preclinical research, patient partners (which includes patients, caregivers, and friends of a particular health condition[29]) are rarely included. Not surprisingly, the same is seen in preclinical systematic reviews. Patient engagement, defined as the meaningful collaboration between researchers and patient partners to co-create research,[30] recognizes patients as the ultimate end-users in research. In addition, the International Association for Public Participation (IAP2) developed three pillars for effective patient engagement processes: Core Values, Code of Ethics, and Spectrum of Public Participation. These pillars were developed with key international stakeholders' inputs and formed the foundation of engagement processes that reflect the interests and concerns of all stakeholders, including the patients. IAP2's Spectrum of Public Participation was designed to assist in the

selection of the level of participation that defines the patient partner's role in any engagement process. The Spectrum is used internationally and is found in many public participation plans.[45]

Patient engagement in clinical research is quickly gaining acceptance to enhance the relevance and quality of studies[46]. The interest is to help align research outcomes with patient priorities through lived experiences. However, clinical research is only a small part of the research process. In preclinical research, examples of patient engagement in laboratory studies are sparse[26]. There exists little guidance for the involvement of patients in this phase of research[47]. However, in Canada, 46% ($472 million) of the total annual Canadian Institute of Health Research's budget – the most significant proportion of federal research funding in Canada – is allocated to preclinical studies, compared to 14% ($140 million) allocated to clinical studies.[48]

Cochrane, internationally recognized for producing high-quality systematic reviews, and NIHR Centre for Engagement and Dissemination (previously known as INVOLVE) have each published one guideline for involving patient partners in systematic reviews.[49,50] Both organizations highlight the benefit of patient partners being engaged in systematic reviews. For instance, Pollock et al. highlight that patient partners with lived experiences of the disease and intervention of the review can be involved in setting the outcomes of the review to emphasize patient priorities, provide unique perspectives on understanding an idea, interpret results, and improve the accessibility of the review.[49] Patient partners can thus be involved in varying degrees and at different stages in the review. The resources also addressed the benefits and potential challenges of engagement. Benefits include considering patient-oriented outcomes, co-learning shared lived experiences and the research process, and reaching a wider audience during dissemination. Identified potential barriers include recruitment of patient partners, ensuring meaningful engagement, dealing with frustration when studies do not include the outcome of interest, and time commitment.[51] Because the resources mainly target clinical systematic reviews, another important potential barrier to consider is patient partners recognizing the importance of being involved in an early stage of the research development process, such as in preclinical research. The unique aspect of preclinical research is that it does not directly involve patients as participants as it is a step removed from human studies. This potential barrier challenges patient engagement in systematic reviews of laboratory-based studies since it requires specific knowledge of a patient-removed topic and the research process. Engagement in

preclinical research then requires the patient partners to not only provide their insight on lived experiences but may also add a layer of complexity. Table 2 provides additional potential barriers to patient engagement in preclinical research. Patient partners may need to invest time in education to develop their knowledge of basic science in order to facilitate meaningful input.[52-57] In addition, challenges on the researcher side include potential lack of training on engagement and proper communication skills geared towards a patient audience. These potential knowledge gaps of how to meaningfully engage patients in preclinical research need to be investigated.

Table 2. Barriers of patient engagement in preclinical research

Barrier	**Explanation**
Non-patient facing studies	Adds a layer of complexity of specific knowledge required of patient partners, which might cause frustration and hesitation in being involved
Structural barriers	Time and budget to support patient engagement can cause a burden to research group
Lack of patient engagement experience	Preclinical researchers typically do not have prior experience with patient engagement, which may make it difficult to incorporate meaning collaboration at the start
Lack of educational background	Lack of educational background of the topic from patient partners, and the lack of experience communicating research to a public/patient audience from preclinical researchers may cause issues in bilateral communication
Clashing patient partner and researcher points of view	Perspective of patient priorities in preclinical research and what is actually feasible might not always align

Despite potential barriers, incorporating patient engagement in the preclinical phase may improve the implementation of patient-oriented objectives from the start of the research development process. Furthermore, allowing key stakeholders, like patient partners, to be part of preclinical research may ultimately allow for more successful translation from animal to human studies.

THESIS OBJECTIVES

The overall objective of this thesis is to evaluate novel knowledge synthesis approaches to better bridge the gap between preclinical studies and clinical trials in the setting up stroke. My thesis will focus on two main studies and the following objectives:

(1) A Preclinical Stroke Systematic Review and Network Meta-Analysis of Alteplase
    - To assess the relative benefits of competing therapies tested in combination with the gold standard treatment, alteplase
    - To determine what preclinical stroke therapy shows the most efficacy in combination with alteplase

(2) A Preclinical Systematic Review and Meta-Analysis with a Panel of Patient Partners
    - To assess the preclinical efficacy and safety of the chosen stroke intervention by the patient partners and researchers, which is C-C motif chemokine receptor 5 (CCR5) inhibitors
    - To assess the potential impact of patient engagement in preclinical systematic reviews and assess the added benefits, barriers, and challenges
    - To assess researchers' and patient partners' outlooks toward patient engagement in preclinical systematic reviews
    - To evaluate patient engagement in a preclinical systematic review from both researchers' and patient partners' perspectives from those involved in the preclinical stroke systematic review

THESIS ORGANIZATION

My thesis is manuscript-based. A brief overview of each chapter is outlined below.

- **Chapter 1:** Provides a background and rationale for the thesis.
- **Chapter 2:** Represents the first article of the thesis, a preclinical systematic review and network meta-analysis aiming to assess the efficacy of potential therapies in combination with alteplase.

- **Chapter 3:** Represents the second article of the thesis, a preclinical systematic review and meta-analysis, co-designed and conducted by a panel of patient partners, analyzing the effect of maraviroc, a CCR5 inhibitor, in stroke animal models.
- **Chapter 4:** Represents the third article of the thesis, a longitudinal survey aimed to gain an understanding of the impact, benefits, and challenges of co-designing a study from the perspectives of both patient partners and researchers.
- **Chapter 5:** Provides a summary of key findings and an integrated discussion.

The novelty of my thesis that incorporates a network meta-analysis and patient engagement approaches in preclinical systematic reviews is that it provides concrete examples and guidance for future preclinical knowledge synthesis studies. Patient engagement and NMA approaches are relatively new in preclinical systematic reviews. Therefore, guidance in this area must be adapted for our needs, especially given that preclinical research is not patient-facing and the potential heterogeneity of preclinical data. This thesis will discuss the incorporation of an NMA in a preclinical systematic review, a patient-led preclinical systematic review, the overall evaluation of the engagement between patient partners and researchers when co-conducting a preclinical systematic review, and finally an integrated discussion of the three studies in this thesis. Ideally, the two systematic reviews incorporated in this thesis will offer guidance in the field of stroke research by filling in the evidence and knowledge gaps when it comes to two promising stroke treatment options currently, alteplase in combination with other therapies and CCR5 inhibitors.

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Chapter 2: Systematic review and Network Meta-Analysis

A preclinical systematic review and network meta-analysis of stroke therapies

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Preface to Chapter 2

This chapter presents the results of a systematic review that uses a network meta-analysis approach to assess preclinical stroke therapies. The results demonstrate the most statistically effective therapies that have been studied preclinically. I was responsible for updating the systematic review following the departure of the statistician who was originally in charge of the analysis. As a result, due to the complexity of the data, the analysis of the NMA had to be simplified, leading to a more straightforward approach. Despite the simplification in methodology, it is deemed adequate for the purpose of this thesis.

ABSTRACT

Background: Given the paucity of treatments available for acute stroke patients, a multitude of therapies have been tested preclinically. Yet, it is difficult to identify the most promising candidates for translation. We performed a systematic review and NMA to identify the most promising preclinical therapeutic candidates.

Methods: We searched MEDLINE, Embase, Web of Science, and CAMARADES databases. Eligible articles met the following criteria: i) *in vivo* models of ischemic stroke, ii) tested therapy in combination with alteplase compared to alteplase alone, iii) therapy delivered within six hours of stroke onset, iv) reported infarct volume. Screening, data extraction and risk of bias assessment were done independently and in duplicate. The primary outcome was infarct volume. A random effects Bayesian network meta-analysis was performed.

Results: A total of 137 studies met our eligibility criteria after screening (n=3,806 animals). In total, 24 distinct interventions were analyzed in the NMA, six of which (AS605240, post-conditioning, normobaric oxygen, minocycline, edaravone, and hypothermia) significantly reduced infarct volume compared to alteplase alone.

Discussion: Our NMA results demonstrate the potential of several therapies that may provide additional therapeutic benefit when delivered in combination with alteplase. These findings provide evidence for potential future therapeutic targets in stroke research.

Keywords: Systematic review; stroke; preclinical; network meta-analysis; translation

INTRODUCTION

Stroke is the second leading cause of death, with six million deaths and an additional five million patients experiencing new permanent disability yearly.[1] In addition to the physical and emotional impact of stroke, the economic burden is significant, with worldwide costs estimated between $266 billion to $1.038 trillion annually.[2] Despite this, intravenous thrombolysis with intravenous alteplase and endovascular thrombectomy are the only clinically accepted therapeutic interventions for acute ischemic stroke.[2] However, both thrombectomy and intravenous thrombolysis have limitations associated with their use (e.g. hemorrhagic transformation, limited window to initiate use),[3] and a minority of stroke patients can be treated with these therapies.[4] In order to address the gap in acute interventions, over 1,000 potentially neuroprotective agents have been tested in a preclinical *in vivo* setting.[5,6] With the multitude of agents being tested preclinically, novel techniques used to identify the most promising preclinical therapeutics are needed in order to potentially reduce research waste and promote translation of these therapeutics.

Systematic reviews and meta-analyses of randomized control trials (RCTs) form the backbone of evidence-based medicine and are integral to impactful healthcare and policy decisions.[7] Clinically, they have been used for decades to inform best practice,[8] and have also recently been gaining traction in preclinical research.[9] There are, however, several acknowledged limitations to the traditional meta-analytic approach,[10,11] which may be further exacerbated when analyzing preclinical data. For example, meta-analyses are limited to traditional pairwise comparisons directly comparing two treatment effects.[10] In many healthcare settings, decisions often involve numerous possible treatment options, many of which have never been directly compared head-to-head in an RCT.[12] This limitation can be overcome with the use of network meta-analysis (NMA), which uses both direct and indirect evidence to compare treatment efficacy.[13]

An NMA can compare multiple treatments, even if they have not all been directly compared in RCTs, as long as the evidence forms a connected network of treatment comparisons. Comparing the relative effectiveness of all relevant treatment options in an NMA can be useful to inform the decision to embark on either additional confirmatory preclinical studies, or clinical trials,[13,14]. As with systematic reviews comparing two treatments [ref 2, 15], systematic reviews with NMAs of multiple treatments have the potential to further reduce the costs and translation failures from preclinical studies by placing individual treatment into context. Despite the increasing popularity of NMA in clinical reviews, it remains rarely used in preclinical systematic reviews, with only two preclinical NMAs being published to date.[15,16] In order to address these knowledge gaps (i.e. identifying promising therapeutics for ischemic stroke and application of NMA methods for preclinical work), our systematic review and NMA aimed to answer the following question, "Amongst *in vivo* models of focal ischemic stroke, what are the relative benefits of competing therapies tested in combination with the gold standard treatment alteplase?"

METHODS

The protocol for this review was developed *a priori*, and published.[17] The final review was prepared in accordance with guidance from the Preferred Reporting Items for Systematic Reviews and Meta-Analyses Network Meta-Analysis extension (PRISMA-NMA),[18] the checklist for which can be found in the Appendix (Appendix 1).

Eligibility Criteria

We included studies that met the following criteria: i) preclinical *in vivo* models of experimentally induced focal ischemia, ii) tested the efficacy of therapies in combination with alteplase compared to alteplase alone, iii) therapy was delivered within six hours of focal ischemia onset, and iv) reported infarct volume. We excluded: i) neonatal models of focal ischemia, ii) animal models of hemorrhagic stroke, global or hemispheric brain ischemia, models of permanent occlusion without reperfusion (i.e. photothrombosis, cauterization), or delayed reperfusion such that it is considered permanent,[19] iii) human and tissue culture studies, iv) alteplase not used as a "foundational" therapy in experimental arm, v) therapy administered more than six hours after onset of focal ischemia, and vi) reviews, commentaries, and conference abstracts.

Outcomes

The primary outcome of this review was infarct size. All methods to ascertain infarct size were included. If multiple timepoints were provided in an included study, the latest timepoint was extracted (i.e. most representative of fully evolved lesion).

Search Strategy

In consultation with an information specialist (Risa Shorr, The Ottawa Hospital), a comprehensive search strategy was developed to search Ovid MEDLINE, Embase, and Web of Science. The

search strategy was also developed and validated through the Peer Review of Electronic Search Strategies (PRESS).[20] The CAMARADES database was searched for relevant studies that were included in previous systematic reviews. No restrictions on language and publication date were applied; articles in foreign languages were translated. The final update of the search was conducted on January 11th, 2022. In addition, we examined reference lists of included studies and relevant reviews identified through the search, to ensure a comprehensive search was performed. The search strategy can be found in the appendix (Appendix 2).

Study Selection Process

All identified citations were uploaded by our team into DistillerSR® (Evidence Partners, Ottawa, Canada) and duplicate studies were removed. Two reviewers independently performed the process of study selection. Title and abstract screening were performed independently and in duplicate using an accelerated screening method (one reviewer required to include, two reviewers required to exclude). A calibration exercise was performed on the first 50 studies to refine the screening question prior to formally commencing the screening process. After initial screening for potentially relevant articles, full-texts were retrieved and screened. This was performed independently by two reviewers. Any conflicts were resolved through discussion with a third team member.

Data Extraction

Two independent reviewers extracted relevant data from included studies using a standardized and pilot-tested data extraction form created in DistillerSR® with iterative input from all members of the study team. Reviewers collected data pertaining to study characteristics (i.e. primary author, country, journal, sample size, etc.), animal model details (i.e. species, strain, sex, etc.), intervention details (i.e. treatment groups, dose of therapy used in combination with alteplase, dose of alteplase,

timing of therapy administration, etc.), outcomes (infarct volume) and risk of bias details. For outcome data, measures of central tendency (e.g. mean, median) and measures of dispersion (e.g. standard deviation, standard error of the mean, 25% and 75% percentiles) were collected as reported. We transformed median and percentiles to approximate mean and standard deviation using recommended methods.[21] Data presented in graphical format only was extracted using Engauge Digitizer (version 12.0).[22] When measures of central tendency and dispersion or sample sizes were missing (or could not be measured digitally), authors were contacted; if authors did not respond, the data were not included. Disagreements were resolved through discussion with a third team member.

Risk of Bias and Construct Validity Assessments

Two reviewers independently assessed the risk of bias for each study using a modified version of the Cochrane Risk of Bias Tool for randomized trials.[23] In addition to the risk of bias, we assessed construct validity for preclinical studies to evaluate the clinical generalizability of the experimental conditions, since a mismatch between the preclinical experimental conditions and the clinical manifestation of the disease provide false estimates of effect.[24] Construct validity was assessed using a tool previously used for models of ischemic stroke.[25,26] Items evaluated in each study included: (1) use of adult animals, (2) use of animals with comorbidities, (3) avoidance of anesthetics with neuroprotective effects (e.g. ketamine, propofol, lidocaine), (4) control of temperature during stroke induction, (5) physiological monitoring during stroke induction, (6) confirmation of ischemic stroke injury via laser doppler of perfusion imaging, and (7) relevance of infarct size. The operationalization of each question can be found in the appendix (Appendix 3). Each item was assigned either a “yes”, “no”, or “unclear”. Disagreements were resolved through discussion with a third team member.

Evidence Synthesis; Exploratory Network Meta-Analysis (NMA)

A NMA uses both direct and indirect evidence to estimate comparisons among various treatments.[13,27] For example, treatment A has been compared to treatment B and C in separate studies. Treatment B and C have never been directly compared. NMA uses evidence from the common comparison of treatment A (i.e. A vs B and A vs C) to compare the effects of B vs C. NMA assumes that the B vs C effect from the direct evidence is similar to the B vs C effect from the indirect evidence (the consistency assumption).[27] Violations of the consistency assumption occur when there are differences in factors that interact with the treatment effects between studies making different comparisons. If there are loops of evidence then the consistency assumption can be checked statistically.[27] NMA produces consistent estimates of the relative effects of all interventions compared to all other interventions and also provides the ability to rank all interventions of interest.[13] The comprehensive summary of evidence produced by NMAs have made them increasingly popular, indeed, 10% of Cochrane systematic reviews published since 2015 have used NMA to analyze data.[28]

We excluded interventions evaluated in only a single study and not substantiated by further studies. Such treatments removed from NMA neither benefit from "borrowing strength" through NMA, nor produce a summary estimate and confidence interval different from what was reported in a single study. For interventions connected to the evidence network with reported (or approximated) mean and standard deviation of infarct size available from at least two studies, we explored the connectivity of the network (i.e. which treatments were compared to which) using network diagrams. We assessed the consistency assumption by fitting unrelated means models to the data.[29]

The raw mean difference scale was used to report the treatment effects. Random effects Bayesian NMAs (both consistency model and unrelated means models) were fitted, and compared to assess the consistency assumption. Time was accounted for and centred at two hours since median time of alteplase administration after stroke was 2 hours and we observed a negative relationship between efficacy and timing of alteplase administration post-stroke. Results are presented from the consistency model.[29] Forest plots of treatment comparisons versus "no treatment" control as well as versus stroke + alteplase were presented with summary estimates of raw mean differences and corresponding 95% confidence intervals. We also report the Surface Under the Cumulative RAnking (SUCRA) rank of each intervention (Appendix 4).[30] NMAs were performed using the R Package BUGSnet package version 1.1.0[31] in R. We used a total of 50,000 sampling iterations, with 25,000 burn-in.

RESULTS

Our literature search returned 3,678 unique citations, 3,045 of which were excluded during the screening of titles and abstracts. A further 399 were excluded during the full-text review stage, leaving a total of 234 citations (Figure 1). Of the 234 citations, 137 citations (325 experimental arms) reported data on infarct volume, our primary outcome of interest, and were included in the NMA. Detailed information on the remaining 72 citations not included in the NMA can be found in the appendix (Appendix 5).

Characteristics of Included Studies

A summary of the characteristics of included studies is presented in Table 1. Studies were published between 1993 and 2021 and were published largely from United States of America (n=59, 42%). The median sample size was 8 (range 5-144). Species studied include rats (n=88, 63%), mice (n=45, 32%), and rabbits (n=5, 4%). Stroke was mainly induced via middle cerebral artery embolism (n=96, 69%) or intraluminal sutures (n=38, 27%). One hundred twenty-five studies (90%) were performed in males only, while four studies (3%) used females only, and three studies (2%) used both sexes; seven studies (5%) provided insufficient information to determine animal sex. Study-level characteristics can be found in the appendix (Appendix 5, additional table 3).

Intervention Characteristics

Detailed characteristics on the alteplase only control arm can be found in Table 2. We observed significant heterogeneity with regards to the timing of administration of alteplase post-stroke and the dosage of alteplase administered. Detailed intervention characteristics can be found in Table 3. The majority (68%) of experiments administered the experimental therapy at the same time as alteplase. However, significant heterogeneity was observed in regard to the timing of

administration of post-stroke interventions, as well as the method of administration, reflective of the wide variety of experimental therapies included.

Risk of Bias and Construct Validity

The risk of bias summary is presented in Figure 2. The majority of included studies were at an unclear risk of bias across the majority of assessed domains. Twenty-two studies (9%) detailed their randomization method, while 12 (5%) detailed allocation concealment techniques and 76 (32%) and 143 (61%) reporting blinding of personnel or outcome assessors, respectively. Ninety-six studies (41%) fully detailed their outcome data, while 36 studies (15%) provided a sample size calculation.

The construct validity summary is presented in Figure 3. When assessing construct validity, 206 (88%) studies used adult animals and 24 (10%) studies used animals with relevant comorbidities (i.e. hypertension, hyperglycemia, etc.) [32,33]. Sixty-six (28%) studies provided sufficient details regarding infarct size to judge its clinical relevance.

Findings from Exploratory Network Meta-Analysis of Infarct Size

Data from 137 articles (n=3,047 animals) representing 24 interventions contributed data to the NMA of infarct volume. The corresponding network diagram (Figure 4) presents the evidence base available for infarct size. Model fit statistics from NMAs are provided in the appendix (Appendix 4).

First, we explored the relationship between the mean difference of infarct size for animals receiving alteplase only compared to animals that received no therapy and the time of alteplase administration after stroke.

Figure 5 displays the mean difference estimates for alteplase plus intervention compared to animals that had stroke induced but did not receive any therapy. When administered in combination with alteplase, six of the 24 interventions demonstrated a statistically significant advantage compared to animals that did not receive any therapy. Compared with no therapy AS605240 saw the largest reduction in infarct size (MD - 66.8, 95% CI -105.6 to -28.3), followed by post-conditioning (MD -60.5, 95% CI -93.6 to -28.1), normobaric oxygen (MD -42.9, 95% CI -63.9 to -22.0), edaravone (MD -37.8, 95% CI -61.7 to -14.0), minocycline (MD 37.6, 95% CI -61.1 to -14.7), hypothermia (MD -28.9, 95% CI -45.4 to -12.6), and estradiol MD -22.6, 95% CI -44.8 to -0.8). Figure 6 displays the mean difference estimates for alteplase plus intervention compared to alteplase alone; SUCRA rankings are reported in the appendix (Appendix 4). Compared with alteplase only, alteplase in combination with AS605240 saw the largest reduction in infarct size (MD -63.1, 95% CI -101.3 to -25.0), followed by post-conditioning (MD -56.8, 95% CI -89.5 to -24.6), normobaric oxygen (MD -39.2, 95% CI -59.8 to -18.5), edaravone (MD -34.1, 95% CI -57.6 to -10.6), minocycline (MD -33.9, 95% CI -56.8 to -11.6), and hypothermia (MD -25.2, 95% CI -41.1 to -9.4). The remaining interventions provided no statistically significant benefit compared to alteplase alone. The corresponding forest plots from NMAs without adjustment for timing of alteplase administration are displayed in the appendix (Appendix 4).

DISCUSSION

Our systematic review and network meta-analysis explored the relative benefits of experimental stroke therapies tested in combination with the current gold standard clinical treatment of alteplase. Our NMA results demonstrate the potential of several therapies that may provide additional therapeutic benefit when delivered in combination with alteplase. These findings provide evidence for potential therapeutic targets in the field of translational stroke research. Additionally, this study represents the largest application of NMA to preclinical data to date. Although there are many noted advantages to this method, it has primarily been applied in the clinical setting and the nuances and challenges associated with implementing such a complex model in a preclinical setting have not been fully explored.

Of the 24 interventions tested in combination with alteplase and included in our NMA, six resulted in statistically significant smaller infarct sizes when compared to alteplase alone. Due to the number of therapeutics identified, priority was given to those with targets effect sizes that were statistically significant. The highest ranked treatment (AS605240) has yet to be tested in clinical trials in humans. Of the remaining five interventions, all have shown some degree of clinical promise by being tested in clinical trials. For example, hypothermia has been the subject of recently published clinical trials;[34,35] however its efficacy remains in question, and a large randomized trial was recently withdrawn from clinicaltrials.gov due to the inability to obtain funding,[36] while the largest prospective trial of hypothermia for stroke patients ever conducted in Europe closed early due to issues related to recruitment and the feasibility issues.[37]

Results of our NMA demonstrated 18 interventions which, when tested in combination with alteplase, did not demonstrate a significant advantage compared to alteplase alone. Interestingly,

eleven of these 18 interventions have been tested in a clinical setting. For example, granulocyte colony-stimulating factor (g-csf) has previously demonstrated efficacy preclinically,[38,39] and clinical trials were undertaken on this basis. Although G-CSF demonstrated safety in a phase IIa clinical trial,[40] it ultimately failed to demonstrate efficacy in the subsequent phase IIb trial[41] and no clinical trials have been undertaken since. Other interventions, such albumin,[42] have had clinical trials terminated for either safety or futility concerns. Given the frequency in which alteplase is currently administered in the acute ischemic stroke population, it is imperative when identifying potential translational targets, that the effects in combination with alteplase be studied. For example, nerinetide (NA1) was identified as a very promising therapeutic candidate based on preclinical studies and early phase safety trials. However, in a large multicenter RCT, NA1 did not improve the proportion of patients achieving meeting the primary outcome (modified Rankin Scale (mRS) score of 0–2).[43] When study authors stratified patients by those which did and did not receive alteplase (with roughly 60% receiving alteplase), it was shown that when NA1 alone was associated with improved outcomes, while no improvement was noted when used in combination with alteplase. Testing potential novel therapeutics in combination with gold-standard therapies may have the potential to identify such issues earlier in the translational pathway, giving trialists and researchers an opportunity to implement strategies to mitigate these issues.

While results from our review and NMA may be able to provide some insight into promising therapeutics for the reduction of infarct volume in acute stroke, results should be interpreted with caution due to risk of bias and construct validity. The field of preclinical stroke research has seen multiple landmark studies demonstrating the association between a lack of reporting of key

methodological items (i.e. randomization and blinding) and an increase in treatment effect sizes.[44,45] Lack of methodological reporting has been hypothesized as a key impediment to reproducibility and thus clinical translation.[46] In our review, less than 10% of included studies adequately described the randomization of animals used in experiments, while less than 30% reporting blinding of experimenters. Results of our risk of bias assessment not only temper the findings of our NMA, but demonstrate the need for an improvement in methodological reporting in the field of preclinical stroke research. The Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines have recently been updated,[47] and authors of future studies are encouraged to consult and utilize these guidelines. Additionally, our assessment of construct validity raised some concerns. In the past, it has been demonstrated that poor construct validity affects reproducibility and therefore downstream clinical translation.[48,49] For example, the large proportion of acute ischemic stroke patients also suffer from hypertension and hyperglycemia, [32,33] yet only 10% of studies in our review utilized animals any comorbidities. Understanding interactions between potential therapies and common underlying comorbidities in the intended population is a crucial component to successful clinical translation. The issues with construct validity identified through our assessment provides additional trepidation when interpreting results of our NMA.

Additional limitations of our review specific to the design and conduct of preclinical studies must be acknowledged. These are important to note as they have not been well characterized in the network meta-analysis literature. First, the heterogeneity of stroke models used may limit the conclusions from our analysis. Studies included in our review used either the middle cerebral artery embolism or intraluminal suture models. These are two well-established animal models; however,

there are inherent differences between the two which have the potential to influence results.[50] We were unable to adjust our analysis for the type of model used given the differences among MCA embolism mechanisms. Second, differing effects of alteplase were noted, as a small number of studies produced larger infarcts with alteplase compared to those animals that stroke induced alone (i.e. no therapy). When performing an analysis to determine the relative benefit of a novel therapy (combination) compared to alteplase alone averaged across all eligible studies, this may overestimate the relative benefit of the novel therapy (combination). We aimed to mitigate this effect by adjusting for the timing of the alteplase administration; however, there are residual biases caused by the setting of the alteplase control group we could not adjust for. Third, our systematic review and NMA focused solely on the effects of therapies on infarct size (for feasibility). Future studies of this nature may which to investigate additional outcomes such as motor control and/or behavioural outcomes, as some potential novel therapeutics may mediate their effects through other mechanisms rather than strictly reducing infarct size. Lastly, although we believe a systematic review and NMA approach can help identify the most promising candidates for translation before translation can be considered, preclinical data must be reliable and generalizable. Many of the top-ranked interventions in our analysis were only investigated in a small handful of studies. More robust preclinical evidence may be needed before translational considerations can be made. When analyzing preclinical data, there is a particular interest in effect measures like normalized mean differences due to their ability to standardize and address heterogeneity. Therefore, there is a need to expand current NMA models to incorporate this effect measure and explore related extensions that can account for the influence of key covariates, such as time. These adaptations would enhance the analytical framework and enable a more comprehensive analysis of preclinical data in NMAs.

Our NMA results demonstrate the potential of several therapies that may provide additional therapeutic benefit when delivered in combination with alteplase. These findings provide evidence for potential future therapeutic targets in stroke research. In addition, our application of NMA methodology demonstrates the potential of this technique in a preclinical setting. We believe a systematic review and NMA approach in areas of high research output (e.g. stroke), can provide researchers with a comprehensive and unbiased tool to select candidates with the highest chances of success when attempting to translate from the bench to the bedside.

Tables and Figures

Table 1. Study Characteristics

Study Characteristics	Number of Studies (n, %)
Year of publication	
1990-1999	5 (3.6%)
2000-2009	21 (15.3%)
2010-2019	85 (62.0%)
2020-2021	26 (19.0%)
Country of corresponding author	
USA	59 (43.1%)
China	25 (18.2%)
Japan	14 (10.2%)
France	9 (6.6%)
Germany	9 (6.6%)
Canada	4 (2.9%)
Spain	3 (2.2%)
Other	14 (10.2%)
Sample size (median, range)	8 (5-144)
Species	
Rat	88 (64.2%)
Mouse	44 (32.1%)
Rabbit	5 (3.6%)
Sex	
Male	123 (89.8%)
Female	4 (2.9%)
Both	3 (2.2%)
Unclear	7 (5.1%)
Model	
MCA embolism	95 (69.3%)
Intraluminal suture	37 (27.0%)
Other	4 (2.9%)
Unclear	1 (0.7%)
Follow-up (from initiation of disease)	
≤1 day	69 (50.4%)
>1 day	68 (49.6%)

Table 2. Alteplase Only Characteristics

Intervention Characteristics	Number of Experiments (n, %)
Time to delivery Post-Stroke (hrs)	
<1	23 (12.8%)
1 – 1.9	27 (15.0%)
2 – 2.9	39 (21.7%)
3 – 3.9	22 (12.2%)
4 – 4.9	44 (24.4%)
5 – 5.9	2 (1.1%)
6	23 (12.8%)
Frequency of administration	
Single	180 (100%)
Multiple	0 (0%)
Dose (mg/kg)	
<1	18 (10.0%)
1 – 1.9	3 (1.7%)
2 – 2.9	5 (2.8%)
3 – 3.9	4 (2.2%)
4 – 4.9	0
5 – 5.9	12 (6.7%)
6 – 6.9	6 (3.3%)
7 – 7.9	0
8 – 8.9	1 (0.6%)
9 – 9.9	4 (2.2%)
10	123 (68.3%)
>10	3 (21.7%)
Unclear	1 (0.6%)
Route of administration	
IV	176 (97.8%)
IA	2 (1.1%)
Unclear	2 (1.1%)

Table 3. Intervention Characteristics

Intervention Characteristics	Number of Experiments (n, %)
Comparative therapy	
Hypothermia	9 (10.8%)
Activated protein-C	7 (8.4%)
Normobaric oxygen	6 (7.2%)
7E3 F(ab')$_2$	5 (6.0%)
Edaravone	5 (6.0%)
Recombinant Annexin-2	5 (6.0%)
Erythropoietin	4 (4.8%)
Minocycline	4 (4.8%)
Albumin	3 (3.6%)
Atorvastatin	3 (3.6%)
Granulocyte-colony stimulating factor	3 (3.6%)
NBQX	3 (3.6%)
Remote ischemic preconditioning	3 (3.6%)
Ultrasound	3 (3.6%)
AcSDKP	2 (2.4%)
AS605240	2 (2.4%)
Bortezomib	2 (2.4%)
Compound 21	2 (2.4%)
Estradiol	2 (2.4%)
Fasudil	2 (2.4%)
Microbubbles + Ultrasound	2 (2.4%)
Matrix metalloproteinase 10	2 (2.4%)
Mesenchymal stromal cells	2 (2.4%)
Post conditioning	2 (2.4%)
Timing of comparative therapy administration	
Before alteplase	27 (32.5%)
With alteplase	46 (55.4%)
After alteplase	10 (12.0%)
Time to delivery Post-Stroke (hrs)	
<1	15 (18.1%)
1 – 1.9	15 (18.1%)
2 – 2.9	10 (12.0%)
3 – 3.9	9 (10.8%)
4 – 4.9	9 (10.8%)
5 – 5.9	1 (1.2%)
6	6 (7.2%)
Multiple timepoints	18 (21.7%)
Frequency of administration	
Single	63 (75.9%)
Multiple	20 (24.1%)

Route of administration	
Intravenous	38 (45.8%)
Intraarticular	1 (1.2%)
Subcutaneous	6 (7.2%)
Intraperitoneal	6 (7.2%)
Other	31 (37.3%)
Not Reported	1 (1.2%)

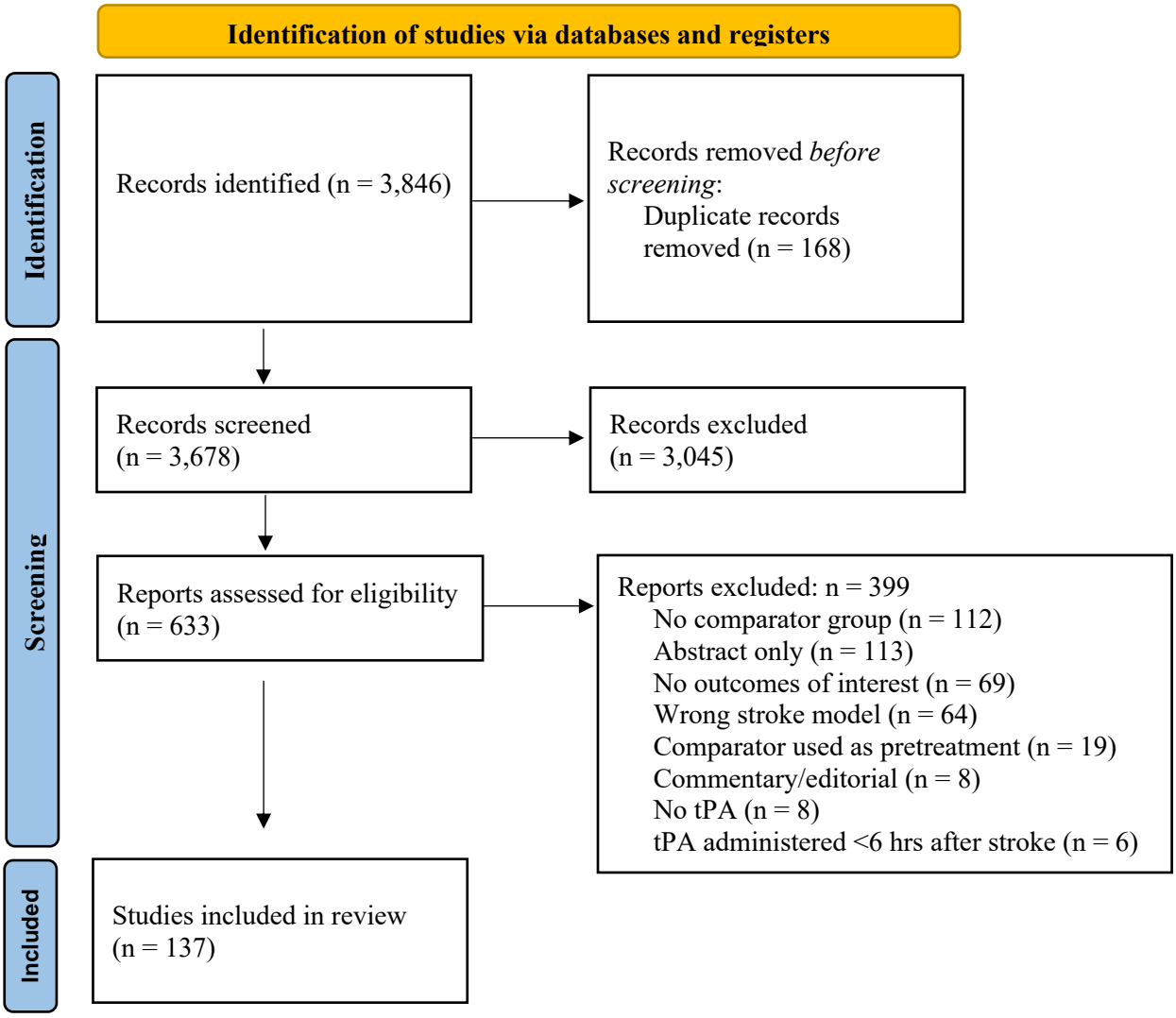

Figure 1. PRISMA flow diagram

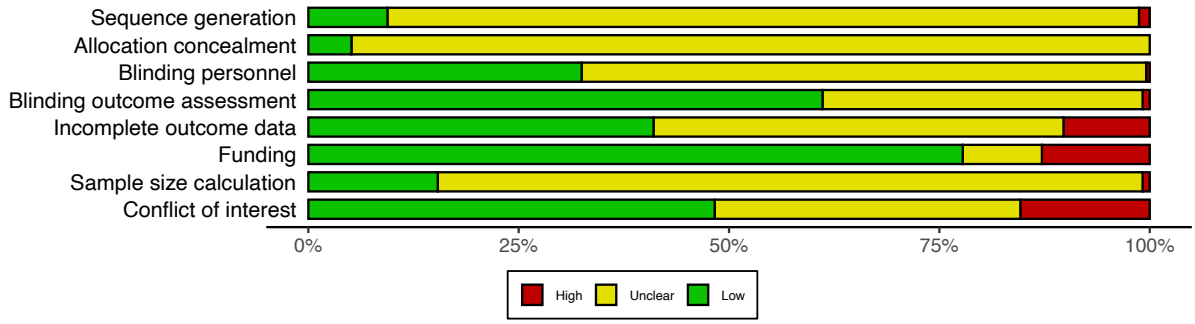

Figure 2. Risk of bias assessment

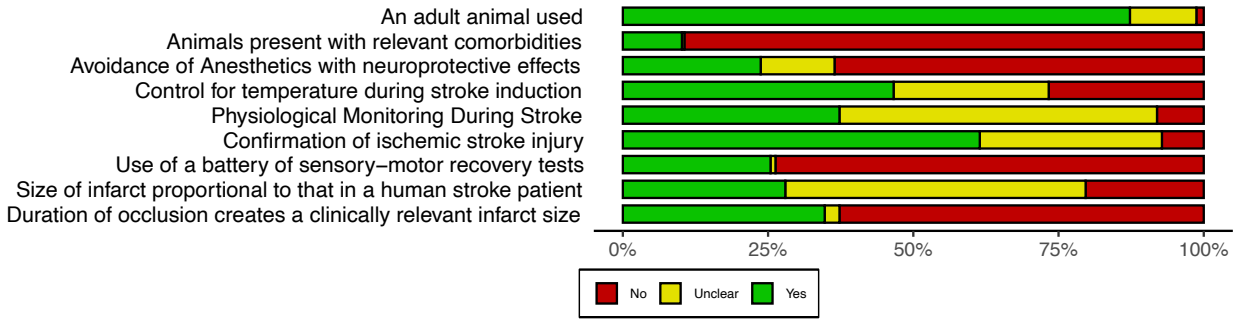

Figure 3. Construct validity assessment

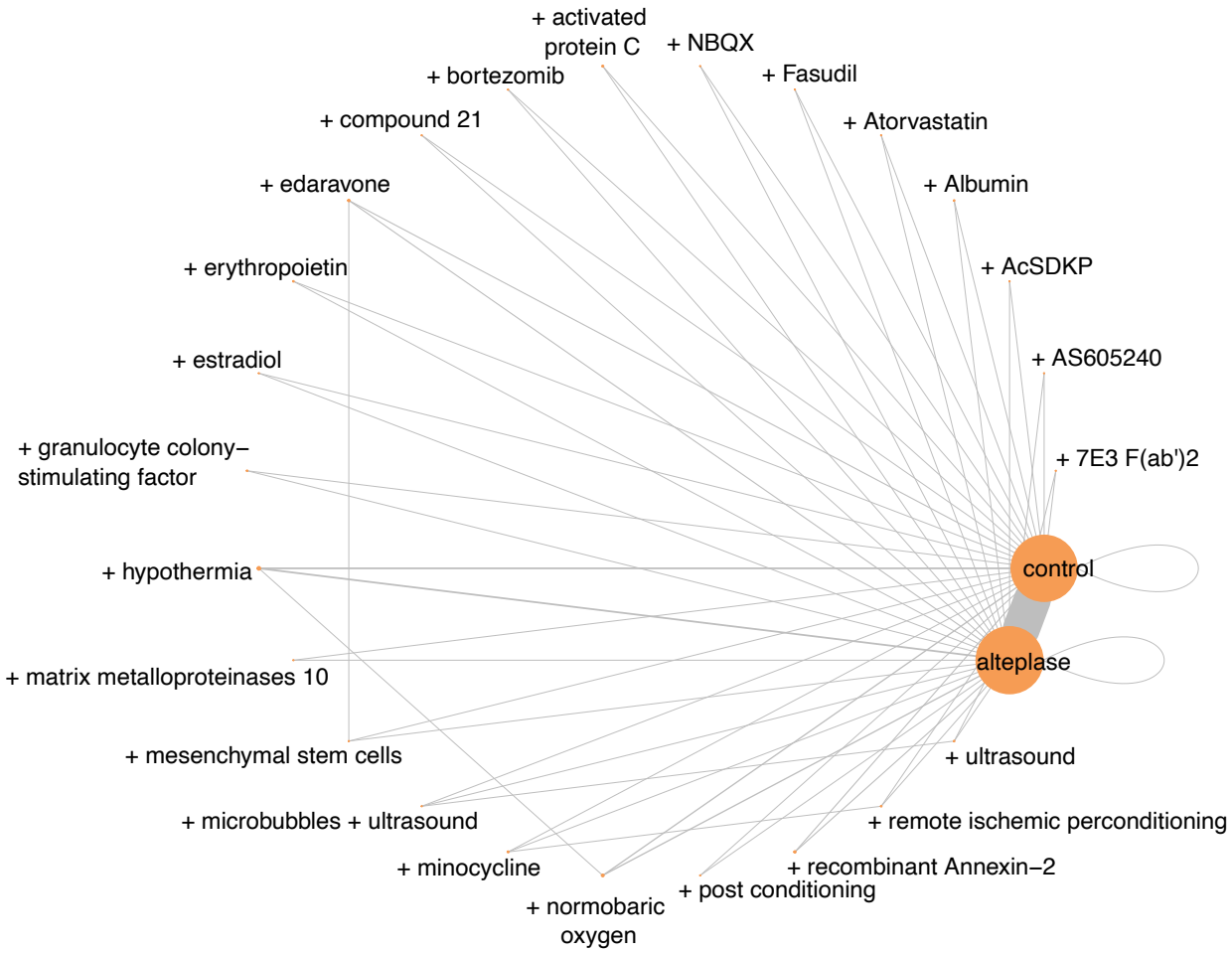

Figure 4. Network diagram for alteplase and alteplase plus interventions that had appeared in at least two studies. Nodes are proportionately sized to reflect the numbers of animals studied with each intervention, while edge width reflects the number of experiments for each pairwise comparison. NBQX: 2,3 dihydroxy-6-nitro-7-sulfamoyl-benzo-quinoxaline. AcSDKP: N-acetyl-seryl-aspartyl-lysyl-proline

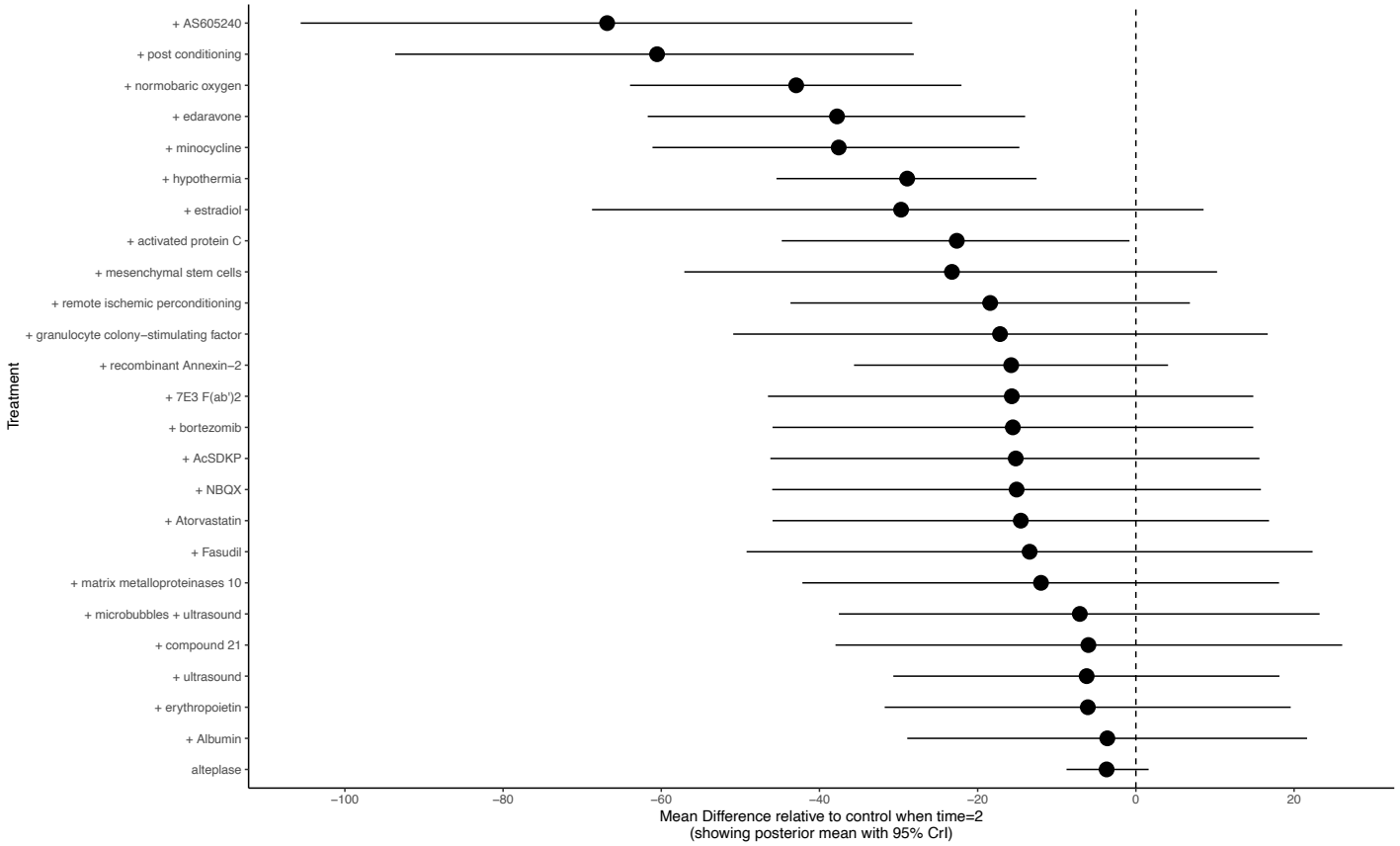

Figure 5. Forest plot of the mean difference for alteplase plus interventions compared to stroke-only control in terms of infarct size, based on the random effects consistency model adjusted for time of alteplase administration. The mean difference estimates assuming alteplase were administered 2 hours after stroke were displayed, and a negative mean difference indicates that alteplase plus intervention led to reduction in the infarct size compared with stroke-only control.

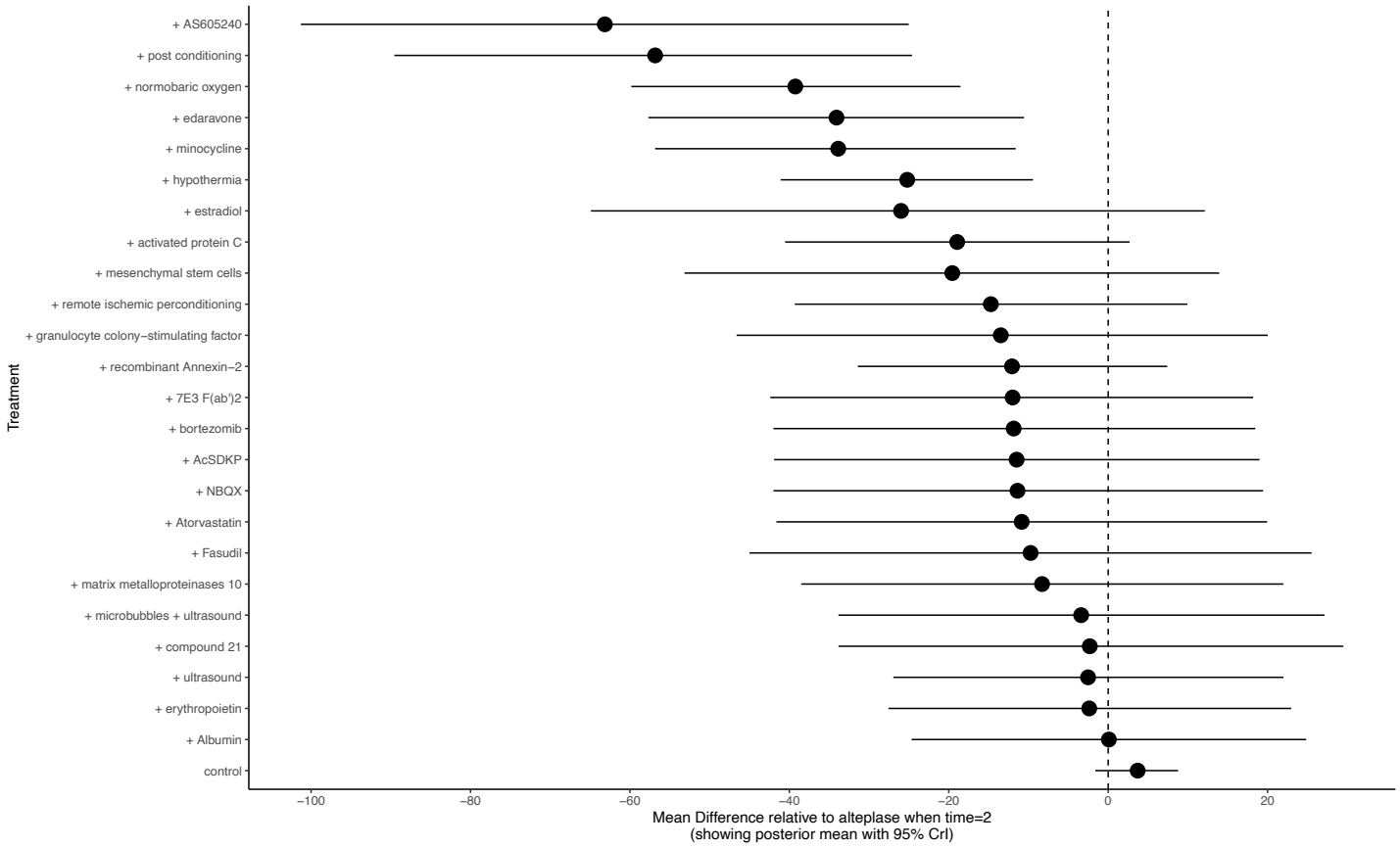

Figure 6. Forest plot of the mean difference for alteplase plus interventions compared with alteplase only in terms of infarct size, based on the random effects consistency model adjusted for time of alteplase administration. The mean difference estimates assuming alteplase were administered 2 hours after stroke were displayed, and a negative mean difference indicates that alteplase plus intervention led to reduction in the infarct size compared with alteplase only.

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Appendices

Appendix 1. PRISMA NMA CHECKLIST

Section/Topic	Item #	Checklist Item	Reported on Page #
TITLE			
Title	1	Identify the report as a systematic review *incorporating a network meta-analysis (or related form of meta-analysis).*	***1***
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: **Background:** main objectives **Methods:** data sources; study eligibility criteria, participants, and interventions; study appraisal; and *synthesis methods, such as network meta-analysis.* **Results:** number of studies and participants identified; summary estimates with corresponding confidence/credible intervals; *treatment rankings may also be discussed. Authors may choose to summarize pairwise comparisons against a chosen treatment included in their analyses for brevity.* **Discussion/Conclusions:** limitations; conclusions and implications of findings. **Other:** primary source of funding; systematic review registration number with registry name.	3
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known*, including mention of why a network meta-analysis has been conducted.*	***5***
Objectives	4	Provide an explicit statement of questions being addressed, with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	6
METHODS			
Protocol and registration	5	Indicate whether a review protocol exists and if and where it can be accessed (e.g., Web address); and, if available, provide registration information, including registration number.	6
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale. *Clearly describe eligible treatments included in the treatment network, and note whether any have been clustered or merged into the same node (with justification).*	***6***
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	7

Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	7
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	7
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	7-8
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	7-8
Geometry of the network	**S1**	Describe methods used to explore the geometry of the treatment network under study and potential biases related to it. This should include how the evidence base has been graphically summarized for presentation, and what characteristics were compiled and used to describe the evidence base to readers.	***9***
Risk of bias within individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	8
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means). *Also describe the use of additional summary measures assessed, such as treatment rankings and surface under the cumulative ranking curve (SUCRA) values, as well as modified approaches used to present summary findings from meta-analyses.*	9
Planned methods of analysis	14	Describe the methods of handling data and combining results of studies for each network meta-analysis. This should include, but not be limited to: • *Handling of multi-arm trials;* • *Selection of variance structure;* • *Selection of prior distributions in Bayesian analyses; and* • *Assessment of model fit.*	9
Assessment of Inconsistency	**S2**	Describe the statistical methods used to evaluate the agreement of direct and indirect evidence in the treatment network(s) studied. Describe efforts taken to address its presence when found.	9
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	**8**
Additional analyses	16	Describe methods of additional analyses if done, indicating which were pre-specified. This may include, but not be limited to, the following: • Sensitivity or subgroup analyses; • Meta-regression analyses; • *Alternative formulations of the treatment network; and*	***9-10***

		• *Use of alternative prior distributions for Bayesian analyses (if applicable).*	
RESULTS†			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	11
Presentation of network structure	**S3**	Provide a network graph of the included studies to enable visualization of the geometry of the treatment network.	***Fig 4***
Summary of network geometry	**S4**	Provide a brief overview of characteristics of the treatment network. This may include commentary on the abundance of trials and randomized patients for the different interventions and pairwise comparisons in the network, gaps of evidence in the treatment network, and potential biases reflected by the network structure.	***12***
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	11
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment.	12
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: 1) simple summary data for each intervention group, and 2) effect estimates and confidence intervals. *Modified approaches may be needed to deal with information from larger networks.*	***12-13***
Synthesis of results	21	Present results of each meta-analysis done, including confidence/credible intervals. *In larger networks, authors may focus on comparisons versus a particular comparator (e.g. placebo or standard care), with full findings presented in an appendix. League tables and forest plots may be considered to summarize pairwise comparisons.* If additional summary measures were explored (such as treatment rankings), these should also be presented.	***12-13***
Exploration for inconsistency	**S5**	Describe results from investigations of inconsistency. This may include such information as measures of model fit to compare consistency and inconsistency models, *P* values from statistical tests, or summary of inconsistency estimates from different parts of the treatment network.	***NR***
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies for the evidence base being studied.	12

Results of additional analyses	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression analyses*, alternative network geometries studied, alternative choice of prior distributions for Bayesian analyses,* and so forth).	***12***
DISCUSSION			
Summary of evidence	24	Summarize the main findings, including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy-makers).	13
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review level (e.g., incomplete retrieval of identified research, reporting bias). *Comment on the validity of the assumptions, such as transitivity and consistency. Comment on any concerns regarding network geometry (e.g., avoidance of certain comparisons).*	15
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	15
FUNDING			2
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review. This should also include information regarding whether funding has been received from manufacturers of treatments in the network and/or whether some of the authors are content experts with professional conflicts of interest that could affect use of treatments in the network.	

PICOS = population, intervention, comparators, outcomes, study design.
* Text in italics indicates wording specific to reporting of network meta-analyses that has been added to guidance from the PRISMA statement.
† Authors may wish to plan for use of appendices to present all relevant information in full detail for items in this section.

Appendix 2. Search Strategy

Databases searched: Embase, Medline, Web of Science, Current Contents, Biosis Previews, Pubmed, CAB abstracts, Society for Neuroscience

Embase Classic+Embase <1947 to 2022 January 10>
Ovid MEDLINE(R) ALL <1946 to January 10, 2022>

1 exp Stroke/ 409365
2 brain ischemia/ or ischemic attack, transient/ 243326
3 (brain isch?em* or cerebral isch?em*).kw. 17255
4 ((brain or cerebral) adj isch?em*).tw. 87835
5 stroke*.tw,kw. 742875
6 ((brain or cerebral) adj infarct*).tw. 57537
7 (brain infarct* or cerebral infarct*).kw. 8320
8 transient isch?em* attack*.tw,kw. 40372
9 or/1-8 985901
10 Tissue Plasminogen Activator/ 50776
11 ttpa.tw,kw. 268
12 (tpa or rtPA or Alteplase or Activase or Actilyse or rt PA).tw,kw. 73572
13 ((t or tissue) adj2 plasminogen activator).tw. 43805
14 (t plasminogen activator or tissue plasminogen activator).kw. 2944
15 or/10-14 114768
16 9 and 15 30994
17 (animals or animal or mice or mus or mouse or murine or woodmouse or rats or rat or murinae or muridae or cottonrat or cottonrats or hamster or hamsters or cricetinae or rodentia or rodent or rodents or pigs or pig or swine or swines or piglets or piglet or boar or boars or "sus scrofa" or ferrets or ferret or polecat or polecats or "mustela putorius" or "guinea pigs" or "guinea pig" or cavia or callithrix or marmoset or marmosets or cebuella or hapale or octodon or chinchilla or chinchillas or gerbillinae or gerbil or gerbils or jird or jirds or merione or meriones or rabbits or rabbit or hares or hare or diptera or flies or fly or dipteral or drosphila or drosophilidae or cats or cat or carus or felis or nematoda or nematode or nematoda or nematode or nematodes or sipunculida or dogs or dog or canine or canines or canis or sheep or sheeps or mouflon or mouflons or ovis or goats or goat or capra or capras or rupicapra or chamois or haplorhini or monkey or monkeys or anthropoidea or anthropoids or saguinus or tamarin or tamarins or leontopithecus or hominidae or ape or apes or pan or paniscus or "pan paniscus" or bonobo or bonobos or troglodytes or "pan troglodytes" or gibbon or gibbons or siamang or siamangs or nomascus or symphalangus or chimpanzee or chimpanzees or prosimians or "bush baby" or prosimian or bush babies or galagos or galago or pongidae or gorilla or gorillas or pongo or pygmaeus or "pongo pygmaeus" or orangutans or pygmaeus or lemur or lemurs or lemuridae or horse or horses or pongo or equus or cow or calf or bull or chicken or chickens or gallus or quail or bird or birds or quails or poultry or poultries or fowl or fowls or reptile or reptilia or reptiles or snakes or snake or lizard or lizards

or alligator or alligators or crocodile or crocodiles or turtle or turtles or amphibian or amphibians or amphibia or frog or frogs or bombina or salientia or toad or toads or "epidalea calamita" or salamander or salamanders or eel or eels or fish or fishes or pisces or catfish or catfishes or siluriformes or arius or heteropneustes or sheatfish or perch or perches or percidae or perca or trout or trouts or char or chars or salvelinus or "fathead minnow" or minnow or cyprinidae or carps or carp or zebrafish or zebrafishes or goldfish or goldfishes or guppy or guppies or chub or chubs or tinca or barbels or barbus or pimephales or promelas or "poecilia reticulata" or mullet or mullets or seahorse or seahorses or mugil curema or atlantic cod or shark or sharks or catshark or anguilla or salmonid or salmonids or whitefish or whitefishes or salmon or salmons or sole or solea or "sea lamprey" or lamprey or lampreys or pumpkinseed or sunfish or sunfishes or tilapia or tilapias or turbot or turbots or flatfish or flatfishes or sciuridae or squirrel or squirrels or chipmunk or chipmunks or suslik or susliks or vole or voles or lemming or lemmings or muskrat or muskrats or lemmus or otter or otters or marten or martens or martes or weasel or badger or badgers or ermine or mink or minks or sable or sables or gulo or gulos or wolverine or wolverines or minks or mustela or llama or llamas or alpaca or alpacas or camelid or camelids or guanaco or guanacos or chiroptera or chiropteras or bat or bats or fox or foxes or iguana or iguanas or xenopus laevis or parakeet or parakeets or parrot or parrots or donkey or donkeys or mule or mules or zebra or zebras or shrew or shrews or bison or bisons or buffalo or buffaloes or deer or deers or bear or bears or panda or pandas or "wild hog" or "wild boar" or fitchew or fitch or beaver or beavers or jerboa or jerboas or capybara or capybaras).tw. 11632034

18 exp "animal experimentation"/ or exp "models, animal"/ or exp "invertebrates"/ or "Animals"/ or exp "animal population groups"/ or "chordata"/ or exp "chordata, nonvertebrate"/ or "vertebrates"/ or exp "amphibians"/ or exp "birds"/ or exp "fishes"/ or exp "reptiles"/ or "mammals"/ or "primates"/ or exp "artiodactyla"/ or exp "carnivora"/ or exp "cetacea"/ or exp "chiroptera"/ or exp "elephants"/ or exp "hyraxes"/ or exp "insectivora"/ or exp "lagomorpha"/ or exp "marsupialia"/ or exp "monotremata"/ or exp "perissodactyla"/ or exp "rodentia"/ or exp "scandentia"/ or exp "sirenia"/ or exp "xenarthra"/ or "haplorhini"/ or exp "strepsirhini"/ or exp "platyrrhini"/ or exp "tarsii"/ or "catarrhini"/ or exp "cercopithecidae"/ or exp "hylobatidae"/ or "hominidae"/ or exp "gorilla gorilla"/ or exp "pan paniscus"/ or exp "pan troglodytes"/ or exp "pongo pygmaeus"/ 37531274

19 17 or 18 38968550

20 16 and 19 20972

21 20 use medall 1595

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23 exp *cerebrovascular accident/ 216234

24 *brain ischemia/ or *transient ischemic attack/ 151440

25 ((brain or cerebral) adj isch?em*).tw. 87835

26 transient isch?em* attack*.tw. 39662

27 stroke*.tw. 726796

28 ((brain or cerebral) adj infarct*).tw. 57537

29 or/23-28 874762

30 *tissue plasminogen activator/ or *Activase/ 30073

31 ttpa.tw.264

32 (tpa or rtPA or Alteplase or Activase or Actilyse or rt PA).tw. 72886

33 ((t or tissue) adj2 plasminogen activator).tw. 43805
34 or/30-33 102882
35 29 and 34 26580
36 exp animal experiment/ or exp animal model/ or exp experimental animal/ or exp transgenic animal/ or exp male animal/ or exp female animal/ or exp juvenile animal/ or animal/ or chordata/ or vertebrate/ or tetrapod/ or exp fish/ or amniote/ or exp amphibia/ or mammal/ or exp reptile/ or exp sauropsid/ or therian/ or exp monotremate/ or placental mammals/ or exp marsupial/ or Euarchontoglires/ or exp Afrotheria/ or exp Boreoeutheria/ or exp Laurasiatheria/ or exp Xenarthra/ or primate/ or exp Dermoptera/ or exp Glires/ or exp Scandentia/ or Haplorhini/ or exp prosimian/ or simian/ or exp tarsiiform/ or Catarrhini/ or exp Platyrrhini/ or ape/ or exp Cercopithecidae/ or hominid/ or exp hylobatidae/ or exp chimpanzee/ or exp gorilla/ or exp orang utan/ 14226388
37 (animal or animals or pisces or fish or fishes or catfish or catfishes or sheatfish or silurus or arius or heteropneustes or clarias or gariepinus or fathead minnow or fathead minnows or pimephales or promelas or cichlidae or trout or trouts or char or chars or salvelinus or salmo or oncorhynchus or guppy or guppies or millionfish or poecilia or goldfish or goldfishes or carassius or auratus or mullet or mullets or mugil or curema or shark or sharks or cod or cods or gadus or morhua or carp or carps or cyprinus or carpio or killifish or eel or eels or anguilla or zander or sander or lucioperca or stizostedion or turbot or turbots or psetta or flatfish or flatfishes or plaice or pleuronectes or platessa or tilapia or tilapias or oreochromis or sarotherodon or common sole or dover sole or solea or zebrafish or zebrafishes or danio or rerio or seabass or dicentrarchus or labrax or morone or lamprey or lampreys or petromyzon or pumpkinseed or pumpkinseeds or lepomis or gibbosus or herring or clupea or harengus or amphibia or amphibian or amphibians or anura or salientia or frog or frogs or rana or toad or toads or bufo or xenopus or laevis or bombina or epidalea or calamita or salamander or salamanders or newt or newts or triturus or reptilia or reptile or reptiles or bearded dragon or pogona or vitticeps or iguana or iguanas or lizard or lizards or anguis fragilis or turtle or turtles or snakes or snake or aves or bird or birds or quail or quails or coturnix or bobwhite or colinus or virginianus or poultry or poultries or fowl or fowls or chicken or chickens or gallus or zebra finch or taeniopygia or guttata or canary).tw. 3899801
38 (canaries or serinus or canaria or parakeet or parakeets or grasskeet or parrot or parrots or psittacine or psittacines or shelduck or tadorna or goose or geese or branta or leucopsis or woodlark or lullula or flycatcher or ficedula or hypoleuca or dove or doves or geopelia or cuneata or duck or ducks or greylag or graylag or anser or harrier or circus pygargus or red knot or great knot or calidris or canutus or godwit or limosa or lapponica or meleagris or gallopavo or jackdaw or corvus or monedula or ruff or philomachus or pugnax or lapwing or peewit or plover or vanellus or swan or cygnus or columbianus or bewickii or gull or chroicocephalus or ridibundus or albifrons or great tit or parus or aythya or fuligula or streptopelia or risoria or spoonbill or platalea or leucorodia or blackbird or turdus or merula or blue tit or cyanistes or pigeon or pigeons or columba or pintail or anas or starling or sturnus or owl or athene noctua or pochard or ferina or cockatiel or nymphicus or hollandicus or skylark or alauda or tern or sterna or teal or crecca or oystercatcher or haematopus or ostralegus or shrew or shrews or sorex or araneus or crocidura or russula or european mole or talpa or chiroptera or bat or bats or eptesicus or serotinus or myotis or dasycneme or daubentonii).tw. 182728
39 (pipistrelle or pipistrellus or cat or cats or felis or catus or feline or dog or dogs or canis or canine or canines or otter or otters or lutra or badger or badgers or meles or fitchew or fitch or foumart or

foulmart or ferrets or ferret or polecat or polecats or mustela or putorius or weasel or weasels or fox or foxes or vulpes or common seal or phoca or vitulina or grey seal or halichoerus or horse or horses or equus or equine or equidae or donkey or donkeys or mule or mules or pig or pigs or swine or swines or hog or hogs or boar or boars or porcine or piglet or piglets or sus or scrofa or llama or llamas or lama or glama or deer or deers or cervus or elaphus or cow or cows or bos taurus or bos indicus or bovine or bull or bulls or cattle or bison or bisons or sheep or sheeps or ovis aries or ovine or lamb or lambs or mouflon or mouflons or goat or goats or capra or caprine or chamois or rupicapra or leporidae or lagomorpha or lagomorph or rabbit or rabbits or oryctolagus).tw. 3282443

40 (cuniculus or laprine or hares or lepus or rodentia or rodent or rodents or murinae or mouse or mice or mus or musculus or murine or woodmouse or apodemus or rat or rats or rattus or norvegicus or guinea pig or guinea pigs or cavia or porcellus or hamster or hamsters or mesocricetus or cricetulus or cricetus or gerbil or gerbils or jird or jirds or meriones or unguiculatus or jerboa or jerboas or jaculus or chinchilla or chinchillas or beaver or beavers or castor fiber or castor canadensis or sciuridae or squirrel or squirrels or sciurus or chipmunk or chipmunks or marmot or marmots or marmota or suslik or susliks or spermophilus or cynomys or cottonrat or cottonrats or sigmodon or vole or voles or microtus or myodes or glareolus or primate or primates or prosimian or prosimians or lemur or lemurs or lemuridae or loris or bush baby or bush babies or bushbaby or bushbabies or galago or galagos or anthropoidea or anthropoids or simian or simians).tw. 6982019

41 (monkey or monkeys or marmoset or marmosets or callithrix or cebuella or tamarin or tamarins or saguinus or leontopithecus or squirrel monkey or squirrel monkeys or saimiri or night monkey or night monkeys or owl monkey or owl monkeys or douroucoulis or aotus or spider monkey or spider monkeys or ateles or baboon or baboons or papio or rhesus monkey or macaque or macaca or mulatta or cynomolgus or fascicularis or green monkey or green monkeys or chlorocebus or vervet or vervets or pygerythrus or hominoidea or ape or apes or hylobatidae or gibbon or gibbons or siamang or siamangs or nomascus or symphalangus or hominidae or orangutan or orangutans or pongo or chimpanzee or chimpanzees or pan troglodytes or bonobo or bonobos or pan paniscus or gorilla or gorillas or troglodytes).tw.309081

42 36 or 37 or 38 or 39 or 40 or 41 16349741

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Appendix 3. Construct Validity Assessment

Question	**Responses**
Was an adult animal used? Rats: ≥ 6 weeks Mice: ≥ 8 weeks Rabbit: ≥ 6 months Sheep: ≥ 38 weeks Dog: ≥ 6 months Cats: ≥ 6 months Minipig: ≥6 months Swine: ≥ 6 months Monkey (Macaques): ≥ 4 years	Yes: Age was explicitly reported Yes: Study only mentions “adult” No: Age was explicitly stated but is under the standard “adult age” Unclear: Age was not reported Unclear: Age and weight unreported (but not labelled as a neonate)
Animals Present with Comorbidities Commonly Associated with Ischemic Stroke?	Yes (Text) No Unclear
Avoidance of Anesthetics with Neuroprotective Effects (i.e. Ketamine)	Yes (Text) No (Text) Unsure Not Reported
Control of temperature during stroke induction	Yes (Text) No (Text) Unsure Not Reported
Physiological Monitoring During Stroke *If yes, indicate which parameters were monitored*	Yes (Text) No Unsure Not Reported
Was the ischemic stroke injury confirmed via laser Doppler or perfusion imaging?	Yes No Not Reported Unclear
Was there use of a battery of sensory-motor recovery tests? These tests include: 1. Walking Tasks (e.g. Beam, Grid Walking or Ladder Tests) 2. Forelimb Asymmetry Tests (e.g. cylinder tests) 3. Skilled Reaching Tests (e.g. Staircase or Single Pellet Reaching Task)	Yes: Multiple tests were used No: Only one test was used No: No sensory motor recovery tests were used Unclear

4. Adhesive Removal Test 5. Neurological Severity Scores (mNSS) 6. Rotarod	
Was the size of infarct proportional to that seen in a human stroke patient?	Yes: Infarct size within reasonable limits (<40%) No: Infarct size was too large (>40%) Unclear: Infarct size was not reported Other (Text)
Did the duration of occlusion create a clinically relevant infarct size?	Yes: Duration of stroke was <90 min No: Duration of stroke was >/= 90 min No: Stroke model was permanent Unclear (Text)

Appendix 4. Additional NMA Details

Additional Table 1: Model fit statistics for the random effects (RE) consistency model adjusted for time of alteplase administration. RE consistency model and unrelated means model adjusting time for time (centred by 2 hours) were compared.

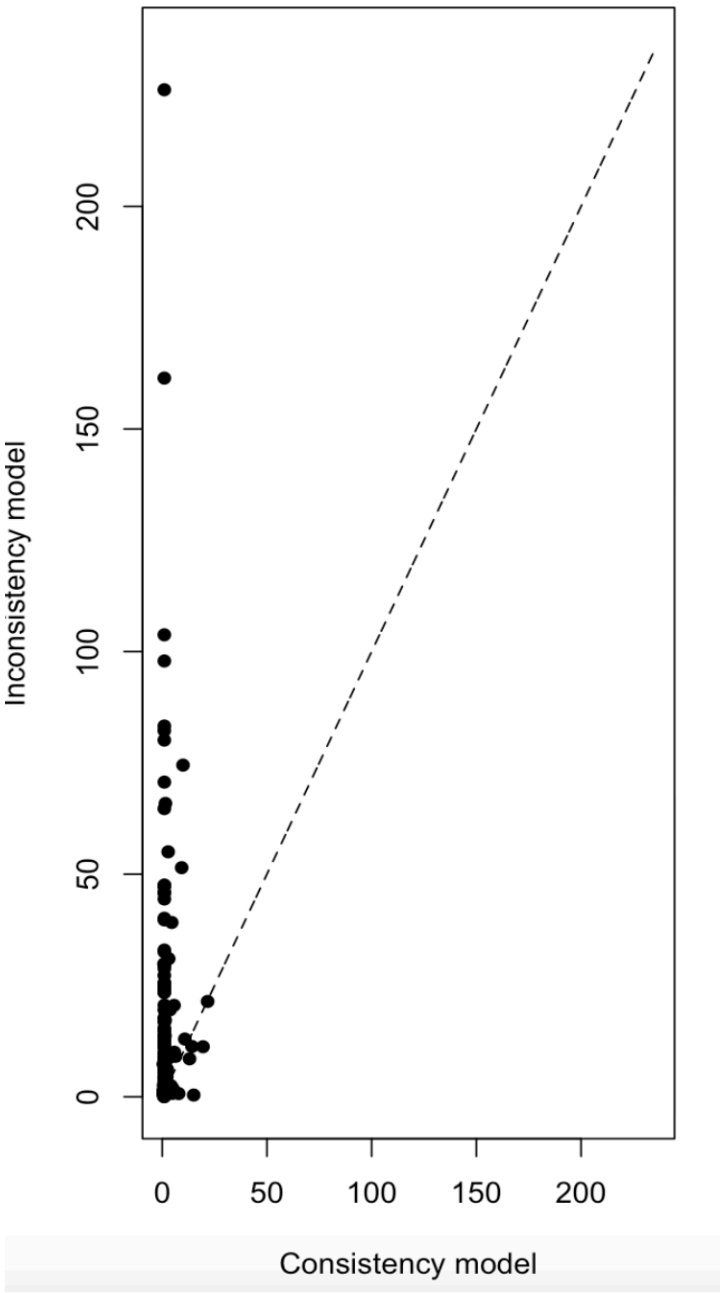
Model	Total Residual Deviance ǂ	Deviance Information Criteria
RE consistency model adjusted for time	548.7	918.5
RE unrelated means model adjusted for time	3251.9	3438.9

Note: ǂ For all NMA, 137 experiments and 325 experimental arms were included.

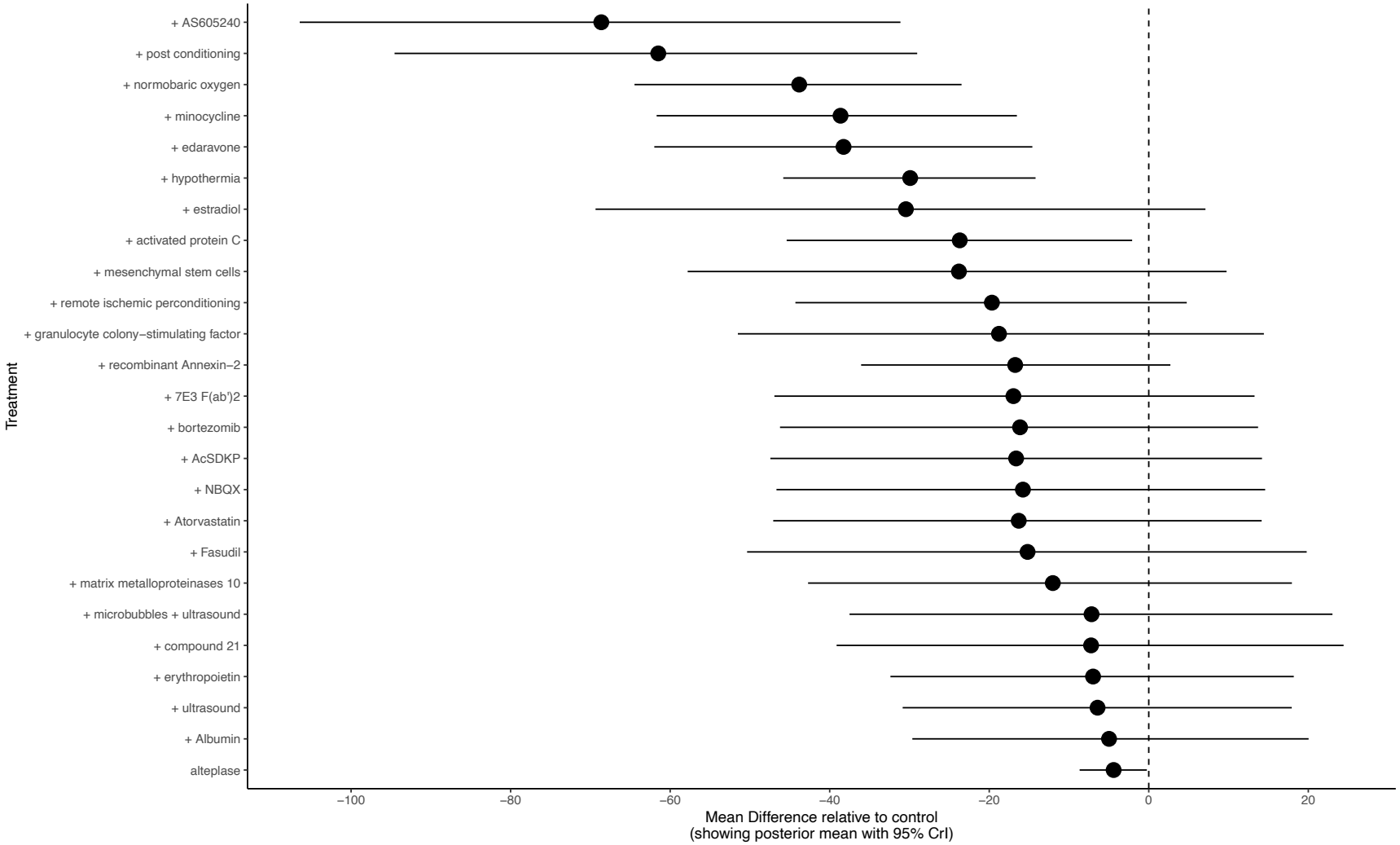
Additional Table 2. Mean SUCRA rank for each treatment based on random effects consistency model adjusted for time of alteplase administration. These secondary measures of effect from network meta-analysis are displayed. Larger values suggest better treatments. SUCRA: the Surface Under the Cumulative RAnking curve (SUCRA) value represents the surface underneath the cumulative ranking curve, which is the posterior probabilities for each drug to be among the n-best options.

	Mean SUCRA
alteplase + AS605240	54.44
alteplase + post conditioning	35.58
alteplase + normobaric oxygen	22.30
alteplase + minocycline	14.69
alteplase + edaravone	13.08
alteplase + hypothermia	10.33
alteplase + estradiol	5.86
alteplase + activated protein C	7.64
alteplase + mesenchymal stem cells	5.94
alteplase + remote ischemic preconditioning	6.03
alteplase + granulocyte colony-stimulating factor	5.03
alteplase + recombinant Annexin-2	7.41
alteplase + 7E3 F(ab')2"	5.40
alteplase + bortezomib	5.58
alteplase + AcSDKP	5.53
alteplase + NBQX	5.23

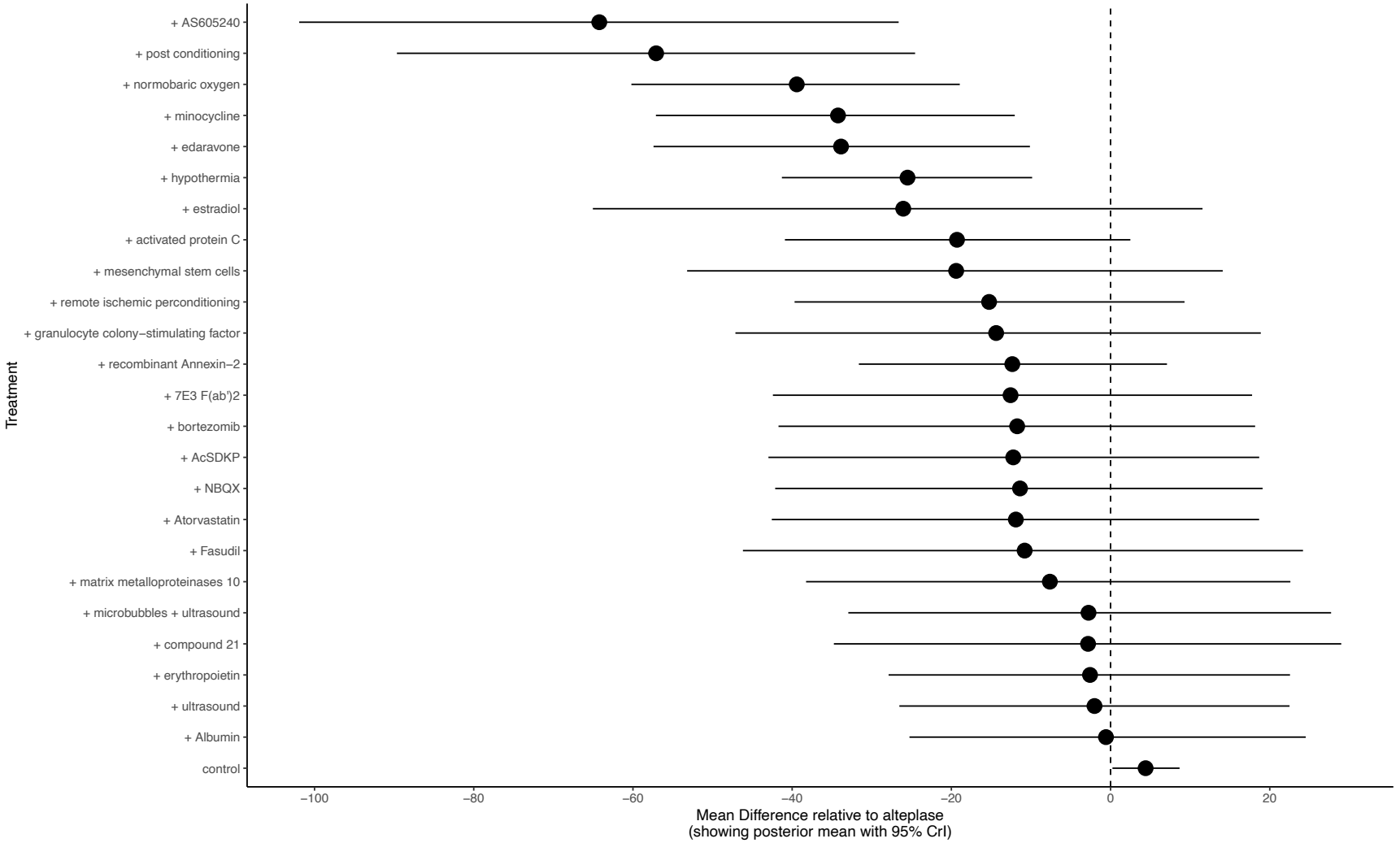
alteplase + Atorvastatin	5.19
alteplase + Fasudil	4.33
alteplase + matrix metalloproteinases 10	5.00
alteplase + microbubbles + ultrasound	5.03
alteplase + compound 21	4.87
alteplase + erythropoietin	5.77
alteplase + ultrasound	6.79
alteplase + albumin	8.52
alteplase	0.72
control: no treatment	2.65



Additional Figure 1. Posterior mean deviance contribution plot



Additional Figure 3. Forest plot of the mean difference for alteplase plus interventions compared with stroke-only control in terms of infarct size, based on the random effects consistency model without adjustment for time of alteplase administration. Negative mean difference indicates that alteplase plus intervention led to reduction in the infarct size compared with stroke-only control. NBQX: 2,3 dihydroxy-6-nitro-7-sulfamoyl-benzo(F)quinoxaline.



Additional Figure 4. Forest plot of the mean difference for alteplase plus interventions compared with alteplase only in terms of infarct size, based on the random effects consistency model without adjustment for time of alteplase administration. Negative mean difference indicates that alteplase plus intervention led to reduction in the infarct size compared with alteplase control. NBQX: 2,3 dihydroxy-6-nitro-7-sulfamoyl-benzo(F)quinoxaline.

Appendix 5. Additional Study Details

Additional Table 3. Individual Study Characteristics

First author	Year of publication	Funding	Country of Corresponding Author	Species	Strain	Sex	Weight Reported (Y/N)	Weight (range in grams)	Age reported (Y/N)	Age (enter range)	Stroke Model	Permanent or transient stroke?	Duration of follow up from initiation of disease state (days)
Jin R *	2019	Government	United States	Rat	SHR	Male	Y	270-310	Y	12-15	MCA Embolism	Transient	3
Dixon A *	2019	Government	United States	Rat	Sprague-Dawley	Male	Y	425-500	N		MCA Embolism	Transient	1
dela Pena I *	2019	Government	United States	Rat	Sprague-Dawley	Male	Y	200-250	Y	9-10	MCA Embolism	Transient	7
Yu Y *	2018	Not Reported	China	Rabbit	Japanese white	Male	Y	2500-3000	N		MCA Embolism	Transient	1
Yang B	2018	Government	United States	Rat	long-Evans	Male	Y	275-300	N		MCA Embolism	Transient	3
Wang Z *	2018	Government	United States	Rat	Sprague-Dawley	Male	Y	280-310	N		MCA Embolism	Transient	2
Shimada Y *	2018	Other	Japan	Mouse	C57BL/6	Male	Y	20-25	Y	10	MCA Clip	Transient	1.125
Schuhmann M *	2018	Foundation	Germany	Mouse	C57BL/6	Male	N		Y	10-14	MCA Clip	Transient	7
Navarro-Oviedo M *	2018	Government	Spain	Mouse	C57BL/6 J	Male	N		Y	12	MCA Embolism	Transient	3
Ma Y *	2018	Foundation	China	Rat	Sprague-Dawley	Male	Y	250-300	N		MCA Embolism	Transient	7

Li, M *	2018	Foundation	China	Rat	Sprague-Dawley	Male	Y	250-280	N		MCA Embolism	Transient	1
Li, M	2018	Foundation	China	Rat	Sprague-Dawley	Male	Y	250-300	N		Intraluminal Suture	Transient	1
Kikuchi, K *	2018	Government	Japan	Rat	Sprague-Dawley	Male	Y	290-310	Y	8	MCA Embolism	Transient	1
Karatas, H	2018	Government	Turkey	Mouse	C57B16	Unclear	N		N		Other	Transient	1
Jin, R *	2018	Government	United States	Rat	Sprague-Dawley	Male	Y	280-340	N		MCA Embolism	Transient	45
Ishrat, T *	2018	Government	United States	Rat	Wistar	Male	Y	320-340	Y	8-10	MCA Embolism	Transient	28
Huang, Y *	2018	Not Reported	Japan	Mouse	ICR	Male	Y	27-30	Y	7	MCA Clip	Transient	1
Hu, J *	2018	Government	United States	Mouse	CD1	Male	N		Y	8	Other	Transient	1
Guo, X *	2018	Government	China	Rat	Sprague-Dawley	Male	Y	250-280	N		MCA Embolism	Transient	1.25
El Amki, M *	2018	Academic Institution	France	Mouse	Swiss albino	Male	Y	25-32	N		MCA Embolism	Transient	1
Deuchar, G *	2018	Government	Scotland	Rat	Wistar	Male	Y	332.8 +/- 21.4	N		MCA Embolism	Transient	1
Deng, J *	2018	Government	China	Rat	Sprague-Dawley	Male	Y	200-230	N		Intraluminal Suture	Transient	1
Chen, S *	2018	Foundation	United States	Mouse	C57BL/6J	Male	Y	18-27	Y	7-11	MCA Embolism	Transient	1

Alawieh, A *	2018	Government	United States	Mouse	C57BL/6	Both	N		Y	12	MCA Embolism	Transient	15
Fan X *	2017	Government	United States	Rat	Wistar	Male	Y	280-300	N		MCA embolism	Transient	1
Mao L *	2017	Government	United States	Mouse	C57BL/6J	Male	N		Y	8-10	Intraluminal suture	Transient	21
Zhang B *	2017	Government	China	Mouse	C57BL/6J	Male	Y	23-26	Y	8	Intraluminal suture	Transient	1
Cai L *	2017	Government	China	Rat	Sprague Dawley	Unclear	Y	300-350	N		MCA embolism	Transient	1
Zarisfi, M *	2017	Academic Institution	Iran	Rat	Wistar	Male	Y	300-350	N		MCA Embolism	Transient	2
Wei, C *	2017	Foundation	China	Mouse	CD1	Male	Y	22-30	Y	8	MCA Embolism	Transient	1
Roncal, C *	2017	Government	Spain	Mouse	C57BL/6J	Male	N		Y	16	MCA Embolism	Transient	1
Niego, B	2017	Government	Australia	Mouse	C57Bl/6	Male	Y	25	Y	8-12	Intraluminal Suture	Transient	1
Li, Q *	2017	Government	China	Rat	Sprague-Dawley	Male	Y	300-350	N		Intraluminal Suture	Transient	1
Haelewyn B *	2016	Government, Industry, Academic	France	Rat	Sprague-Dawley	Male	Y	250-275	N		MCA embolism	Transient	1
Jiang Y *	2016	Government	United States	Rat	Wistar	Male	Y	280-330	N		MCA embolism	Transient	28

Nakazaki M *	2016	Government	Japan	Rat	Sprague-Dawley	Male	Y	280-330	N		Intraluminal suture	Transient	28
Wang, R *	2016	Foundation	China	Rat	Sprague-Dawley	Male	Y	280 +/- 30	N		Intraluminal Suture	Transient	1.08
Kim EJ	2015	Government	South Korea	Rat	Sprague-Dawley	Male	Y	280-320	N		Intraluminal suture	Transient	1
Eidizadeh A *	2015	Government	Germany	Mouse	C57BL/6N	Male	Y	24.3-27.1	Y	8-11	Intraluminal suture	Transient	2
Tan Z	2015	Government	United States	Rat	Sprague Dawley	Female	N		Y	72-80	MCA embolism	Transient	1
Esmaeeli-Nadimi A *	2015	Academic institution	Iran	Rat	Wistar	Male	Y	200-250	N		MCA embolism	Transient	2
Cechmanek BK *	2015	Government	Canada	Mouse	C57BL/6	Male	Y	25-35	Y	12	Intraluminal suture	Transient	3
Culp WC	2015	Government	United States	Rabbit	New Zealand	Both	Y	3400-4700	N		MCA embolism	Transient	1
Chen HS	2015	Government	China	Rat	Sprague Dawley	Male	Y	260-290	N		Intraluminal suture	Transient	1
Andreou AP *	2015	Foundation	United Kingdom	Mouse	C57BL/6	Male	N		N		Intraluminal suture	Transient	2
Cai L	2015	Government	China	Rat	Sprague-Dawley	Unclear	Y	280-340	N		MCA embolism	Transient	1

Liang J *	2015	Government	China	Rat	Sprague Dawley	Male	Y	290-320	N		Intraluminal suture	Transient	0.29
Lapchak PA	2015	Government	United States	Rabbit	New Zealand	Male	Y	2200-2500	N		MCA embolism	Transient	2
Wyseure T	2015	Government	Belgium	Mouse	SWISS	Male	Y	30-35	N		MCA embolism	Transient	1
dela Pena IC *	2015	Government	United States	Rat	Sprague Dawley	Male	Y	200-250	Y	9-10	Intraluminal suture	Transient	1
Gleeson EM	2015	Government, Industry, Foundation	United Kingdom	Mouse	C57BL/6	Unclear	N		Y	16	MCA embolism	Transient	1
Hoda MN *	2014	Government	United States	Mouse	C57BL/6J	Male	N		Y	47-49	MCA embolism	Transient	2
Durand A	2014	Industry	France	Mouse	Swiss	Male	Y	25-30	N		MCA embolism	Transient	1
Zhu W	2014	Government	United States	Mouse	DR2-Tg	Male	Y	20.1-27.7	Y	8-12	Intraluminal suture	Transient	4
Tan Z	2014	Government	United States	Rat	Sprague-Dawley	Female	N		Y	72-80	MCA embolism	Transient	1
Kawamura K	2014	Government	Japan	Rat	Sprague-Dawley	Male	Y	250-300	N		MCA embolism	Transient	1
Gautier S	2014	Government	France	Rat	Spontaneously Hypertensive	Male	Y	270-320	Y	10	Intraluminal suture	Transient	3
Ding G	2014	Government	United States	Rat	Wistar	Male	Y	300-350	Y	8-12	MCA embolism	Transient	6

Zuo W	2014	Government	China	Rat	Sprague Dawley	Male	Y	300-320	N		MCA embolism	Transient	3
Houng AK	2014	Government	United States	Mouse	C57BL/6J	Male	Y	29-35	N		MCA embolism	Transient	0.25
Zhang L *	2014	Government	United States	Rat	Wistar	Male	Y	350-400	N		MCA embolism	Transient	7
Hoda MN *	2014	Government	United States	Mouse	C57BL/6J	Female	N		Y	18-22	MCA embolism	Transient	1
Wang X	2014	Government	United States	Rat	Wistar	Male	Y	280-330	N		MCA embolism	Transient	28
Won S	2014	Government	United States	Rat	Sprague Dawley	Male	Y	300-350	Y	12	Intraluminal suture	Transient	1
Lapchak PA	2013	Government	United States	Rabbit	New Zealand	Male	Y	2000-2500	N		MCA embolism	Transient	2
Cai A	2013	Industry	Germany	Mouse	C57BL/6	Unclear	N		Y	10-12	Intraluminal suture	Transient	1
Tang XN *	2013	Government	United States	Mouse	C57BL/6	Male	Y	25-30	N		Intraluminal suture	Transient	1
Ishrat T *	2013	Government	United States	Rat	Wistar	Male	Y	330-350	N		MCA embolism	Transient	2
Tan Z	2013	Government	United States	Rat	Sprague Dawley	Female	N		Y	72-80	MCA embolism	Transient	21
Wang Y *	2013	Government	United States	Rat	Spontaneously Hypertensive	Male	N		Y	9-10	MCA embolism	Transient	7
Campos M *	2013	Government	Spain	Rat	Spontaneously Hypertensive	Male	Y	300-325	N		MCA embolism	Transient	1

Tian FF *	2013	Government	Japan	Mouse	C57BL/6J	Male	Y	20-23	Y	8	Intraluminal suture	Transient	2
Goebel S	2013	Other	Germany	Mouse	C57BL/6J	Male	Y	21-29	Y	8-12	Intraluminal suture	Transient	3
Lapergue B *	2013	Government	France	Rat	Sprague Dawley	Male	Y	300-350	N		Intraluminal suture	Transient	1
Fan X *	2013	Government	United States	Rat	Wistar	Male	N		Y	14	MCA embolism	Transient	1
Campos F *	2013	Government	Ireland	Mouse	C57BL/6	Male	Y	25-30	N		MCA embolism	Transient	3
Wang L	2013	Government	China	Mouse	C57BL/6J	Male	Y	23-26	Y	10	Intraluminal suture	Transient	1
Fan X	2013	Government	United States	Rat	Wistar	Male	N		Y	14	MCA embolism	Transient	1
Simard JM	2012	Government	United States	Rat	Wistar	Male	Y	250-275	N		Other	Transient	2
Kallmunzer B *	2012	Foundation	Germany	Rat	Wistar	Male	Y	280-320	N		MCA embolism	Transient	1
Doeppner TR	2012	Foundation	Germany	Mouse	C57BL/6N	Male	N		N		Intraluminal suture	Transient	4
Tiebosch IACW	2012	Foundation	Netherlands	Rat	Wistar	Male	Y	351	N		MCA embolism	Transient	7
Hoda MN *	2012	Government, Industry, Academic	United States	Mouse	C57BL/6J	Male	N		Y	20	MCA embolism	Transient	2
Montagne A *	2012	Government	France	Mouse	Swiss	Male	Y	36-42	N		MCA embolism	Transient	2

Takamiya M *	2012	Government	Australia	Mouse	C57BL/6	Male	Y	24-27	Y	8	Intraluminal suture	Transient	2
Turner RJ *	2012	Government	Australia	Rat	Sprague Dawley	Male	Y	365-395	N		Intraluminal suture	Transient	7
Zhang L *	2012	Government	France	Rat	Wistar	Male	Y	450-700	Y	64-72	MCA embolism	Transient	7
Ishiguro M *	2012	Not reported	France	Mouse	ddY	Male	Y	22-28	Y	4	Intraluminal suture	Transient	7
David HN *	2012	Government, Industry, Academic	France	Rat	Sprague Dawley	Male	Y	250-275	N		MCA embolism	Transient	1
Lu HT *	2012	Government	China	Rat	Sprague Dawley	Male	Y	350-400	N		MCA embolism	Transient	14
Deguchi K *	2012	Government	Japan	Rat	Wistar	Male	Y	250-280	Y	12	Intraluminal suture	Transient	4
Jullienne A *	2011	Government	France	Mouse	Swiss	Male	Y	29-35	N		MCA embolism	Transient	1
Michalski D	2011	Academic institution	Germany	Rat	Wistar	Male	Y	316	N		MCA embolism	Transient	1
Krakovsky M *	2011	Not reported	Israel	Rat	Sprague Dawley	Male	Y	300-350	Y	12	MCA embolism	Transient	1
Won SJ	2011	Government	United States	Rat	Sprague Dawley	Male	Y	250-350	N		Other	Transient	3

Haelewyn B	2011	Government, Industry, Academic	France	Rat	Sprague Dawley	Male	Y	250-275	N		MCA embolism	Transient	1
Gakuba C	2011	Government	France	Mouse	Swiss	Male	Y	30-33	N		MCA embolism	Transient	1
Li M *	2011	Government	Italy	Rat	Wistar	Female	Y	250-280	N		Intraluminal suture	Transient	1
Shehadah A	2011	Government	United States	Rat	Wistar	Male	Y	350-450	N		MCA embolism	Transient	7
Tomasi S	2011	Government	Italy	Rat	Sprague Dawley	Male	Y	270-350	N		MCA embolism	Transient	1
Kuppers-Tiedt L *	2011	Academic institution	Germany	Rat	Wistar	Male	Y	295-355	N		MCA embolism	Transient	0.25
Culp WC *	2011	Government	United States	Rabbit	New Zealand	Unclear	Y	5200	N		MCA embolism	Transient	1
Macrez R *	2011	Government	France	Mouse	Swiss	Male	Y	28-30	N		MCA embolism	Transient	1
Walvick RP *	2011	Government	United States	Rat	Wistar	Male	Y	291-345	N		MCA embolism	Transient	0.15
Flores R *	2011	Government	United States	Rabbit	New Zealand	Both	Y	5200	N		MCA embolism	Transient	1
Kanazawa M *	2011	Government	Japan	Rat	Sprague Dawley	Male	Y	300-390	N		MCA embolism	Transient	1
Brown AT	2011	Government	United States	Rabbit	New Zealand	Unclear	Y	5200	N		MCA embolism	Transient	1
Kollmar R *	2010	Not reported	Germany	Rat	Wistar	Male	Y	280-320	N		MCA embolism	Transient	1

Ishiguro M *	2010	Academic institution	Japan	Mouse	ddY	Male	Y	22-28	Y	4	Intraluminal suture	Transient	7
Jia L *	2010	Government	United States	Rat	Wistar	Male	Y	350-450	N		MCA embolism	Transient	7
Hernandez-Guillamon M	2010	Government	Spain	Rat	Sprague Dawley	Male	Y	250-300	N		MCA embolism	Transient	1
Nedelmann M	2010	Foundation	Germany	Rat	Wistar	Male	Y	291-331	N		Intraluminal suture	Transient	1
Lapchak PA	2010	Government	United States	Rabbit	New Zealand	Male	Y	2000-2500	N		MCA embolism	Transient	1
Sun L	2010	Foundation	Germany	Rat	Spontaneously Hypertensive	Male	Y	300-350	N		MCA embolism	Transient	1
Zechariah A *	2010	Foundation	Germany	Mouse	C57BL/6J	Male	Y	20-25	N		Intraluminal suture	Transient	1
David HN *	2010	Government, Industry, Academic	France	Rat	Sprague Dawley	Male	Y	250-275	N		MCA embolism	Transient	2
Zhu H *	2010	Government	United States	Rat	Wistar	Male	Y	280-330	N		MCA embolism	Transient	1
Liu R *	2010	Government	United States	Rat	Sprague Dawley	Female	Y	250	N		MCA embolism	Transient	1
Lapchak PA	2010	Other	United States	Rabbit	New Zealand	Male	Y	2000-2500	N		MCA embolism	Transient	1
Noor R *	2010	Government, Industry, Foundation	Canada	Rat	Sprague Dawley	Male	Y	300-350	N		MCA embolism	Transient	1

Zhang L *	2010	Government	United States	Rat	Wistar	Male	N		Y	72-80	MCA embolism	Transient	7
Fujiwara N *	2009	Government	United States	Rat	Spontaneously Hypertensive	Male	N		N		MCA embolism	Transient	1
Michalski D	2009	Not reported	Germany	Rat	Wistar	Male	Y	277-396	N		MCA embolism	Transient	28
Zhang L *	2009	Government	United States	Rat	Wistar	Male	Y	350-450	N		MCA embolism	Transient	7
Machado LS *	2009	Government	United States	Rat	Wistar	Male	Y	270-300	N		Intraluminal suture	Transient	1
Liu W	2009	Government	United States	Rat	Sprague Dawley	Male	Y	290-325	N		Intraluminal suture	Transient	1
Tan Z	2009	Government	United States	Rat	Sprague Dawley	Female	N		Y	12-16	MCA embolism	Transient	1
Tang J *	2009	Not reported	China	Rat	Sprague Dawley	Male	Y	280-350	N		Intraluminal suture	Transient	1
Yamashita T *	2009	Government	Japan	Rat	Spontaneously Hypertensive	Male	Y	250-280	Y	11	Intraluminal suture	Transient	1.19
Yagi K *	2009	Industry	Japan	Rat	Wistar	Male	Y	250-280	N		Intraluminal suture	Transient	1
Lapchak PA	2009	Government	United States	Rabbit	New Zealand	Male	Y	2000-2500	N		MCA embolism	Transient	1
Henninger N *	2009	Academic institution	United States	Rat	Sprague Dawley	Male	Y	270-330	N		MCA embolism	Transient	1

Murata Y *	2008	Government	United States	Rat	Spontaneously Hypertensive	Male	N		N		MCA embolism	Transient	1
Wiegler K	2008	Government	Switzerland	Mouse	ICR-CD1	Male	Y	19-30	N		Intraluminal suture	Transient	2
Nedelmann M	2008	Government	Germany	Rat	Wistar	Male	Y	286-350	N		Intraluminal suture	Transient	1
Zhang RL	2008	Industry	United States	Rat	Wistar	Male	Y	375-400	N		MCA embolism	Transient	7
Copin JC	2008	Government	Switzerland	Rat	Sprague Dawley	Male	Y	275-350	N		MCA embolism	Transient	1
Schatlo B	2008	Government	United States	Rat	Sprague Dawley	Male	Y	300-400	N		Intraluminal suture	Transient	2
Li YD	2008	Government	China	Rat	Sprague Dawley	Male	Y	300-450	N		MCA embolism	Transient	14
Sayedi S	2007	Not reported	Iran	Rat	NR	Male	Y	200-250	N		MCA embolism	Not Reported	2
Zhang L	2007	Government	United States	Rat	Wistar	Male	Y	350-450	N		MCA embolism	Transient	7
Strbian D	2007	Academic institution, Foundation	Finland	Rat	Wistar	Male	Y	290-340	N		Intraluminal suture	Transient	1
Tanaka Y	2007	Not reported	Japan	Rat	Sprague Dawley	Male	N		Y	9	MCA embolism	Transient	1
Lapchak PA	2007	Government	United States	Rabbit	New Zealand	Male	Y	2200-2600	N		MCA embolism	Transient	1

Okubo S	2007	Government	Japan	Rat	Sprague Dawley	Male	Y	290-320	N		MCA embolism	Transient	1
Wilhelm-Schwenkmezger T	2007	Government	Germany	Rat	Wistar	Male	Y	307-415	N		MCA embolism	Transient	7
Romanos E	2007	Government	Spain	Rat	Sprague Dawley	Male	Y	306-350	N		MCA embolism	Transient	1
Cheng T *	2006	Government	United States	Rat	Wistar	Male	Y	350-450	N		MCA embolism	Transient	7
Armstead WM *	2006	Government	United States	Rat	Sprague Dawley	Unclear	Y	250	N		MCA embolism	Transient	1
de Lecinana MA	2006	Government	Spain	Rat	Long Evans	Male	Y	250-350	N		MCA embolism	Transient	3
Ding G *	2006	Government	United States	Rat	Wistar	Male	Y	300-400	N		MCA embolism	Transient	2
Omura T	2006	Not reported	Japan	Monkey	Cynomolgus	Male	Y	3000-5000	N		MCA embolism	Transient	1
Zhang L	2006	Government	United States	Rat	Wistar	Male	Y	350-450	N		MCA embolism	Transient	7
Lapchak PA	2006	Government	United States	Rabbit	New Zealand	Male	Y	2200-2600	N		MCA embolism	Transient	1
Kilic E *	2005	Foundation	Switzerland	Mouse	C57BL/6	Male	Y	21-26	N		Intraluminal suture	Transient	1
Zhang L *	2005	Government	United States	Rat	Wistar	Male	Y	350-450	N		MCA embolism	Transient	7
Zlokovic BV *	2005	Government	United States	Mouse	C57BL/6	Male	Y	28-32	N		MCA embolism	Transient	7
Wang CX	2005	Not reported	Canada	Rat	Wistar	Male	Y	300-350	N		MCA embolism	Transient	2

Kilic E	2005	Government	Switzerland	Mouse	C57BL/6J	Male	Y	21-26	N		Intraluminal suture	Transient	1
Lian L *	2005	Government	United States	Rat	Wistar	Male	Y	300-350	N		MCA embolism	Transient	2
Ding G	2005	Government	United States	Rat	Wistar	Male	Y	300-350	N		MCA embolism	Transient	2
Kilic E	2005	Government	Switzerland	Mouse	C57BL/6	Male	Y	20-25	N		Intraluminal suture	Transient	1
Liu D *	2004	Government	United States	Mouse	C57BL/6	Unclear	N		N		Intraluminal suture	Transient	1
Kollmar R *	2004	Not reported	Germany	Rat	Wistar	Male	Y	280-320	N		MCA embolism	Transient	1
Lapchak PA	2004	Government	United States	Rabbit	New Zealand	Male	N		N		MCA embolism	Transient	1
Zhang W *	2004	Government	Japan	Rat	Wistar	Male	Y	250-280	N		Intraluminal suture	Transient	7
Gautier S	2003	Academic institution	France	Rat	Spontaneously Hypertensive	Male	Y	270-320	N		Intraluminal suture	Transient	1
Rasmussen RS	2003	Industry	Denmark	Rat	Sprague Dawley	Male	Y	340-404	Y	8-12	MCA embolism	Transient	3
Lapchak PA	2003	Government	United States	Rabbit	New Zealand	Male	N		N		MCA embolism	Transient	1
Zhang L	2003	Government	United States	Rat	Wistar	Male	Y	320-400	N		MCA embolism	Transient	7
Zhang L *	2003	Government	United States	Rat	Wistar	Male	Y	300-350	N		MCA embolism	Transient	7
Yang Y	2003	Not reported	Canada	Rat	Wistar	Male	Y	250-350	N		MCA embolism	Transient	1

Suzuki M	2003	Not reported	Japan	Rat	Sprague Dawley	Male	Y	280-350	N		MCA embolism	Transient	1
Zhang Z	2002	Government	United States	Rat	Wistar	Male	Y	300-350	N		MCA embolism	Transient	1
Lapchak PA	2002	Government	United States	Rabbit	New Zealand	Male	Y	2000-3000	N		MCA embolism	Transient	2
Shuaib A *	2002	Not reported	Canada	Rat	Wistar	Male	Y	350-400	N		MCA embolism	Transient	3
Lapchak PA	2002	Government, Industry	United States	Rabbit	New Zealand	Male	Y	2000-3000	N		MCA embolism	Transient	2
Zhang L	2001	Other	United States	Rat	Wistar	Male	Y	320-380	N		MCA embolism	Transient	7
Morris DC	2001	Industry	United States	Rat	Wistar	Male	Y	320-380	N		MCA embolism	Transient	2
Lapchak PA *	2001	Government	United States	Rabbit	New Zealand	Male	Y	2000-3000	N		MCA embolism	Transient	2
Lapchak PA	2000	Government, Industry	United States	Rabbit	New Zealand	Male	Y	2000-3000	N		MCA embolism	Transient	2
Asahi M	2000	Government	United States	Rat	Spontaneously Hypertensive	Male	N		N		MCA embolism	Transient	1
Zhang RL *	1999	Government	United States	Rat	Wistar	Male	Y	300-350	N		MCA embolism	Transient	2
Zhang RL *	1999	Government, Industry	United States	Rat	Wistar	Male	N		N		MCA embolism	Transient	7
Hoffman P	1998	Not reported	France	Rat	Sprague Dawley	Male	Y	350-400	N		MCA embolism	Transient	1

Lekieffre D	1997	Not reported	France	Rat	Sprague Dawley	Male	Y	280-320	N		MCA embolism	Transient	1
Yenari MA	1997	Government, Industry, Foundation	United States	Rabbit	New Zealand White	Male	Y	2500-3000	N		MCA embolism	Transient	0.25
Gross CE	1997	Government, Industry	United States	Rabbit	New Zealand White	Both	Y	3000-3500	N		MCA embolism	Transient	0.33
Meden P	1996	Industry, Foundation	Denmark	Rat	Sprague Dawley	Male	Y	308-474	N		MCA embolism	Transient	2
Orozco J *	1995	Government	United States	Rabbit	New Zealand White	Unclear	Y	3000	N		MCA embolism	Transient	0.25
Bowes MP	1995	Not reported	United States	Rabbit	New Zealand White	Unclear	Y	2000-3000	N		MCA embolism	Transient	0.75
Meden P	1994	Foundation	Denmark	Rat	Sprague Dawley	Male	Y	300-400	N		MCA embolism	Transient	2
Meden P *	1993	Industry, Foundation	Denmark	Rat	Sprague Dawley	Male	Y	300-400	N		MCA embolism	Transient	3
Sereghy T	1993	Foundation	Czech Republic	Rat	Sprague Dawley	Male	Y	300-400	N		MCA embolism	Transient	2
Bowes MP	1993	Not reported	Unclear	Rabbit	New Zealand White	Unclear	Y	2000-3000	N		MCA embolism	Transient	1

Overgaard K *	1993	Industry, Foundation	Denmark	Rat	Sprague Dawley	Male	Y	300-400	N		MCA embolism	Transient	2
Carter LP	1992	Government	United States	Rabbit	New Zealand White	Both	Y	3000	N		MCA embolism	Transient	0.25
Overgaard K	1992	Foundation	Denmark	Rat	Sprague Dawley	Male	Y	300-400	N		MCA embolism	Transient	2
Zivin JA	1991	Government, Industry	United States	Rabbit	New Zealand White	Unclear	Y	2000-3000	N		MCA embolism	Transient	1
Zenych, A	2021	Academic Institution	France	Mouse	Swiss wild-type	Male	Y	35-45	Y	8-9	MCA Embolism	4	Zenych, A
Salman, M	2021	Government	United States	Mouse	C57Bl/6	Male	N		Y	8-12	MCA Embolism	1	Salman, M
Orset, C	2021	Government	Sweden	Mouse	C57 black/6J	Male	Y	20-30	N		MCA Embolism	1	Orset, C
Mohammadi, M	2021	Academic Institution	Iran	Rat	Wistar	Female	Y	200-250	N		MCA Embolism	2	Mohammadi, M
Liu, D	2021	Government	China	Mouse	C57BL/6	Male	Y	23-27	N		Intraluminal Suture	3	Liu, D
Li, C	2021	Government	United States	Rat	Wistar	Male	Y	350-400	N		MCA Embolism	7	Li, C
Kong, L	2021	Government	China	Rat	Sprague-Dawley	Male	Y	260-280	N		common carotid artery electrocoagulation thrombosis	1	Kong, L

Kong, L	2021	Government	China	Rat	Sprague–Dawley	Male	Y	260-280	N		common carotid artery thrombosis	7	Kong, L
Ismael, S	2021	Government	United States	Mouse	C57Bl/6	Male	Y	22-25	Y	8-10	Intraluminal Suture	1	Ismael, S
Igarashi, T	2021	Government	United States	Rat	Wistar	Male	Y	290-330	N		Intraluminal Suture	2	Igarashi, T
Fu, Y	2021	Government	China	Rat	Sprague Dawley	Unclear	Y	320-400	N		MCA Embolism	1	Fu, Y
Cheng, G	2021	Government	China	Rat	Wistar	Male	Y	300-350	Y	10-12	MCA Embolism	1	Cheng, G
Braun, T	2021	Academic Institution	Germany	Rat	Wistar	Male	Y	263-313	N		MCA Embolism	1	Braun, T
Zhang, X	2020	Government	China	Rat	Sprague-Dawley	Male	Y	300-340	N		MCA Embolism	1	Zhang, X
Zhang, L	2020	Government	China	Rat	Sprague Dawley	Male	Y	250-280	N		MCA Embolism	1.25	Zhang, L
Wu, D	2020	Government	China	Monkey	Rhesus monkeys (Macaca mulatta)	Male	Y	7000-10600	Y	364-572	MCA Embolism	30	Wu, D
Shi, K	2021	Government	China	Rat	Wistar	Both	N		N		MCA Embolism	28	Shi, K
Sasaki, R	2020	Government	Japan	Rat	SHR/Izm	Male	Y	280-310	Y	12	Intraluminal Suture	1	Sasaki, R

Pan, R	2020	Government	China	Rat	Sprague-Dawley	Male	Y	270-330	N		MCA Embolism	1	Pan, R
Mertens, J	2020	Government	Belgium	Rat	Sprague-Dawley	Male	Y	320-400	N		Intraluminal Suture	1	Mertens, J
Kuo, P	2020	Government	United States	Mouse	C57Bl/6	Male	N		Y	12-16	Intraluminal Suture	1	Kuo, P
Kuo, P	2020	Government	United States	Mouse	C57Bl/6	Male	N		Y	72-96	Intraluminal Suture	1	Kuo, P
Jin, R	2020	Government	United States	Rat	Sprague-Dawley	Male	Y	280-340	N		MCA Embolism	35	Jin, R
Hassanipour, M	2020	Academic Institution	Iran	Rat	Wistar	Male	Y	200-250	N		MCA Embolism	2	Hassanipour, M
Halder, S	2020	Government	Japan	Mouse	C57Bl/6J	Male	Y	20-24	Y	6	Intraluminal Suture	1	Halder, S
Dhanesha, N	2020	Government	United States	Mouse	C57Bl/6J	Male	N		Y	10-14	MCA Embolism	1	Dhanesha, N
Cai, L	2020	Not Reported	China	Rat	Sprague-Dawley	Unclear	N		N		MCA Embolism	1	Cai, L
Boese, A	2020	Government	United States	Mouse	C57Bl/6J	Male	N		Y	50-52	Intraluminal Suture	2	Boese, A
Alawieh, A	2020	Government	United States	Mouse	C57BL/6	Male	N		Y	12	Microembolic	30	Alawieh, A
Alawieh, A	2020	Government	United States	Mouse	C57Bl/6	Male	N		Y	12	Microembolic	3	Alawieh, A

Ahmad, S	2019	Academic Institution	United States	Mouse	C57Bl/6J	Male	Y	28-32	Y	20	MCA Embolism	2	Ahmad, S
Zheng, Y	2019	Government	United States	Mouse	C57Bl/6	Male	N		N		Intraluminal Suture	1	Zheng, Y
Wang, T	2019	Government	China	Rat	Sprague-Dawley	Male	Y	250-280	N		Intraluminal Suture	1	Wang, T
Wang, C	2019	Government	United States	Rat	Wistar	Male	Y	350-400	Y	8-12	MCA Embolism	7	Wang, C
Schuhmann, M	2019	Government	Germany	Mouse	C57Bl/6	Male	N		Y	10-12	Not Reported	1	Schuhmann, M
Luo, H	2019	Government	China	Mouse	C57Bl/6J	Male	Y	21-28	Y	8-10	Intraluminal Suture	1	Luo, H
Li, Y	2019	Government	China	Mouse	C57Bl/6	Male	Y	25-30	Y	8-12	Intraluminal Suture	1	Li, Y
Li, C	2019	Government	United States	Rat	Wistar	Male	N		Y	72	MCA Embolism	7	Li, C

* indicates inclusion in the NMA

Chapter 3: Systematic Review with a Panel of Patient Partners

Effect of CCR5 Antagonists on Stroke Recovery in Animal Stroke Models: A Systematic Review and Meta-Analysis

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Preface to Chapter 3

This chapter presents the results of a systematic review and meta-analysis co-designed with a panel of patient partners to assess the effect of CCR5 antagonists on stroke recovery in animal stroke models. The results evaluate the effect of CCR5 antagonist reported in preclinical stroke animal models. The patient engagement aspect is described in Chapter 4.

ABSTRACT

Background: C-C chemokine receptor type 5 (CCR5) antagonists have emerged as a promising therapeutic approach for stroke recovery patients. Despite their application in ongoing clinical trials, gaps remain in our understanding of the preclinical evidence supporting their use. To bridge this gap, we conducted a systematic review of preclinical studies that assessed the effects of CCR5 antagonists on motor and cognitive impairment in animal models of stroke.

Methods: In collaboration with a panel of individuals with lived experiences of stroke, we conducted a systematic search of MEDLINE, Web of Science, and Embase databases, to identify studies of animal models of focal ischemic and intracerebral hemorrhagic stroke that administered a CCR5 antagonist drug. Integrated patient priorities included drug therapies for the chronic phase of stroke, consideration of comorbidities, physical therapy along with drug administration, and spasticity. All studies were screened, and data were extracted in duplicate to ensure thoroughness and accuracy. Random-effects meta-analyses focusing on motor and cognitive outcomes and infarct volumes were conducted. Subgroup analysis was performed for infarct volume in experiments that administered the drug pre- and post-stroke induction. We utilized the Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE) tool and the Stroke Treatment Academic Industry Roundtable (STAIR) XI Consolidated Recommendations to appraise the risk of bias and ascertain the certainty of evidence with clinical relevance.

Results: We screened 166 articles, and five met our eligibility criteria. In all tests, behavioural outcomes demonstrated an overall improvement with CCR5 antagonist drug administration. Similarly, the pooled analysis for infarct volume also favoured CCR5 antagonist drug administration. Subgroup analysis for post-stroke and pre-stroke drug administration also exhibited beneficial outcomes with the use of CCR5 antagonists, and demonstrated that the two subgroups were not significantly different. Overall, a high risk of bias was observed in all included studies. When applying STAIR XI, 9/18 (50%) of the recommendations were satisfied by the overall evidence from the included preclinical studies. The a priori patient important design elements of assessing the effects of extended drug administration, consideration of physical therapy in tandem with drug administration, the inclusion of stroke-relevant comorbidities, spasticity, and motor and cognitive outcomes were either not reported or reported in a very limited manner.

Discussion: CCR5 antagonists have been showing promising signs of aiding stroke recovery, with notable improvements in behavioural outcomes and a decrease in infarct volumes in animal models. The findings of the STAIR XI analysis and the included studies suggest the possibility of future clinical relevance. However, it emphasizes the necessity for further robust and transparent preclinical studies that closely align with clinical trial protocols. Additionally, future studies are encouraged to prioritize patient outcomes, ensuring that the benefits of the research

are directly applicable and valuable to those receiving treatment. This approach is an opportunity to refine research methods and enhance the effectiveness of potential treatments.

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Keywords: Stroke; Animals; CCR5 antagonists; maraviroc; Patient Engagement; People with Lived Experience; Preclinical; Systematic Review; Stroke Treatment Academic Industry Roundtable; STAIR; CAMAROS

INTRODUCTION

Stroke is a significant health concern, leading to new motor and cognitive impairments for more than 12 million people every year worldwide.[1,2] C-C chemokine receptor type 5 (CCR5) antagonist, a class of antiretroviral drugs, show promise in blocking the CCR5 receptor, which is believed to play a role in the inflammatory processes that exacerbate stroke-induced damage.[3] Given the burgeoning interest in such antagonists, it is necessary to examine the existing evidence to better understand their potential role in stroke recovery.

Although ongoing clinical trials like The Canadian Maraviroc RCT To Augment Rehabilitation Outcomes After Stroke (CAMAROS) study (ClinicalTrials.gov Identifier: NCT04789616) are now investigating the use of CCR5 antagonists in individuals with stroke,[4-6] a comprehensive overview of preclinical evidence has not been conducted. To examine the evidence in support of a clinical trial, a preclinical systematic review of CCR5 antagonists is needed to thoroughly assess the available preclinical data and provide a comprehensive analysis of its quality and relevance. To address this knowledge gap, we conducted a systematic review and meta-analysis focused on preclinical studies using animal stroke models. Our primary research question evaluated the effects of CCR5 antagonists on motor and cognitive impairment in these models. We used the Stroke Treatment Academic Industry Roundtable (STAIR) XI Consolidated Recommendations[7] as a tool to assess the certainty of evidence derived from the included studies.

To foster a patient-centred approach throughout the review process, we collaborated with a panel of patients and caregivers with lived experience with stroke. This collaboration enabled us to

incorporate their perspectives and priorities from the study's inception. By incorporating patient and caregiver input, we sought to ensure that the systematic review addressed their specific concerns and needs, ultimately contributing to a more complete understanding of CCR5 antagonists in stroke recovery.

METHODS

The protocol for this review was developed and published on the International Prospective Register of Systematic Reviews (PROSPERO ID: CRD42023393438).[8] The final review was prepared in accordance with guidance from the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA; see Appendix 1).[9] A panel of patient partners was engaged in this study; thus, we completed a Guidance for Reporting the Involvement of Patients and the Public (GRIPP2)[10] checklist to summarize engagement activities and outcomes (Appendix 2).

Eligibility Criteria

Eligibility criteria for study inclusion were created *a priori* and defined according to five parameters: animal, model, intervention, comparator, outcomes, and study design (AMICOS). Predefining these parameters enabled an unbiased search, inclusion, and assessment of pertinent articles. The reasons for the exclusion criteria can be found in Appendix 3.

Animals

We included any preclinical *in vivo* animal models of adult stroke. Human studies, invertebrate animals, *in vitro*, *ex vivo*, and neonatal preclinical studies were excluded.

Model

All focal ischemic or intracerebral hemorrhagic stroke models were included, while animal models of global ischemia were excluded.

Intervention

Studies must have administered a CCR5 antagonist drug for inclusion (e.g. maraviroc, DAPTA, TAK-779). All routes, doses, timing, and frequency of administration were included. Studies in which a CCR5 antagonist drug was not administered to animals directly (i.e. CCR5 knockout) were excluded.

Comparator

All stroke-induced comparators that were vehicle-treated were included. Groups treated with a CCR5 antagonist without a stroke and control groups with a stroke, but without a vehicle administered were excluded.

Outcome

We included studies that reported on at least one of the following: behavioural tests, infarct size, mortality, adverse events, and spasticity.

Study Design and Publication Characteristics

We included controlled interventional studies (randomized, pseudo-randomized, or non-randomized) published as full journal articles. Abstracts, review articles, opinion-based letters/editorials, and unpublished grey literature were excluded. Studies were not excluded based on language or publication date.

Information Sources and Search Strategy

In collaboration with an information specialist with experience in preclinical systematic searches, a search strategy was developed and validated through the Peer Review of Electronic Search Strategies (PRESS).[11] To provide an accurate literature search, keywords related to the focus of

the systematic review were implemented, such as using precise vocabulary related to CCR5 antagonists (e.g. maraviroc). Additional validated search filters specific to identifying preclinical animal studies were applied, and modifications to search filters were made on a per-database basis. Three databases were searched, MEDLINE (OVID interface, including In-Process and Epub Ahead of Print), Web of Science, and Embase (OVID interface). The final update of the search of the databases was conducted from inception to October 20th, 2022. The full search strategy can be found in Appendix 4.

Selection Process

All identified citations were uploaded into DistillerSR® (Evidence Partners, Ottawa, Canada) and duplicate studies were removed. Title and abstract screening were performed independently and in duplicate using an accelerated screening method (one reviewer was required to include, and two reviewers were required to exclude). A calibration exercise was performed on the first 10 studies to refine the screening question prior to formally commencing the screening process. After initial screening for potentially relevant articles, full-text articles were retrieved and screened in duplicate. Any conflicts were resolved through discussion with an experienced third team member.

Data Collection Process

Two independent reviewers extracted relevant text data from included studies using a standardized and pilot-tested data extraction form created in DistillerSR® with iterative input from all members of the study team. Data presented in graphical format was extracted using Engauge Digitizer. For numerical data from graphs, one reviewer extracted, and another reviewer audited the extraction. For all other data, two reviewers were responsible for extracting

data independently from each study. Data extraction was piloted with one study to ensure extraction was consistent. After piloting, any disagreement surrounding the inclusion/exclusion criteria was resolved through discussion between reviewers. Data extraction for all studies was audited by an experienced third reviewer, with conflicts resolved by a consensus discussion.

Data Items

Extraction items included study characteristics (e.g. primary author, country, journal, sample size), animal model details (e.g. species, strain, sex), intervention details (e.g. treatment groups, the dose of therapy, the timing of therapy administration), outcomes (behavioural tests, infarct volume), risk of bias details, and quality of reporting details. A full list of data extraction items can be found in Appendix 5.

Study Risk of Bias and Reporting Bias Assessment

Risk of bias was assessed using the Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE)[12] risk of bias tool. The SYRCLE tool features 10 different parameters including randomization, blinding and outcome reporting, each of which was assessed as having a low, high, or unclear risk of bias for each study. The risk of bias for each study was assessed independently by two reviewers. Two reviewers audited the responses. A modified risk of bias visualization was used to include four categories (Low Risk, Unclear Risk, Some Concerns of Risk, and High Risk). 'Some Concerns of Risk' indicates reporting of a domain (i.e. randomization), but lack of details of the methodology (i.e. no details of how the study was randomized). This differs from 'Unclear Risk of Bias' since a domain was categorized as unclear when no mention of the domain (i.e. randomization) was reported in the study.

Effect Measures and Data Synthesis

All analyses of behaviour tests and infarct volumes were performed using the R (version 4.1.2; R Core Team 2022)[13] "metafor" package (version 4.0.0; Viechtbauer 2010)[14]. Analyses were reported using forest plots. Continuous outcome measures were expressed as standardized mean differences (SMDs) with 95% confidence intervals (CIs) with inverse variance random effects modelling. The standardized mean difference was chosen due to the expected variability of measurement techniques and reported scales used for the outcomes of interest. Statistical heterogeneity of effect sizes was assessed using the Cochrane I^2 statistic. Subgroup analyses included subgroups based on the timing of intervention (hyper-acute, acute, early subacute, late subacute/chronic – e.g. <1 day, 1-5 days, 5-30 days, >30 days)[15], intervention dose, route of intervention, model of stroke, stroke type, species, type of behavioural outcome being tested (i.e. motor, cognitive), and comorbidities. Planned subgroup analysis from the protocol on study quality, specific regions/areas of the brain, and post-stroke rehabilitation paradigms was not done due to a lack of heterogeneity in these categories.

Preclinical Assessment of the Quality of Evidence for Stroke Treatments

We evaluated the included studies using the updated Stroke Treatment Academic Industry Roundtable (STAIR) XI Consolidated Recommendations.[7] STAIR XI was developed to improve the quality of preclinical stroke research and enhance its translatability into the clinic. It provides a set of guidelines to ensure high-quality design, conduct, and reporting of preclinical studies, with the goal of reducing bias and increasing the likelihood that effective treatments are identified and successfully translated into clinically effective therapies. We used STAIR XI to grade both the overall body of evidence of included studies and the individual studies separately. STAIR XI comprises domains separated into two experimental purposes, which are the

intervention methodology of the studies (e.g. dose, timing of dose, outcomes) and the prediction of success in clinical trials (e.g. age, sex, sample size, animal type). Relevant items were extracted by two reviewers and audited by a third reviewer. Differences were resolved by consensus discussion. Subsequently, one reviewer determined if the extraction data satisfied each of the domains in STAIR XI were satisfied from the overall body of included studies.

Comparison of Preclinical Studies and Clinical Trials

Guided by domains from STAIR XI, the body of evidence from the preclinical studies was assessed to determine if it supports active clinical trials testing a CCR5 antagonist in patients with stroke. The assessed domains were dose response, time window, outcomes, age, sex, comorbidities, the use of multiple laboratories, non-human primates, and circadian effects.

There are three clinical trials of CCR5 antagonists being tested in people with stroke; however, one (ClinicalTrials.gov Identifier: NCT03172026) was terminated due to poor recruitment, partly related to the Covid epidemic, and one (ClinicalTrials.gov Identifier: NCT04966429) primarily focused on the effect of the CCR5 antagonist with dementia. Therefore, the preclinical studies were assessed to determine if the included studies were clinically relevant to the remaining CAMAROS trial (ClinicalTrials.gov Identifier: NCT04789616).[4]

Patient Engagement in this Research Study from Individuals with Lived Experience of Stroke

A panel of eight patients and caregivers with lived experience of a stroke informed project development (e.g. research question development, review protocols, search strategy development) and were actively involved in the research conduct (screening, data extraction, analysis, interpretation). Monthly meetings occurred with the patients and caregivers to provide educational sessions of background knowledge of

preclinical stroke, systematic review conduct, and discuss research findings as the review progressed. We co-developed a terms of reference document *a priori* to document details of the engagement (i.e., roles, responsibilities, expectations, project goals, etc.). The patient partners co-developed the research question to address patient interests, including chronic stroke recovery (i.e. to evaluate the effects of extended drug administration), consideration of physical therapy in tandem with drug administration, inclusion of stroke-relevant comorbidities, spasticity, and motor and cognitive outcomes. Co-authorship and financial compensation were agreed upon with the patients and caregivers and offered as a method of acknowledgement according to the SPOR Evidence Alliance Patient Partner Appreciation Policy.[16] The next chapter (Chapter 4) describes the patient engagement of this review in more detail.

RESULTS

Study Selection

Our search retrieved 263 studies. After removing duplicates, 166 studies were screened and assessed for eligibility. Five articles representing 10 experiments met the eligibility criteria.[17-21] Screening results are presented using a PRISMA flow diagram (Figure 1).

Study and Animal Model Characteristics

The baseline and animal model characteristics of the five included studies are reported in Table 1. Most studies utilized ischemic stroke models (n=4; 80%), with one investigating hemorrhagic stroke (n=1; 20%). Ischemic stroke was induced using permanent middle cerebral artery occlusion via cauterization (n=1; 20%), photothrombosis (n=1; 20%), and intraluminal suture (n=2; 40%), whereas hemorrhage was induced via autologous whole blood injection (n=1; 20%). All studies used mouse (n=4; 80%) or rat (n=1; 20%) models, where one study used a ddY mouse strain, which is susceptible to IgA nephropathy (n=1; 20%). Relevant stroke comorbidities (e.g. hypertension, diabetes) highlighted by co-authors with lived experience with stroke were not seen in the included studies. All studies used male animals exclusively (n=5; 100%).

Intervention Characteristics

The intervention characteristics of the five included studies representing 10 experiments are reported in Table 2. Maraviroc was used as the CCR5 antagonist drug in six of the experiments (n=6; 60%), TAK-779 in three of the experiments (n=3; 30%), and D-Ala-Peptide T-Amide (DAPTA) in one (n=1; 10%). There were various routes of administrations used to inject the CCR5 antagonist drug, which include intraperitoneally (n=4; 40%), intranasally (n=2; 120%),

subcutaneously (n=1; 10%), intracerebroventricular (n=2; 20%), and intravenously (n=1; 10%). Doses ranged from 0.01 to 100 mg/kg with a median of 12.5 mg/kg. Most treatment administrations were initiated after stroke induction (n=7, 70%) and ranged from one hour to three weeks post-stroke induction for the first administration, while the remaining experiments administered treatment pre-stroke induction (n=3; 30%) ranging from 10 to 15 minutes pre-stroke induction. This range of timing for stroke treatment does not fall in the chronic stage of recovery (two months post-stroke), which was a specific interest to the patient partners. The evaluation of the effects of extended drug administration for chronicity raised by co-authors with lived experience with stroke was limited. Most studies delivered a single dose of the drug (n=6; 60%); experiments with multiple administrations (n=4; 40%) ranged from 3 – 63 doses. Other a priori patient priority interests of consideration of physical therapy alongside drug administration, the inclusion of stroke-relevant comorbidities, and spasticity were not reported in the included studies.

Meta-Analysis of Infarct Volume

Infarct volume was reported in six experiments (60%) from four different studies with an overall pooled analysis demonstrating marked improvements with CCR5 antagonist drug administration (SMD -1.02, 95% CI -1.58 to -0.46, I^2=34%) (Figure 2). Taking into consideration when the drug was administered in relation to stroke onset, infarct volume for post-stroke drug administration was reported in three experiments (30%) from three different articles where the pooled analysis favoured CCR5 antagonist drug administration (SMD -0.80, 95% CI -1.35 to -0.25, I^2=0%). Infarct volume for pre-stroke drug administration was reported in three experiments (30%) from two different studies with a pooled analysis favouring CCR5 antagonist drug administration (SMD -1.48, 95% CI -2.83 to -0.14, I^2=70%). Timing of CCR5

administration (pre- vs. post-stroke) did not result in statistically significant differences in infarct volume (P=0.47). A sensitivity analysis was conducted, removing the significant experiment that administered treatment at 15 minutes pre-stroke because of the large heterogeneity, and the overall pooled pre-stroke treatment of the two other experiments remained statistically significant (SMD -0.83, 95% CI -1.58 to -0.46).

From combined post- and pre-stroke drug administration subgroup analysis, there was no observed difference in effect size when considering the timing of drug treatment, route of administration, stroke model, species type, drug, dose, whether behaviour tests were assessed, or if comorbidities were present (Figure 3). Similarly, subgroup analysis for only post-stroke studies demonstrated no difference in effect size when considering any of the subgroups (Figure 4). However the subgroup analysis for pre-stroke studies, infarct volume was reduced to a greater extent by the intraluminal suture stroke model (P=0.04), in rat models (P=0.01), with DAPTA (P=0.01), and when behaviour tests were performed (P=0.01) compared to other types of stroke models, mice, using TAK-779, and when no behaviour tests were performed (Figure 5).

Meta-Analysis of Behavioural Tests

Motor behavioural tests were reported in six experiments from three studies and represented 15 outcome measures, and a cognitive behavioural test was applied in one study. CCR5 inhibition was effective and statistically significant in 11 of 16 behavioural outcomes tested (Figure 6). Meta-analysis and planned subgroup analysis were not conducted due to an inadequate number of studies for each given outcome measure (i.e. 1 study) for each test reported.

Yan et al. demonstrated that treatment was less effective the further administration was delayed from stroke onset (i.e. one-hour post-stroke compared to 24 hours post-stroke). A similar trend was seen for the Joy et al. experiments post-stroke for the cylinder tasks was less effective the further out the first administration was given from stroke onset (i.e. 24 hours post-stroke compared to 28 days post-stroke). For the grid walking tasks, treatment was effective regardless of administration time.

One study of pre-stroke drug administration was reported where motor functions improved when administered a CCR5 antagonist drug, but the effect was not statistically significant (Li et al., 2016; SMD -1.04, 95% CI -2.36 to 0.28). A cognitive behavioural test was only applied in one study, where probe quadrant duration was measured as part of a Morris Water Maze test, which showed improvements in the treated group in spatial learning and memory compared to the control group (Yan et al., 2021; SMD -2.43, 95% CI -3.73 to -1.14).

Risk of Bias in Studies

All articles were deemed as having a 'high' risk of bias, and the majority across most domains being deemed as 'unclear' risk of bias (Figure 7).

One study (20%) reported details of randomization and allocation concealment, and two studies (40%) reported all expected outcomes and were deemed 'low' risk of bias for randomization, allocation concealment, and selective reporting. Four studies (80%) reported randomization but did not provide details, and three (60%) reported blinding investigators and outcome assessors but did not provide details and were deemed to have 'some concerns' of bias.

The risk of bias was 'unclear' across all studies for the domains of baseline characteristics because of missing data in the studies, random housing because no details on this domain were reported, and random outcome assessment because no details of how cohorts of animals were selected to perform certain outcomes nor how the order of outcome assessment proceeded. Four studies did not report on allocation concealment, and two studies did not report on blinding investigators and outcome assessors and were deemed as having an 'unclear' risk of bias.

Incomplete outcome data was assessed by whether the sample size was consistent between the methods and results sections. All studies exhibited a 'high' risk for incomplete outcome data, and similarly, three studies (60%) had a 'high' risk of selective outcome reporting since all expected outcomes discussed in the methods of the articles did not align with their results. Appendix 6 provides a detailed rationale for the categorization of risk of biases for each category.

Lastly, other potential sources of bias considered included the source of funding (industry funded), contamination of pooling drugs (additional treatment which might influence or bias the result), unit error analysis (all animals receiving the same intervention are caged together, but analysis was conducted as if every single animal was one experimental unit), design-specific risks of bias (reporting details of which animals performed the same or different outcomes), and the addition of new animals to replace dropouts from the original population. Two studies (40%) had a 'high' risk in at least one of these additional categories.

Preclinical Assessment of the Quality of Evidence for Stroke Treatments

All five articles were assessed individually and overall for their quality of evidence based on the STAIR XI Consolidated Recommendations[7] checklist (Table 3).

In the five domains in assessing the intervention methodology, the overall body of evidence for all domains was satisfied except for ensuring the drug enters the brain. Individually, all studies tested both behavioural and histological outcomes, and the drug causes expected physiological effects. One study (20%) ensured that the drug entered the brain. Three studies (60%) tested at clinically relevant delayed times between one and 4.5 hours after stroke onset. Overall, seven different doses were evaluated with three studies (60%) evaluating more than one dose.

In the 13 domains assessing the preclinical assessment and validation of the studies, four domains were satisfied, which were effective stroke induction, clinically relevant ages of animals used (aging/adult animals), evidence in two or more laboratories, and reporting conflict of interests. Individually, all studies reported effective stroke induction in animals and their conflicts of interest. Three studies (60%) reported the ages of the animals used and since that represents the majority, this domain for the overall body of evidence is satisfied. One (20%) study reported details on randomization, allocation concealment, and exclusion of animals, therefore this domain for the overall body of evidence is not satisfied. None of the studies adequately supported the recommendations for justification for sample size used, details of blinding outcome assessors, used both male and female animals, used animals with relevant stroke comorbidities, had independent replications of their findings, used non-human primates, or considered circadian effects.

Comparison of Preclinical Studies with Clinical Trials

Guided by recommendations by STAIR XI, domains were assessed to see if the overall body of evidence from the preclinical studies were relevant to the active clinical trial of maraviroc on

patients with stroke, the CAMAROS trial[4]. A delated rationale for each domain is provided in Table 4. The clinical trial used a significantly higher dose than any of the preclinical trials. The highest dose preclinically used was 100 mg/kg, where the availability of the drug in cerebrospinal fluid is seen to be about half the levels of the human therapeutic range of 300 mg given twice daily in the clinical trial.[19] This conversion was reported in the Joy et al. study by mass spectrometry. Thus, the domain for dose amount was not satisfied, as no preclinical study tested a similar dose to that of human trials. Similarly, the domain for behavioural and histological outcomes cannot be satisfied since the outcomes assessed, even though they were behavioural and histological outcomes, cannot be directly compared between the clinical trial and preclinical studies due to inherent differences in experimental conditions. For instance, behavioural outcomes in animals are not directly analogous to human experiences and capacities, and histological outcomes may not reflect the exact physiological responses observed in humans due to differences in biology and disease progression. A preclinical study did administer the drug during similar timing of the clinical trial; thus, the domain for timing is satisfied.

The use of adult-aged animals and conduct in multiple laboratories reported overall in the preclinical studies align with the clinical trial, making these domains satisfied. It should be noted that, no replications of the same experimental protocols were reported independently by multiple laboratories, which should be considered for future experiments. The use of only male animals, lack of relevant comorbidities, non-human primates, and testing during the awake phase of animals does not align with the clinical trial, and subsequently, these domains were not satisfied.

DISCUSSION

Our main findings indicate that CCR5 antagonists were efficacious in reducing infarct volume and improving behavioural assessments in rodent models. This illustrates the potential of innovative drug therapy for stroke recovery. Our study also represents the first comprehensive preclinical review of CCR5 data co-designed with individuals with lived experience of stroke, providing unique input and perspective from project conception to the dissemination of findings. This collaboration comes at a critical time, given the ongoing clinical trials on CCR5 antagonists.[4]

Our findings indicate consistent beneficial outcomes associated with CCR5 antagonism, despite the diverse experimental approaches and characteristics of the included studies. While our findings are promising, there are limitations. The included studies often lacked robust and comprehensive methods required to produce more definitive and less biased conclusions. For instance, studies overlooked key patient priority outcomes such as the inclusion of stroke-relevant comorbidities, the long-term impact on physical and cognitive abilities, and the effectiveness of combining physical therapy with drug administration; this limits the applicability of the findings to clinical practice. Furthermore, the potential biases, limited number of studies, and the fact that animal models are not fully analogous to human pathophysiology and outcomes, all impact the strength of our conclusions.[22] These weaknesses emphasize the need for future research to address identified knowledge gaps to produce more robust and generalizable preclinical evidence.

The incorporation of patient partners within our research team was valuable. The panel of eight patients and caregivers with lived experiences of stroke had active involvement in every stage of the research process. Their input shaped the research question to cover critical patient interests, such as the evaluation of chronic stroke recovery, the combination of physical therapy with drug administration, the inclusion of stroke-relevant comorbidities, and the assessment of motor and cognitive outcomes. However, we noted that patient priority outcomes were either minimally highlighted or completely absent in the included studies. This discrepancy highlights the need for future preclinical studies to better align with patient priorities, thereby enhancing the relevance and impact of research outcomes. However, we recognize this is not always feasible, given that animal models are not always analogous to human conditions, presenting challenges in translating research findings.

The adoption of the STAIR XI criteria as a tool for assessing the quality of evidence in our systematic review adds a novel dimension to our research.[7] This standard provided a rigorous framework for our review and facilitated a thorough evaluation of the current evidence. In our adherence to the STAIR XI criteria, we found that half the studies did not meet the guidelines, highlighting areas for improvement in preclinical stroke research.

Our findings share similarities and differences with the ongoing CAMAROS trial evaluating maraviroc in stroke recovery patients.[4] The trial, like our study, recognizes the therapeutic potential of CCR5 antagonism for stroke. However, the applicability of our preclinical findings to the CAMAROS trial context is limited by a few important differences between the trial and preclinical studies that preceded it. For instance, the dose where the highest preclinical dose

reported in the studies we identified was half of the mean concentration used in the CAMAROS trial.[4] Nonetheless, overall, our results corroborate the therapeutic promise of CCR5 antagonism in stroke recovery, aligning with the ongoing CAMAROS trial.

Moreover, it must be recognized that the evidence bases to support clinical trials such as CAMAROS extends beyond these preclinical studies to include other animal and human investigations not specifically focused on stroke or studies not captured within the scope of this systematic review. Therefore, while our preclinical findings contribute to the supportive evidence for the use of maraviroc in the CAMAROS trial, a thorough appraisal of all available evidence is required to ensure a fully informed clinical trial. For instance, in vitro studies have illuminated the cellular and molecular mechanisms of CCR5 antagonism, which, though not part of our preclinical animal-based review, can provide valuable context and potential therapeutic justification for its use in the treatment of stroke.[3]

Our systematic review highlights the potential therapeutic benefits of CCR5 antagonism. Yet, we find it essential to acknowledge the existence of gaps and limitations within the preclinical evidence base. Our belief, shared among leaders in the stroke research field based on the STAIR XI criteria, is that such limitations should be ideally addressed prior to advancing to a clinical trial stage. These concerns echo a broader consensus emphasizing the need for high-quality, robust evidence to support and justify the initiation of clinical trials. Thus, in alignment with the principles of STAIR XI, we propose that future research efforts should prioritize enhancing the quality and robustness of preclinical evidence for CCR5 antagonists. This objective extends beyond simply comprehending the role of CCR5 antagonists in stroke therapy, but rather

advocates for the demonstration of robust, reproducible, and reliable functional outcomes that are important to patients and support clinical translation. By adhering to this standard, we believe we can pave the way for transformative advancements in stroke recovery treatments that are both evidence-based and patient-centric.

Tables and Figures

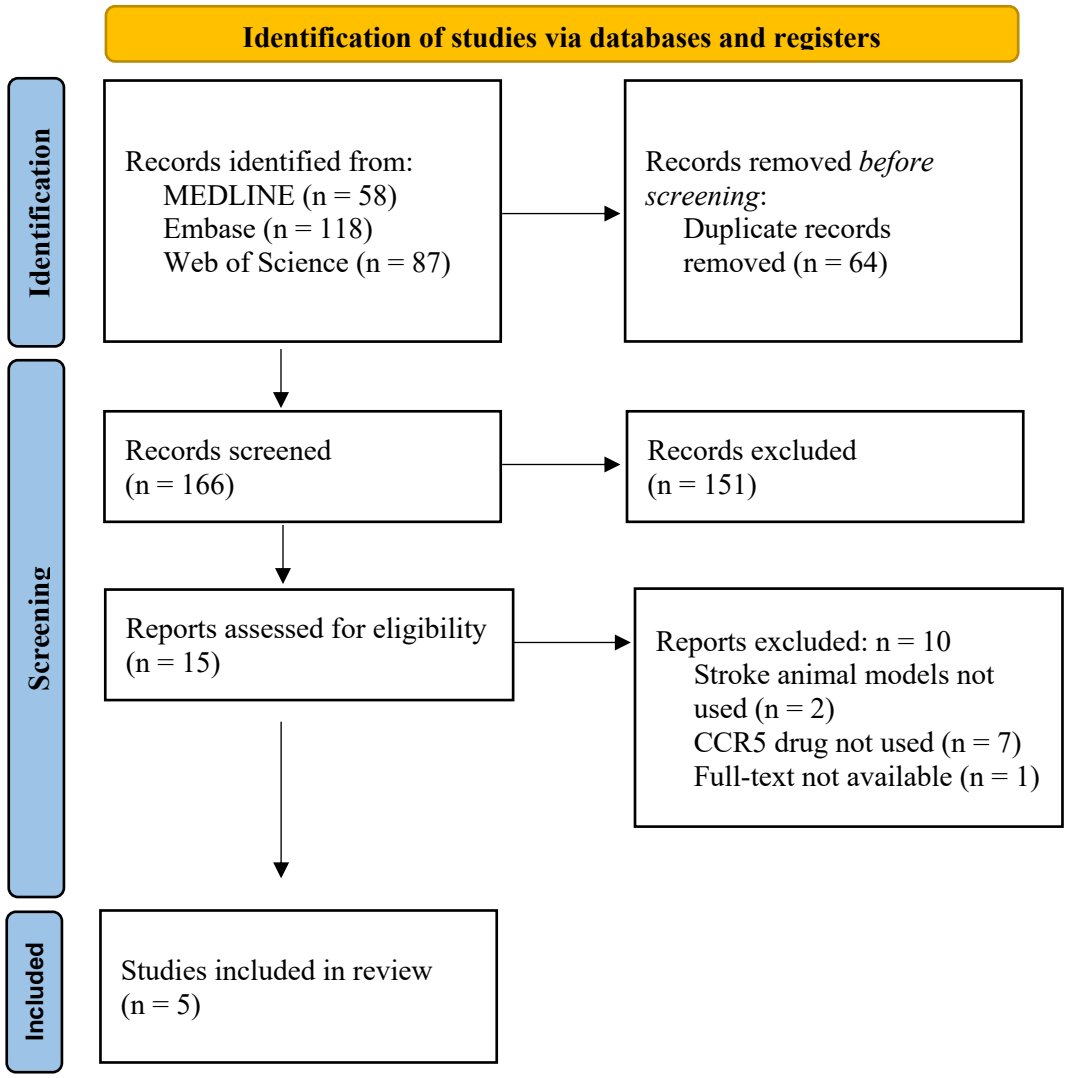

Figure 1. Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) flow diagram detailing study screening and selection

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

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

*Susceptible to IgA nephropathy (comorbidity)

Table 2. Summary of intervention characteristics of all included studies

Author	Drug	Dose (mg/kg)	Route of Administration	Timing of Drug Administration	Number of Administrations	Outcomes Measured (Treatment n/Control n)	Time of Outcome Measure
Chen et al.	Maraviroc	20	Intraperitoneally	1.5 h, 24 h, and 48 h post-stroke	3	- Infarct volume (5/5) - Longa score (5/5)* - Neurological deficit score (5/5)* - TTC staining	72 h post-stroke
Yan et al.	Maraviroc	0.15	Intranasally	1 h post-stroke	1	- Garcia test (6/6) - Limb placement (6/6) - Corner turn test (6/6) - Immunofluorescence staining	72 h post-stroke
						- Foot fault (8/8) - Rotarod (8/8)	3 weeks post-stroke
						- Probe quadrant duration (8/8) - Nissl staining	25 days pos-stroke
				24 h post-stroke		- Garcia test (6/6) - Limb placement (6/6) - Corner turn test (6/6) - Tunnel staining	72 h post-stroke
Joy et al.	Maraviroc	100	Intraperitoneally	24 h post-stroke through daily injections for 9 weeks	63	- Infarct volume (5/5)	9 weeks post-stroke
						- Grid walk (10/10) - Forelimb (10/10) - GFAP and IBA-1 immunoreactivity	8 weeks post-stroke
				24 h post-stroke through daily injections for 3 weeks	21	- Grid walk (10/10) - Cylinder test (9/8)	9 weeks post-stroke
				3 weeks post-stroke through	56	- Grid walk (9/9) - Cylinder test (9/9)	11 weeks post-stroke

				daily injections for 11 weeks			
Li et al.	DAPTA (D-Ala-Peptide T-Amide)	0.01	Subcutaneously	15 min pre-stroke	1	- Infarct volume (5/5) - Neurological deficit score (5/5) - TTC and H & E staining	24 h post-stroke
Takami et al.	TAK-779	5	Intracerebroventricular	10 min pre-stroke	1	- Infarct volume (10/6)	48 h post-stroke
		50	Intracerebroventricular	10 min pre-stroke		- Infarct volume (13/6)	
		0.25	Intravenously	50 min post-stroke		- Infarct volume (17/18) - Immunostaining	

*Chen et al. reported motor behavioural outcomes without standard deviations or standard errors, and thus could not be included in the meta-analysis. Contact with the authors via email was attempted, without success.

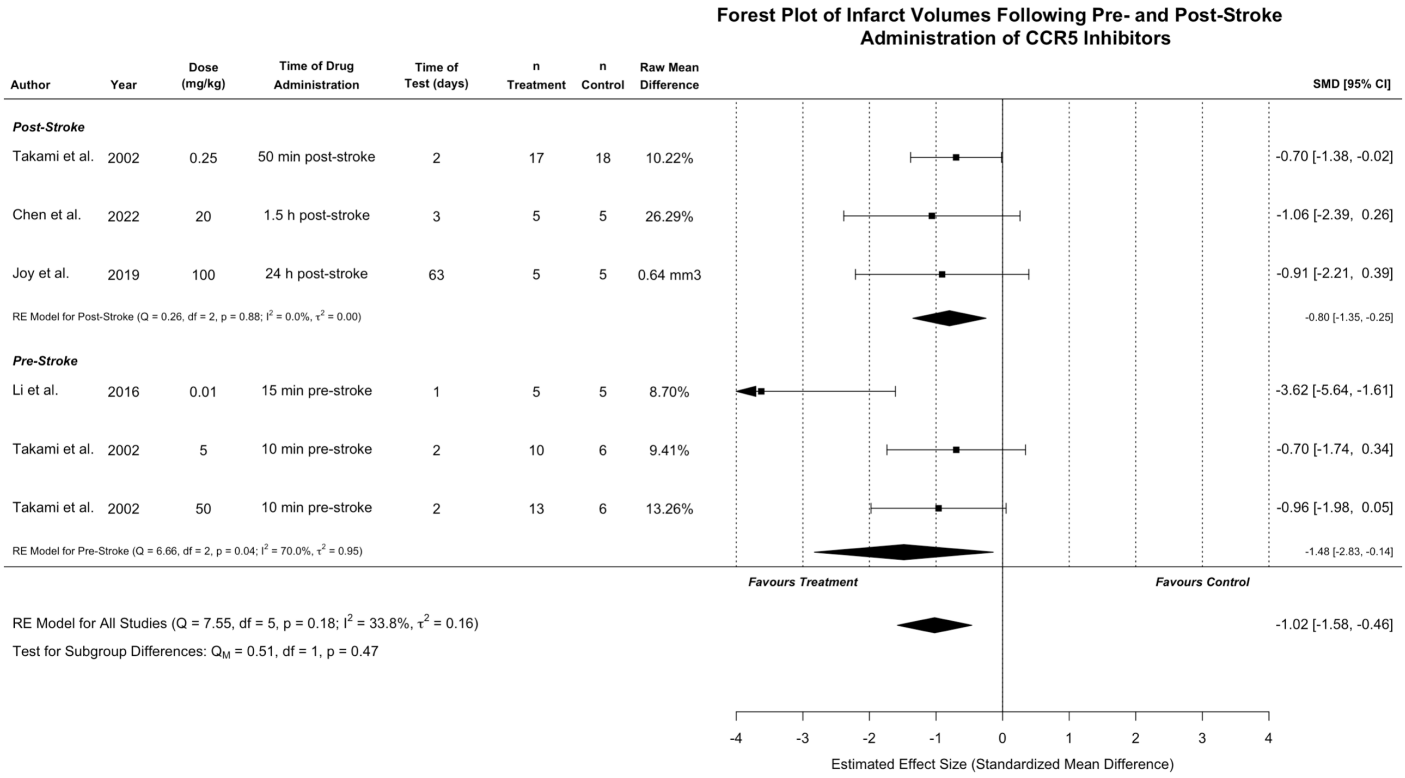

Figure 2. Meta-analysis for all included studies of pre-stroke and post-stroke drug administration of a CCR5 antagonist that reported infarct volume. Data is presented as a forest plot with a standardized mean difference and 95% confidence intervals. Effect sizes <0 favours drug treatment and >0 favours control. 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 drug administration of a CCR5 antagonist. The I^2 value represents the statistical heterogeneity.

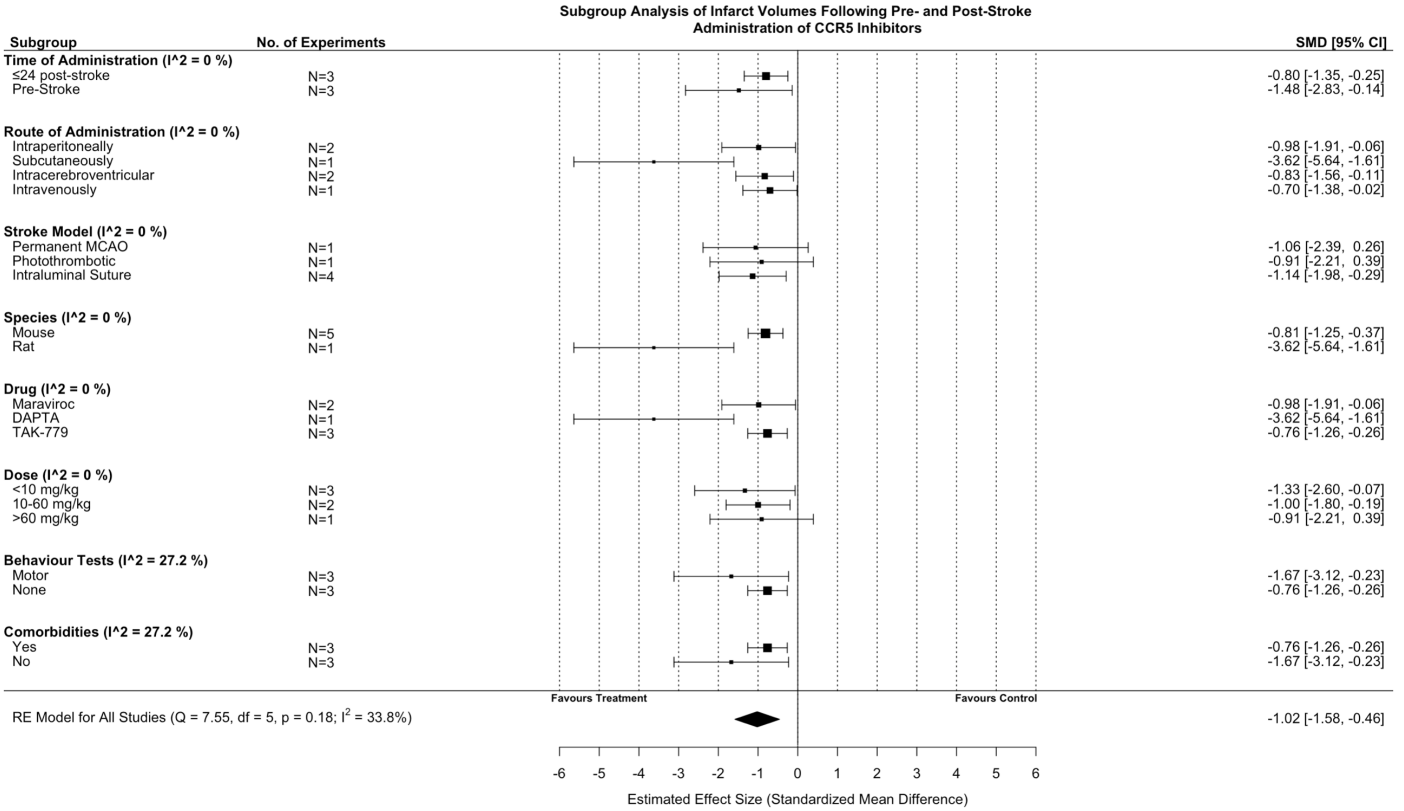

Figure 3. Subgroup analysis for all included studies of pre-stroke and post-stroke drug administration of a CCR5 antagonist that reported infarct volume. Each row represents pooled estimate data from studies within that subgroup. Data is presented as a forest plot with a standardized mean difference and 95% confidence intervals. The I^2 value represents the statistical heterogeneity within each subgroup. Effect sizes <0 favours drug treatment and >0 favours control. The 'RE Model for All Studies' represents a pooled estimate of the CCR5 antagonist drug effect on infarct volume from all studies combined.

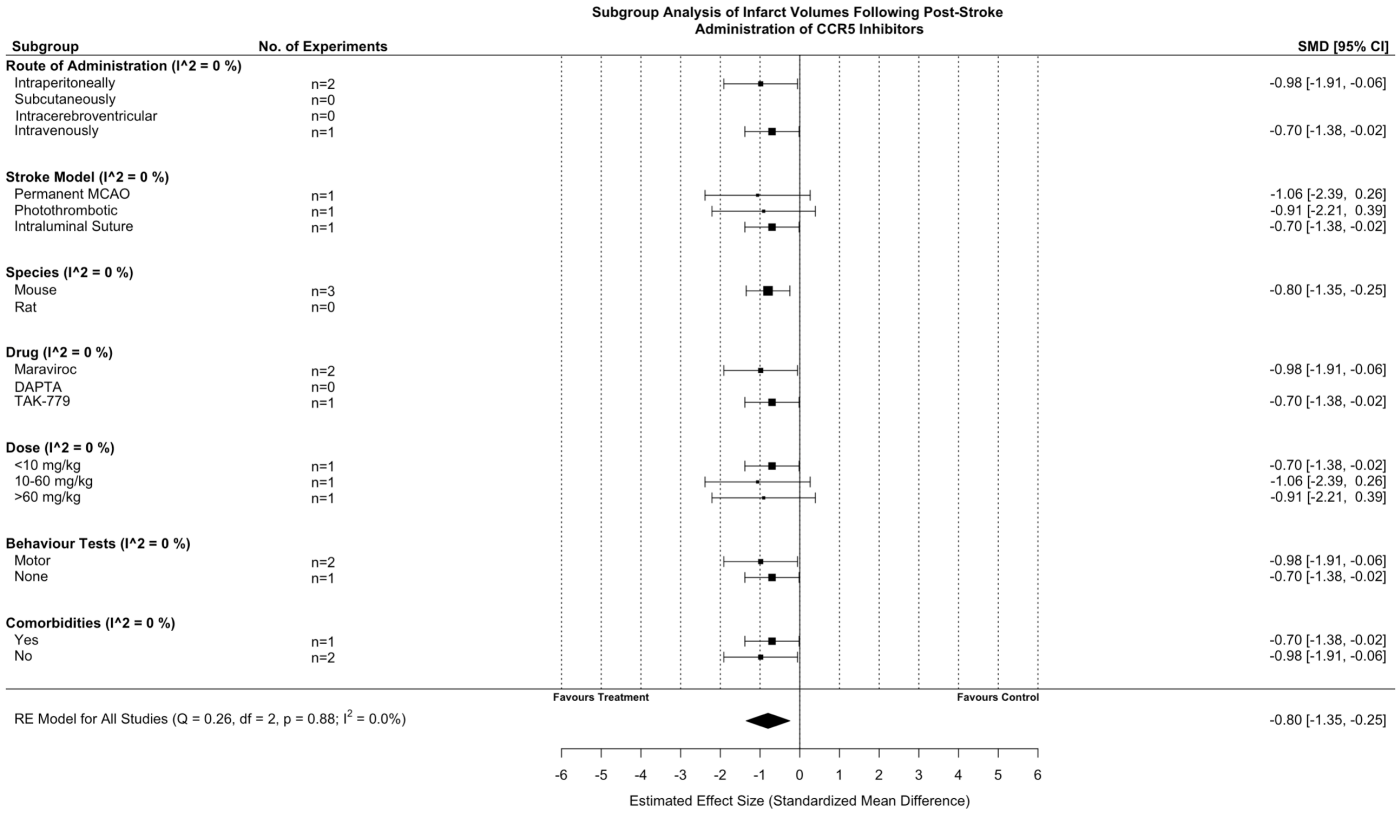

Figure 4. Subgroup analysis for all included studies of post-stroke drug administration of a CCR5 antagonist that reported infarct volume. Each row represents pooled estimate data from studies within that subgroup. Data is presented as a forest plot with a standardized mean difference and 95% confidence intervals. The I^2 value represents the statistical heterogeneity within each subgroup. Effect sizes <0 favours drug treatment and >0 favours control. The 'RE Model for All Studies' represents a pooled estimate of the CCR5 antagonist drug effect on infarct volume from all post-stroke drug administration of a CCR5 antagonist studies combined.

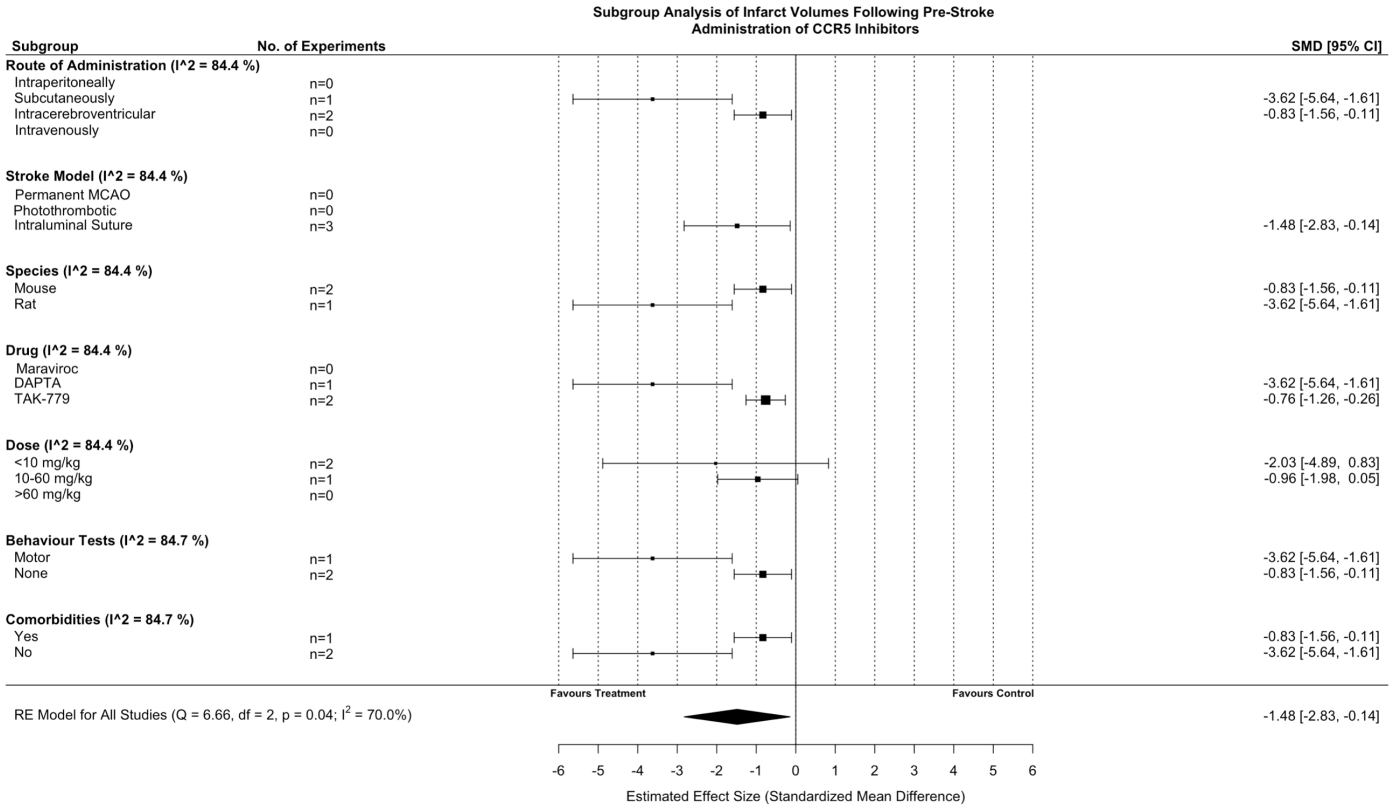

Figure 5. Subgroup analysis for all included studies of pre-stroke drug administration of a CCR5 antagonist that reported infarct volume. Each row represents pooled estimate data from studies within that subgroup. Data is presented as a forest plot with a standardized mean difference and 95% confidence intervals. The I^2 value represents the statistical heterogeneity within each subgroup. Effect sizes <0 favours drug treatment and >0 favours control. The 'RE Model for All Studies' represents a pooled estimate of the CCR5 antagonist drug effect on infarct volume from all pre-stroke drug administration of a CCR5 antagonist studies combined.

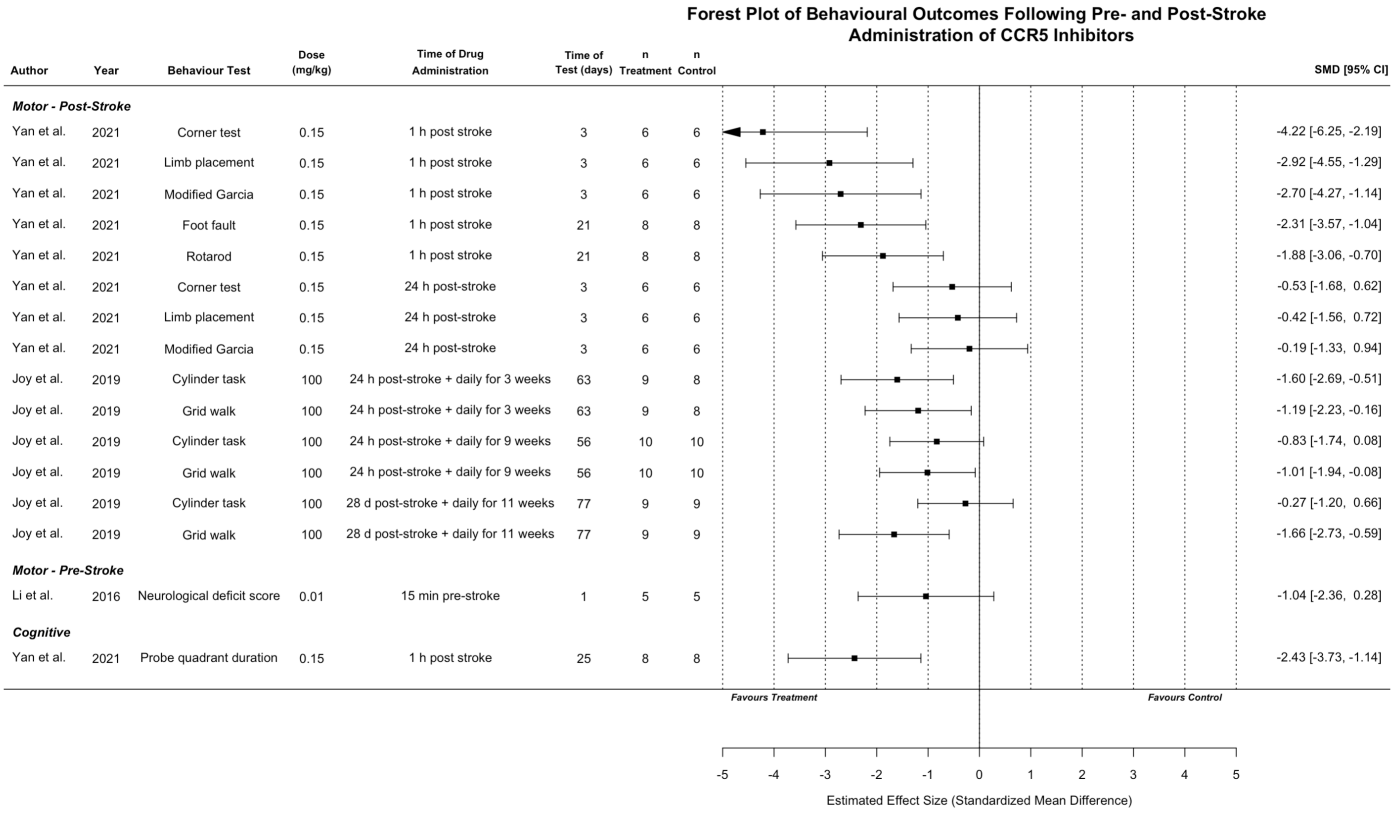

Figure 6. Meta-analysis for all included studies of pre-stroke and post-stroke drug administration of a CCR5 antagonist that reported motor and cognitive behavioural outcomes. Data is presented as a forest plot with a standardized mean difference and 95% confidence intervals. Effect sizes <0 favours drug treatment and >0 favours control.

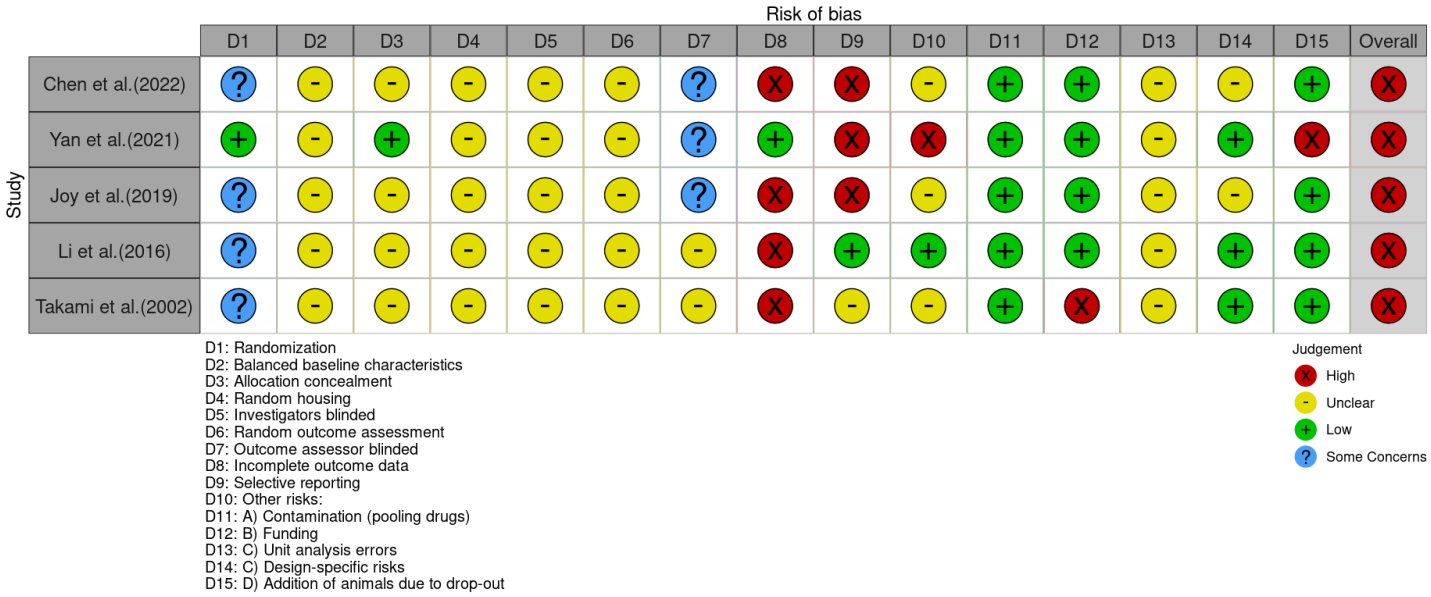

Figure 7. Modified risk of bias traffic light plot in accordance with the SYRCLE tool. Yellow represents an unclear risk of bias, green represents a low risk of bias, and red represents a high risk of bias. Blue represents some concerns of a risk of bias.

Table 3. Preclinical assessment in accordance with STAIR XI Consolidated 2021 Recommendations of all included studies. Green indicates the recommendation was satisfied and red indicates the recommendation was not satisfied.

	Chen et al. (2022)	Yan et al. (2021)	Joy et al. (2019)	Li et al. (2016)	Takami et al. (2002)	Overall	Notes
Candidate Treatment Qualification							
(1) Testing at least two doses of the drug							Doses across all studies ranged from 0.01 to 100 mg/kg
(2) Treatment given after clinically relevant delayed times (one to 4.5 hours post-stroke)							All studies except Li et al. and Takami et al. administered the drug at least one-hour post-stroke
(3) Both histologic and behavioural outcomes							Effects of behaviour and histological damage were measured in most studies
(4) Treatment reaches presumed target and causes expected physiological effects							Histological measurements showed that the drug reached the brain target and caused expected physiological stroke effects, but this was not done for all experiments in the studies
(5) Drug enters the brain							Mass spectrometry and histological measurements were assessed to determine the drug reaching the brain only in the Joy et al. paper
Preclinical Assessment and Validation							
(1) Sample size							No protocol nor known or assumed SD and predicted effect size were reported to prespecify sample size
(2) Effective stroke induction							Stroke procedures were verified to be effective through Laser Doppler or other flowmetry
(3) Randomization							Randomization was reported in all studies, but not all studies reported details of their randomization
(4) Allocation concealment							Only one study (Yan et al.) reported blinded surgeon and group assignment
(5) Reported on excluded animals							Studies did not report on excluded animals or justification of animals lost at each experimental step after randomization
(6) Blinding of outcome assessors							All studies either did not report or provide details of blinding, where

							investigators remained unaware of treatment assignment during all assessments
(7) Reporting on age							Adult animals were used from those that reported age
(8) Male and female animals							Only male animals were assessed, where male and females should have been assessed
(9) Animals with co-morbidities							ddY mouse models, used in one study by Takami et al., are prone to IgA nephropathy (Berger's disease – kidney disease), which is not a common stroke comorbidity (e.g. diabetes or hypertension)
(10) Evidence from two or more laboratories							All articles were conducted in different facilities
(11) Species other than rodents							All animals used were mice and rats, but nonhuman primates should be used to contribute to predicting clinical efficacy
(12) Circadian effects considered							One study by Yan et al. reported conducting experiments during the sleep phase of the rodents, the rest did not report (default preclinical studies occur during the sleep phase)
(13) Reporting and managing conflicts of interests							Investigator and institution conflicts are reported and managed

Table 4. Comparison of included preclinical studies and CAMAROS clinical trial, guided by recommendations with STAIR XI Consolidated 2021 Recommendations.

	Relevant to the CAMAROS Trial	Notes
Candidate Treatment Qualification		
(1) Testing at least two doses of the drug	X	• CAMAROS - Participants take 300 mg doses twice daily • Preclinical - highest dose of 100 mg/kg used by Joy et al. reported the availability of maraviroc in the brain from cerebrospinal fluid to be about half the levels to the human therapeutic range for this drug • We should have preclinical studies administer an equivalent dose of 200 mg/kg in mice twice daily to be more clinically relevant
(2) Treatment given after clinically relevant delayed times	✓	• CAMAROS - Participants were recruited within ≥5 days and 6 weeks of stroke, during the acute/subacute phase of recovery • Preclinical - One experiment by Joy et al. started administration at 20 days from stroke onset, which is during the subacute phase of recovery
(3) Both histologic and behavioural outcomes	X	• 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) • Preclinical - The timepoint of all tests ranged from 3 days (acute) to 11 weeks (chronic) post-stroke for all included studies • While motor function is being assessed in the both the animal studies and trial, the preclinical tasks focus on spontaneous movements or fine motor coordination, while the human assessments are more structured and focus on specific functional movements and tasks. We recognize, direct comparisons are challenging due to the inherent differences in anatomy and behaviour between humans and rodents. Using more clinical relevant animal models or using more complex behavioural tests in rodents might be solutions
Preclinical Assessment and Validation		
(1) Aging/adult age	✓	• CAMAROS - Age ≥18 year old adult participants were recruited • Preclinical – Ages ranged from four to 20 weeks for rodents, which are considered adults
(2) Male and female animals	X	• CAMAROS - Both sexes were recruited • Preclinical – Only males used in the included studies • Preclinical studies should assess both male and female animals to be more clinically relevant, where dose-response differences between sexes should be determined
(3) Animals with co-morbidities	X	• CAMAROS - Participants with renal issues were excluded, as well as excluded participants with other comorbidities (i.e. dementia, hepatitis, Parkinson's, HIV) • Preclinical – None of the most common stroke comorbidities (hypertension, diabetes, and other cardiovascular factors) were used in the included

		studies. The trial did not exclude these comorbidities, but the preclinical studies did not include these comorbidities in their animals • Future preclinical studies should include relevant stroke comorbidities in their animals to align with the clinical trial
(4) Evidence from two or more laboratories	✓	• CAMAROS - Studies under the trials are occurring across facilities in Canada • Preclinical – Overall, all five studies were conducted in different laboratories; however, no replications of the same experimental protocols independently by multiple laboratories. • Relevant preclinical experiments should be conducted across different laboratories
(5) Gyrencephalic species	X	• CAMAROS – humans are a gyrencephalic species. • Preclinical – all studies conducted in lissencephalic (rodent) species. • No studies conducted in gyrencephalic species, particularly nonhuman primates, which may contribute to predicting clinical efficacy; future studies should consider this
(6) Tests during the awake phase of animals	X	• CAMAROS - follow-up occurs during the awake phase of the participants • Preclinical - all studies occurred during the sleep phase of the rodents • We should have preclinical studies occur during the awake phase

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Appendices

Appendix 1: PRISMA 2020 Checklist

Section and Topic	Item #	Checklist item	Location where item is reported
TITLE			
Title	1	Identify the report as a systematic review.	1
ABSTRACT			
Abstract	2	See the PRISMA 2020 for Abstracts checklist.	2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of existing knowledge.	3-4
Objectives	4	Provide an explicit statement of the objective(s) or question(s) the review addresses.	4
METHODS			
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.	5
Information sources	6	Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted.	5-6
Search strategy	7	Present the full search strategies for all databases, registers, and websites, including any filters and limits used.	39-53
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process.	6
Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process.	7
Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g. for all measures, time points, analyses), and if not, the methods used to decide which results to collect.	7
	10b	List and define all other variables for which data were sought (e.g. participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information.	7
Study risk of bias assessment	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.	7-8

Appendix 1: PRISMA 2020 Checklist

Section and Topic	Item #	Checklist item	Location where item is reported
Effect measures	12	Specify for each outcome the effect measure(s) (e.g. risk ratio, mean difference) used in the synthesis or presentation of results.	8
Synthesis methods	13a	Describe the processes used to decide which studies were eligible for each synthesis (e.g. tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item #5)).	8
	13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions.	8
	13c	Describe any methods used to tabulate or visually display results of individual studies and syntheses.	8
	13d	Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used.	8
	13e	Describe any methods used to explore possible causes of heterogeneity among study results (e.g. subgroup analysis, meta-regression).	8
	13f	Describe any sensitivity analyses conducted to assess robustness of the synthesized results.	8
Reporting bias assessment	14	Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases).	7-8
Certainty assessment	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.	8-9
RESULTS			
Study selection	16a	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram.	11, 19
	16b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded.	11, 19
Study characteristics	17	Cite each included study and present its characteristics.	11-12, 20-21
Risk of bias in studies	18	Present assessments of risk of bias for each included study.	14, 29-30
Results of individual	19	For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (e.g. confidence/credible interval), ideally using structured tables or plots.	12-13, 22-28

Appendix 1: PRISMA 2020 Checklist

Section and Topic	Item #	Checklist item	Location where item is reported
studies			
Results of syntheses	20a	For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies.	12-13, 22-28
	20b	Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g. confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect.	12-13, 22-28
	20c	Present results of all investigations of possible causes of heterogeneity among study results.	12-13, 22-28
	20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results.	12-13, 22-28
Reporting biases	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed.	14, 29-30
Certainty of evidence	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed.	14-15, 31
DISCUSSION			
Discussion	23a	Provide a general interpretation of the results in the context of other evidence.	16-17
	23b	Discuss any limitations of the evidence included in the review.	16-17
	23c	Discuss any limitations of the review processes used.	16-17
	23d	Discuss implications of the results for practice, policy, and future research.	16-17
OTHER INFORMATION			
Registration and protocol	24a	Provide registration information for the review, including register name and registration number, or state that the review was not registered.	1, 5
	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared.	5
	24c	Describe and explain any amendments to information provided at registration or in the protocol.	5
Support	25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review.	18
Competing	26	Declare any competing interests of review authors.	18

Appendix 1: PRISMA 2020 Checklist

Section and Topic	Item #	Checklist item	Location where item is reported
interests			
Availability of data, code, and other materials	27	Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review.	18

Appendix 2: GRIPP2 Short Form Checklist

Section and topic	Item
1: Aim	Conduct a preclinical systematic review to assess the effects of C-C chemokine receptor type 5 (CCR5) Antagonists on motor and cognitive impairment. To collaborate with a panel of patients and caregivers with lived experience of stroke throughout the development and conduct of the preclinical systematic review.
2: Methods	A panel of eight patients and caregivers of lived experience of a stroke were recruited to join the research team through the Heart & Stroke Foundation and the Patient and Family Advocacy Program at The Ottawa Hospital by an advertisement distributed to both organizations. The patients and caregivers were involved in developing the research question and the protocol; defining stroke type, phase of care, intervention, and outcomes; identifying data items for extraction; conduct of the review including screening and extraction; analyzing and interpreting the review results; and contributed to edits to the final manuscript. Patients and caregivers attended monthly virtual meetings to provide educational sessions of background knowledge of preclinical stroke, systematic review conduct, and discuss research findings as the review progressed. Patients and caregivers were offered financial compensation and co-authorship in recognition of their contributions to the research project.
3: Results	Patient engagement contributed to the study in several ways, including: - Informing the research question with the patient partner experience: patient partners have lived experience of stroke and provided patient priorities to analyze in our review - Defining our primary outcome (motor chronic recovery)

	- Providing insight on the development of the protocol to include non-technical language and incorporate additional patient priorities (certain regions of the brain effected) - Editing screening and extraction forms - Participating as reviewers in abstract and full-text level screening - Participating as reviewers in extraction - Participated in an international stroke conference - Presented barriers and limitation to incorporate in the discussion of the systematic review proposal
4: Discussion	Overall, patient engagement was successful in informing review development and conduct. Additionally, the research team learned a lot about the patient partner experience with stroke and the priorities in place for persons with lived experiences in early drug development. We recognize patient partners for their expertise through frequent and regular meetings. Patient partners also participated in the conduct phase of the systematic review, which allowed for more meaningful discussion through our meetings. The systematic review was conducted within a year. At the beginning of the project, we co-developed a timeline and budget to reflect the number of hours that patient partners devoted to the project. In the future, we will refer back to this timeline at the mid-term mark to ensure that the number of hours budgeted for were accurate.
5: Reflections	Engagement was embedded within the research project from the very start of the study to end, where patient partners were members of the larger team. Patient partners directed us to outcomes for the systematic review that critical clinically to patients in the chronic phase of stroke recovery. Their participation in the conduct of the review facilitated meaningful collaborations and discussion of tangible goals. These outcomes would not have been possible without the involvement of our patient partners.

PPI = patient and public involvement

Appendix 3: Reasoning for Exclusion Criteria

Exclusion Criteria	Reason
Ex vivo	Not physiologically representative of a living organism
In vitro	Not physiologically representative of a living organism
Neonatal animal models	Neonates are different and complex compared to adult models; not representative of the majority of individuals with stroke
Global ischemia stroke animal models	Not representative of typical human strokes
Invertebrate animal models	Different anatomy than humans, and not physiologically representative of humans
Human studies	Focus of this review is preclinical studies
Uncontrolled study designs	Uncontrolled studies introduce potentially a lot of bias that can greatly skew the results
Abstracts, review articles, opinion-based letters/editorials, and unpublished grey literature	These publications typically do not provide the level of detailed experiments needed to extract for a systematic review and meta-analysis

Appendix 4: Search Strategy

Medline : 58
Embase : 118
Web of Science : 87

Total: 230
Total after duplicates: 166
Duplicates: 64

Embase Classic+Embase <1947 to 2022 October 24>
Ovid MEDLINE(R) ALL <1946 to October 24, 2022>

1 exp animal experimentation/ or exp models, animal/ or animals/ or exp animal population groups/ or chordata/ or vertebrates/ or exp amphibians/ or exp birds/ or exp fishes/ or exp reptiles/ or mammals/ or primates/ or eutheria/ or exp artiodactyla/ or exp carnivore/ or exp cephalopoda/ or exp cetacea/ or exp chiroptera/ or exp elephants/ or exp hyraxes/ or exp insectivora/ or exp lagomorpha/ or marsupialia/ or exp monotremata/ or exp perissodactyla/ or proboscidea mammal/ or exp rodentia/ or exp scandentia/ or exp sirenia/ or exp cingulata/ or haplorhini/ or exp strepsirhini/ or exp platyrrhini/ or exp tarsii/ or catarrhini/ or exp cercopithecidae/ or exp hylobatidae/ or hominidae/ or exp gorilla gorilla/ or exp pan paniscus/ or exp pan troglodytes/ or exp pongo/ 38941083
2 (rat or rats or animal or animals or mice or in vivo or mouse or rabbit or rabbits or murine or pig or pigs or dog or dogs or bovine or fish or vertebrate or vertebrates or cat or cats or rodent or rodents or mammal or mammals or chicken or chickens or monkey or monkeys or sheep or canine or canines or porcine or cattle or bird or birds or hamster or hamsters or primate or primates or cow or cows or chick or horse or horses or avian or avians or calf or swine or swines or xenopus or turkeys or bear or bears or frog or frogs or zebrafish or goat or goats or equine or calves or poultry or macaque or macaques or mole or moles or ovine or lamb or lambs or fishes or diptera or amphibian or amphibians or snake or snakes or ruminant or ruminants or hen or hens or piglet or piglets or feline or felines or simian or simians or laevis or trout or trouts or teleost or teleosts or salmon or salmons or seal or seals or bull or bulls or ewe or ewes or hedgehog or hedgehogs or macaca or macacas or proteus or pigeon or pigeons or bat or bats or duck or ducks or chimpanzee or chimpanzees or baboon or baboons or deer or rana or ranas or carp or carps or heifer or swallow or swallows or lizard or lizards or canis or sow or sows or cynomolgus or quail or quails or reptile or reptiles or turtle or turtles or buffalo or gerbil or gerbils or boar or boars or squirrel or squirrels or oncorhynchus or mus or toad or toads or fowl or fowls or rerio or danio or ara or aras or musculus or tadpole or tadpoles or mulatta or salmo or ram or eagle or eagles or ferret or ferrets or goldfish or catfish or whale or whales or fox or foxes or ape or apes or elephant or elephants or bos or marmoset or marmosets or cod or cods or shark or sharks or wolf or eel or eels or auratus or rattus or zebra or zebras or tilapia or tilapias or gilt or camel or camels or squid or gallus or marsupial or marsupials or vole or voles or fascicularis or ovis or salmonid or salmonids or tiger or tigers or dolphin or dolphins or robin or robins or carpio or opossum or opossums or cyprinus or salamander or salamanders or felis or mink or minks or swan or swans or norvegicus or bufo or torpedo or bass or lamprey or lampreys or sus or python or pythons or tetrapod or tetrapods or shrew or shrews or lion or lions or hog or hogs or songbird or songbirds or oreochromis or starling or starlings or caprine or carassius or owl or owls or newt or newts or papio or scrofa or hare or hares or gorilla or gorillas or flounder or

flounders or goose or herring or herrings or therian or buffaloes or canary or sparrow or sparrows or microtus or octopus or troglodytes or tuna or amphibia or chinchilla or chinchillas or ide or oryzias or cervus or kangaroo or kangaroos or armadillo or armadillos or callithrix or pan troglodytes or saimiri or cichlid or cichlids or donkey or donkeys or bream or char or chars or finch or raccoon or raccoons or bothrops or anguilla or perch or cricetus or seabird or seabirds or buck or bucks or naja or coturnix or salmonids or geese or minnow or minnows or raptor or raptors or merione or meriones or rodentia or elaphus or amniote or amniotes or elasmobranch or emu or emus or peromyscus or hominid or hominids or bubalus or crotalus or gull or gulls or anas or anura or lemur or lemurs or crow or crows or camelus or gibbon or gibbons or waterfowl or parrot or parrots or eels or cob or stickleback or sticklebacks or columba or mesocricetus or ambystoma or raven or ravens or gadus or penguin or penguins or orangutan or orangutans or sturgeon or sturgeons or cuniculus or aves or virginianus or cephalopod or cephalopods or cebus or sparus or tortoise or tortoises or guttata or morhua or unguiculatus or dogfish or vulpes or mallard or mallards or apodemus or alligator or alligators or oryctolagus or llama or llamas or reindeer or mustela or duckling or ducklings or wolves or sander or amazona or zebu or badger or badgers or dove or doves or ictalurus or capra or capras or equus or camelid or camelids or poecilia or mule or mules or perciformes or salvelinus or labrax or cyprinidae or ariidae or crocodile or crocodiles or fundulus or dicentrarchus or clarias or cercopithecus or chiroptera or alpaca or alpacas or pike or pikes or paralichthys or puma or pumas or didelphis or pisces or macropus or triturus or bison or bisons or epinephelus or gasterosteus or panthera or acipenser or mackerel or mackerels or tamarin or tamarins or ostrich or anolis or vervet or vervets or wallaby or glareolus or beaver or beavers or dromedary or catus or killifish or pimephales or promelas or aotus or phoca or panda or pandas or porpoise or porpoises or myotis or yak or yaks or agkistrodon or vipera or otter or otters or turbot or turbots or squamate or carnivora or mullet or mullets or hawk or hawks or taeniopygia or seahorse or seahorses or poecilia reticulata or falcon or falcons or prosimian or prosimians or parus or perca or fingerling or fingerlings or antelope or antelopes or tupaia or passeriformes or sepia or saguinus or coyote or coyotes or pongo or meleagris or reptilia or lepus or psittacine or hagfish or warbler or warblers or russell's viper or russell's vipers or smolt or smolts or budgerigar or sardine or sardines or cavia or cavias or hyla or pleurodeles or siluriformes or great tit or great tits or guppy or bonobo or bonobos or rutilus or trichosurus or muridae or phodopus or channa or squalus or lynx or sturnus or petromyzon or vitulina or monodelphis or cuttlefish or adder or adders or lepomis or canaria or gambusia or guppies or xiphophorus or flatfish or koala or koalas or labeo or stingray or stingrays or chelonia or lampetra or spermophilus or crocodilian or passer domesticus or sciurus or artiodactyla or ranidae or corvus or necturus or platypus or canaries or bovid or lagopus or trimeresurus or gariepinus or marten or martens or drosophilidae or mugil or sunfish or porcellus or cypriniformes or alouatta or scophthalmus or anser or electrophorus or putorius or iguana or iguanas or lama or lamas or takifugu or circus or eptesicus or flycatcher or galago or galagos or trachemys or lungfish or characiformes or shorebird or shorebirds or giraffe or giraffes or micropterus or scyliorhinus or cichlidae or loligo or porcupine or porcupines or chub or chubs or solea or pleuronectes or hylidae or viperidae or echis or sorex or anchovy or lagomorph or ostriches or vulture or vultures or whitefish or araneus or jird or jirds or tern or esox or drake or drakes or elapidae or gallopavo or chordata or myodes or caretta or serinus or grouse or misgurnus or meles or blackbird or blackbirds or coregonus or bobwhite or bobwhites or heteropneustes or mammoth or mammoths or turdus or rhinella or ateles or characidae or clupea or bungarus or brill or struthio camelus or sloth or sloths or pteropus or sculpin or anthropoids or

pollock or pollocks or morone or pan paniscus or litoria or chipmunk or chipmunks or balaenoptera or marmota or melopsittacus or hyrax or lemming or lemmings or halibut or hylobates or lates or caiman or caimans or sigmodon or stenella or barbel or barbels or sterna or parakeet or parakeets or phocoena or leptodactylus or canidae or buteo or harengus or gopher or gophers or marmot or marmots or gosling or goslings or platichthys or gar or gars or sebastes or marsupialia or notophthalmus or gazelle or gazelles or insectivora or paridae or felidae or russula or galliformes or bombina or colobus or echidna or echidnas or seabass or syncerus or plaice or blue tit or blue tits or pagrus or catfishes or cetacea or barbus or cygnus or ficedula or chamois or colubridae or perches or coelacanth or fitch or urodela or cynops or martes or halichoerus or aix or salmonidae or leuciscus or magpie or magpies or silurus or whiting or whitings or anseriformes or colinus or rhea or chlorocebus or octodon or acinonyx or mouflon or mouflons or ibex or tetraodon or bufonidae or equidae or jackal or cephalopoda or dendroaspis or glama or muskrat or muskrats or sable or sables or wildebeest or streptopelia or albifrons or vespertilionidae or woodpecker or woodpeckers or muntjac or muntjacs or archosaur or branta or cricetulus or megalobrama or poeciliidae or desmodus or snakehead or snakeheads or tench or teal or teals or bandicoot or bandicoots or apteronotus or phyllostomidae or crocidura or buzzard or buzzards or larimichthys or cercocebus or pipistrellus or erithacus or impala or impalas or rousettus or haddock or haddocks or tinca or ratite or calidris or cynoglossus or hypophthalmichthys or bullock or bullocks or dromedaries or alectoris or filly or salamandra or cingulata or bitis or grus or ammodytes or macaw or macaws or hypoleuca or sapajus or cyprinodontiformes or hippopotamus or pelophylax or capybara or capybaras or weasel or weasels or cairina or cynomys or lutra or cockatoo or cockatoos or lachesis or lagomorpha or rupicapra or daboia or orang utan or orang utans or platyrrhini or charadriiformes or micrurus or psittaciformes or spalax or loris or mustelidae or sylvilagus or vitticeps or cockatiel or mustelus or cottus or erythrocebus or dipodomys or platessa or callicebus or loricariidae or catostomus or cuneata or cyanistes or cyprinodon or sigmodontinae or elasmobranchii or trichechus or sauropsid or xenarthra or dormouse or perissodactyla or nautilus or cirrhinus or gulo or tragelaphus or merula or numida or sciaenidae or cerastes or sciuridae or gibbosus or octopuses or eland or elands or phyllomedusa or pogona or walrus or agamidae or leptodactylidae or ridibundus or leontopithecus or anteater or anteaters or pelodiscus or cebidae or columbianus or pelteobagrus fulvidraco or hominoidea or mandrillus or zonotrichia leucophrys or agama or gobiocypris or bearded dragon or bearded dragons or sarotherodon or talpa or discoglossus or hagfishes or sphenodon or gudgeon or amphiuma or aythya or tenrec or tenrec or hominidae or risoria or salamandridae or camelidae or columbiformes or latimeria or plover or plovers or afrotheria or falco sparverius or polecat or polecats or crotalinae or salvadora or tarsier or lucioperca or anchovies or lungfishes or terrapin or dromaius novaehollandiae or lateolabrax or eigenmannia or pelamis or theropithecus or murinae or gander or gymnotus or pseudacris or gymnophiona or gymnotiformes or laticauda or falconiformes or dugong or dugongs or pintail or pintails or rook or rooks or lasiurus or catshark or catsharks or micropogonias or red junglefowl or paddlefish or ophiophagus or hollandicus or nymphicus or pimelodidae or aepyceros or cobitidae or strigiformes or cobitis or dormice or alytes or calloselasma or guanaco or phasianidae or round goby or trichogaster or catarrhini or eelpout or eelpouts or galaxias or gaur or pungitius or suslik or susliks or flatfishes or percidae or caprinae or todarodes or osmerus or ameiurus or anthropoidea or castor canadensis or pouting or poutings or tetraodontiformes or arvicolinae or siamang or siamangs or castor fiber or nomascus or red knot or red knots or syngnathidae or iguanidae or eretmochelys or ursidae or callimico or columbidae or

microhylidae or anaxyrus or menidia or pipistrelle or greylag or pipidae or scandentia or bowfin or bowfins or dendrobatidae or zenaida or bushbaby or harrier or harriers or macropodidae or pygerythrus or clupeidae or odorrana or corvidae or jerboa or jerboas or canutus or hylobatidae or clupeiformes or great cormorant or great cormorants or scorpaeniformes or chondrostean or garfish or proboscidea or psetta or diapsid or serotinus or tetrao or walruses or carcharhiniformes or leucoraja or pumpkinseed or dosidicus or acipenseriformes or daubentonii or emberizidae or gadiformes or hyraxes or stizostedion or wolverine or wolverines or lissotriton or acanthurus or centrarchidae or gloydius or laurasiatheria or limosa or psittacula or leporidae or proteidae or zander or zanders or arapaima or bagridae or cyprinodontidae or mithun or pandion or jackdaw or jackdaws or procyonidae or carus or jaculus or salmoniformes or common sole or common soles or protobothrops or calamita or brachyteles or trionyx or turdidae or boidae or luscinia or pugnax or euarchontoglires or saithe or saithes or symphalangus or aardvark or aardvarks or oystercatcher or oystercatchers or arius or corydoras or poacher or poachers or aurochs or cebuella or crecca or lemuridae or sirenia or lemmus or perdix or glires or lepidosaur or muskox or deinagkistrodon or pholidota or holocephali or cercopithecinae or clariidae or agapornis or doryteuthis or tyrannidae or dicroglossidae or godwit or godwits or monedula or pongidae or atheriniformes or colobinae or lophocebus or atelidae or cottidae or leucopsis or acanthuridae or didelphimorphia or elver or elvers or lapponica or dermoptera or european hake or european hakes or gerbillinae or banteng or hartebeest or hartebeests or hogget or haematopus or anguis fragilis or grey heron or grey herons or blue whiting or blue whitings or furnariidae or macrovipera or esocidae or lapwing or lapwings or mylopharyngodon or wallabia or beloniformes or potoroo or potoroos or athene noctua or pleuronectidae or bushbabies or muscicapidae or alligatoridae or fuligula or bush baby or guineafowl or spoonbill or spoonbills or viverridae or catostomidae or zebrafishes or ibexes or vendace or estrildidae or monotremata or sepiella or ambystomatidae or shelduck or shelducks or treeshrew or treeshrews or hoplobatrachus or pochard or hoolock or hoolocks or lynxes or antilope or antilopes or blackbuck or blackbucks or cricetinae or paramisgurnus or skylark or skylarks or soleidae or allobates or northern wheatear or northern wheatears or pitheciidae or takin or theria or vanellus or galaxiidae or lorisidae or ostralegus or palaeognathae or stone loach or alauda or callitrichinae or caniformia or duttaphrynus or ictaluridae or osteoglossiformes or poultries or curema or ruddy turnstone or ruddy turnstones or sheatfish or sunfishes or centropomidae or hemachatus or platalea or thamnophilidae or song thrush or atherinopsidae or siluridae or tadorna or chroicocephalus or ermine or ermines or gavialis or ruff or tupaiidae or diprotodontia or hyaenidae or antilopinae or crocodylidae or herpestidae or hippopotamidae or northern shoveler or round gobies or cheirogaleidae or indriidae or fundulidae or pythonidae or rhynchocephalia or anodorhynchus or red-backed shrike or red-backed shrikes or triakidae or phalangeridae or aoudad or boreoeutheria or eurasianjay or eurasian jays or feliformia or haplorhini or osteoglossidae or paenungulata or struthioniformes or ferina or sanderling or sanderlings or spheniscidae or cuttlefishes or cygnet or dasycneme or gadwall or gadwalls or pelobates fuscus or wryneck or wrynecks or afrosoricida or culaea or dover sole or dover soles or paralichthyidae or passeridae or osteolaemus or song thrushes or bluethroat or bluethroats or hydrophiidae or megrim or mephitidae or strepsirhini or tomistoma or epidalea or osmeriformes or bush babies or tarsiiform or atelinae or bufotes or eurasian coot or eurasian coots or galagidae or geopelia or philomachus or tubulidentata or bombinatoridae or pelobatidae or tachysurus or ailuridae or woodlark or woodlarks or alcelaphinae or redshank or redshanks or salientia or sand smelt or sand smelts or woodmice or woodmouse or dasyproctidae or eurasian wigeon or eurasian

wigeons or garganey or garganeys or lemon sole or lemon soles or common dab or common dabs or graylag or graylags or leucorodia or osphronemidae or bewickii or common moorhen or common moorhens or decapodiformes or gobbler or gobblers or odontophoridae or paddlefishes or eutheria or salmonine or esociformes or eurasian woodcock or eurasian woodcocks or european smelt or european smelts or goldfishes or tenches or tyranni or common chaffinch or common chaffinchs or common redstart or common redstarts or common roach or common roachs or great knot or great knots or potoroidae or alytidae or coregonine or dipteral or leveret or poeciliopsis gracilis or amphiumidae or batrachoidiformes or bighead goby or heteropneustidae or lullula or norway pout or norway pouts or sipunculida or dogfishes or sebastidae or tarsiidae or alethinophidia or common nase or common nases or common sandpiper or common sandpipers or eurasian blackcap or eurasian blackcaps or pterocnemia or syngnathiformes or common chaffinches or eupleridae or octopodiformes or phascolarctidae or scophthalmidae or starry smooth-hound or starry smooth-hounds or whitefishes or cuniculidae or european sprat or european sprats or rosy bitterling or rosy bitterlings or common dace or common daces or lesser weever or lesser weevers or scaldfish or water rail or water rails or alouattinae or centrarchiformes or common whitethroat or common whitethroats or gavialidae or grey gurnard or grey gurnards or lateolabracidae or rheiformes or tubgurnard or tub gurnards or common chiffchaff or common chiffchaffs or garfishes or lesser whitethroat or lesser whitethroats or myoxidae or seabasses or spariformes or umbridae or yellow boxfish or anabantiformes or aotidae or common bleak or common bleaks or common rudd or common rudds or greater pipefish or hapale or nandiniidae or stone loaches or whinchat or whinchats or acanthuriformes or brotula barbata or common ling or common lings or common roaches or cottonrat or cottonrats or douroucoulis or dromaiidae or fitches or fitchew or galaxiiformes or laprine or saimiriinae or solenette or tarsii or tompot blenny or common dragonet or common dragonets or longspined bullhead or longspined bullheads or monotremate or monotremates or pempheriformes or perdicinae or presbytini or smegmamorpha or bighead gobies or carangaria incertae sedis or coiidae or fivebeard rockling or foulmart or foumart or grasskeet or greater pipefishes or ibices or millionfish or muguliformes or norwegian topknot or peewit or red sea sailfin tang or rupicapras or sheatfishes or tompot blennies or twait shad or yellow boxfishes).tw. 14176107

3 medline.st. 29904626

4 2 not 3 8398417

5 1 or 4 40352035

6 "Receptors, CCR5"/ 18466

7 (cc chemokine receptor* 5 or cc-ckr5 or ccr5 or cd195 antigen* or ckr5 receptor*).tw,kf. 22382

8 exp CCR5 Receptor Antagonists/ 8211

9 C-C chemokine receptor type 5.tw,kf. 434

10 (md6p741w8a or maraviroc or selzentry or Celsentri or uk 427,857 or uk-427,857 or uk427,857).tw,kf. 3408

11 Vicriviroc.mp. 728

12 (Aplaviroc or ancriviroc or aplaviroc or leronlimab or mavorixafor).mp. 866

13 INCB009471.mp. 2

14 TBR 652.mp. 37

15 Pro 140.mp. 297

16 HGS004.mp. 15

17 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 33160
18 5 and 17 23661
19 exp cerebrovascular disorders/ or stroke/ 1287736
20 stroke*.tw,kf. 792358
21 transient isch?em* attack*.tw,kf. 42566
22 brain injur*.tw,kf. 199884
23 ((brain or cereb* or intracereb*) adj2 (isch?em* or infarct* or h?emorrhag*)).tw,kf. 261843
24 or/19-23 1714778
25 18 and 24 373
26 25 use medall 58
27 exp animal experiment/ or exp animal model/ or exp experimental animal/ or exp transgenic animal/ or exp male animal/ or exp female animal/ or exp juvenile animal/ or animal/ or chordata/ or vertebrate/ or tetrapod/ or exp fish/ or amniote/ or exp amphibia/ or mammal/ or exp reptile/ or exp sauropsid/ or therian/ or exp monotreme/ or placental mammal/ or exp marsupial/ or Euarchontoglires/ or exp Afrotheria/ or exp Boreoeutheria/ or exp Laurasiatheria/ or exp Xenarthra/ or primate/ or exp Dermoptera/ or exp Glires/ or exp Scandentia/ or Haplorhini/ or exp prosimian/ or simian/ or exp tarsiiform/ or Catarrhini/ or exp Platyrrhini/ or ape/ or exp Cercopithecidae/ or hominid/ or exp hylobatidae/ or exp chimpanzee/ or exp gorilla/ or exp orang utan/ or exp cephalopod/ 14616185
28 (rat or rats or animal or animals or mice or "in vivo" or mouse or rabbit or rabbits or murine or pig or pigs or dog or dogs or bovine or fish or vertebrate or vertebrates or cat or cats or rodent or rodents or mammal or mammals or chicken or chickens or monkey or monkeys or sheep or canine or canines or porcine or cattle or bird or birds or hamster or hamsters or primate or primates or cow or cows or chick or horse or horses or avian or avians or calf or swine or swines or xenopus or turkeys or bear or bears or frog or frogs or zebrafish or goat or goats or equine or calves or poultry or macaque or macaques or mole or moles or ovine or lamb or lambs or fishes or diptera or amphibian or amphibians or snake or snakes or ruminant or ruminants or hen or hens or piglet or piglets or feline or felines or simian or simians or laevis or trout or trouts or teleost or teleosts or salmon or salmons or seal or seals or bull or bulls or ewe or ewes or hedgehog or hedgehogs or macaca or macacas or proteus or pigeon or pigeons or bat or bats or duck or ducks or chimpanzee or chimpanzees or baboon or baboons or deer or deers or rana or ranas or carp or carps or heifer or swallow or swallows or lizard or lizards or canis or sow or sows or cynomolgus or quail or quails or reptile or reptiles or turtle or turtles or buffalo or gerbil or gerbils or boar or boars or squirrel or squirrels or oncorhynchus or mus or toad or toads or fowl or fowls or rerio or danio or ara or aras or musculus or tadpole or tadpoles or mulatta or salmo or ram or eagle or eagles or ferret or ferrets or goldfish or catfish or whale or whales or fox or foxes or ape or apes or elephant or elephants or bos or marmoset or marmosets or cod or cods or shark or sharks or wolf or eel or eels or auratus or rattus or zebra or zebras or tilapia or tilapias or gilt or camel or camels or squid or gallus or marsupial or marsupials or vole or voles or fascicularis or ovis or salmonid or salmonids or tiger or tigers or dolphin or dolphins or robin or robins or carpio or opossum or opossums or cyprinus or salamander or salamanders or felis or mink or minks or swan or swans or norvegicus or bufo or torpedo or bass or lamprey or lampreys or sus or python or pythons or tetrapod or tetrapods or shrew or shrews or lion or lions or hog or hogs or songbird or songbirds or oreochromis or starling or starlings or caprine or carassius or owl or owls or newt or newts or papio or scrofa or hare or hares or gorilla or gorillas or flounder

or flounders or goose or herring or herrings or therian or buffaloes or canary or sparrow or sparrows or microtus or octopus or troglodytes or tuna or amphibia or chinchilla or chinchillas or ide or oryzias or cervus or kangaroo or kangaroos or armadillo or armadillos or callithrix or "pan troglodytes" or saimiri or cichlid or cichlids or donkey or donkeys or bream or char or chars or finch or raccoon or raccoons or bothrops or anguilla or perch or cricetus or seabird or seabirds or buck or bucks or naja or coturnix or salmonids or geese or minnow or minnows or raptor or raptors or merione or meriones or rodentia or elaphus or amniote or amniotes or elasmobranch or emu or emus or peromyscus or hominid or hominids or bubalus or crotalus or gull or gulls or anas or anura or lemur or lemurs or crow or crows or camelus or gibbon or gibbons or waterfowl or parrot or parrots or eels or cob or stickleback or sticklebacks or columba or mesocricetus or ambystoma or raven or ravens or gadus or penguin or penguins or orangutan or orangutans or sturgeon or sturgeons or cuniculus or aves or virginianus or cephalopod or cephalopods or cebus or sparus or tortoise or tortoises or guttata or morhua or unguiculatus or dogfish or vulpes or mallard or mallards or apodemus or alligator or alligators or oryctolagus or llama or llamas or reindeer or mustela or duckling or ducklings or wolves or sander or amazona or zebu or badger or badgers or dove or doves or ictalurus or capra or capras or equus or camelid or camelids or poecilia or mule or mules or perciformes or salvelinus or labrax or cyprinidae or ariidae or crocodile or crocodiles or fundulus or dicentrarchus or clarias or cercopithecus or chiroptera or alpaca or alpacas or pike or pikes or paralichthys or puma or pumas or didelphis or pisces or macropus or triturus or bison or bisons or epinephelus or gasterosteus or panthera or acipenser or mackerel or mackerels or tamarin or tamarins or ostrich or anolis or vervet or vervets or wallaby or glareolus or beaver or beavers or dromedary or catus or killifish or pimephales or promelas or aotus or phoca or panda or pandas or porpoise or porpoises or myotis or yak or yaks or agkistrodon or vipera or otter or otters or turbot or turbots or squamate or carnivora or mullet or mullets or hawk or hawks or taeniopygia or seahorse or seahorses or "poecilia reticulata" or falcon or falcons or prosimian or prosimians or parus or perca or fingerling or fingerlings or antelope or antelopes or tupaia or passeriformes or sepia or saguinus or coyote or coyotes or pongo or meleagris or reptilia or lepus or psittacine or hagfish or warbler or warblers or "russell s viper" or "russell s vipers" or smolt or smolts or budgerigar or sardine or sardines or cavia or cavias or hyla or pleurodeles or siluriformes or "great tit" or "great tits" or guppy or bonobo or bonobos or rutilus or trichosurus or muridae or phodopus or channa or squalus or lynx or sturnus or petromyzon or vitulina or monodelphis or cuttlefish or adder or adders or lepomis or canaria or gambusia or guppies).tw. 14111285

29 (xiphophorus or flatfish or koala or koalas or labeo or stingray or stingrays or chelonia or lampetra or spermophilus or crocodilian or "passer domesticus" or sciurus or artiodactyla or ranidae or corvus or necturus or platypus or canaries or bovid or lagopus or trimeresurus or gariepinus or marten or martens or drosophilidae or mugil or sunfish or porcellus or cypriniformes or alouatta or scophthalmus or anser or electrophorus or putorius or iguana or iguanas or lama or lamas or takifugu or circus or eptesicus or flycatcher or galago or galagos or trachemys or lungfish or characiformes or shorebird or shorebirds or giraffe or giraffes or micropterus or scyliorhinus or cichlidae or loligo or porcupine or porcupines or chub or chubs or solea or pleuronectes or hylidae or viperidae or echis or sorex or anchovy or lagomorph or ostriches or vulture or vultures or whitefish or araneus or jird or jirds or tern or esox or drake or drakes or elapidae or gallopavo or chordata or myodes or caretta or serinus or grouse or misgurnus or meles or blackbird or blackbirds or coregonus or bobwhite or bobwhites or heteropneustes or mammoth or mammoths or turdus or rhinella or ateles or characidae or clupea

or bungarus or brill or "struthio camelus" or sloth or sloths or pteropus or sculpin or anthropoids or pollock or pollocks or morone or "pan paniscus" or litoria or chipmunk or chipmunks or balaenoptera or marmota or melopsittacus or hyrax or lemming or lemmings or halibut or hylobates or lates or caiman or caimans or sigmodon or stenella or barbel or barbels or sterna or parakeet or parakeets or phocoena or leptodactylus or canidae or buteo or harengus or gopher or gophers or marmot or marmots or gosling or goslings or platichthys or gar or gars or sebastes or marsupialia or notophthalmus or gazelle or gazelles or insectivora or paridae or felidae or russula or galliformes or bombina or colobus or echidna or echidnas or seabass or syncerus or plaice or "blue tit" or "blue tits" or pagrus or catfishes or cetacea or barbus or cygnus or ficedula or chamois or colubridae or perches or coelacanth or fitch or urodela or cynops or martes or halichoerus or aix or salmonidae or leuciscus or magpie or magpies or silurus or whiting or whitings or anseriformes or colinus or rhea or chlorocebus or octodon or acinonyx or mouflon or mouflons or ibex or tetraodon or bufonidae or equidae or jackal or cephalopoda or dendroaspis or glama or muskrat or muskrats or sable or sables or wildebeest or streptopelia or albifrons or vespertilionidae or woodpecker or woodpeckers or muntjac or muntjacs or archosaur or branta or cricetulus or megalobrama or poeciliidae or desmodus or snakehead or snakeheads or tench or teal or teals or bandicoot or bandicoots or apteronotus or phyllostomidae or crocidura or buzzard or buzzards or larimichthys or cercocebus or pipistrellus or erithacus or impala or impalas or rousettus or haddock or haddocks or tinca or ratite or calidris or cynoglossus or hypophthalmichthys or bullock or bullocks or dromedaries or alectoris or filly or salamandra or cingulata or bitis or grus or ammodytes or macaw or macaws or hypoleuca or sapajus or cyprinodontiformes or hippopotamus or pelophylax or capybara or capybaras or weasel or weasels or cairina or cynomys or lutra or cockatoo or cockatoos or lachesis or lagomorpha or rupicapra or daboia or "orang utan" or "orang utans" or platyrrhini or charadriiformes or micrurus or psittaciformes or spalax or loris or mustelidae or sylvilagus or vitticeps or cockatiel or mustelus or cottus or erythrocebus or dipodomys or platessa or callicebus or loricariidae or catostomus or cuneata or cyanistes or cyprinodon or sigmodontinae or elasmobranchii or trichechus or sauropsid or xenarthra or dormouse or perissodactyla or nautilus or cirrhinus or gulo or gulos or tragelaphus or merula or numida or sciaenidae or cerastes or sciuridae or gibbosus or octopuses or eland or elands or phyllomedusa or pogona or walrus or agamidae or leptodactylidae or ridibundus or leontopithecus or anteater or anteaters or pelodiscus or cebidae or columbianus or "pelteobagrus fulvidraco" or hominoidea or mandrillus or "zonotrichia leucophrys" or agama or gobiocypris or "bearded dragon" or "bearded dragons" or sarotherodon or talpa or discoglossus or hagfishes or sphenodon or gudgeon or amphiuma or aythya or tenrec or tenrec or hominidae or risoria or salamandridae or camelidae or columbiformes or latimeria or plover or plovers or afrotheria or "falco sparverius" or polecat or polecats or crotalinae or salvadora or tarsier or lucioperca or anchovies or lungfishes or terrapin or "dromaius novaehollandiae" or lateolabrax or eigenmannia or pelamis or theropithecus or murinae or gander or gymnotus or pseudacris or gymnophiona or gymnotiformes or laticauda or falconiformes or dugong or dugongs or pintail or pintails or rook or rooks or lasiurus or catshark or catsharks or micropogonias or "red junglefowl" or paddlefish or ophiophagus or hollandicus or nymphicus or pimelodidae or aepyceros or cobitidae or strigiformes or cobitis or dormice or alytes or calloselasma or guanaco or guanacos or phasianidae or "round goby" or trichogaster or catarrhini or eelpout or eelpouts or galaxias or gaur or pungitius or suslik or susliks or flatfishes or percidae or caprinae or todarodes or osmerus or ameiurus or anthropoidea or "castor canadensis" or pouting or poutings or tetraodontiformes or arvicolinae or siamang or siamangs or

"castor fiber" or nomascus or "red knot" or "red knots" or syngnathidae or iguanidae or eretmochelys or ursidae or callimico or columbidae or microhylidae or anaxyrus or menidia or pipistrelle or greylag or pipidae or scandentia or bowfin or bowfins or dendrobatidae or zenaida or bushbaby or harrier or harriers or macropodidae or pygerythrus or clupeidae or odorrana or corvidae or jerboa or jerboas or canutus or hylobatidae or clupeiformes or "great cormorant" or "great cormorants" or scorpaeniformes or chondrostean or garfish or proboscidea or psetta or diapsid or serotinus or tetrao or walruses or carcharhiniformes or leucoraja or pumpkinseed or dosidicus or acipenseriformes or daubentonii or emberizidae or gadiformes or hyraxes or stizostedion or wolverine or wolverines or lissotriton or acanthurus or centrarchidae or gloydius or laurasiatheria or limosa or psittacula or leporidae or proteidae or zander or zanders or arapaima or bagridae or cyprinodontidae or mithun or pandion or jackdaw or jackdaws or procyonidae or carus or jaculus or salmoniformes or "common sole" or "common soles" or protobothrops or calamita or brachyteles or trionyx or turdidae or boidae or luscinia or pugnax or euarchontoglires or saithe or saithes or symphalangus or aardvark).tw. 272058

30 (aardvarks or oystercatcher or oystercatchers or arius or corydoras or poacher or poachers or aurochs or cebuella or crecca or lemuridae or sirenia or lemmus or perdix or glires or lepidosaur or muskox or deinagkistrodon or pholidota or holocephali or cercopithecinae or clariidae or agapornis or doryteuthis or tyrannidae or dicroglossidae or godwit or godwits or monedula or pongidae or atheriniformes or colobinae or lophocebus or atelidae or cottidae or leucopsis or acanthuridae or didelphimorphia or elver or elvers or lapponica or dermoptera or "european hake" or "european hakes" or gerbillinae or banteng or hartebeest or hartebeests or hogget or haematopus or "anguis fragilis" or "grey heron" or "grey herons" or "blue whiting" or "blue whitings" or furnariidae or macrovipera or esocidae or lapwing or lapwings or mylopharyngodon or wallabia or beloniformes or potoroo or potoroos or "athene noctua" or pleuronectidae or bushbabies or muscicapidae or alligatoridae or fuligula or "bush baby" or guineafowl or spoonbill or spoonbills or viverridae or catostomidae or zebrafishes or ibexes or vendace or estrildidae or monotremata or sepiella or ambystomatidae or shelduck or shelducks or treeshrew or treeshrews or hoplobatrachus or pochard or hoolock or hoolocks or lynxes or antilope or antilopes or blackbuck or blackbucks or cricetinae or paramisgurnus or skylark or skylarks or soleidae or allobates or "northern wheatear" or "northern wheatears" or pitheciidae or takin or theria or vanellus or galaxiidae or lorisidae or ostralegus or palaeognathae or "stone loach" or alauda or callitrichinae or caniformia or duttaphrynus or ictaluridae or osteoglossiformes or poultries or curema or "ruddy turnstone" or "ruddy turnstones" or sheatfish or sunfishes or centropomidae or hemachatus or platalea or thamnophilidae or "song thrush" or atherinopsidae or siluridae or tadorna or chroicocephalus or ermine or ermines or gavialis or ruff or tupaiidae or diprotodontia or hyaenidae or antilopinae or crocodylidae or herpestidae or hippopotamidae or "northern shoveler" or "round gobies" or cheirogaleidae or indriidae or fundulidae or pythonidae or rhynchocephalia or anodorhynchus or "red-backed shrike" or "red-backed shrikes" or triakidae or phalangeridae or aoudad or boreoeutheria or "eurasian jay" or "eurasian jays" or feliformia or haplorhini or osteoglossidae or paenungulata or struthioniformes or ferina or sanderling or sanderlings or spheniscidae or cuttlefishes or cygnet or dasycneme or gadwall or gadwalls or "pelobates fuscus" or wryneck or wrynecks or afrosoricida or culaea or "dover sole" or "dover soles" or paralichthyidae or passeridae or osteolaemus or "song thrushes" or bluethroat or bluethroats or hydrophiidae or megrim or mephitidae or strepsirhini or tomistoma or epidalea or osmeriformes or "bush babies" or tarsiiform or atelinae or bufotes or "eurasian coot" or "eurasian coots" or galagidae or geopelia or philomachus or tubulidentata or

bombinatoridae or pelobatidae or tachysurus or ailuridae or woodlark or woodlarks or alcelaphinae or redshank or redshanks or salientia or "sand smelt" or "sand smelts" or woodmice or woodmouse or dasyproctidae or "eurasian wigeon" or "eurasian wigeons" or garganey or garganeys or "lemon sole" or "lemon soles" or "common dab" or "common dabs" or graylag or graylags or leucorodia or osphronemidae or bewickii or "common moorhen" or "common moorhens" or decapodiformes or gobbler or gobblers or odontophoridae or paddlefishes or eutheria or salmonine or esociformes or "eurasian woodcock" or "eurasian woodcocks" or "european smelt" or "european smelts" or goldfishes or tenches or tyranni or "common chaffinch" or "common chaffinchs" or "common redstart" or "common redstarts" or "common roach" or "common roachs" or "great knot" or "great knots" or potoroidae or alytidae or coregonine or dipteral or leveret or "poeciliopsis gracilis" or amphiumidae or batrachoidiformes or "bighead goby" or heteropneustidae or lullula or "norway pout" or "norway pouts" or sipunculida or dogfishes or sebastidae or tarsiidae or alethinophidia or "common nase" or "common nases" or "common sandpiper" or "common sandpipers" or "eurasian blackcap" or "eurasian blackcaps" or pterocnemia or syngnathiformes or "common chaffinches" or eupleridae or octopodiformes or phascolarctidae or scophthalmidae or "starry smooth-hound" or "starry smooth-hounds" or whitefishes or cuniculidae or "european sprat" or "european sprats" or "rosy bitterling" or "rosy bitterlings" or "common dace" or "common daces" or "lesser weever" or "lesser weevers" or scaldfish or "water rail" or "water rails" or alouattinae or centrarchiformes or "common whitethroat" or "common whitethroats" or gavialidae or "grey gurnard" or "grey gurnards" or lateolabracidae or rheiformes or "tub gurnard" or "tub gurnards" or "common chiffchaff" or "common chiffchaffs" or garfishes or "lesser whitethroat" or "lesser whitethroats" or myoxidae or seabasses or spariformes or umbridae or "yellow boxfish" or anabantiformes or aotidae or "common bleak" or "common bleaks" or "common rudd" or "common rudds" or "greater pipefish" or hapale or nandiniidae or "stone loaches" or whinchat or whinchats or acanthuriformes or "brotula barbata" or "common ling" or "common lings" or "common roaches" or cottonrat or cottonrats or douroucoulis or dromaiidae or fitches or fitchew or galaxiiformes or laprine or saimiriinae or solenette or tarsii or "tompot blenny" or "common dragonet" or "common dragonets" or "longspined bullhead" or "longspined bullheads" or monotremate or monotremates or pempheriformes or perdicinae or presbytini or smegmamorpha or "bighead gobies" or "carangaria incertae sedis" or coiidae or "fivebeard rockling" or foulmart or foumart or grasskeet or "greater pipefishes" or ibices or millionfish or muguliformes or "norwegian topknot" or peewit or "red sea sailfin tang" or rupicapras or sheatfishes or "tompot blennies" or "twait shad" or "yellow boxfishes").tw. 21723

31 or/27-30 18002118

32 exp chemokine receptor CCR5 antagonist/ 6682

33 (cc chemokine receptor* 5 or cc-ckr5 or ccr5 or cd195 antigen* or ckr5 receptor* or C-C chemokine receptor type 5).tw. 22155

34 (md6p741w8a or maraviroc or selzentry or Celsentri or uk 427,857 or uk-427,857 or uk427,857).tw. 3329

35 Vicriviroc.mp. 728

36 (Aplaviroc or ancriviroc or aplaviroc or leronlimab or mavorixafor).mp. 866

37 INCB009471.mp. 2

38 TBR 652.mp. 37

39 Pro 140.mp. 297

40 HGS004.mp. 15

41 chemokine receptor CCR5/ 12698
42 or/32-41 31956
43 31 and 42 10275
44 exp cerebrovascular disease/ or exp cerebrovascular accident/ 1207681
45 stroke*.tw. 771477
46 ((brain or cereb* or intracereb*) adj2 (isch?em* or infarct* or h?emorrhag*)).tw. 247502
47 (transient isch?em* attack* or brain injur*).tw. 231020
48 44 or 45 or 46 or 47 1675247
49 43 and 48 172
50 49 use emczd 118
51 26 or 50 176

Web of Science – October 25, 2022

Web of Science Search Strategy (v0.1)

Entitlements:

- WOS.SSCI: 1900 to 2022
- WOS.AHCI: 1975 to 2022
- WOS.ISTP: 1990 to 2022
- WOS.ESCI: 2005 to 2022
- WOS.SCI: 1900 to 2022
- WOS.ISSHP: 1990 to 2022

Searches:

1: CCR5 (Topic) Results: 11678

2: TS=(cc chemokine receptor* 5) OR TS=(C-C chemokine receptor type 5)
Results: 1796

3: TS=(cc-ckr5) Results: 9

4: TS=(Vicriviroc) Results: 158

5: TS=(((md6p741w8a OR maraviroc OR selzentry OR Celsentri)))
Results: 1542

6: ALL=((Aplaviroc or ancriviroc or aplaviroc or leronlimab or mavorixafor))
Results: 97

7: (((TS=(INCB009471)) OR TS=(TBR 652)) OR TS=(Pro 140)) OR TS=(HGS004)
Results: 972

8: #7 OR #6 OR #5 OR #4 OR #3 OR #2 OR #1 Results: 14241

9: (TS=(stroke*)) OR TS=("transient isch?em* attack*") Results: 430668

10: TS=((brain or cereb* or intracereb*) NEAR/2 ischem*) Results: 77919

11: TS=(((brain or cereb* or intracereb*) NEAR/2 ischaem*))
Results: 5389

12: TS=(((brain or cereb* or intracereb*) NEAR/2 hemorrhag*))
Results: 33959

13: TS=(((brain or cereb* or intracereb*) NEAR/2 haemorrhag*))
Results: 4867

14: TS="cerebrovascular disorder*" Results: 3993

15: TS="brain injur*" Results: 121601

16: #15 OR #14 OR #13 OR #12 OR #11 OR #10 OR #9 Results: 591495

17: #8 AND #16 Results: 136

18: TS=(rat OR rats OR animal OR animals OR mice OR "in vivo" OR mouse OR rabbit OR rabbits OR murine OR pig OR pigs OR dog OR dogs OR bovine OR fish OR vertebrate OR vertebrates OR cat OR cats OR rodent OR rodents OR mammal OR mammals OR chicken OR chickens OR monkey OR monkeys OR sheep OR canine OR canines OR porcine OR cattle OR bird OR birds OR hamster OR hamsters OR primate OR primates OR cow OR cows OR chick OR horse OR horses OR avian OR avians OR calf OR swine OR swines OR xenopus OR turkeys OR bear OR bears OR frog OR frogs OR zebrafish OR goat OR goats OR equine OR calves OR poultry OR macaque OR macaques OR mole OR moles OR ovine OR lamb OR lambs OR fishes OR diptera OR amphibian OR amphibians OR snake OR snakes OR ruminant OR ruminants OR hen OR hens OR piglet OR piglets OR feline OR felines OR simian OR simians OR laevis OR trout OR trouts OR teleost OR teleosts OR salmon OR salmons OR seal OR seals OR bull OR bulls OR ewe OR ewes OR hedgehog OR hedgehogs OR macaca OR macacas OR proteus OR pigeon ORpigeons OR bat OR bats OR duck OR ducks OR chimpanzee OR chimpanzees OR baboon OR baboons OR deer OR rana OR ranas OR carp OR carps OR heifer OR swallow OR swallows OR lizard OR lizards OR canis OR sow OR sows OR cynomolgus OR quail OR quails OR reptile OR reptiles OR turtle OR turtles OR buffalo OR gerbil OR gerbils OR boar OR boars OR squirrel OR squirrels OR oncorhynchus OR mus OR toad OR toads OR fowl OR fowls OR rerio OR danio OR ara OR aras OR musculus OR tadpole OR tadpoles OR mulatta OR salmo OR ram OR eagle OR eagles OR ferret OR ferrets OR goldfish OR catfish OR whale OR whales OR fox OR foxes OR ape OR apes OR elephant OR elephants OR bos OR marmoset OR marmosets OR cod OR cods OR shark OR sharks OR wolf OR eel OR eels OR auratus OR rattus OR zebra OR zebras OR tilapia OR tilapias OR gilt OR camel OR camels OR squid OR gallus OR marsupial OR marsupials OR vole OR voles OR fascicularis OR ovis OR salmonid OR salmonids OR tiger OR tigers OR dolphin OR dolphins OR robin OR robins OR carpio OR opossumOR opossums OR cyprinus OR salamander OR salamanders OR felis OR mink OR minks OR swan OR swans OR norvegicus OR bufo OR torpedo OR bass OR lamprey OR lampreys OR sus OR python OR pythons OR tetrapod OR tetrapods OR shrew OR shrews OR lion OR lions OR hogOR hogs OR songbird OR songbirds OR oreochromis OR starling OR starlings OR caprine OR carassius OR owl OR owls OR newt OR newts OR papio OR scrofa OR hare OR hares OR gorilla OR gorillas OR flounder OR flounders OR goose OR herring OR herrings OR therianOR buffaloes OR canary OR sparrow OR sparrows OR microtus OR octopus OR troglodytes OR tuna OR amphibia OR chinchilla OR chinchillas OR ide OR oryzias OR cervus OR kangaroo OR kangaroos OR armadillo OR armadillos OR callithrix OR "pan troglodytes" OR saimiri OR cichlid OR cichlids OR donkey OR donkeys OR bream OR char OR chars OR finch OR raccoon OR raccoons OR

bothrops OR anguilla OR perch OR cricetus OR seabird OR seabirds OR buck OR bucks OR naja OR coturnix OR salmonids OR geese OR minnow OR minnows ORraptor OR raptors OR merione OR meriones OR rodentia OR elaphus OR amniote OR amniotes OR elasmobranch OR emu OR emus OR peromyscus OR hominid OR hominids OR bubalus OR crotalus OR gull OR gulls OR anas OR anura OR lemur OR lemurs OR crow OR crows OR camelus OR gibbon OR gibbons OR waterfowl OR parrot OR parrots OR eels OR cob OR stickleback OR sticklebacks OR columba OR mesocricetus OR ambystoma OR raven OR ravens OR gadus OR penguin OR penguins OR orangutan OR orangutans OR sturgeon OR sturgeons OR cuniculus OR aves OR virginianus OR cephalopod OR cephalopods OR cebus OR sparus OR tortoise OR tortoises OR guttata OR morhua OR unguiculatus OR dogfish OR vulpes OR mallard OR mallards OR apodemus OR alligator OR alligators OR oryctolagus OR llama OR llamas OR reindeer OR mustela OR duckling OR ducklings OR wolves OR sander OR amazona OR zebu OR badger OR badgers OR dove OR doves OR ictalurus OR capra OR capras OR equus OR camelid OR camelids OR poecilia OR mule OR mules OR perciformes OR salvelinus OR labrax OR cyprinidae OR ariidae OR crocodile OR crocodiles OR fundulus OR dicentrarchus OR clarias OR cercopithecus OR chiroptera OR alpaca OR alpacas OR pike OR pikes OR paralichthys OR puma OR pumas OR didelphis OR pisces OR macropus OR triturus OR bison OR bisons OR epinephelus OR gasterosteus OR panthera OR acipenser OR mackerel OR mackerels OR tamarin OR tamarins OR ostrich OR anolis OR vervet OR vervets OR wallaby OR glareolus OR beaver OR beavers OR dromedary OR catus OR killifish OR pimephales OR promelas OR aotus OR phoca OR panda OR pandas OR porpoise OR porpoises OR myotis OR yak OR yaks OR agkistrodon OR vipera OR otter OR otters OR turbot OR turbots OR squamate OR carnivora OR mullet OR mullets OR hawk OR hawks OR taeniopygia OR seahorse OR seahorses OR "poecilia reticulata" OR falcon OR falcons OR prosimian OR prosimians OR parus OR perca OR fingerling OR fingerlings OR antelope OR antelopes OR tupaia OR passeriformes OR sepia OR saguinus OR coyote OR coyotes OR pongo OR meleagris OR reptilia OR lepus OR psittacine OR hagfish OR warbler OR warblers OR "russell s viper" OR "russell s vipers" OR smolt OR smolts OR budgerigar OR sardine OR sardines OR cavia OR cavias OR hyla OR pleurodeles OR siluriformes OR "great tit" OR "great tits" OR guppy OR bonobo OR bonobos OR rutilus OR trichosurus OR muridae OR phodopus OR channa OR squalus OR lynx OR sturnus OR petromyzon OR vitulina OR monodelphis OR cuttlefish OR adder OR adders OR lepomis OR canaria OR gambusia OR guppies OR xiphophorus OR flatfish OR koala ORkoalas OR labeo OR stingray OR stingrays OR chelonia OR lampetra OR spermophilus OR crocodilian OR "passer domesticus" OR sciurus OR artiodactyla OR ranidae OR corvus OR necturus OR platypus OR canaries OR bovid OR lagopus OR trimeresurus OR gariepinus ORmarten OR martens OR drosophilidae OR mugil OR sunfish OR porcellus OR cypriniformes OR alouatta OR scophthalmus OR anser OR electrophorus OR putorius OR iguana OR iguanas OR lama OR lamas OR takifugu OR circus OR eptesicus OR flycatcher OR galago OR galagos OR trachemys OR lungfish OR characiformes OR shorebird OR shorebirds OR giraffe OR giraffes OR micropterus OR scyliorhinus OR cichlidae OR loligo OR porcupine OR porcupines OR chub OR chubs OR solea OR pleuronectes OR hylidae OR viperidae OR echis OR sorex OR anchovy OR lagomorph OR ostriches OR vulture OR

vultures OR whitefish OR araneus OR jird OR jirds OR tern OR esox OR drake OR drakes OR elapidae OR gallopavo OR chordata OR myodes OR caretta OR serinus OR grouse OR misgurnus OR meles OR blackbird OR blackbirds OR coregonus OR bobwhite OR bobwhites OR heteropneustes OR

mammoth OR mammoths OR turdus OR rhinella OR ateles OR characidae OR clupea OR bungarus OR brill OR "struthio camelus" OR sloth OR sloths OR pteropus OR sculpin OR anthropoids OR pollock OR pollocks OR morone OR "pan paniscus" OR litoria OR chipmunk OR chipmunks OR balaenoptera OR marmota OR melopsittacus OR hyrax OR lemming OR lemmings OR halibut OR hylobates OR lates OR caiman OR caimans OR sigmodon OR stenella OR barbel OR barbels ORsterna OR parakeet OR parakeets OR phocoena OR leptodactylus OR canidae OR buteo OR harengus OR gopher OR gophers OR marmot OR marmots OR gosling OR goslings OR platichthys OR gar OR gars OR sebastes OR marsupialia OR notophthalmus OR gazelle OR gazelles OR insectivora OR paridae OR felidae OR russula OR galliformes OR bombina OR colobus OR echidna OR echidnas OR seabass OR syncerus OR plaice OR "blue tit" OR "blue tits" OR pagrus OR catfishes OR cetacea OR barbus OR cygnus OR ficedula OR chamois OR colubridae OR perches OR coelacanth OR fitch OR urodela OR cynops OR martes OR halichoerus OR aix OR salmonidae OR leuciscus OR magpie OR magpies OR silurus OR whiting OR whitings OR anseriformes OR colinus OR rhea OR chlorocebus OR octodon OR acinonyx OR mouflon OR mouflons OR ibex OR tetraodon OR bufonidae OR equidae OR jackal OR cephalopoda OR dendroaspis OR glama OR muskrat OR muskrats OR sable OR sables OR wildebeest OR streptopelia OR albifrons OR vespertilionidae OR woodpecker OR woodpeckers OR muntjac OR muntjacs OR archosaur OR branta OR cricetulus OR megalobrama OR poeciliidae OR desmodus OR snakehead OR snakeheads OR tench OR teal OR teals OR bandicoot OR bandicoots OR apteronotus OR phyllostomidae OR crocidura OR buzzard OR buzzards OR larimichthys OR cercocebus OR pipistrellus OR erithacus OR impala OR impalas OR rousettus OR haddock OR haddocks OR tinca OR ratite OR calidris OR cynoglossus OR hypophthalmichthys OR bullock OR bullocks OR dromedaries OR alectoris OR filly OR salamandra OR cingulata OR bitis OR grus OR ammodytes OR macaw OR macaws OR hypoleuca OR sapajus OR cyprinodontiformes OR hippopotamus OR pelophylax OR capybara OR capybaras OR weasel OR weasels OR cairina OR cynomys OR lutra OR cockatoo OR cockatoos OR lachesis OR lagomorpha OR rupicapra OR daboia OR "orang utan" OR "orang utans" OR platyrrhini OR charadriiformes OR micrurus OR psittaciformes OR spalax OR loris OR mustelidae OR sylvilagus OR vitticeps OR cockatiel OR mustelus OR cottus OR erythrocebus OR dipodomys OR platessa OR callicebus OR loricariidae OR catostomus OR cuneata OR cyanistes OR cyprinodon OR sigmodontinae OR elasmobranchii OR trichechus OR sauropsid OR xenarthra OR dormouse OR perissodactyla OR nautilus OR cirrhinus OR gulo OR gulos OR tragelaphus OR merula OR numidaOR sciaenidae OR cerastes OR sciuridae OR gibbosus OR octopuses OR eland OR elands OR phyllomedusa OR pogona OR walrus OR agamidae OR leptodactylidae OR ridibundus OR leontopithecus OR anteater OR anteaters OR pelodiscus OR cebidae OR columbianus OR "pelteobagrus fulvidraco" OR hominoidea OR mandrillus OR "zonotrichia leucophrys" OR agama OR gobiocypris OR "bearded dragon" OR "bearded dragons" OR sarotherodon OR

talpa OR discoglossus OR hagfishes OR sphenodon OR gudgeon OR amphiuma OR aythya OR tenrec OR tenrec OR hominidae OR risoria OR salamandridae OR camelidae OR columbiformes OR latimeria OR plover OR plovers OR afrotheria OR "falco sparverius" OR polecat OR polecats OR crotalinae OR salvadora OR tarsier OR lucioperca OR anchovies OR lungfishes OR terrapin OR "dromaius novaehollandiae" OR lateolabrax OR eigenmannia OR pelamis OR theropithecus OR murinae OR gander OR gymnotus OR pseudacris OR gymnophiona OR gymnotiformes OR laticauda OR falconiformes OR dugong OR dugongs OR pintail OR pintails OR rook ORrooks OR lasiurus OR catshark OR catsharks OR micropogonias OR "red junglefowl" OR paddlefish OR eutheria OR ophiophagus OR hollandicus OR nymphicus OR pimelodidae OR aepyceros OR cobitidae OR strigiformes OR cobitis OR dormice OR alytes OR calloselasma OR guanaco OR guanacos OR phasianidae OR "round goby" OR trichogaster OR catarrhini OR eelpout OR eelpouts OR galaxias OR gaur OR pungitius OR suslik OR susliks OR flatfishes OR percidae OR caprinae OR todarodes OR osmerus OR ameiurus OR anthropoidea OR "castor canadensis" OR pouting OR poutings OR tetraodontiformes OR arvicolinae OR siamang OR siamangs OR "castor fiber" OR nomascus OR "red knot" OR "red knots" OR syngnathidae OR iguanidae OR eretmochelys OR ursidae OR callimico OR columbidae OR microhylidaeOR anaxyrus OR menidia OR pipistrelle OR greylag OR pipidae OR scandentia OR bowfin OR bowfins OR dendrobatidae OR zenaida OR bushbaby OR harrier OR harriers OR macropodidae OR pygerythrus OR clupeidae OR odorrana OR corvidae OR jerboa OR jerboas OR canutus OR hylobatidae OR clupeiformes OR "great cormorant" OR "great cormorants" OR scorpaeniformes OR chondrostean OR garfish OR proboscidea OR psetta OR diapsid OR serotinus OR tetrao OR walruses OR carcharhiniformes OR leucoraja OR pumpkinseed OR dosidicus OR acipenseriformes OR daubentonii OR emberizidae OR gadiformes OR hyraxes OR stizostedion OR wolverine OR wolverines OR lissotriton OR acanthurus OR centrarchidae OR gloydius OR laurasiatheria OR limosa OR psittacula OR leporidae OR proteidae OR zander ORzanders OR arapaima OR bagridae OR cyprinodontidae OR mithun OR pandion OR jackdaw OR jackdaws OR procyonidae OR carus OR jaculus OR salmoniformes OR "common sole" OR "common soles" OR protobothrops OR calamita OR brachyteles OR trionyx OR turdidae OR boidae OR luscinia OR pugnax OR euarchontoglires OR saithe OR saithes OR symphalangus OR aardvark OR aardvarks OR oystercatcher OR oystercatchers OR arius OR corydoras OR poacher OR poachers OR aurochs OR cebuella OR crecca OR lemuridae OR sirenia OR lemmus OR perdix OR glires OR lepidosaur OR muskox OR deinagkistrodon OR pholidota OR holocephali OR cercopithecinae OR clariidae OR agapornis OR doryteuthis OR tyrannidae OR dicroglossidae OR godwit OR godwits OR monedula OR pongidae OR atheriniformes OR colobinae OR lophocebus OR atelidae OR cottidae OR leucopsis OR acanthuridae OR didelphimorphia OR elver OR elvers OR lapponica OR dermoptera OR "european hake" OR "european hakes" OR gerbillinae OR banteng OR hartebeest OR hartebeests OR hogget OR haematopus OR "anguis fragilis" OR "grey heron" OR "grey herons" OR "blue whiting" OR "blue whitings" OR furnariidae OR macrovipera OR esocidae OR lapwing OR lapwings OR mylopharyngodon OR wallabia OR beloniformes OR potoroo OR potoroos OR "athene noctua" OR pleuronectidae OR bushbabies OR muscicapidae OR alligatoridae OR fuligula OR "bush baby" OR guineafowl OR

spoonbill OR spoonbills OR viverridae OR catostomidae OR zebrafishes OR ibexes OR vendace OR estrildidae OR monotremata OR sepiella OR ambystomatidae OR shelduck OR shelducks OR treeshrew OR treeshrews OR hoplobatrachus OR pochard OR hoolock OR hoolocks OR lynxes OR antilope OR antilopes OR blackbuck OR blackbucks OR cricetinae OR paramisgurnus OR skylark OR skylarks OR soleidae OR allobates OR "northern wheatear" OR "northern wheatears" OR pitheciidae OR takin OR theria OR vanellus OR galaxiidae OR lorisidae OR ostralegus OR palaeognathae OR "stone loach" OR alauda OR callitrichinae OR caniformia OR duttaphrynus OR ictaluridae OR osteoglossiformes OR poultries OR curema OR "ruddy turnstone" OR

"ruddy turnstones" OR sheatfish OR sunfishes OR centropomidae OR hemachatus OR platalea OR thamnophilidae OR "song thrush" OR atherinopsidae OR siluridae OR tadorna OR chroicocephalus OR ermine OR ermines OR gavialis OR ruffe OR tupaiidae OR diprotodontia OR hyaenidae OR antilopinae OR crocodylidae OR herpestidae OR hippopotamidae OR "northern shoveler" OR "round gobies" OR cheirogaleidae OR indriidae OR fundulidae OR pythonidae OR rhynchocephalia OR anodorhynchus OR "red-backed shrike" OR "red-backed shrikes" OR triakidae OR phalangeridae OR aoudad OR boreoeutheria OR "eurasian jay" OR "eurasian jays" OR feliformia OR haplorhini OR osteoglossidae OR paenungulata OR struthioniformes OR ferina OR sanderling OR sanderlings OR spheniscidae OR cuttlefishes OR cygnet OR dasycneme OR gadwall OR gadwalls OR "pelobates fuscus" OR wryneck OR wrynecks OR afrosoricida OR culaea OR "dover sole" OR "dover soles" OR paralichthyidae OR passeridae OR osteolaemus OR "song thrushes" OR bluethroat OR bluethroats OR hydrophiidae OR megrim OR mephitidae OR strepsirhini OR tomistoma OR epidalea OR osmeriformes OR "bush babies" OR tarsiiform OR atelinae OR bufotes OR "eurasian coot" OR "eurasian coots" OR galagidae OR geopelia OR philomachus OR tubulidentata OR bombinatoridae OR pelobatidae OR tachysurus OR ailuridae OR woodlark OR woodlarks OR alcelaphinae OR redshank OR redshanks OR salientia OR "sand smelt" OR "sand smelts" OR woodmice OR woodmouse OR dasyproctidae OR "eurasian wigeon" OR "eurasian wigeons" OR garganey OR garganeys OR "lemon sole" OR "lemon soles" OR "common dab" OR "common dabs" OR graylag OR graylags OR leucorodia OR osphronemidae OR bewickii OR "common moorhen" OR "common moorhens" OR decapodiformes OR gobbler OR gobblers OR odontophoridae OR paddlefishes OR salmonine OR esociformes OR "eurasian woodcock" OR "eurasian woodcocks" OR "european smelt" OR "european smelts" OR goldfishes OR tenches OR tyranni OR "common chaffinch" OR "common chaffinchs" OR "common redstart" OR "common redstarts" OR "common roach" OR "common roachs" OR "great knot" OR "great knots" OR potoroidae OR alytidae OR coregonine OR dipteral OR leveret OR "poeciliopsis gracilis" OR amphiumidae OR batrachoidiformes OR "bighead goby" OR heteropneustidae OR lullula OR "norway pout" OR "norway pouts" OR sipunculida OR dogfishes OR sebastidae OR tarsiidae OR alethinophidia OR "common nase" OR "common nases" OR "common sandpiper" OR "common sandpipers" OR "eurasian blackcap" OR "eurasian blackcaps" OR pterocnemia OR syngnathiformes OR "common chaffinches" OR eupleridae OR octopodiformes OR phascolarctidae OR scophthalmidae OR "starry smooth-hound" OR "starry smooth-hounds" OR whitefishes OR cuniculidae OR "european sprat"

OR "european sprats" OR "rosy bitterling" OR "rosy bitterlings" OR "common dace" OR "common daces" OR "lesser weever" OR "lesser weevers" OR scaldfish OR "water rail" OR "water rails" OR alouattinae OR centrarchiformes OR "common whitethroat" OR "common whitethroats" OR gavialidae OR "grey gurnard" OR "greygurnards" OR lateolabracidae OR rheiformes OR "tub gurnard" OR "tub gurnards" OR "common chiffchaff" OR "common chiffchaffs" OR garfishes OR "lesser whitethroat" OR "lesser whitethroats" OR myoxidae OR seabasses OR spariformes OR umbridae OR "yellow boxfish" OR anabantiformes OR aotidae OR "common bleak" OR "common bleaks" OR "common rudd" OR "common rudds" OR "greater pipefish" OR hapale OR nandiniidae OR "stone loaches" OR whinchat OR whinchats OR acanthuriformes OR "brotula barbata" OR "common ling" OR "common lings" OR "common roaches" OR cottonrat OR cottonrats OR douroucoulis OR dromaiidae OR fitches OR fitchew OR galaxiiformes OR laprine OR saimiriinae OR solenette OR tarsii OR "tompot blenny" OR "common dragonet" OR "common dragonets" OR "longspinedbullhead" OR "longspined bullheads" OR monotremate OR monotremates OR pempheriformes OR perdicinae OR presbytini OR smegmamorpha OR "bighead gobies" OR "carangaria incertae sedis" OR coiidae OR "fivebeard rockling" OR foulmart OR foumart OR grasskeet OR "greater pipefishes" OR ibices OR millionfish OR muguliformes OR "norwegian topknot" OR peewit OR "red sea sailfin tang" OR rupicapras OR sheatfishes OR "tompot blennies" OR "twait shad" OR "yellow boxfishes")
Results: 10177702

19: #17 AND #18 Results: 87

Data Extraction Sheets

Study Characteristics

1. What is the year of publication?
2. What journal is the article published in?
3. Name of the first author? (Full last name, initial of first name - e.g. Smith, K)
4. Name and email of the corresponding author? (e.g. Smith K, smithk89@uottawa.ca)
5. What is the source of funding?
    a. Not Reported
    b. Government
    c. Industry
    d. Academic Institution
    e. Charity/Foundation
    f. Other
    g. Unclear
6. What country is the corresponding author from (look at corresponding address)?

Animal Model

1. What is the species?
    a. Mouse
    b. Rat
    c. Monkey
    d. Rabbit
    e. Other
2. What type of stroke is being studied?
    a. Ischemic
    b. Hemorrhagic
    c. Other
3. What type of stroke model is being used?
    a. Intraluminal suture (e.g. Filament, distal, and transient middle cerebral artery occlusion)
        i. What is the number of minutes of occlusion time (relevant to lesion severity)?
    b. Permanent middle cerebral artery occlusion (i.e. cauterization, permanent clip, permanent distal middle cerebral artery ligation)
    c. Photothrombotic
    d. Embolism
    e. Modified 3 vessel occlusion
    f. Endothelin-1
    g. Microvascular embolic
    h. L-NIO
    i. Intracerebral hemorrhagic
    j. Other (please specify)

4. What are the sexes of the animals?
    a. Male
    b. Female
    c. Male and female
    d. Not reported
5. What is the weight in grams and, if applicable, specify when the weight(s) was measured?
6. What is the age in weeks of the species?
7. Are there comorbidities in the animals have (e.g. aged, obesity, diabetes/hyperglycemia, hypertension)?
    a. Yes (please specify)
    b. No
8. What specific area of the brain was the stroke induced in?
    a. Frontal lobe
    b. Parietal lobe
    c. Temporal lobe
    d. Occipital lobe
    e. Cerebellum
    f. Thalamus
    g. Basal ganglia
    h. Other (please describe)
    i. Not reported
9. Were there any animals excluded?
    a. Yes (please explain why i.e. death, no impairment from stroke, etc.)
    b. No
10. What was the total number of animals excluded from the study?

<u>Study Intervention</u>

1. What agent is used?
    a. Maraviroc
    b. Selzentry
    c. Celsentri
    d. Leronlimab
    e. Aplaviroc
    f. Vicriviroc
    g. Ancriviroc
    h. Other (please specify)
2. What is the dose (numeric value)?
3. What is the unit used for the dose?
4. What is the route of administration?
5. What was the agent prepared/diluted in?
6. When was the treatment administered?
    a. Pre-stroke
    b. Post-stroke

7. What is the timing in hours of intervention post-stroke (first adminsteration if applicable)?
8. Was the treatment administered at multiple timepoints?
    a. Yes (please specify the other timepoints in hours)
    b. No
9. How is time zero defined in the study?
10. Was the intervention paired with any other therapy (e.g. post-stroke rehabilitation paradigms)?
    a. Yes (please describe)
    b. No

Outcomes

1. Are motor behaviour outcomes (e.g. motor skills) being assessed? Please check all that apply
    a. Yes
        i. Neurological deficit score
        ii. Rotarod
        iii. Adhesive removal test
        iv. Cylinder task
        v. Foot fault & paw placement tests (e.g. tapered beam, ladder, grid walking)
        vi. Montoya staircase
        vii. Reaching task (e.g. tray, pellet, pasta matrix)
        viii. Corner test
        ix. Staircase test
        x. Forelimb placing test
        xi. Wire hanging test
        xii. Other
    b. No
2. Are cognitive behavioural outcomes (e.g. cognitive skills) being assessed? Please check all that apply
    a. Yes
        i. Morris water maze
        ii. Y-maze test
        iii. Novel object recognition test
        iv. Elevated plus maze
        v. Sucrose preference test
        vi. Tail suspension test
        vii. Open field test
        viii. Forced swim test
        ix. Other
    b. No
3. Was infarct volume/size measured?
    a. Yes

i. What is the mean infarct size in mm3 or % of the hemisphere for the treatment animals?
ii. What is the mean infarct size in mm3 or % of the hemisphere for the control animals?
iii. What is the method for measuring infarct size? Please check all that apply
    1. Triphenyltetrazolium chloride (TTC)
    2. Cresyl violet (CV)
    3. Hematoxylin and eosin (H&E)
    4. Magnetic resonance imaging (MRI)
    5. Other (please specify)
iv. What is the (first, if applicable) time of the post-stroke measure of infarct size in days?
v. Was infarct size measured multiple times?
    1. Yes (please list the other times in days)
    2. No

b. No

4. How many animals are in the treatment and control groups?
5. What number of animals died for control and treatment groups?
6. What was the adverse effect (e.g. muscle fatigue, reduced mobility) for control and treatment groups?
7. Were tissue outcomes (e.g. immunohistochemical staining, axonal neurofilament staining, pharmacogenetic techniques, axonal tracing techniques, genetic markers identification) measured or visualized?
    a. Yes, please explain
    b. No
8. Were brain imaging or biomarkers used to measure animal neural connectivity?
    a. Yes
        i. Diffusion tensor imaging (DTI)
        ii. Resting state functional MRI (rsFMRI)
        iii. Task-based functional MRI
        iv. Other (please specify)
    b. No

Risk of Bias

"Yes" indicates low risk of bias; "no" indicates high risk of bias; and "unclear" indicates an unclear risk of bias. If one of the relevant signaling questions is answered with "no," this indicates high risk of bias for that specific entry.

1. Was the allocation sequence adequately generated and applied?

*Did the investigators describe a random component in the sequence generation process such as:

- Referring to a random number table;
- Using a computer random number generator.
    a. Yes
    b. No

c. Unclear

Additional info:
Examples of a non-random approach:

- Allocation by judgement or by investigator's preference;
- Allocation based on the results of a laboratory test or a series of tests;
- Allocation by availability of the intervention;
- Sequence generated by odd or even date of birth;
- Sequence generated by some rule based on animal number or cage number.

2. Were the groups similar at baseline or were they adjusted for confounders in the analysis?
   *Was the distribution of relevant baseline characteristics balanced for the intervention and control groups?
   a. Yes
   b. No
   c. Unclear

   *If relevant, did the investigators adequately adjust for unequal distribution of some relevant baseline characteristics in the analysis?
   a. Yes
   b. No
   c. Unclear

   *Was the timing of disease induction adequate?
   a. Yes
   b. No
   c. Unclear

Additional info:
The number and type of baseline characteristics are dependent on the review question. Before starting their risk of bias assessment, therefore, reviewers need to discuss which baseline characteristics need to be comparable between the groups. In an SR investigating the effects of hypothermia on infarct size, for example, gender distribution, left ventricular weight and heart rate and blood pressure should be similar between the groups at the start of the study.
A description of baseline characteristics and/or confounders usually contains:

- The sex, age, and weight of the animals
- Baseline values of the outcomes which are of interest in the study

Timing of disease induction:
In some prevention studies, the disease is induced after allocation of the intervention. For example, in an experiment on preventive probiotic supplementation in acute pancreatitis,

pancreatitis is induced after allocation of the animals to the probiotic or control group. To reduce baseline imbalance, the timing of disease induction should be equal for both treatment groups. Examples of adequate timing of disease induction:

- The disease was induced before randomization of the intervention.
- The disease was induced after randomization of the intervention, but the timing of disease induction was at random, and the individual inducing the disease was adequately blinded from knowing which intervention each animal received.

3. Was the allocation to the different groups adequately concealed during?
   *Could the investigator allocating the animals to intervention or control group not foresee assignment due to one of the following or equivalent methods?
   - Third-party coding of experimental and control group allocation Central randomization by a third party; Sequentially numbered opaque, sealed envelopes

   a. Yes
   b. No
   c. Unclear

   Additional info:
   Examples of investigators allocating the animals being possibly able to foresee assignments:
   - Open randomization schedule
   - Envelopes without appropriate safeguard
   - Alternation or rotation
   - Allocation based on date of birth
   - Allocation based on animal number
   - Any other explicitly unconcealed procedure of a non-random approach

4. Were the animals randomly housed during the experiment?
   *Did the authors randomly place the cages or animals within the animal room/facility?
   a. Yes
   b. No
   c. Unclear
   - Animals were selected at random during outcome assessment (use signaling questions of entry 6).

   *Is it unlikely that the outcome or the outcome measurement was influenced by not randomly housing the animals?
   a. Yes
   b. No
   c. Unclear

The animals from the various experimental groups live together in one cage/pasture (e.g., housing conditions are identical).
Additional info:
Examples of investigators using a non-random approach when placing the cages:

- Experimental groups were studied on various locations (e.g., group A in lab A or on shelf A; Group B in Lab B or on shelf B).

5. Were the caregivers and/or investigators blinded from knowledge which intervention each animal received during the experiment?
   *Was blinding of caregivers and investigators ensured, and was it unlikely that their blinding could have been broken?
   a. Yes
   b. No
   c. Unclear

   - ID cards of individual animals, or cage/animal labels are coded and identical in appearance.
   - Sequentially numbered drug containers are identical in appearance.
   - The circumstances during the intervention are specified and similar in both groups (#).
   - Housing conditions of the animals during the experiment are randomized within the room (use criteria of entry 4).

   Additional info:
   Examples of inappropriate blinding:
   - Colored cage labels (red for group A, yellow group B)
   - Expected differences in visible effects between control and experimental groups
   - Housing conditions of the animals are not randomized within the room during the experiment; use criteria of entry 4
   - The individual who prepares the experiment is the same as the one who conducts and analyses the experiment
   - Circumstances during the intervention are not similar in both groups (#)
   - Examples where circumstances during the intervention were not similar:
   - Timing of administration of the placebo and exp drug was different.
   - Instruments used to conduct experiment differ between experimental and control group (e.g., experiment about effects abdominal pressure; exp group receives operation and needle to increase pressure, while control group only has the operation).

   **The relevance of the above-mentioned items depends on the experiment. Authors of the review need to judge for themselves which of the above-mentioned items could cause bias in the results when not similar. These should be assessed.

6. Were animals selected at random for outcome assessment?

*Did the investigators randomly pick an animal during outcome assessment, or did they use a random component in the sequence generation for outcome assessment?

a. Yes
b. No
c. Unclear

- Referring to a random number table;
- Using a computer random number generator;
- Etc.

7. Was the outcome assessor blinded?
*Was blinding of the outcome assessor ensured, and was it unlikely that blinding could have been broken?

a. Yes
b. No
c. Unclear

- Outcome assessment methods were the same in both groups.
- Animals were selected at random during outcome assessment (use signaling questions of entry 6).

*Was the outcome assessor not blinded, but do review authors judge that the outcome is not likely to be influenced by lack of blinding?

a. Yes
b. No
c. Unclear

(e.g., mortality)
Additional info:
This item needs to be assessed for each main outcome.

8. Were incomplete outcome data adequately addressed? (*)
*Were all animals included in the analysis?

a. Yes
b. No
c. Unclear

*Were the reasons for missing outcome data unlikely to be related to true outcome? (e.g., technical failure)

a. Yes
b. No
c. Unclear

*Are missing outcome data balanced in numbers across intervention groups, with similar reasons for missing data across groups?

a. Yes
b. No
c. Unclear

*Are missing outcome data imputed using appropriate methods?

a. Yes
b. No
c. Unclear

9. Are reports of the study free of selective outcome reporting? (*)
*Was the study protocol available and were all of the study's pre-specified primary and secondary outcomes reported in the current manuscript?

a. Yes
b. No
c. Unclear

*Was the study protocol not available, but was it clear that the published report included all expected outcomes (i.e. comparing methods and results section)?

a. Yes
b. No
c. Unclear

Additional info:
Selective outcome reporting:
- Not all of the study's pre-specified primary outcomes have been reported;
- One or more primary outcomes have been reported using measurements, analysis methods or data subsets (e.g., subscales) that were not pre-specified in the protocol;
- One or more reported primary outcomes were not pre-specified (unless clear justification for their reporting has been provided, such as an unexpected adverse effect);
- The study report fails to include results for a key outcome that would be expected to have been reported for such a study.

10. Was the study apparently free of other problems that could result in high risk of bias? (*)
*Was the study free of contamination (pooling drugs)?

a. Yes
b. No
c. Unclear

*Was the study free of inappropriate influence of funders?

a. Yes
b. No
c. Unclear

*Was the study free of unit of analysis errors?

a. Yes
b. No
c. Unclear

*Were design-specific risks of bias absent?

a. Yes
b. No
c. Unclear

*Were new animals added to the control and experimental groups to replace drop-outs from the original population?

a. Yes
b. No
c. Unclear

Additional info:
The relevance of the signaling questions (Table 3) depends on the experiment. Review authors need to judge for themselves which of the items could cause bias in their results and should be assessed.
Contamination/pooling drugs:
Experiments in which animals receive – besides the intervention drug – additional treatment or drugs which might influence or bias the result.
Unit of analysis errors:

- Interventions to parts of the body within one participant (i.e., one eye exp; one eye control).
- All animals receiving the same intervention are caged together, but analysis was conducted as if every single animal was one experimental unit.
- Design-specific risks of bias:
- Crossover design that was not suitable (intervention with no temporary effect, or the disease is not stable over time)
- Crossover design with risk of carry-over effect
- Crossover design with only first period data being available
- Crossover design with many animals not receiving 2nd or following treatment due to large number of drop-outs probably due to longer duration of study
- Crossover design in which all animals received same order of interventions
- Multi-arm study in which the same comparisons of groups are not reported for all outcomes (selective outcome reporting)

- Multi-arm study in which results of different arms are combined (all data should be presented per group)
- Cluster randomized trial not taking clustering into account during statistical analysis (unit of analysis error)
- Crossover design in which paired analysis of the results is not taken into account

Validity

1. Does the treatment effect vary with dose?
    a. Yes
    b. No
    c. Not applicable (only tested one dose)
    d. Unsure
2. Does the treatment remain effective when administered at clinically relevant delayed times?
    a. Yes (tested after 6 hours and the CCR5 antagonist was still effective)
    b. No (tested after 6 hours, but the CCR5 antagonist was not effective)
    c. Not applicable (not tested after 6 hours)
    d. Unsure
3. Does the treatment cause expected physiological effects?
    a. Yes
    b. No
    c. Unsure
4. Does the treatment penetrate the blood brain barrier?
    a. Yes
    b. No
    c. Unsure
5. What the tests done across multiple laboratories?
    a. Yes
    b. No
    c. Unsure
6. Was testing done on gyrencephalic species (non-human primates i.e. monkeys, dogs, pigs, etc.)
    a. Yes
    b. No
    c. Unsure
7. Did the preclinical testing of therapy occur during the awake phase for the animal model (during the dark phase for rodents i.e. was the test done during the nighttime)?
    a. Yes
    b. No
    c. Unsure

Chapter 4: Patient Engagement Process

Involvement of Individuals with Lived Experiences of Stroke in a Preclinical Systematic Review and Meta-Analysis

Preface to Chapter 4

This chapter provides the findings of the patient engagement assessment conducted with patient partners and researchers who participated in the systematic review and meta-analysis presented in Chapter 3. The assessment presented in this chapter reflects my own viewpoint rather than representing the collective perspective of the entire team of patient partners and other researchers.

ABSTRACT

Background: Preclinical (i.e. animal-based) systematic reviews can be a valuable component in early-stage drug development process as they offer significant opportunities for informing and guiding the development of new drugs. Engaging individuals with lived experiences as partners in the review process can provide valuable insights into patient priorities and help ensure a patient-centred approach, which is well-known in clinical reviews, but not as explored in preclinical ones. In this project, we implemented a novel approach that engaged patient partners with lived experience of stroke in the planning and conduct of a preclinical systematic review of a stroke therapy. We aimed to engage the patient partners in all stages of the preclinical stroke systematic review and evaluate the engagement process.

Methods: We recruited eight patient partners with lived experiences of stroke. Patient partners actively participated in all stages of the systematic review, including topic and question generation, protocol development, screening, extraction, interpretation and analysis of results, and dissemination. Surveys were administered after each investigator meeting to reach a consensus on components of each stage of the systematic review. For screening and extraction done by the patient partners, their abstractions were compared to those of the researchers. Activities such as investigator meetings, educational sessions, and surveys were used to facilitate engagement and foster communication. The impact of engagement was assessed using the Public and Patient Engagement Evaluation Tool (PPEET).

Results: We co-developed a systematic review to answer the following research question: *In preclinical studies of animal stroke models, what are the effects of CCR5 inhibitors on motor and cognitive impairment?* In the analysis of results, the patient partners highlighted gaps in the reported outcomes of the included studies (i.e. only male animals used in studies), and the lack of patient priority items emphasized (i.e. spasticity, relevant comorbidities included in animals, paired physical therapy with intervention, and chronicity of intervention to address chronic recovery). In terms of screening and data extraction, patient partners actively engaged in the review process and demonstrated good performance, with accuracy rates ranging from 75% to 83% for study screening and data extraction (83% median). The PPEET questionnaires indicated positive feedback from patient partners and researchers, highlighting the value of engagement. Challenges such as maintaining engagement, managing feasibility, and budgeting were addressed through careful planning, ongoing communication, and resource allocation.

Discussion: Patient partners with lived experience of stroke were successfully engaged in all stages of a preclinical systematic review, and provided valuable contributions highlighting the importance of a patient-centred approach. Their involvement helped shape the topic and research question. Patients also successfully participated in the technical conduct of the systematic review and assisted with the analysis and dissemination of findings. Despite some challenges, patient and caregiver involvement proved to be feasible in a preclinical systematic review. We believe the involvement of patient partners ultimately resulted in a more patient-centred review of early drug development.

Keywords: Patient engagement; systematic reviews; preclinical; animal; stroke; individuals with lived experiences

INTRODUCTION

Internationally recognized organizations like Cochrane and the NIHR Centre for Engagement and Dissemination have published guidelines on involving patient partners in systematic reviews[1,2] and highlight the benefits of including patient partners. Patient partners with lived experiences of a particular health condition and/or intervention can contribute to setting review outcomes, providing unique perspectives, interpreting results, and improving review accessibility.[1] The guidelines also address the potential challenges of engagement, including recruitment, meaningful involvement, dealing with frustration, and time commitment.[3]

It is important to note that these resources focus on clinical systematic reviews, and no guidance has been provided on engaging patient partners in the conduct of preclinical systematic reviews. Preclinical research differs from clinical studies as it does not directly involve patients as participants but rather focuses on laboratory-based studies. This poses a barrier to patient engagement in preclinical research, and it requires patient partners to possess specific knowledge, including biology, pathophysiology, chemistry, and laboratory-based experimentation. Indeed, meaningful engagement in preclinical research may require patient partners to invest some time in education to develop their familiarity and understanding of basic science.[4-9] Furthermore, preclinical researchers may face challenges in engaging patients, including a lack of training in engagement and effective communication skills tailored to a patient audience. These potential knowledge gaps highlight the need for further investigation into how to meaningfully engage patients in preclinical research, including preclinical systematic reviews.

To begin to address these gaps, we co-designed a preclinical systematic review with patient partners with lived experience of stroke. Their involvement spanned the entire process, including topic and question generation, protocol development, conduct, synthesis, interpretation of results, and dissemination. By incorporating patient partners, we aimed to enhance the quality and relevance of the systematic review by incorporating patient perspectives, and evaluate the engagement process between researchers and patient partners throughout the development and conduct of the preclinical stroke systematic review.

METHODS

Recruitment of Patient Partners

To incorporate patient partner perspectives from study onset (proposal development) to completion (dissemination of research findings), patient partners across Canada were recruited to be involved in the co-design and conduct of a preclinical stroke systematic review and meta-analysis. Patients were recruited through the Canadian Heart & Stroke Foundation and the Patient and Family Advocacy Program at The Ottawa Hospital through an advertisement we distributed through both organizations (Appendix 1). The eligibility criteria of patient partners living in Canada were individuals with a lived experience of a stroke, or caregivers, family, and friends of patients with a stroke who were able to communicate in English. For brevity, throughout this manuscript, we use the term patient partner to reflect patients, family, friends, and caregivers. There were no explicit exclusion criteria.

Onboarding Process and Team Structure

All patient partners were onboarded to the research team, which included senior scientists, physicians, clinician scientists, and graduate and medical students. The onboarding process included a virtual information session with the researcher team and recruited patient partners. The session reviewed what research is and the steps it entails, why and how patient partners can be involved, what preclinical research is, the details and steps of a systematic review, and how our project will relate to stroke. There was an opportunity for discussion to address any questions the patient partners had. We also allocated a portion of the meeting to discuss patient partners' expectations, time commitment, and what they hoped to gain from the experience.

Terms of Reference

After the information session, patient partners decided on their level of involvement in the review. We then co-developed a Terms of Reference document (see Appendix 2). This document was intended to establish clear roles, responsibilities, compensation, and expectations from the researchers and patient partners. We discussed the document with the patient partners individually and collectively, and they

were given an opportunity to provide feedback on it. All patient partners have indicated their preferred method of compensation and accepted the conditions of the document.

Planned Engagement

To ensure meaningful collaboration, potential areas for involvement were identified in advance among the research team by following guidance resources for engagement in systematic reviews by Cochrane[1] and INVOLVE[2], including topic and question development, technical conduct, and dissemination. However, we purposely left specific details open for discussion, as patient partners needed to play a role in the planning process. This included determining the frequency of educational sessions and training opportunities, focusing on interventions and outcomes for question generation, involvement in screening and extraction, and analyzation of the results of the review. Additionally, given the novelty of involving patients in a preclinical systematic review, we wanted to allow organic engagement to address unanticipated issues.

To facilitate engagement, we scheduled monthly team meetings that aligned with the main stages of the review process, including question generation, protocol development, screening, data extraction, data synthesis, data analysis, manuscript development, and dissemination. During these meetings, we encouraged open discussion and active participation from all team members, including patients and caregivers, by having stopping points throughout the meeting where discussion points were prepared. During meetings, to ensure the comfort of all members, we offered the option of either speaking aloud or using the chat feature to express thoughts and ideas. This allowed participants to communicate in a manner that suited their preferences and fostered inclusivity. We provided training and resources to ensure that patients and caregivers had the necessary knowledge and skills to engage meaningfully in the review process. Patient partners were equipped with educational materials, such as literature reviews and relevant research articles, to enhance their understanding of the preclinical stroke research landscape. Additionally, interactive training sessions were conducted, covering topics such as systematic review methodology, critical appraisal of scientific papers, and the interpretation of study findings. These

sessions involved presentations, hands-on exercises, and discussions to enhance the patient partners' ability to actively engage in screening, data extraction, and data synthesis tasks. The research team offered ongoing support and guidance, with regular check-ins and opportunities for patient partners to seek clarification and ask questions. The provision of these training and resources aimed to empower patient partners and ensure their meaningful and informed participation throughout the systematic review process.

Our approach to planned engagement was designed to foster a collaborative and inclusive environment that valued the perspectives of patients and caregivers. By involving patients and caregivers throughout the review process, we wanted to ensure that our research was relevant and meaningful to those who ultimately would benefit from our findings.

Evaluation of Engagement

To systematically assess the perceptions of engagement from both the researchers and patient partners in the preclinical stroke systematic review, we used the Public and Patient Engagement Evaluation Tool (PPEET)[10] to survey both groups. The PPEET is a validated and commonly used tool to evaluate the planning, execution, and impact of patient engagement initiatives; it contains two relevant parts: the participant and project questionnaires. These questionnaires allowed us to obtain patient partner and researcher assessments of the patient engagement initiative; assess the planning, execution, and impact of engagement; and assess how engagement is carried out. An online version of the PPEET was administered using the platform LimeSurvey (LimeSurvey GmbH, Germany).

The engagement tool is a three-part series of questionnaires administered to both the patient partners and researchers. The first round of questionnaires was administered at the beginning of the review when the research question was being generated, the second during the start of the technical phase of screening and extraction, and the last round near the end of the review when the results of the review were analyzed.

Level of Participation of Patient Partners

In order to gauge engagement from the patient partners we used guidance from the International Association for Public Participation Framework[11], which outlines five levels of participation with increasing impact on decision-making: Inform, Consult, Involve, Collaborate, and Empower. Throughout the various phases of the systematic review, patient partners were involved at different levels of engagement. For example, the initial onboarding session held with the patient partners would be considered an activity at the Inform level, as it was an opportunity for them to learn about the project and its goals. In contrast, discussions surrounding the research question and prioritization of outcomes occurred at the Collaborate level, as patient partners worked closely with the research team to make decisions about the review's direction.

Patient partners were given the opportunity to be involved in each stage of the systematic review, including screening and extraction, and their contributions were considered during the decision-making processes. While the level of participation varied throughout the engagement process, we aimed to empower the patient partners to take an active role in the review. By involving them in the decision-making process, we were able to ensure that their perspectives and priorities were taken into account.

RESULTS

We recruited a panel of eight patient partners at the start of the review and held a total of 12 virtual team meetings and discussions over the course of 12 months, focusing on different stages of the systematic review (Table 1). In addition, one recorded educational session was provided. Of the 13 sessions, five were dedicated to topic and question generation, two to search strategy and protocol development, five to technical conduct (i.e. screening and extraction), and one to analysis and interpretation of our results (Table 2). PowerPoint presentations were utilized to enhance discussions and provide visual aids. To ensure patient partners had the necessary training, educational sessions were incorporated that provided an overview of the systematic review process.

Educational Sessions and Communication

We recognized the need to provide foundational knowledge for the patient partners as this was their first experience conducting a systematic review as well as their first exposure to preclinical research. Thus, we tried to ensure educational sessions were incorporated in our meetings on animal studies, systematic review methodology, and stroke research to allow for meaningful collaboration among team members. There was also an opportunity for two patient partners to participate in a four-week course on Knowledge Synthesis organized by the Strategy for Patient-Oriented Research (SPOR) Evidence Alliance. This provided deeper insight into the methodology of systematic reviews for a patient and public audience. Patient partners expressed that the course provided more insight into the technical stages of a systematic review, which set them up for the current and coming stages of our review. Five patient partners also virtually attended an international three-day scientific conference on stroke recovery (i.e. the Advances in Stroke Recovery Scientific Conference 2023[12], an abstract was submitted for the project where all patient partners were co-authors). Patient partners provided positive feedback on the conference, noting that it was well-organized and provided a valuable opportunity to learn more about stroke recovery research. While some talks were highly technical, other talks were presented in a way that was more accessible to a non-expert audience. The partners appreciated the chance to ask questions related to their own personal situations and expressed interest in attending similar events in the future.

In addition, we developed and distributed regular newsletters to serve as a platform to update on research progress, highlight team members (i.e. pictures and biographies of the researchers and patient partners), and provide relevant studies that might be of interest. The newsletters were well-received by the team, and patient partners and researchers expressed their appreciation for the regular updates and the opportunity to be informed about the team's progress. Furthermore, the newsletters facilitated a sense of community among the team members, and helped to maintain project enthusiasm and motivation (See Appendix 3 for an example).

Research Question Development

One of the crucial roles played by patient partners in our study was leading the development of the topic and research question. Together, we co-developed the research question for the systematic review through a series of 5 interactive virtual discussions and meetings. During these discussions, stroke recovery and spasticity emerged as clear priorities of the patient partners, which were explicitly raised and thoroughly discussed. To reach a consensus on various items of the research question, such as animal models, interventions, and outcomes, we engaged in a series of discussions and surveys. Surveys were administered to the team to vote on the areas that were voiced by the patient partners and researchers. The surveys allowed patient partners to have equal input with the researchers in the decision-making process. The patient partners highlighted the importance of assessing patient-priority outcomes, such as spasticity and the inclusion of relevant comorbidities in animal models. These priorities stemmed from their personal experiences and expectations of stroke recovery. Emphasizing the chronic nature of stroke recovery, patient partners underscored the need to evaluate the effects of extended drug administration, recommending chronic administration. This emphasis recognized the continuity of stroke recovery in human patients and our partners wanted to see continuity investigated in animal models. Patient partners also identified the importance of considering physical therapy in tandem with drug administration in both the control and treatment groups. Furthermore, the patient partners advised a broadened examination of outcomes to include both motor and cognitive learning, reflecting the comprehensive impact of stroke on

patients' lives. These contributions from our patient partners served to ensure the relevance of our systematic review to both clinical practice and patient experience.

In the end, we decided on the following research question: *In preclinical studies of animal stroke models, what are the effects of CCR5 inhibitors on motor and cognitive impairment?*. The patient partners' input was instrumental in refining the research topic and question, and the surveys helped ensure that everyone's opinion was considered. This approach allowed us to develop a research question that was sensitive to the needs of the patient community and reflective of the values of all team members. In addition, when the final results were presented and discussed, there were outcomes of interest to the patient partners that were not studied in our identified studies. Patient partners identified areas requiring future research attention, such as the inclusion of female animals, inclusion of cognitive behavioural outcomes, and drug treatment options for those who have had stroke several months or years in the past. The development of our systematic review was presented at two local conferences (University of Ottawa Faculty of Medicine Research Day, and the Ottawa Hospital Research Institute Research Day) and one international conference (Advances in Stroke Recovery Scientific Conference 2023), where patient partners were co-authors on all three.

Protocol Development

Once the research question was established, the team co-developed a protocol[13] with patient partners. The protocol was then registered. To ensure further clarification of the protocol from a patient/public perspective, we sought a champion to go through the details and serve as a representative. We asked patient partners if anyone would be willing to take on this role. Fortunately, a patient partner with professional editing experience volunteered, providing valuable feedback on the protocol, specifically on areas that needed to be clearer for a patient/public audience, which results in overall shaped the protocol to be more in plain language, where additional explanations were added to provide more comprehensive background knowledge to orient a lay audience. The patient partner also provided refinement and suggestions of our included outcomes. To recognize the significant contribution and expertise she brought

to the table, we also compensated her for her time and effort. In addition, they helped construct a section in the protocol on patient engagement, which is unique to preclinical protocols. After incorporating the patient partner's feedback, the refined protocol was presented to the rest of the patient partners and researchers, and they reviewed it to provide additional feedback.

Screening and Extraction of Articles

On the technical conduct of the systematic review, patient partners were involved as reviewers in screening and extraction alongside researchers. After undergoing practice sessions with the partners, we were able to refine the screening and extraction questions to clarify how they needed to be answered (i.e. modifying the wording of questions, adding additional useful notes, or removing questions). Seven of the eight patient partners participated in the title and abstract stage of screening, where each partner had two pilot screening rounds of four to five articles and then a third screening round of 25 articles. The median time for screening the set of 25 articles was 60 minutes, with an accuracy of 78% compared to the screening results of the researchers. Six patient partners participated in the full-text stage of screening, where we held a live virtual meeting to go through an example article, and then partners were given two full-text articles to screen. The median time for screening two full-text articles was 37.5 minutes, with an accuracy of 75% compared to the screening results of the researchers. Four patient partners participated in the extraction phase of the systematic review, where we held a live virtual meeting to go through an example article to extract, and then partners were given one article to extract. The median time for extracting a full-text article was 43 minutes, with an overall accuracy of 83% compared to the extraction results of the researchers. The researchers discussed any discrepancies in the studies that were included with the patient partners, providing explanations of why certain items were extracted from the perspectives of the researchers, and all team members discussed the reasoning for potentially differing answers.

Analysis

Throughout the interpretation of results and discussion points for the review, patient partners identified areas that require researcher attention in the future. These areas included, integrating and evaluating outcomes that align with patient interests, including both sexes in preclinical stroke studies, focusing on long-term drug therapy options in the recovery phase (i.e. administering the drug weeks to months out from stroke onset), and evaluating the economic impact of treatment. The patient partners, and many patients that suffered a stroke, are living with the effects months to years afterwards with no drug option for them, and the hope of our systematic review was to address those concerns. However, most of the studies identified focused on the acute phase of care (i.e. hours to days after stroke onset).

Assessment of Engagement

To assess the engagement of the project, the Public and Patient Engagement Evaluation Tool (PPEET) was utilized. Questionnaires were administered at different time points to gather feedback from both patient partners and researchers, and results were collected (Appendix 4). The initiation round was administered at the start of the study during question generation, which identified beneficial ways of improving our engagement process from the start. From the patient partners, there was a need for clarity on the purpose of the review and the benefits of animal research. Since then, we have emphasized the purpose of our review in our newsletters and implemented more educational sessions. From the researchers' perspectives, there were concerns about consistent communication of the patient engagement process. This was another reason for implementing the newsletters, and also, moving forward, we ensured all researchers were informed about the engagement aspect through individual meetings or via other modes of communication (i.e. email).

Subsequent rounds of the PPEET showed positive results. The second round of the PPEET questionnaire was administered after the co-developed protocol was finalized. Partners expressed that we were able to create a positive environment where they felt supported and that their views would be acted upon.

"I have been able to express the areas of research in which I am interested. I have been able to review and provide feedback on presentation documentation of the project. These involvements make me feel like I have substantial and sufficient influence."

"Outstanding leadership and a broad range of patient partners/participants"

"Opportunity to comment and discuss components and outcomes of the review. Good team management. Good medical expert intervention."

"I believe having Zoom meetings has helped with promoting individual influence in the study."

"My years (71) of lived experience and seven years as a stroke survivor have always been taken into consideration when I share my views. Comments that I have offered has always been considered before moving forward."

"All opinions are considered."

From the perspectives of researchers, engagement also improved and was largely positive. Researchers were made more aware of the logistics of engagement and the concrete benefit it provided to the scope of the project.

The last round of the PPEET was administered after our last scheduled meeting to discuss the analysis and interpretation of results. The results from the patient partners continued to show an overall positive benefit from their involvement in the review, but also a deeper understanding of what they would like from future preclinical studies also was highlighted.

"I really enjoyed working on this project and hope that we will be able to build on this study. Much research has been done on a variety of stroke medications and treatment, but nothing has really been done to improve the recovery process. For example, we know CCR5 inhibitors help in the short term after a stroke, but will continued use of these medications help the brain to improved neural function, such as problem solving, improvement of speech, etc.? It would be wonderful if the

research team could look into helping people who are dealing with the after effects of a stroke. Also, more research needs to include females in the studies. Only looking at half of the population is foolish."

"Now that we are almost finished our study, the paper still has to be written, we should build on what we know and begin more cutting edge research."

"My hope is that when this project is finished that we can reconvene and study aspects of stroke recovery."

Benefits and their contributions from this research were also expressed by the patient partners.

"We looked at CCR5 inhibitors that may improve stroke outcomes. Without this type of research we will not be able to get the medicines to the people it can help. Certainly, more needs to be done and I would like to continue working on the projects in order to change the lives of patients."

"It was great that the team listened to and considered our personal interests, comments, and questions. I was surprised that a scientific study team would take the time to do that. I hope we didn't waste too much of your time."

"I was able to suggest some aspects of interest in the fine tuning of the subject matter. I provided feedback on the lack of content/scope/data in some aspects of pre-clinical trials based on reading the material."

"As a stroke survivor for over 43 and one is who is continuing a remarkable recovery, I think my unique experience and viewpoints have been looked upon with great interest and respect."

"My opinions were heard, discussed and acted upon as appropriate."

"We looked at CCR5 inhibitors that may improve stroke outcomes. Without this type of research we will not be able to get the medicines to the people it can help. Certainly, more needs to be done and I would like to continue working on the projects in order to change the lives of patients."

"The primary strength of the Stroke Review is the introduction of the Systematic Review Process to a neophyte group of Patient Partners (PP). Over the course of the completion of the review process the loosely associated group of PP and Academic Staff formed into a cohesive team."

"Incorporating patient and patient partner input in the pre-clinical trial review process which provides some specific focus of personal interest. As a side benefit, you helped interested patients and patient partners understand the pre-clinical trial process and understand some of the trial results as it relates to their personal situation."

There were also improvements that the patient partners suggested for enhanced collaboration in future projects and the challenges faced throughout the review.

"More time should be spent in the initial stages of the review process ensuring that all PP and other team members understand their role in the process."

"The extraction of the complete documents was challenging. I am not a medical practitioner so some of the language was difficult. Thank goodness for Google, it helped a lot. Patient partners look at the documents differently from medical professionals and I think this is a good thing."

"The pace at which the research project progressed was my biggest challenge. The excessive amount of time between meetings often lead to having to continually go over what was covered in the last meeting before moving forward."

"Due to my unique personal and professional life as both a freelance writer and a care-giver for my 92 year-old mother, I work many hours and often have serious lack of sleep issues."

"This is my first experience as a PP on a Research Project. I found it very rewarding and frustrating at the same time. It was rewarding because of my introduction to the Systematic Review Process, the opportunity to take courses (activate my 72 yr old brain) and exposure to new and exciting activities. It was frustrating due to the overall slow pace of the project. My background is completely different from the healthcare / academic area of study."

The final round of the PPEET administered to the researchers focused on assessing the impact of the engagement component within the project, yielded significant findings. In the initial two rounds, researchers expressed some uncertainty regarding the necessity and potential benefits of involving patient partners. However, this third round conclusively demonstrated that the inclusion of patient partners had a noticeable influence on the overall project outcomes, leading to a distinct alteration in the process compared to scenarios where they were not involved. This finding highlights the importance of engaging patient partners in research endeavours, as acknowledged by the researchers themselves.

> *"Patient partners formed valuable members of the team, and delivered feedback that I hadn't considered prior."*

> *"This project provides a good example to learn from and inform future engagements in basic science / systematic review in order to continue to refine and streamline the process. This was a beneficial project, but as with any initial attempt at a new research process, gaps and inefficiencies in the system were revealed that could be improved upon in future iterations."*

Obstacles and Challenges

While patient engagement is valuable, it also presents a number of challenges and obstacles. One of the biggest challenges we faced in this preclinical systematic review was the amount of planning, time, and coordination it took to engage eight patient partners. From recruitment to onboarding to engagement activities, the process required significant administrative effort and resources. It was important to ensure that patients and caregivers were adequately prepared and supported throughout the process, which required a substantial investment of time and effort from the research team to prepare documents and slides, schedule meetings with all team members, follow-up with surveys, and incorporate all feedback from members.

Due to scheduling conflicts and other commitments, it was not always possible for all patient partners and senior researchers to attend all meetings, which was a challenge for maintaining consistent communication and engagement. As a result, catch-up one-on-one meetings were necessary, organized and presented by the lead research assistant (AS) as part of her thesis projects, to ensure that everyone was up to date on the project's progress and decisions. There was also varying engagement of partners, which we recognized was normal for any team, and we adapted according to the needs and interests of the partners. For instance, one patient partner dropped out from being involved because of other commitments. Another example is one patient partner opted not to be involved in the full-text screening and extraction of the articles because of limited time commitment, but rather would be present at the meetings explaining those stages and results, while another patient partner was eager to be actively involved in all parts of screening, extraction, and results outcomes at each of the stages. These highlighted the importance of being flexible and accommodating when working with patients and caregivers.

Budgeting for patient and public involvement can also be a challenge, particularly when engaging a relatively large number of patient partners. In our case, we went over budget, which required us to seek additional funding to continue the engagement process. This highlights the importance of careful planning and budgeting when incorporating patient engagement into research projects.

At times it was a challenge to manage the feasibility of the review and balance patient partners' expectations with the available resources and time frame. Some patient partners were interested in exploring a broader range of research questions and outcomes than what was feasible within the scope of the review. Additionally, learning the scope of the benefits and limitations of animal research was novel for the patient partners, and there were discussions of a desire to focus on human research instead. These challenges required ongoing communication and education to help patients and caregivers understand the rationale for the review and the specific focus on animal research.

Finally, it was a significant effort to navigate the involvement of patient partners in the screening and extraction phases of the systematic review, particularly with the review topic being preclinical. This was a novel area with little guidance in the literature; however, including training, regular meetings, and constant communication ensured that the patient partners felt comfortable with their roles and responsibilities.

Despite these challenges, we were able to successfully engage patient partners in the preclinical systematic review and obtain valuable input and feedback that helped to shape the research question, design, conduct, and dissemination. By being responsive to the needs and challenges of the patient partners, we were able to build a collaborative and inclusive research environment that valued the perspectives of all team members.

DISCUSSION

We co-designed and executed a preclinical stroke systematic review with patient partners. Our findings highlight the feasibility and value of patient engagement in this form of preclinical research. Through their active participation and leadership, patient partners played a crucial role in co-developing the research question, co-developing the protocol, involvement in screening and extraction, interpretation of the analysis, and co-authorship in our disseminated work. Regular surveys following team meetings provided an excellent opportunity for patient partners and researchers to contribute to decision-making, resulting in a systematic review that genuinely reflected the needs and values of the patient panel.

The first-hand experience of patients reviewing, screening, and extracting data from articles helped inform the interpretation and discussion of the results in our team meeting, where points and issues were raised regarding the lack of outcomes covered in the included studies that were initially highlighted during question generation (i.e. chronic drug administration). This comprehensive engagement enhanced the relevance and patient-centeredness of the review for the discussion of results, paving the way for addressing identified gaps and prioritizing patient perspectives. To our knowledge, our preclinical systematic review is the first to involve patient partners in the data extraction stage. A scoping review that investigated stakeholder engagement in systematic reviews[14] identified one review[15] that involved patient partners in all stages of the review except for extraction. Building on this comprehensive scoping review on engagement in systematic reviews[14], the authors constructed a resource, Patient and Public Involvement (PPI) in Systematic Reviews[1], that emphasizes the importance of involving patients and the public throughout the systematic review process to ensure research relevance and patient-centeredness. This aligns with our experience, as we actively engaged patient partners from the initial stages that helped orient the final product of the review.

In the initial stages, we recognized that preclinical systematic reviews were unique compared to clinical systematic reviews given their reliance on understanding multiple basic sciences along with the technicalities of animal experimentation. As a result, to build a knowledge foundation among the patient

partners, we incorporated educational sessions on animal studies of stroke along with systematic review methodology before starting the review and throughout the project. These sessions aimed to enhance the understanding of the research process and foster shared learning. We also offered additional training courses led by patient engagement experts for the patient partners to attend, providing them with the opportunity to further develop their knowledge and skills for the review. Furthermore, we encouraged patient partners to attend conferences where they could gain exposure to the wider research community and broaden their perspectives. A resource by INVOLVE[4], Public Involvement in Systematic Reviews: Supplement to the briefing notes for researchers, emphasizes the crucial need to focus on education, training, and support for patient engagement in research. According to INVOLVE, training should encompass various methods such as group sessions with a trainer, high-quality written materials and guidance, on-the-job learning, attending conferences, networking and shared learning with peers, and online activities. They state the importance of tailoring the educational session to the specific context and should be provided to both the patient partners and researchers on the team. In our project, we aimed to educate the researchers on best practices for engaging patient partners in the review. However, it is important to acknowledge that the existing resource primarily focuses on patient engagement in clinical systematic reviews. Our study expands upon this by exploring patient engagement in a preclinical systematic review, which presents unique challenges and considerations such as the absence of direct patient involvement. Nonetheless, by implementing the educational sessions, offering additional training opportunities, addressing practical and emotional support needs, and maintaining ongoing communication and guidance we incorporated the principles highlighted by INVOLVE. This aided in creating a knowledge foundation from the beginning and throughout the review to create informed decisions for the team.

Lastly, given the novelty of patient engagement in the preclinical space, we faced challenges such as educating patients on what preclinical clinical studies can or cannot demonstrate compared to clinical studies. Since animal studies that use rodents cannot be directly related to patient studies in every aspect,

the educational sessions led by preclinical stroke experts aided in understanding the limitations of preclinical studies. In addition, having patient partners involved in reading articles for screening and extraction was also helpful. Of note, being adaptive and incorporating additional sessions when we recognized a need or interest, required additional planning, time investment, budget management, and coordination from the researchers. We made a concerted effort to address practical and financial issues that patient partners may face throughout the review process, so the lead researcher (AS) took the initiative to hold multiple follow-up meetings with individual patient partners to clarify any issues or concerns and provide guidance as needed. The adaptation needed from the INVOLVE resource for additional training and educational sessions was unanticipated but helped strengthen the study for collaboration from all team members. We recognized the importance of providing additional and improved educational sessions to build up knowledge in future preclinical systematic reviews. Moving forward, it is fundamental to allocate more time for education and training sessions for successful engagement.

By adopting these improvements and incorporating patient perspectives into preclinical research, we can foster a more inclusive and patient-centred approach to systematic reviews.

Tables and Figures

Table 1. Overview of outcomes from categorized meetings

Virtual Team Meetings and Discussions			
Topic and Question Generation	**Search Strategy and Protocol Development**	**Technical Conduct**	**Analysis and Interpretation**
• Identification of research priorities • Determining research topic • Establishing inclusion criteria • Determining consensus on each part of the research question (Animal, Model, Intervention, Outcomes)	• Developing a comprehensive search strategy • Establishing eligibility criteria • Writing and refining the protocol • Incorporating feedback from patient partners on protocol • Identifying conflicts of interest	• Refining screening and extraction questions • Pilot screening rounds for patient partners • Full-text screening for patient partners • Extraction phase for patient partners	• Discussing discrepancies among included studies • Synthesizing and interpreting the results

Table 2. Details of each meeting held with patient partners

Meeting Number	Meeting Topic	Discussion Points	Outcomes
1	Topic and Question Generation	Discussion of what the project is and expectation of roles	Onboarding session of an overview of the project
2	Topic and Question Generation	Brainstormed potential topics	Determined that drug treatment during stroke recovery was the priority topic
3	Topic and Question Generation	Decided on specific class of drugs to investigate	Determined that maraviroc and CCR5 inhibitors were of most interest
4	Topic and Question Generation	Educational session	Provided more in-depth information on animal research and the difference compared to human research
5	Topic and Question Generation	Refining the research question and identifying outcomes of interest	Developed the final research question and identified key outcomes
6	Search Strategy and Protocol Development	Information specialist discussed developing the search strategy	Finalized the search strategy and protocol with input from patient partners
7	Search Strategy and Protocol Development	Discussed details of the protocol and incorporated changes feedback from patient partners	Finalized the search strategy and protocol with input from patient partners
8	Technical Conduct	Reviewed the screening process and providing guidance to patient partners on title and abstract screening	Refined and clarified the screening process, and provided training sessions and practice rounds of five examples for patient partners
9	Technical Conduct	Monitored progress, m addressed any issues and provided additional training on title and abstract screening	Addressed any questions or concerns regarding the screening process and provided training sessions for an

			additional four example for patient partners
10	Technical Conduct	Expert in preclinical stroke research addressed issues with technicalities of stroke models	Addressed any questions or concerns regarding the screening process
11	Technical Conduct	Discussed discrepancies among included studies, trained patient partners on full-text screening	Discussed any discrepancies in study inclusion and resolved any issues and trained on the second stage (full text) of screening; Determined that patient partners were able to effectively screen
12	Technical Conduct	Trained patient partners on the extraction process and refined extraction questions	Refined and clarified the extraction question and process, and provided training session an example for patient partners
13	Analysis and Interpretation	Discussed discrepancies of extracted articles and the findings and implications for future research	Patient partners successfully extracted data from included studies; Analyzed the data and discussed the implications for future research

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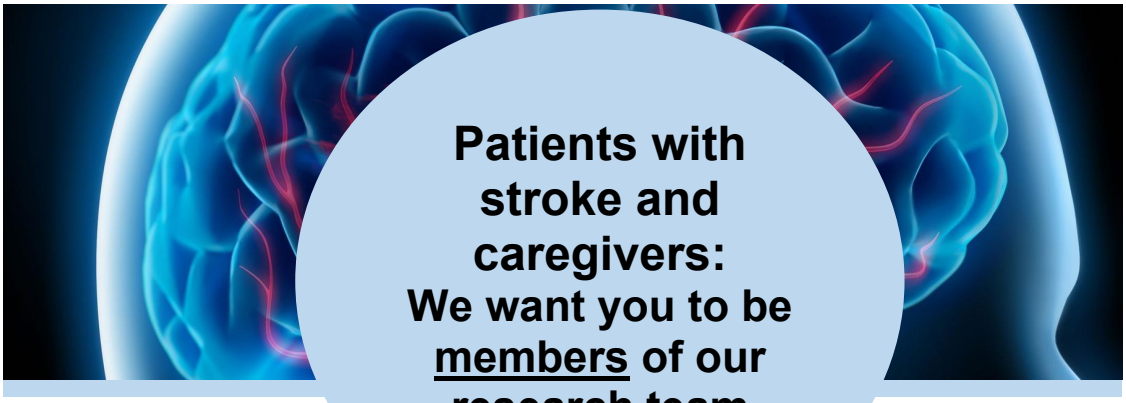
Appendices

Appendix 1: Recruitment Advertisement



Patients with stroke and caregivers: We want you to be members of our research team

We need your input to review laboratory studies testing treatments for stroke.

This review of lab studies of stroke will help researchers decide when it is time to test a new treatment in human patients for the first time.

Your involvement will make sure that the review focuses on topics most important to patients.

Together, we will:

- Determine what treatments and outcomes the review should focus on
- Interpret results from a patient perspective, draw conclusions, and suggest future work
- Co-develop a non-technical summary of the review, share the findings to reach a wider audience. Patient partners will also have the option to be co-authors on the paper we write.

Are you eligible?

- We are looking for individuals with personal experience of stroke OR informal caregivers, including family and friends.
- If you are interested in research and helping guide a project, we would love to have you on our team.

Time Commitment

- Commitment is flexible to your schedule
- Approximately six 90 minute meetings over 12 months

Compensation

- $25/hour, including preparation time (if you wish to receive it)

If you're interested in learning more, please contact:

- Dr. Manoj Lalu
- Associate Scientist, The Ottawa Hospital
- mlalu@toh.ca

The Ottawa Hospital | L'Hôpital d'Ottawa
Blueprint Translational Research Group | Groupe de recherche translationnelle Blueprint

'A Review of Laboratory Studies in Stroke' Terms of Reference for Patient Panel

Name of Group: A Review of Laboratory Studies in Stroke Team

Purpose of this document: This terms of reference document is intended to be co-developed by all team members to help guide how we plan to work together. This document is also meant to be a 'living' document, which means that elements can be updated throughout the project as needed to suit the team's needs and provide the most up-to-date information.

Purpose/role of group

- The Objective of the Review of Laboratory Studies in Stroke
  The Review of Laboratory Studies in Stroke team comprises researchers and Patient Partners in Canada with experience in stroke or patient engagement. The objective of the review is to work together to:
  1) Conduct a systematic review of laboratory studies ('preclinical') of therapies for stroke
  2) Assess how researchers and Patient Partners collaborated on the review

- What are the aims/responsibilities of the Patient Partner group?
  Provide support to, and insight on, the preclinical systematic review. Patient Partner input will be based on lived experiences with stroke, as well as other expertise they may wish to contribute. Patient Partner input is anticipated during the development of the following components (*note: planned opportunities for involvement may be updated throughout the course of the project, there is no expectation for Patient Partners to be involved in all activities of the review; engagement will be based on individual Patient Partner interest and availability):

  - **Systematic Review:** Our team plans to co-develop a systematic review of the published preclinical (laboratory-based) stroke literature. Patient Partners may help with co-developing the study question, co-developing the protocol, and/or co-developing the search strategy. In addition, Patient Partners may aid in screening and extracting relevant information from the articles, provide input on how to present the results (e.g. how to organize tables, how to narratively present results, co-identify and provide feedback on key themes), co-develop a non-technical summary and manuscript, aid in the dissemination of results to networks and organizations, and co-present the results.

  - **Surveys:** We will administer surveys to the Patient Partners and research team members alike to evaluate the patient engagement process of the review. This will allow us to obtain both perspectives of the patient engagement initiative; assess the

planning, execution, and impact of engagement; and assess key features of the engagement activity or activities that Patient Partners have participated in.

- **Relationships between team members**
  Within the team, we aim to develop meaningful relationships where all team members are confident in sharing their input and feel a shared sense of accomplishment in the results.

Opportunities for Patient Partner Involvement

Preclinical research is a novel area for patient engagement. We plan to co-identify how best to work together and activities that work towards the goals and priorities of all members. To date, we have had several discussions to brainstorm ideas for patient engagement. In general, activities will aim to allow Patient Partners to be the ultimate decision-makers in the review and provide lived experience when developing the deliverables below. We also note that involvement can be based on interest and availability to ensure feasibility.

Terms of Reference: A Terms of Reference document will be co-developed by team members to outline a set of shared expectations. An initial draft will be created by Ayni Sharif (Research Assistant), Raj Bapuji (Research Assistant), Dean Fergusson (Principal Investigator), and Manoj Lalu (Principal Investigator) using a team template. The draft will then be circulated to all panel members for input. We will plan to update the terms of reference as the roles of Patient Partners and researchers change.

Patient Partner Membership

Recruitment Strategy: Patient Partners have been recruited through the Heart and Stroke Foundation and the Patient and Family Advisory Council at The Ottawa Hospital.

- Who is the membership of the group open to?
  1) Patients with lived experience of stroke
  2) Caregivers or family members of individuals with lived experiences of stroke

- Planned number of Patient Partners

Currently, the panel consists of eight Patient Partners.

- Information Session

Upon recruitment, new Patient Partners will meet with at least one research team member for an information session. At this meeting, background on the research project and its overall goals and

components will be provided for the Patient Partners so they can gain an understanding of the project and how they can become involved.

- Length of the Patient Partnership Membership
    - *How many hours per month?*
      Patient Partners will be given the opportunity to attend meetings that align with the main stages of the review process that are most of interest to them. We will aim to have a minimum of one meeting a month for the project. If Patient Partners cannot attend, they may book a one-on-one meeting with Ayni Sharif (Research Assistant) for an update. Meeting minutes will also be circulated to the group.

    - *How many months of involvement?*
      Development of the proposal began in December 2021. Work on the funded project started in May 2022 and will continue until April 2023. The project timeline may vary depending on the nature of the review question. The Patient Partners are free to change their level of participation at any time for any reason (e.g. increase, decrease, or stop participation at any point in the project). Patient Partners can contact one of the Research Assistants if they wish to change their level of participation.

Accountability

Ayni Sharif (aysharif@ohri.ca) and Raj Bapuji (rabapuji@ohri.ca) (Research Assistants) will be the main contacts for the Patient Partners. Please allow for up to one to three business days for a response, but we will do our best to get back to you as soon as we can. **Note:** Due to the collaborative nature of research teams, it is possible that research team members may join throughout the course of the project. This new person will be introduced at a team meeting, and their name and role will be added to the Appendix.

Review:

- Patient Engagement

All team members will be asked to complete The Public and Patient Engagement Evaluation Tool (PPEET) three times over the course of the project. This is a validated survey that can be used to gather feedback on the team's patient engagement strategy and help identify areas for improvement.

Working methods/ways of working together:

- Meetings
    - *How will meeting topics be generated?*

Topics will be generated at least one week before the scheduled meeting and all members will be informed. Upon circulating meeting minutes from the previous meeting, team members will be encouraged to respond with any potential discussion topics for the next meeting.

- *How and when will meeting documents be circulated?*
  Meeting agendas and documents will be distributed in advance of the meeting by Ayni or Raj. Ayni or Raj will also circulate action plans developed after meetings or any meeting minutes following the meeting.

- *What will be the format of the meetings?*
  The format of the meetings will be online and include a combination of presentations, project updates, and discussions. Meetings will occur on Zoom, which will allow the presenter to share their screen so everyone can see the presentation on their computer screens. If Zoom fails or is challenging, a research team member will be available to provide technical support and guidance. To ensure understanding of each team member, a clear overview of relevant terms and scientific background and scientific background will be provided at the start of each meeting. Our team strives to create a supportive environment, respectful of the fact that members may have different backgrounds and lived experiences. As such, we encourage everyone to ask any questions they have at any time.

- *Will non-members be invited to group meetings, and if so, under what circumstances?*
  Only group members have attended the meetings so far. However, if a non-member were to attend a team meeting, all members would be notified in advance when the meeting agenda is distributed.

- *Who will keep documentation for group meetings?*
  A research team member will keep records of meetings in the form of meeting minutes. In addition, another research team member will chair the meetings.

- <u>Sharing of information and resources (including confidential materials)</u>

Information and resources will be predominantly shared through e-mail. All members of the research team will use their institutional email addresses to comply with The Ottawa Hospital confidentiality mandates.

How will confidential materials and copyright issues be identified and dealt with?

All members of the team are expected to keep shared materials (e.g. slides, meeting minutes) and discussions confidential (private, within the group) as these may involve personal ideas and details (e.g. medical history). If any issues regarding confidentiality arise, they will be discussed at the team meetings, and the terms of reference will be updated to solve and prevent future issues.

- <u>Compensation/Honoria</u>

Compensation will be discussed individually. We will follow compensation recommendations put forth by the Strategy for Patient Oriented-Research (SPOR) Evidence Alliance[1], the SPOR Networks in Chronic Diseases, and the PICHI Network[2]. If you would like to be compensated in an alternative way (e.g. receiving the amount in form of a gift card, etc.), that would be possible.

- Reimbursement of Expenses

On the chance that the Patient Partners anticipate any monetary expenses to attend team meetings or fulfil team activities, they should email one of the Research Assistants ahead of time to discuss the potential for reimbursement.

Definition of Terms

An onboarding meeting will take place for any new Patient Partners to discuss the overall goal and components of the 'A Review of Laboratory Studies in Stroke' project. The Patient Partners are highly encouraged to ask or email for clarification if they are unsure about anything related to the project. Patient Partners may also request further one-on-one meetings with the research staff and project leaders if they wish to learn more or want clarification on a topic. An overview of terms/background information in clear language will be presented at the beginning of each meeting to ensure the understanding of all team members. Below, several key terms have been defined.

Study-Related Terms

Patient: As per the Canadian Institutes of Health Research (CIHR), "patient" refers to "individuals with personal experience of a health issue and informal caregivers, including family and friends."[3]

Patient Engagement: Patient engagement in research refers to an active partnership between patients and members of the public and researchers (e.g. collaboration between patients and researchers). This is different than when a patient participates in a research project. Examples of engagement activities include (but are not limited to): priority-setting, co-designing a research experiment, helping conduct an experiment, providing perspective on study results, co-developing a manuscript, and helping to disseminate study results.

Preclinical Research: We will use the following definition: research using animals to determine if a drug, procedure, or treatment is likely to be useful; preclinical studies also take place to inform human testing.[4]

Systematic Review: A comprehensive review of the literature. A systematic review aims to identify records (e.g. articles, reports, blogs) to help answer a clearly formulated question by running a search of all related published and unpublished literature.[5] Before beginning the systematic review, a plan of the details of the review will be written down in what we call a protocol.

Team Member Terms

Co-Principal Investigator: Senior-level researchers who are leading the project.

Co-Investigator: Senior-level researchers who provide expertise and input throughout the project.

Patient Partner: Patient, caregiver, or member of the public who has partnered with the research team to provide input of their expertise through lived experience throughout the research project.

Research Assistant/Technician: A researcher who provides assistance throughout/coordinates a project. Research assistants/coordinators may be undergraduate and graduate students or have already completed their Masters or Ph.D. Research assistants/coordinators work under the supervision of principal investigators or co-investigators, and research associates.

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THE BLUEPRINT POST

October 14, 2022 issue 3

The Ottawa Hospital

Blueprint Translational Research Group

Affiliated with uOttawa

About this Newsletter

This is the third edition of our newsletter. You will find updates on our timeline, research question, in addition to our regular feature on our team's patient partners and researchers. Also included is a study that we hope you will find interesting.

Progress Timeline

We have narrowed down our intervention to focus on CCR5 inhibitors, like maraviroc. Maraviroc helps surviving brain cells ('neurons') to make new connections, which can promote brain repair following stroke. What makes maraviroc interesting is that there are active clinical trials of the drug in Canada!

Now that we've decided on all the different elements of our research question, we are ready to write our protocol (a detailed plan) to find and analyze laboratory studies of maraviroc.

Meet your Fellow Patient Partner

Each issue, we will be sharing some of your fellow patient partners' profiles. This time, we are pleased to introduce **Patient Partner 1 (Name, Image, and Biography removed for privacy)**.

Meet the Researchers

We will also be sharing some profiles of members of the research team. This time, here are Stuart and Matthew!

Stuart Nicholls (he/him), BSc (Hons), MSc, MRes, PhD

Dr. Stuart Nicholls is the Strategy for Patient-Oriented Research (SPOR) Program Facilitator in the Office for Patient Engagement in Research Activities (OPERA) at the Ottawa Methods Centre. In his capacity as SPOR Program facilitator he consults with researchers to provide methodological guidance regarding all

Matthew Jeffers BSc, MSc, PhD (s)

Matthew is a PhD student with the Blueprint Translational Research Group, led by Dr. Manoj Lalu and Dr. Dean Fergusson at the Ottawa Hospital Research Institute (OHRI). Matthew conducted 9 years of research in preclinical

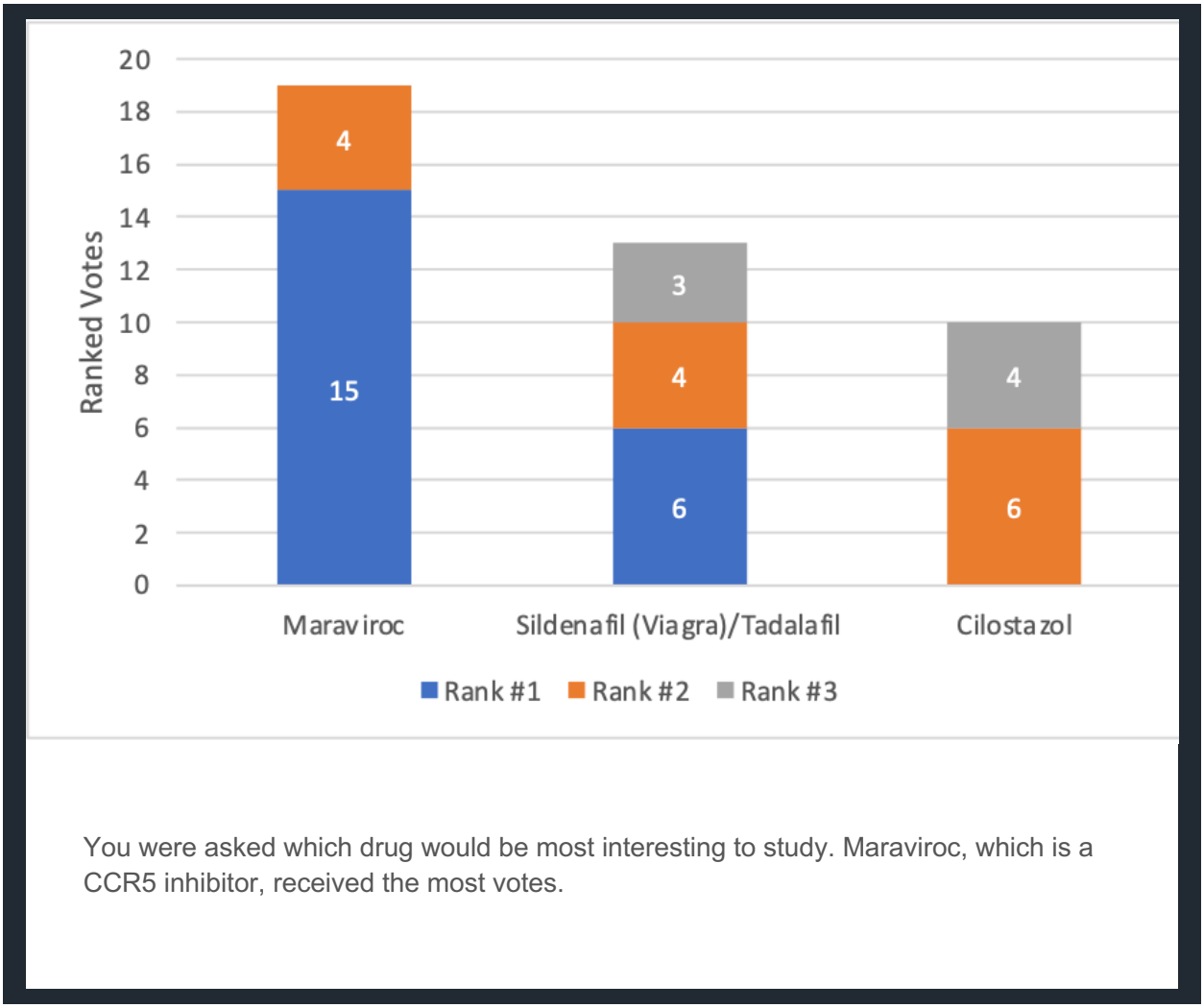
aspects of Patient-Oriented Research. In addition, he provides training and education on patient engagement in research and actively contributes to research in this field. His research includes exploration of parent experiences of consent, public attitudes to genomics, and work to explore the impact of patient engagement on research. Dr. Nicholls was a member of the Canadian Clinical Trials Coordinating Centre (CCTCC)/Health Canada working group on developing a pan-Canadian accreditation system for Research Ethics Boards reviewing clinical trials. He is also a member of the Health Canada/Public Health Agency of Canada Research Ethics Board.

models of stroke recovery and rehabilitation as Laboratory Manager to Dr. Dale Corbett and Dr. Gergely Silasi at the University of Ottawa. Matthew will be helping to facilitate the Stroke Review.

Previously, he completed an Honours BSc in Psychology at the University of Manitoba and a MSc in Neurosciences at Memorial University of Newfoundland.

Survey Results

Appendix 3: Example of Newsletter



You were asked which drug would be most interesting to study. Maraviroc, which is a CCR5 inhibitor, received the most votes.

Appendix 3: Example of Newsletter

In the Spotlight

Check out this Canadian clinical trial happening with maraviroc on patients with stroke by clicking Find out more!

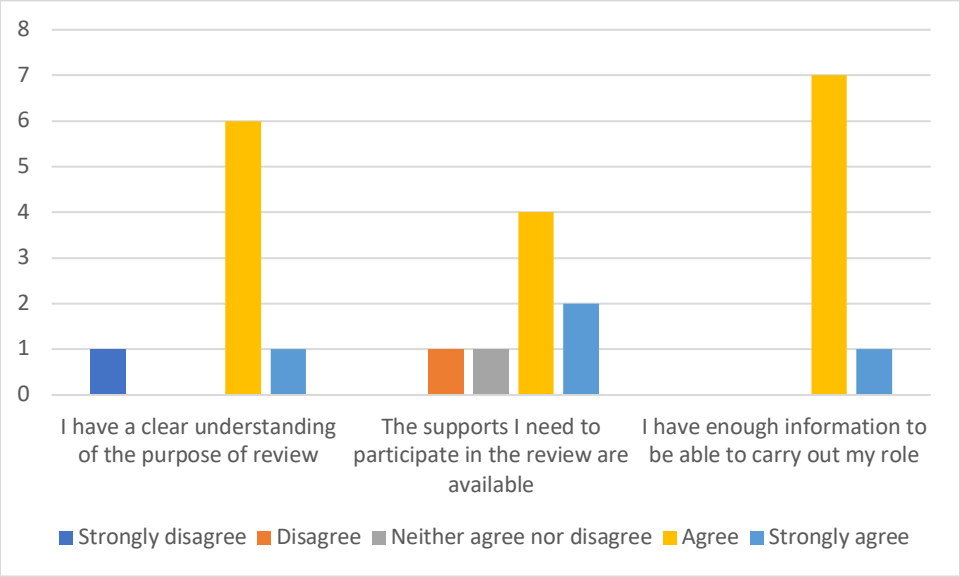
Find out more

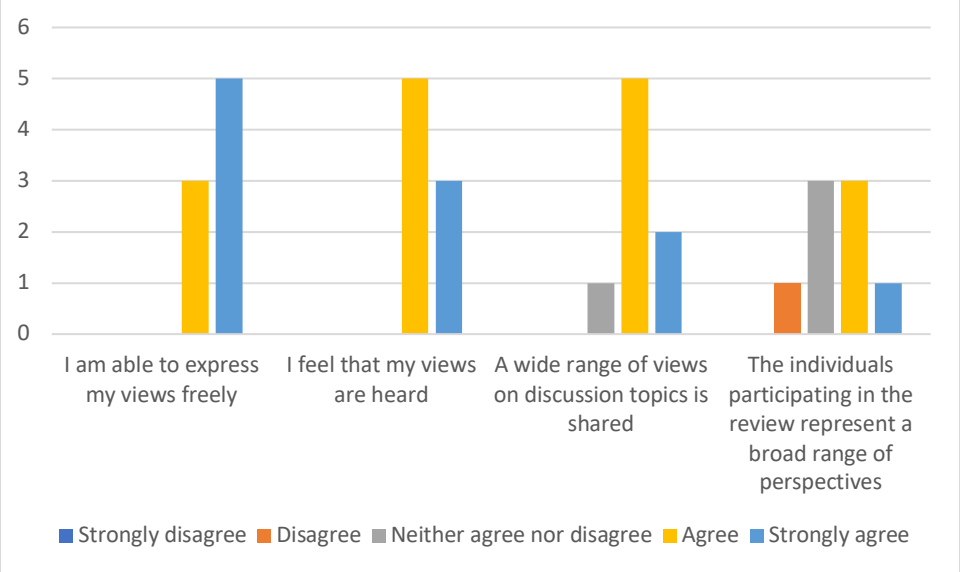
Socials

Appendix 3: Example of Newsletter

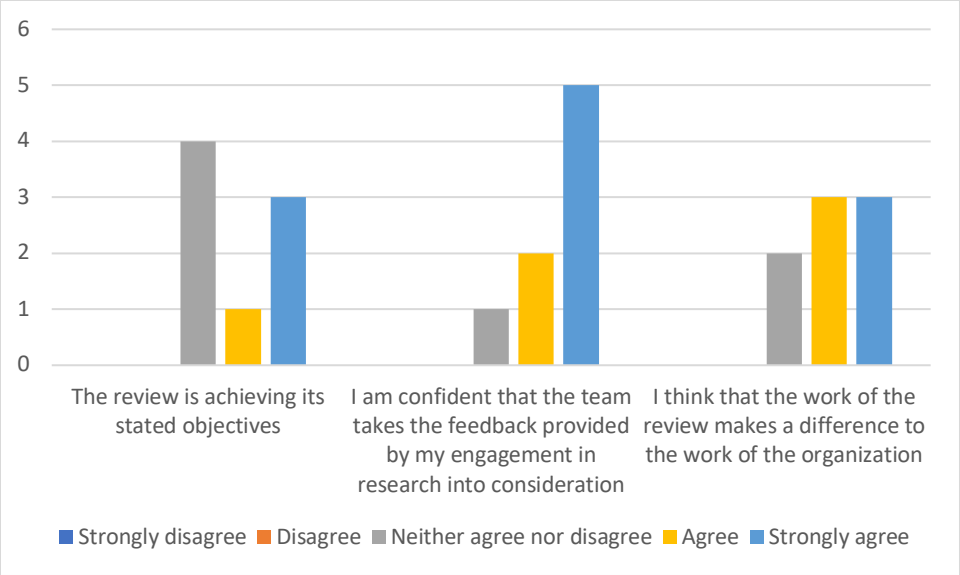
Appendix 4: PPEET Results from Patient Partners and Researchers

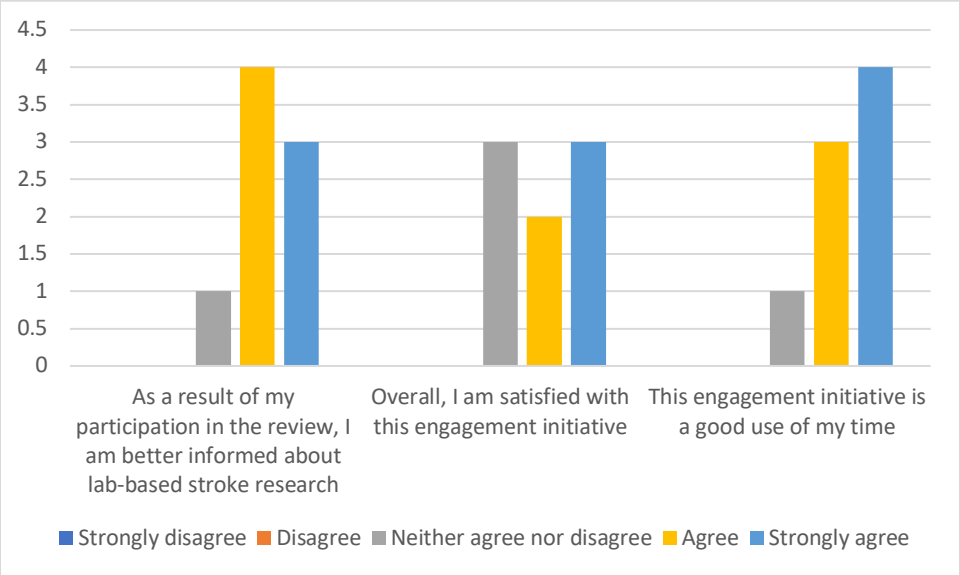
Patient Partner Questionnaire 1 Results:





Appendix 4: PPEET Results from Patient Partners and Researchers





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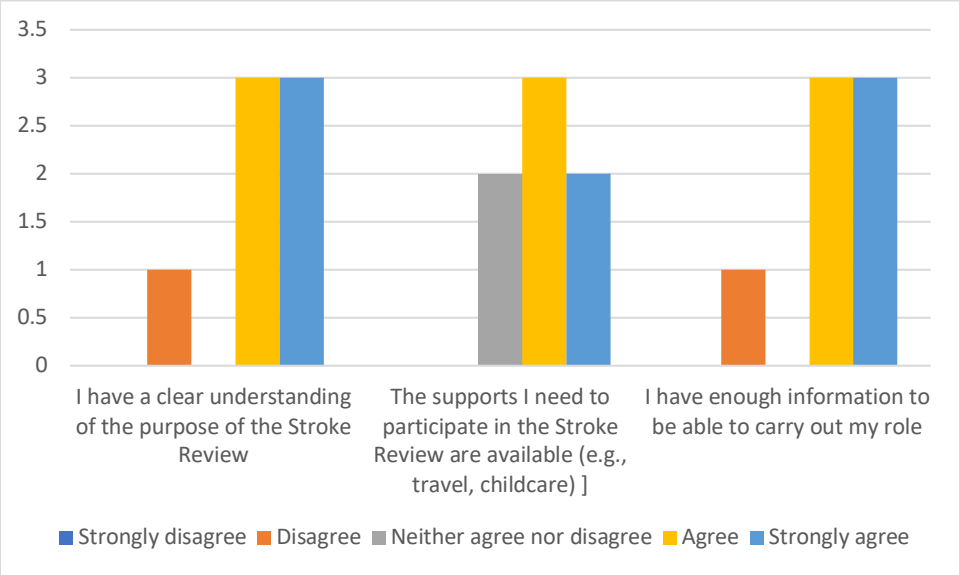

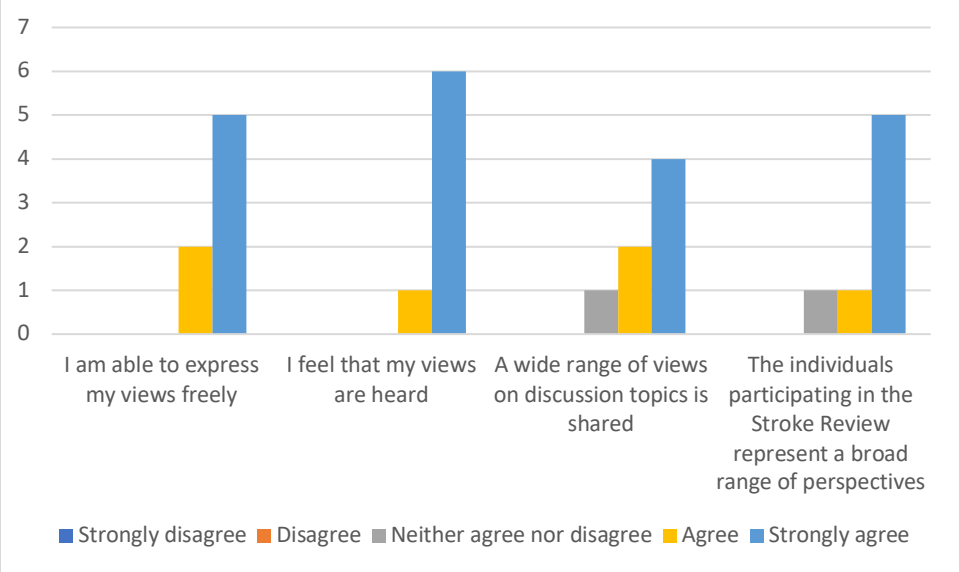

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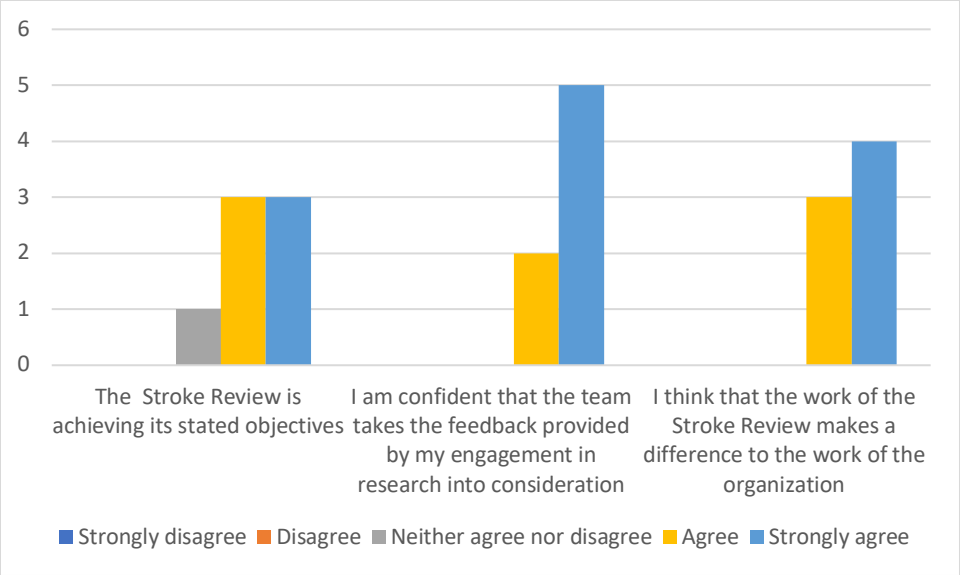

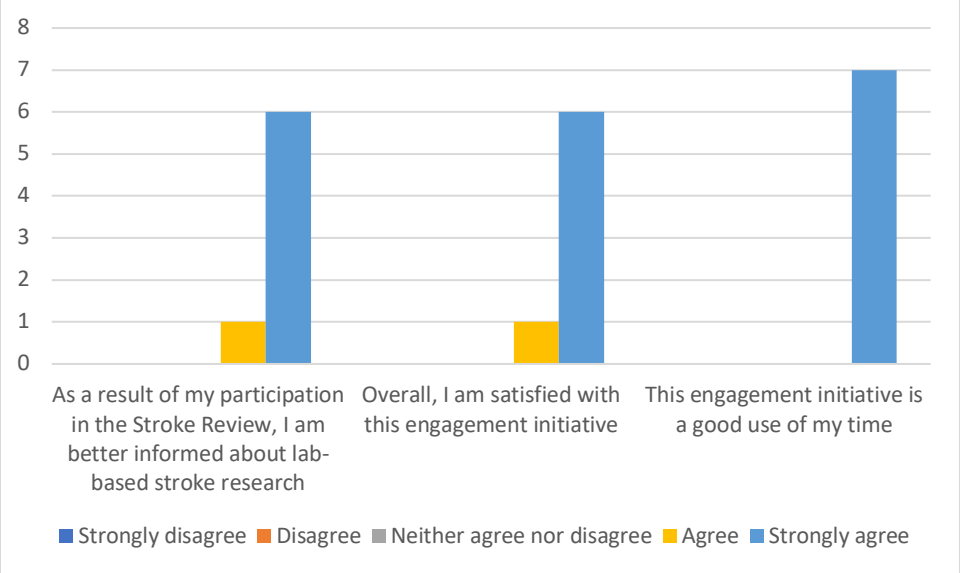

Appendix 4: PPEET Results from Patient Partners and Researchers

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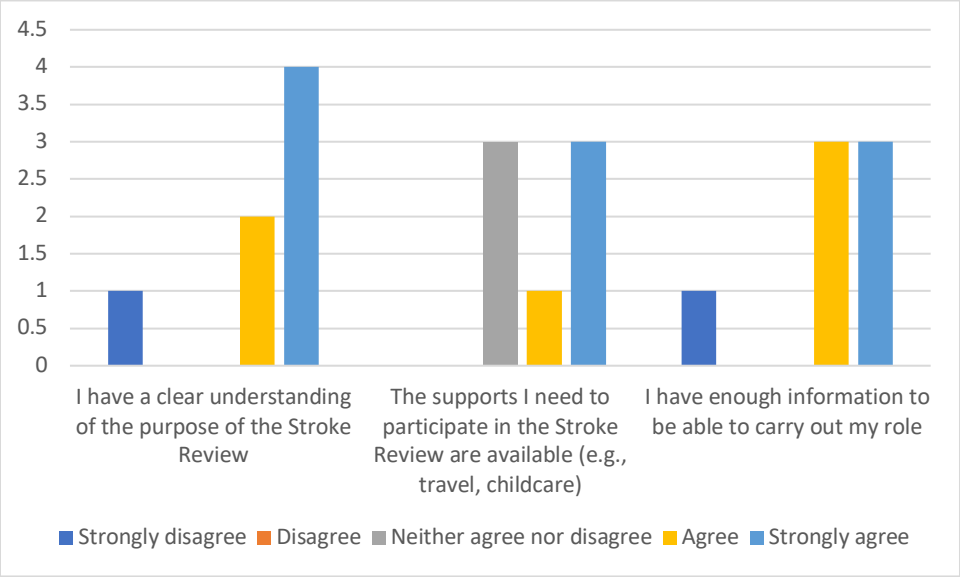

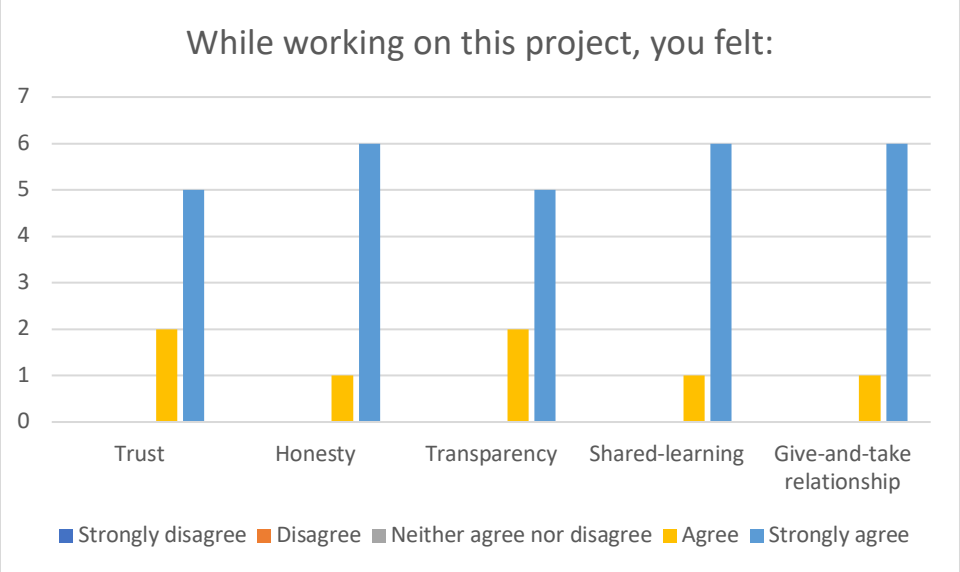

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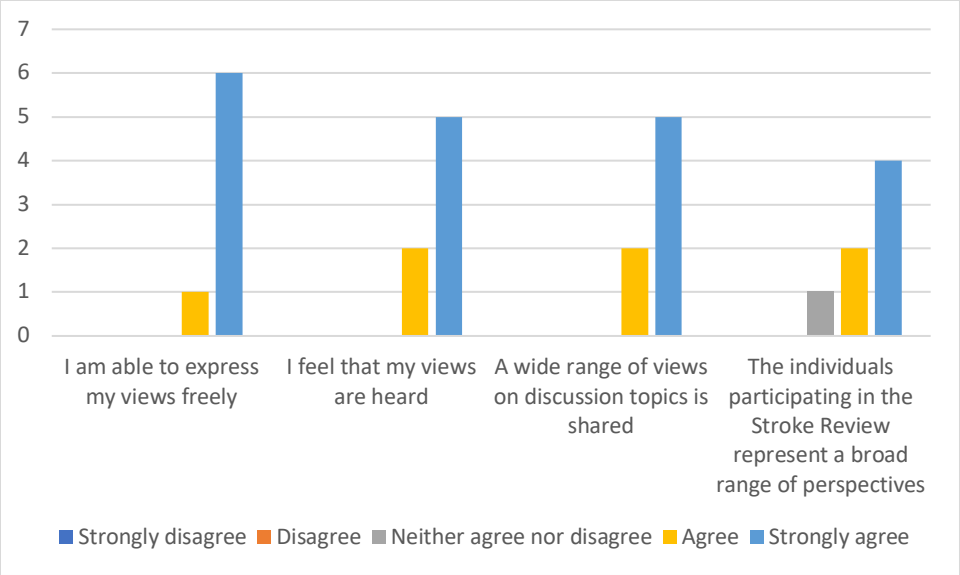

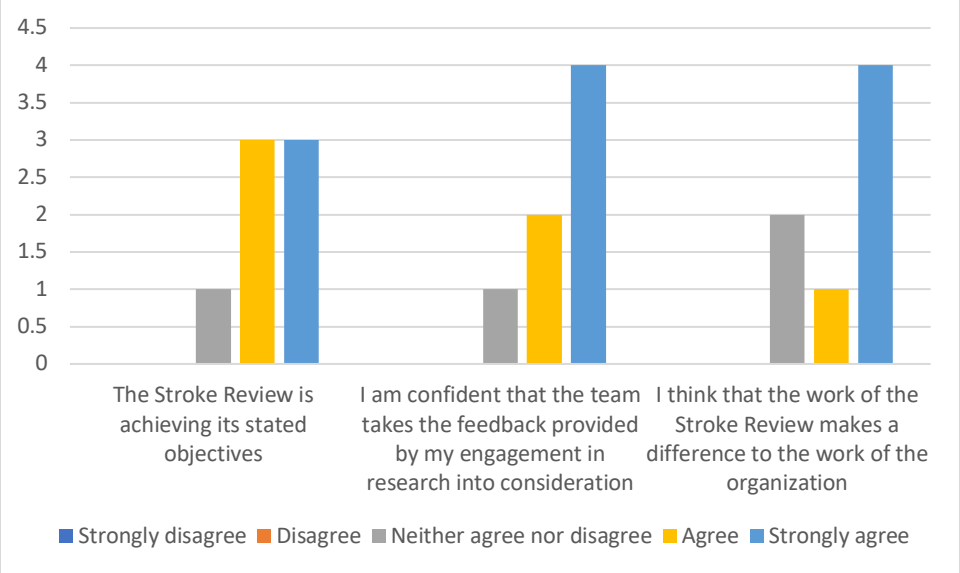

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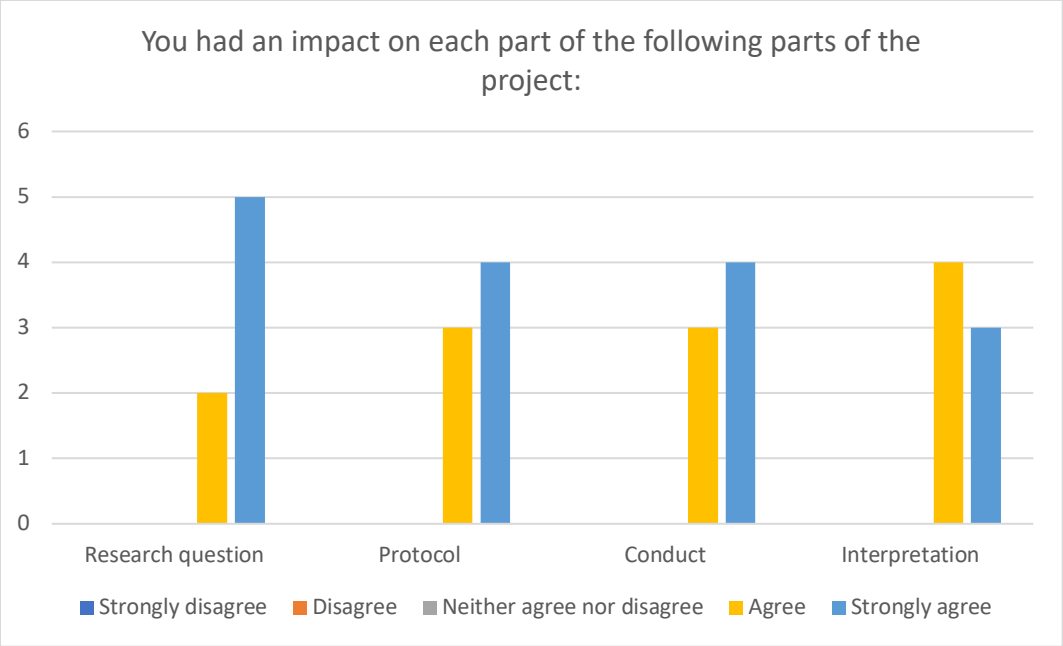

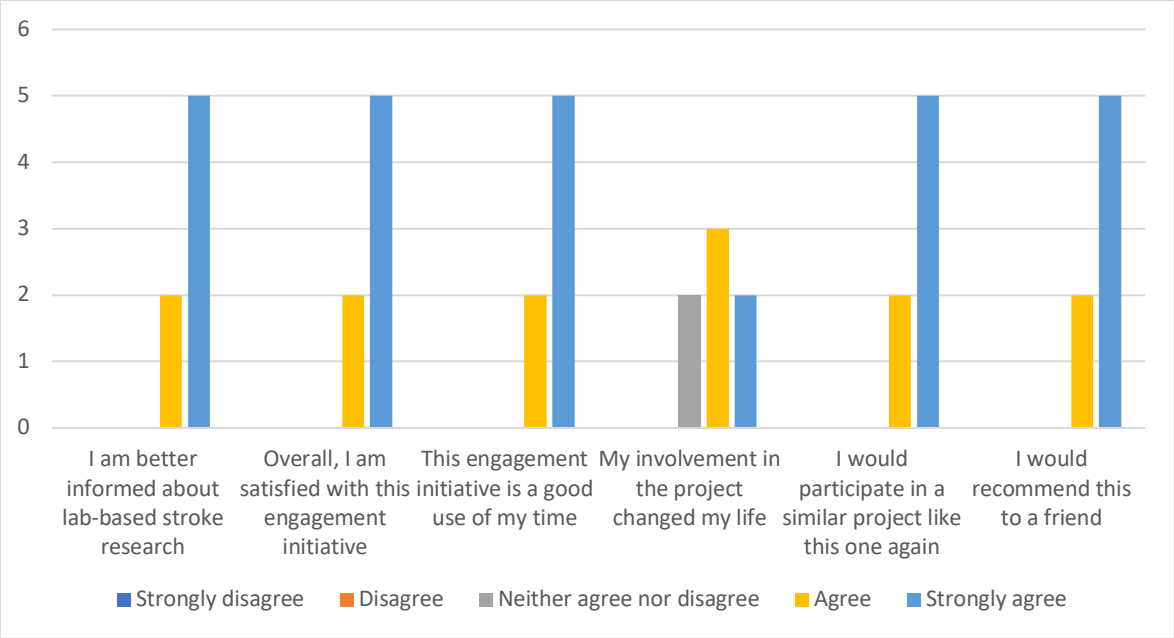

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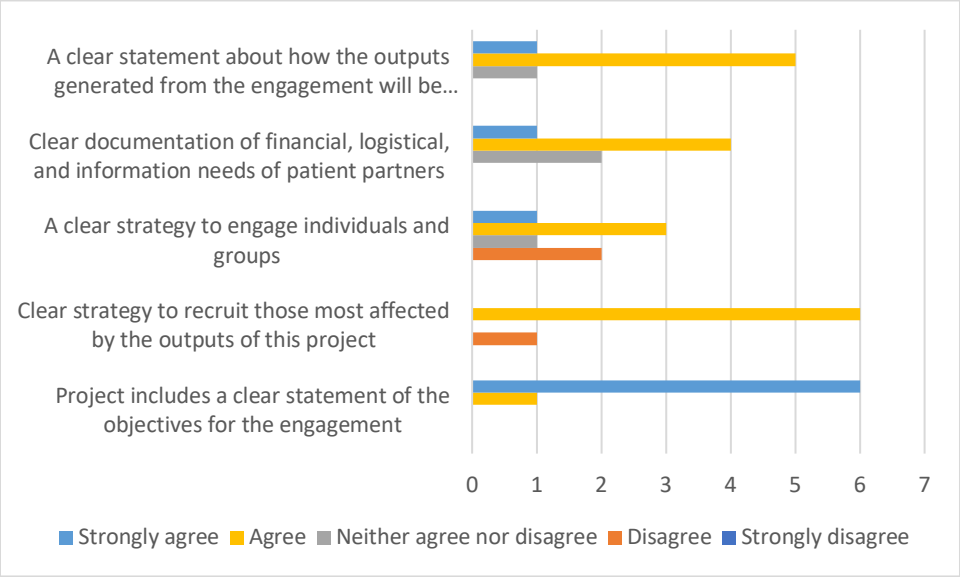

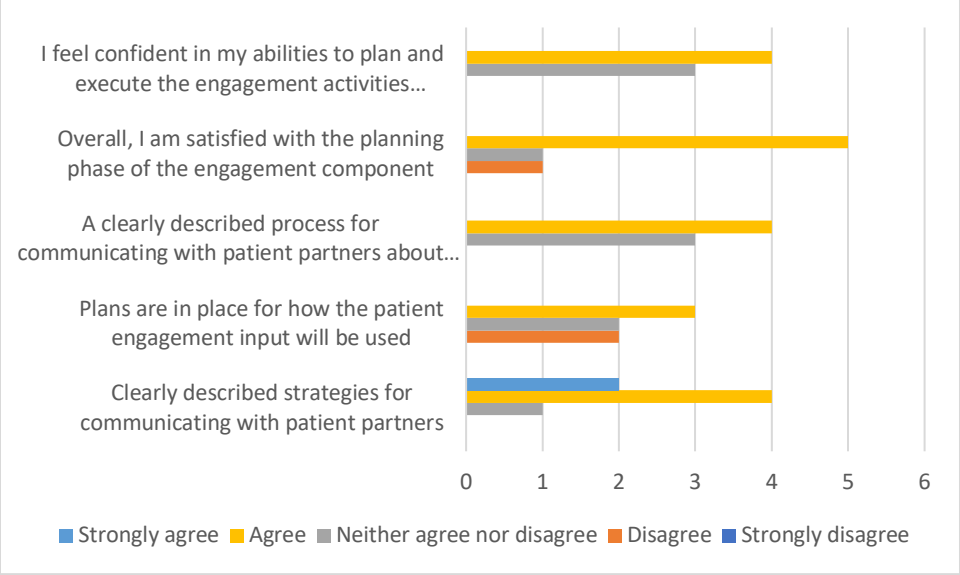

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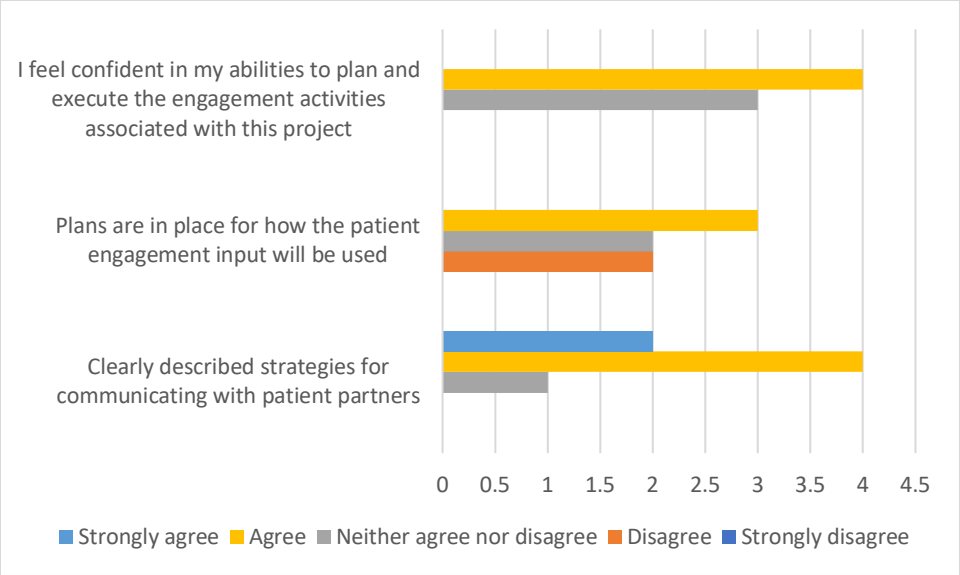

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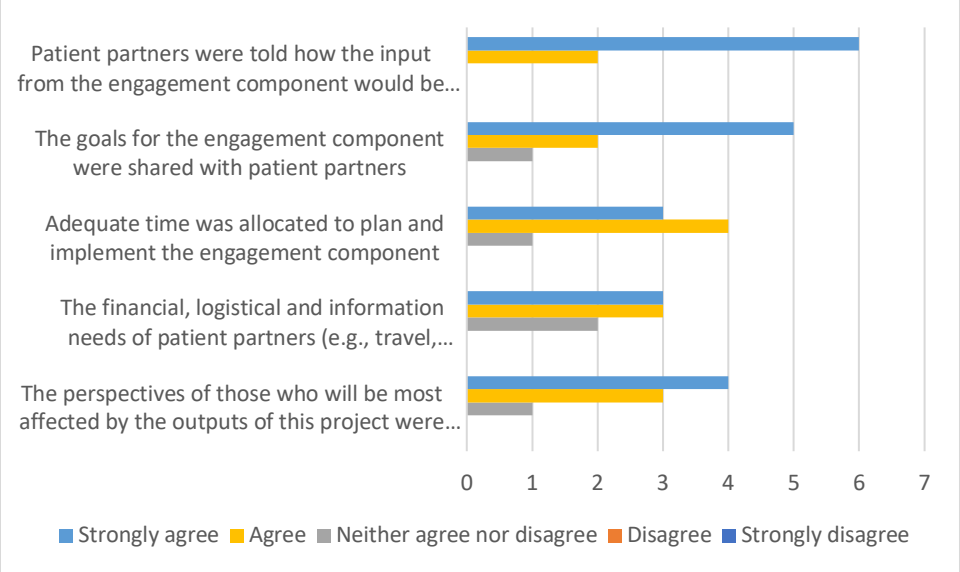

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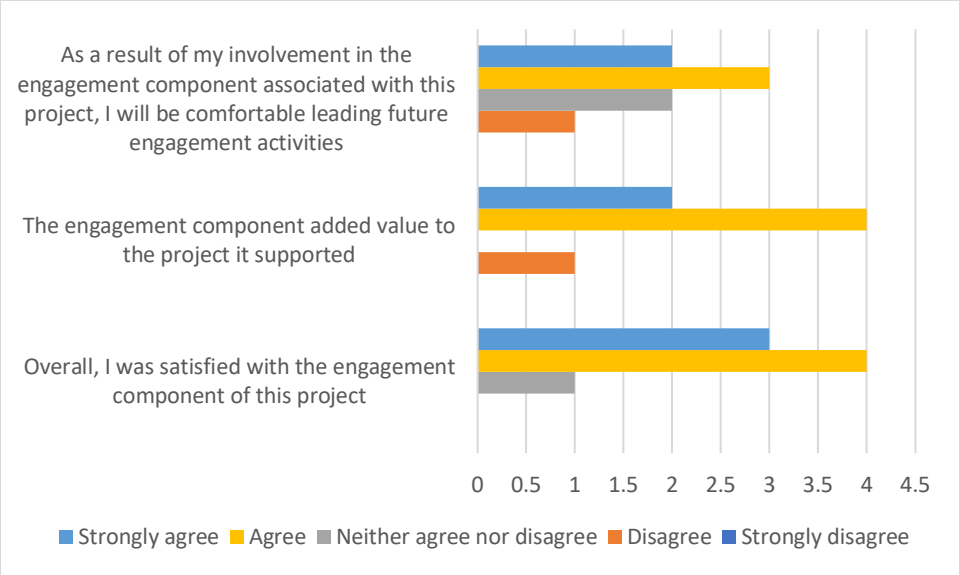

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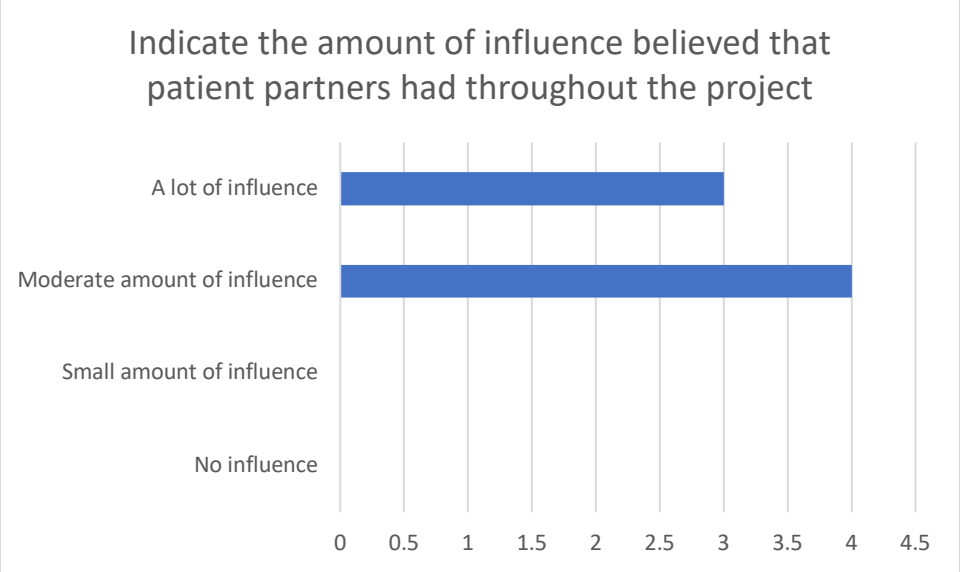

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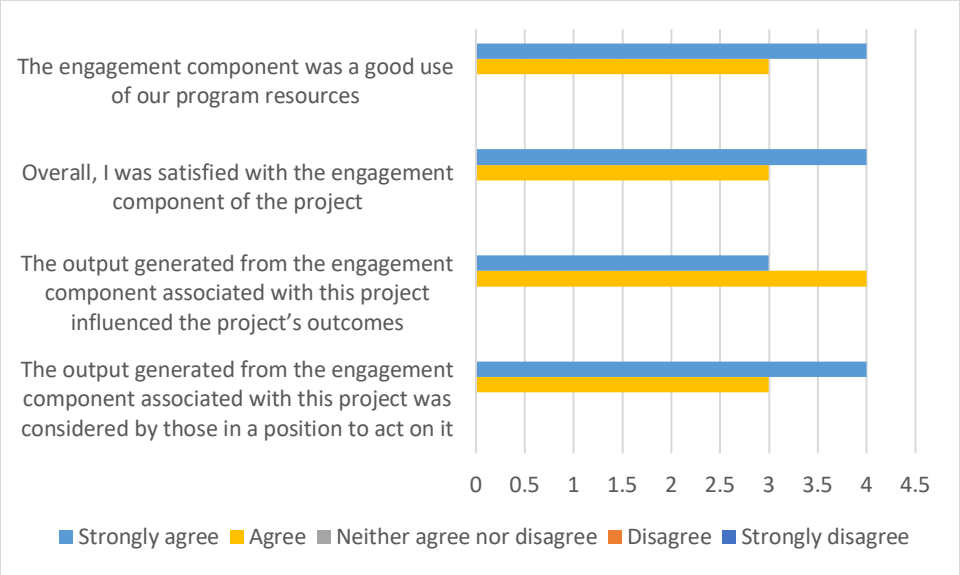

Chapter 5: Discussion

Introduction

This chapter provides an overview and synthesis of the findings from Chapters 2, 3, and 4. I also place my findings within the relevant broader scientific literature that has reported on these topics. This discussion outlines my contributions made to stroke research and preclinical patient engagement. Finally, I reflect on potential implications for practice, education, leadership, and future research.

Summary of Key Findings

I investigated and evaluated novel knowledge synthesis methods and approaches to better bridge the evidence gaps between preclinical studies and clinical trials in the setting of stroke. In Chapter 2, a preclinical stroke systematic review with network meta-analysis of stroke therapies was conducted. It identified the potential of several drug therapies that may provide additional therapeutic benefits when delivered in conjunction with alteplase, a current standard therapy. These findings offer avenues for future exploration by laying the groundwork for subsequent therapy options in acute stroke treatment. Chapter 3 pivots to an examination of CCR5 inhibitors through a preclinical systematic review and meta-analysis conducted with a panel of patient partners. This study revealed that CCR5 antagonist drugs demonstrated efficacy in preclinical stroke treatment. However, the study also highlighted challenges related to the quality and quantity of available studies, suggesting that further rigorous investigations may need to be considered before translating these therapies into clinical trials. In Chapter 4, I assessed patient engagement in the context of a preclinical systematic review. These findings demonstrated that patient partners guided the review toward more patient-oriented outcomes, thereby enhancing its relevance to the target patient population. Furthermore, the inclusion of patient partners and the engagement process was positively received by both patients and researchers, highlighting the value of such collaborations in advancing patient-centred research. Taken together, these chapters explore avenues to bridge the gap between preclinical and clinical research in stroke from improving preclinical studies, exploring different analysis, and incorporating different perspectives.

Integrated Findings with Broader Literature

The Potential of Network Meta-Analysis and Patient Engagement in Preclinical Research

The successful application of novel methodologies of a Network Meta-Analysis (NMA) and patient engagement in preclinical research, as demonstrated across the three articles in this thesis, attests to the potential of these approaches to provide fresh perspectives on preclinical data. NMA, recognized as one of the most comprehensive methods of data analysis, offers a robust tool for the investigation of potential stroke therapies. Particularly in chapter two, a comparative evaluation against a gold standard therapy was used, thereby offering a reference point often lacking in many systematic reviews. The omission of a reference standard in most systematic reviews might contribute to an overestimation of the effectiveness of novel therapies, thereby potentially skewing conclusions drawn from such studies.[1] Furthermore, the inclusion of patient partners in research uniquely grounds research processes in the lived experiences of those most directly impacted by the conditions being studied. This has several key impacts that enhance the relevance of these studies to real-world clinical outcomes. Firstly, patient partners offer crucial insights derived from their personal experiences with stroke. They can highlight aspects of the disease or its treatment that may not be immediately apparent to researchers but are directly impactful on patients' daily lives.[2] For instance, patient partners brought attention to the issues of focusing on acute care rather than chronic recovery. Also, the involvement of patient partners helps ensure that the outcomes studied are of direct relevance and importance to patients. While researchers might focus on traditional clinically-driven outcomes such as infarct volume or rates of specific clinical events, patients may place greater value on outcomes that directly affect their quality of life, such as fatigue, mobility, or cognitive function.[2] By integrating patient perspectives into the research process, these patient-relevant outcomes can be prioritized, ensuring that the study findings have real-world applicability and significance. The demonstrated success of these innovative methodologies in the included studies strongly suggests their potential for broader application in preclinical research. Given the complexities and challenges associated with bridging the translation of preclinical studies to clinical trials, the introduction and validation of such methods represent a step forward that may enhance the translatability of preclinical findings.[3]

Progress Towards Human Testing

While the investigations in Chapters 2 and 3 have demonstrated encouraging results for several therapies, including combination treatments and CCR5 inhibitors in preclinical stroke models, these findings should be viewed as preliminary steps toward human testing. Indeed, many of these potential therapies remain untested in humans, and some are only now entering the initial stages of clinical trials.[4] It is critical to acknowledge the inherent limitations and challenges that accompany the translation of these preclinical findings into successful human applications.[5] Factors such as interspecies differences, variations in disease progression and responses to treatment, and the complexity of human pathophysiology often pose significant hurdles to the direct translation of preclinical results to clinical settings.[6] Thus, while the preclinical results are promising and contribute to the growing body of knowledge in stroke research, they should be considered as a guide for future research and clinical trials rather than definitive evidence of success in human applications. This aligns with the broader literature emphasizing the essential role of rigorous preclinical testing in identifying the most promising therapies for further exploration in clinical trials.[7,8] Therefore, continued focus on rigorous preclinical research and knowledge synthesis is key to providing the necessary direction and groundwork for future clinical testing, potentially leading to more effective stroke treatments.

Need for Comprehensive Evaluation Beyond Efficacy in Preclinical Studies

The findings from both the NMA and the systematic review on CCR5 inhibitors highlight that efficacy in preclinical studies does not always translate to success in clinical trials. More than half of the therapies deemed effective in preclinical animal models from the NMA failed to replicate their success in human trials. This discrepancy calls attention to the need for a more comprehensive evaluation in preclinical studies that extend beyond efficacy. Aspects such as adherence to preclinical guidelines for increased translational potential, mitigation of potential risks and bias, and alignment with patient-relevant outcomes may significantly enhance the predictive value of preclinical studies for success in clinical trials.[3,9] However, the studies in Chapters 2 and 3 have also highlighted a challenge regarding the consistency in methodological reporting in preclinical studies. The observed heterogeneity in reporting

poses a barrier to the accurate comparison and synthesis of findings across different studies. A lack of standardization and transparency in reporting, as well as inconsistency in the outcomes measured within a study, undermines the reliability of preclinical stroke research.[3,10] This presents an opportunity for further improvement in preclinical research methods and reporting. One specific avenue is adhering to Stroke Therapy Academic Industry Roundtable (STAIR) XI recommendations. This provides a blueprint for enhancing the methodological rigour and consistency of preclinical studies, which can facilitate better alignment with clinical trials, as these guidelines were developed with a specific emphasis on improving the translatability of preclinical stroke research into successful human trials.[11] The broader literature echoes the need for enhanced methodological rigour and transparency in preclinical research.[3,10,12] It emphasizes the potential of rigorous methodological guidelines, comprehensive evaluation metrics beyond efficacy, and consistent reporting to significantly improve the predictiveness of preclinical studies for successful clinical translation. Addressing these issues may begin to narrow the gap between preclinical studies and clinical trials, and thereby facilitate successful translation of new stroke therapies.

Limitations

There are limitations inherent to the integrated approach taken in this thesis. Firstly, while the NMA presents a more robust comparison of treatments, it still relies on the quality and completeness of the underlying data from preclinical studies.[13] There is an assumption when using the NMA approach that the studies being compared are similar in terms of certain key characteristics, such as methods used in the study and the population of animals; however, this is not the case, given that preclinical studies vary considerably because there is no set standardization procedure. Given the notable inconsistency in reporting and the heterogeneity in preclinical research methodology, the analysis may be impacted by these variations.

Secondly, the approach of patient engagement, while successful, faced a unique challenge. In the realm of preclinical systematic reviews, there is limited established guidance for integrating patient partners, which led to an organic evolving approach in this thesis. While this flexibility helped ensure feasibility, it also

implied that some aspects of engagement might not have been optimally structured. Consequently, there could be potential missed opportunities for even more impactful patient contribution. Future work would benefit from a more structured approach to patient engagement in preclinical systematic reviews, building on the lessons learned from this pioneering effort.

While these limitations do not undermine the overall contributions of my thesis, they do highlight the complexities and challenges inherent to the translation of preclinical research to human trials and suggest areas for future investigation and improvement.

Implications Of Findings

Enhancing the Relevance and Translatability of Preclinical Stroke Research

To improve the translatability of preclinical stroke research, a multifaceted approach is essential. Firstly, there is a need to standardize methodologies and increase adherence to reporting guidelines to better ensure consistency and transparency in preclinical stroke studies. Secondly, prioritizing patient-relevant outcomes and involving patient partners can provide valuable insights into the disease's realities and bridge the gap between laboratory research and patient needs. Integrating these elements can facilitate the design of personalized therapeutic strategies and ultimately enhance patient outcomes. Initiatives should focus on implementing consistent reporting protocols and fostering collaboration between researchers, clinicians, and patient partners to better understand successful translation, the long-term effects of treatments, and rigorous reporting. By taking these actionable steps, the relevance and translatability of preclinical stroke research can be significantly improved, leading to better outcomes for patients with stroke.

Conclusion

With a multi-study design, I have shed light on the potential of various therapies, highlighted the need for more robust evaluations beyond efficacy, and demonstrated the value of patient engagement in shaping research toward patient-important outcomes. This work exposed critical areas for improvement in preclinical stroke research, specifically around the consistency in reporting and methodological

standardization. It advocates for an increased focus on patient-relevant outcomes and adherence to guidelines, thereby informing future research directions. These original contributions help bridge the translational gap in stroke research, with implications that extend to researchers and patients involved in stroke research and recovery.

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