PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Efficacy and Safety of Fingolimod in Stroke: A Systemic Review and Meta-analysis.	1
ABSTRACT			
Structured summary	2	*Background and Objective*: The incidence of stroke increases significantly during the coronavirus disease 2019 pandemic. The meta-analysis of animal experiments concluded that fingolimod could treat stroke in animal models via effectively reducing lymphocyte infiltration. Herein, we conducted a systemic review and meta-analysis of recent randomized controlled trials to confirm our hypothesis that fingolimod could promote reduction of infarction lesion or hematoma and improve neurological prognosis in patients with acute ischemic stroke or intracerebral hematoma via effectively and safely reducing lymphocyte infiltration. *Methods*: Studies were identified after literature search of PubMed, Embase and Cochrane. Data extraction of treatment effect included counts of $CD8^+$ T cells($\times 10^6$/ml), lesion volume(infarction or hematoma, ml), and modified Barthel indexes. The data extraction of safety was risk ratio(RR). The overall standard mean difference(SMD) with its 95% confidence interal（95%CI） and pooled effect with its 95%CI were calculated by fixed effects model. I-square (I^2) was used to test the heterogeneity. To check the stability of overall results, sensitivity analysis was conducted by recalculating the overall SMD or pooled effect of the remaining studies after omitting the study with the highest quality or the fixed effects model was switched to random effects model. Funnel plot symmetry and Egger's regression were used to evaluate publication	1

		bias. *Results*: Four randomized controlled trials with high quality were included. There was significant difference in $CD8^+$ T cell counts (I^2=0, overall SMD=-3.59, 95%CI=-4.37~-2.80, *p*=0.737) and modified Barthel index (I^2=0, overall SMD=2.42, 95%CI=1.63~3.21, *p*=0.290) between fingolimod and control groups. However, there was no significant difference in lesion volume (I^2=10.6%, overall SMD=-0.17, 95%CI=-0.75~0.42, *p*=0.917), and, RRs of fever (pooled RR=0.93, 95%CI=0.97-2.32, *p*=0.864), suspected lung infection (pooled RR=0.90, 95%CI=0.33-2.43, *p*=0.876) and all of events with at least one adverse event (pooled RR=0.82, 95%CI=0.36-1.87, *p*=0.995) between fingolimod and control groups. There was no publication bias. All of results were stable as revealed by sensitivity analysis. *Conclusion*: Fingolimod might improve neurological function in stroke patients via reducing lymphocyte infiltration in brain effectively, however, we did not find the evidence that it could promote infarction lesion or hematoma absorption. In general, it is safe for stroke patients to take fingolimod orally (0.5mg/d, 3 consecutive days) except for patients with rare severe adverse events.	
INTRODUCTION			
Rationale	3	Stroke, which mainly consists of acute ischemic stroke(AIS) with cerebral infarction and hemorrhagic stroke with intracerebral hematoma(ICH), is a common neurological disease with high mortality and disability rate. Especially, during the coronavirus disease 2019 pandemic, related coagulopathy might be associated with ischemic stroke, cerebral hemorrhage and cerebral venous thrombosis, which aggravated	2

		the financial burden in the world[22][17]. For severe stroke patients whose condition has deteriorated rapidly, surgical intervention should be performed, which is considered the best way to save lives[12]. Thus, to reduce the risk of disease deterioration and promote the recovery of nervous system function, it is important to perform timely intervention at the early stage of stroke. Fingolimod is a newly approved drug for the treatment of multiple sclerosis. It can be metabolized to the active metabolite of fingolimod-phosphate via sphingosine kinase. Fingolimod-phosphate has high affinity for sphingosine-1-phosphate receptor 1, 3, 4 and 5 [10]. Sphingosine-1-phosphate and its receptors can regulate lymphocyte migration via upstream cell signaling pathways. Thus, fingolimod-phosphate can block the ability of lymphocytes to enter or exit the lymph nodes, which can reduce the number of lymphocytes in peripheral blood[16]. Although fingolimod can be used for the treatment of multiple sclerosis with unknown mechanism, its role as immunomodulator might involve the reduction of the migration of lymphocytes to central nervous system[3]. Brain tissue injury in stroke patients involves inflammation around infarction lesion or hematoma, which is an important reason for disease deterioration and can result in poor prognosis[24]. The meta-analysis of animal experiments has concluded that fingolimod could treat animal models of stroke via effectively reducing lymphocyte infiltration[4]. However, the efficacy and safety of fingolimod in stroke patients has not been found in evidence-based medicine.	
Objectives	4	We hypothesize that fingolimod could effectively and safely promote reduction of infarction lesion or hematoma and improve neurological prognosis in AIS or ICH patients via reducing lymphocyte infiltration. In this study, we performed a systemic review and meta-analysis of recent randomized controlled trials(RCTs) to confirm this hypothesis.	2
METHODS			
Protocol and registration	5	This systemic review and meta-analysis was performed referring to the protocol published on the database	2

		of International Platform of Registered Systematic Review and Meta-analysis Protocols(INPLASY, https://inplasy.com/, registration number: INPLASY202140130, DOI number: 10.37766/inplasy2021.4.0130.)	
Eligibility criteria	6	The articles involving the effect and safety of stroke patients treated by fingolimod were screened. The inclusion criteria were: (1) Language and regions of articles were not restricted; (2) Date of publication was up to December 31, 2020; (3) RCTs; (4) Patients suffered from AIS or ICH; (5) Fingolimod was used as the clinical intervention; (6) Outcomes involved assessment of immune cells, lesion volume, neurological function and adverse events. The exclusion criteria were: (1) Duplicated publications; (2) Reviews, comments, letters, case reports, protocols of clinic trials and conference papers; (3) Animal experiments; (4) Articles with data irrelevant to this meta-analysis.	2
Information sources	7	Literature search was performed in three public electronic databases of PubMed, Embase and Cochrane.	2
Search	8	The strategy of literature search was as follows: ("Fingolimod Hydrochloride"[Mesh]) OR (((((2-Amino-2-(2-(4-octylphenyl)ethyl)-1,3-propanediol hydrochloride[Title/Abstract]) OR (FTY?720[Title/Abstract])) OR (Gilenya[Title/Abstract])) OR (Gilenia[Title/Abstract])) OR (Fingolimod[Title/Abstract])) AND (("Stroke"[Mesh]) OR ((((((((((((((((Stroke*[Title/Abstract]) OR (Cerebrovascular Accident*[Title/Abstract])) OR (CVA* [Title/Abstract])) OR (Cerebrovascular Apoplexy[Title/Abstract])) OR (Apoplexy, Cerebrovascular[Title/Abstract])) OR (Vascular Accident, Brain[Title/Abstract])) OR (Brain Vascular Accident*[Title/Abstract])) OR (Vascular Accidents, Brain[Title/Abstract])) OR (Cerebrovascular Stroke*[Title/Abstract])) OR (Stroke*, Cerebrovascular[Title/Abstract])) OR (Apoplexy[Title/Abstract])) OR (Cerebral Stroke*[Title/Abstract])) OR (Stroke*, Cerebral[Title/Abstract])) OR (Stroke*, Acute[Title/Abstract])) OR (Acute Stroke*[Title/Abstract])) OR (Cerebrovascular Accident*, Acute[Title/Abstract])) OR (Acute Cerebrovascular Accident*[Title/Abstract]))).	3

Study selection	9	The articles involving the effect and safety of stroke patients treated by fingolimod were screened. The inclusion criteria were: (1) Language and regions of articles were not restricted; (2) Date of publication was up to December 31, 2020; (3) RCTs; (4) Patients suffered from AIS or ICH; (5) Fingolimod was used as the clinical intervention; (6) Outcomes involved assessment of immune cells, lesion volume, neurological function and adverse events. The exclusion criteria were: (1) Duplicated publications; (2) Reviews, comments, letters, case reports, protocols of clinic trials and conference papers; (3) Animal experiments; (4) Articles with data irrelevant to this meta-analysis.	3
Data collection process	10	In consideration of the pharmacological action of fingolimod, absolute immunological cell counts ($\times 10^6$/ml) in blood might be the most intuitive index. Especially, T-lymphocytes with cluster of differentiation 8 expression ($CD8^+$ T cells) played key role in damaging the nervous system due to its cell killing effect, which could result in inflammatory edema[5]. The edema around infarction or hematoma could result in serious neurologic impairment in stroke patients[15][20]. Thus, rapid reduction of absolute infarction or hematoma volume might be the key of treatment in stroke patients. Lesion volume (ml) is defined as absolute infarction volume in AIS patients or absolute hematoma in ICH patients. National Institute of Health stroke scale (NIHSS) is the specialized assessment to measure recovery of neurological function[1]. Modified Barthel index (mBI) is one of the earliest and the most commonly used methods for assessing ADL (activity of daily living) in rehabilitation medicine, which has high validity and reliability in assessment of stroke[19]. Though risk ratio (RR) is often used as an effect quantity in cohort studies, it might be significant to assess the probability of adverse events like the number of patients need to be harmed in clinical trials[9].	3
Data items	11	In this study, the data extracted included $CD8^+$ T cell counts, lesion volume (infarction or hematoma), NIHSS, mBI and RR of safety.	3
Risk of bias in individual studies	12	The data at time points near baseline, which might reflect condition of stroke at early treatment, were extracted. In addition, some confounders, which might result in errors, were adjusted, including characteristics of participation, period of treatment and other factors. If the original data could not be acquired, other data were also extracted such as the difference between data at baseline and data at other time point.	3

Summary measures	13	Mean and standard deviation (SD) was used to represent continuous variables of normal distribution. Statistical difference of data before treatment in intervention and control groups was tested by one way Analysis of Variance (ANOVA) using SigmaStat version 4.0. If there was no statistical difference, data after treatment were directly used for meta-analysis. Relative numbers and their 95% confidence intervals (95%CI) were used to describe count data. Meta-analysis was performed using corresponding modules in Software for Statistics and Data Science (Stata, version 15.1; College Station, Texas 77845 USA). The overall standard mean difference (SMD) with its 95%CI and pooled effect with its 95%CI were calculated by fixed effects model. I-square (I^2) was used to test the heterogeneity. Sensitivity analysis was performed to evaluate the stability of overall results by recalculating the overall SMD or pooled effect of the remaining studies after omitting the study with the highest quality or the fixed effects model was switched to random effects model. Funnel plot symmetry and Egger's regression were used to evaluate publication bias. The method to reduce heterogeneity and enhance sensitivity were the deletion of studies with publication bias or studies with the lowest quality and subgroup analysis. All *p* values were two-sided with a significant level at 0.05.	3
Synthesis of results	14	Meta-analysis was performed using corresponding modules in Software for Statistics and Data Science (Stata, version 15.1; College Station, Texas 77845 USA). The overall standard mean difference (SMD) with its 95%CI and pooled effect with its 95%CI were calculated by fixed effects model. I-square (I^2) was used to test the heterogeneity. Sensitivity analysis was performed to evaluate the stability of overall results by recalculating the overall SMD or pooled effect of the remaining studies after omitting the study with the highest quality or the fixed effects model was switched to random effects model. Funnel plot symmetry and Egger's regression were used to evaluate publication bias. The method to reduce heterogeneity and enhance sensitivity were the deletion of studies with publication bias or studies with the lowest quality and subgroup analysis.	4

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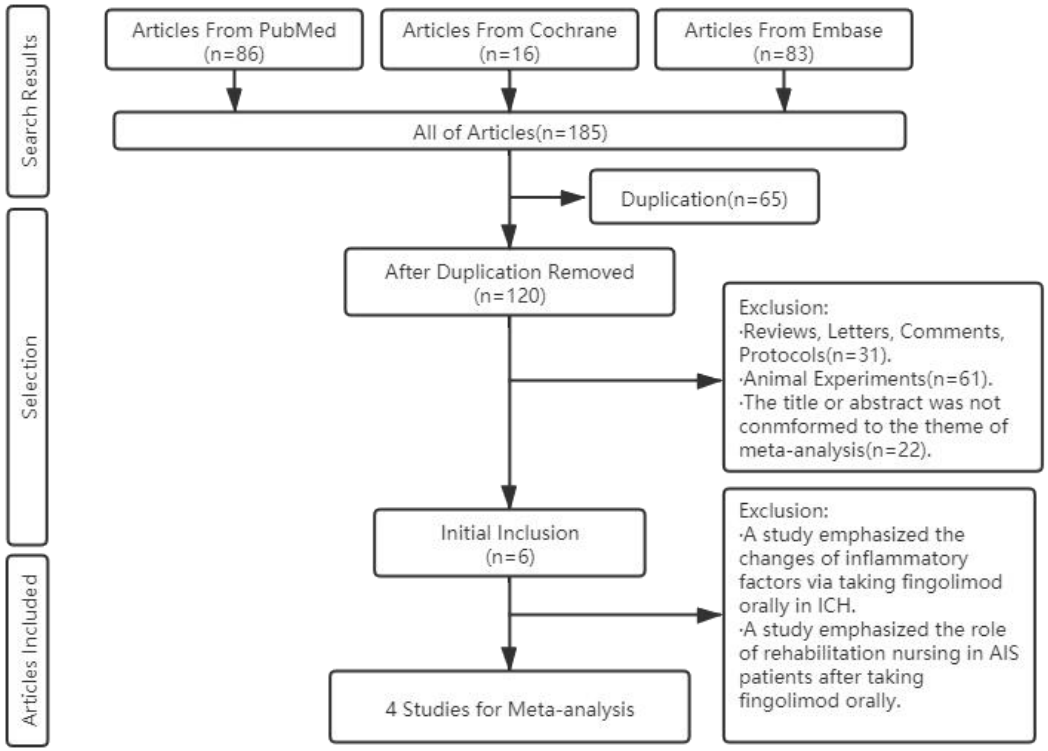
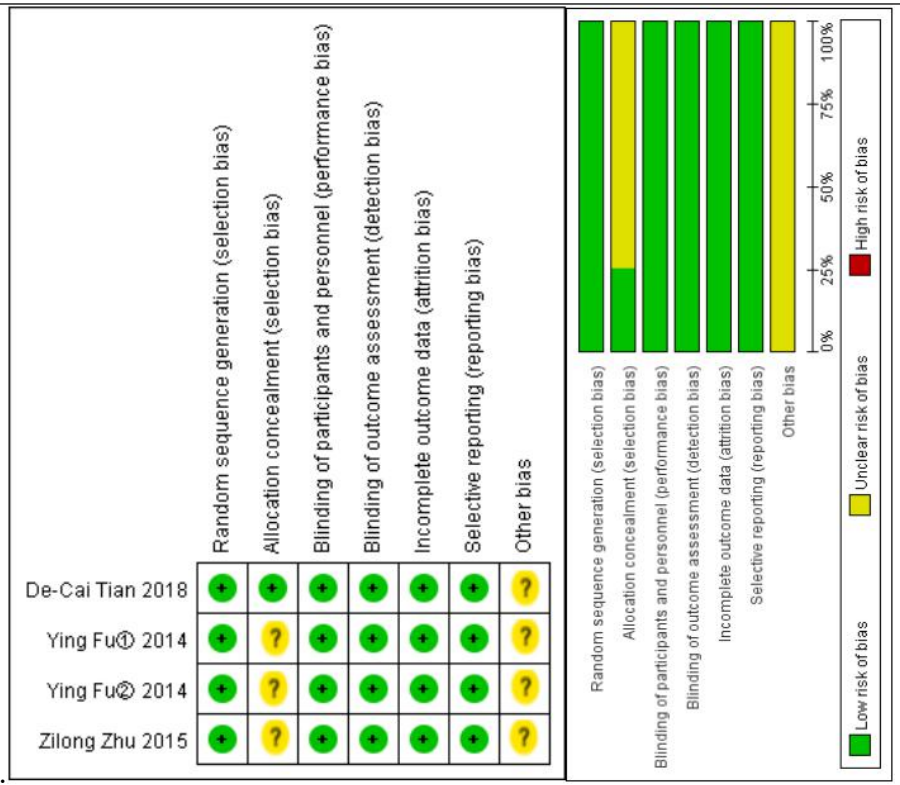
Risk of bias across studies	15	The quality assessment of included articles was performed via the Cochrane Collaboration's Tool of Assessing Risk of Bias by the software Review Manager 5.3 before data extraction.	3
Additional analyses	16	Funnel plot symmetry and Egger's regression were used to evaluate publication bias.	4
RESULTS			
Study selection	17	Totally, 185 articles were retrieved from 3 databases according to the strategy. After screening according to the inclusion and exclusion criteria, four articles[7][6][26][21] of RCTs were enrolled ultimately (**Figure 1**). One study emphasized the changes of inflammatory factors after taking fingolimod orally in patients with ICH[13]. One study emphasized the role of rehabilitation	4

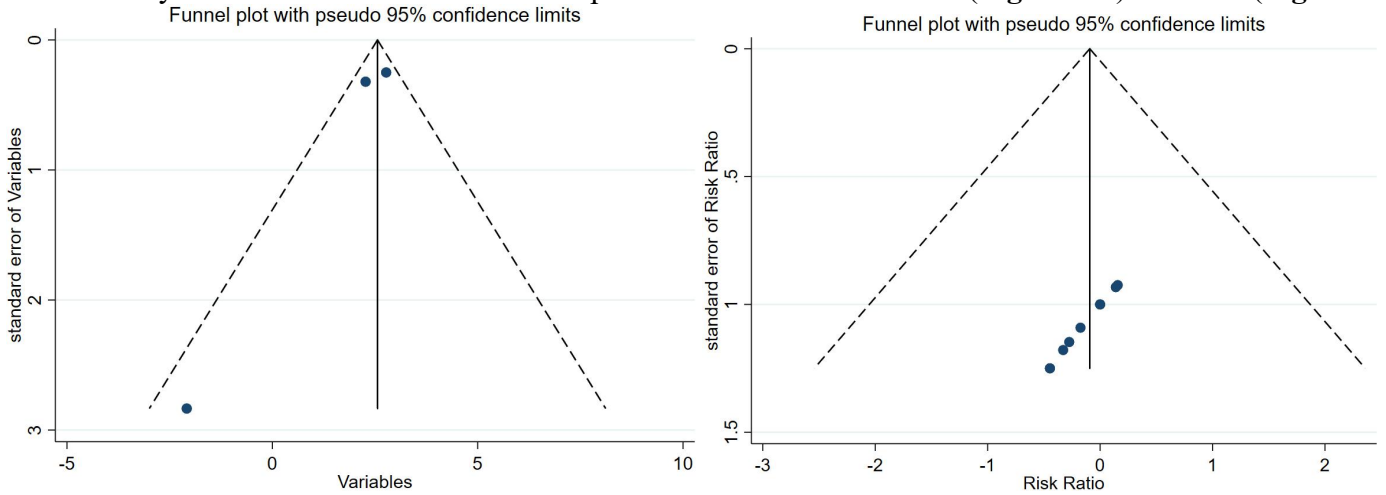
		nursing in AIS patients after taking fingolimod orally[14].  	
Study characteristics	18	Articles with final decision were published in 2014 to 2018 (**Table 1**). There were 67 stroke patients, including 56 AIS patients and 11 ICH patients, in experiment group, whose age range were 49 to 74 years. The period of oral fingolimod were 3 consecutive days after hospitalization. The oral dose was 0.5mg/d. Conclusion with the same tendency was fingolimod could effectively and safely treat AIS or ICH.	4
Risk of bias within studies	19	The assessment of article quality via Cochrane Collaboration's Tool of Assessing Risk of Bias was shown in **Figure 2**. Three studies (Ying Fu①, et al. 2014, Ying Fu②, et al. 2014, Zilong Zhu, et al. 2015) were evaluated as unclear risk bias in allocation concealment except for low risk in one study (De-cai Tian, et al 2018). There was no bias in performance, detection, attrition and	4

		reporting. All studies had unclear risk biases in other bias. 	
Results of individual studies	20		6

The First Author	Publication	Fingolimod Group	Types of Stroke	Age (mean±SD)	Sex (Male%)	Dose of Fingolimod	Conclusion
Ying Fu①	2014	11	AIS	62.3±8.0	73	0.5mg/d orally, 3 consecutive days after hospitalization	It could safely limit secondary tissue injury, decrease microvascular permeability, attenuate neurological deficits and promote recovery.
g Fu②	2014	11	ICH	60.7±12.3	36	0.5mg/d orally, 3 consecutive days after hospitalization	It could safely reduce PHE, attenuate neurologic deficits and promote recovery.
Zilong Zhu	2015	22	AIS	60.0±2.5	59	0.5mg/d orally, 3 consecutive days after hospitalization	In this pilot study, combination therapy of fingolimod and alteplase was well tolerated, which attenuated reperfusion injury and improved clinical outcomes in AIS patients.
De-Cai Tian	2018	23	AIS	67±6.7	39	0.5mg/d orally, 3 consecutive days after hospitalization	Fingolimod may enhance the efficacy of alteplase administration in the 4.5- to 6-hour time window in patients with a proximal cerebral arterial occlusion and salvageable penumbral tissue by promoting both anterograde reperfusion and retrograde collateral flow.

"①②" were used to distinguish two different articles with the same first author's name. "①" represented the article: Impact of An Immune Modulator Fingolimod on Acute Ischemic Stroke. "②" represented the article: Fingolimod for the Treatment of Intracerebral Hemorrhage A 2-Arm Proof-of-Concept Study.

Synthesis of results	21	Analysis with fixed effects model showed that there was no heterogeneity (I^2=0, **Figure 3**) in the 2 studies used for overall SMD difference in $CD8^+$ T cell counts and lesion volume except for 2 articles used for overall SMD difference in mBI scores(I^2=10.6%, **Figure 3**). There was significant difference in $CD8^+$ T cell counts (overall SMD=-3.59, 95%CI=-4.37~-2.80, *p*=0.737, **Figure 3**) and mBI (overall SMD=2.42, 95%CI=1.63~3.21, *p*=0.290, **Figure 3**) between fingolimod and control groups. However, there was no significant difference in lesion volume between fingolimod and control groups (overall SMD=-0.17, 95%CI=-0.75~0.42, *p*=0.917, **Figure 3**). Pooled RRs and their 95%CIs were analyzed with fixed effects model. The RRs of fever (I^2=0, pooled RR=0.93, 95%CI=0.97-2.32, *p*=0.864, **Figure 4**), suspected lung infection (I^2=0, pooled RR=0.90, 95%CI=0.33-2.43, *p*=0.876,	7

		Figure 4) and all of events with at least one adverse event (I^2=0, pooled RR=0.82, 95%CI=0.36-1.87, *p*=0.995, **Figure 4**) showed no significant difference between fingolimod and control groups. Meta-analysis was not performed in RRs of other serious events because there was only one study reported hemorrhage of digestive tract or cerebral hernia and RRs of bradycardia, atrial flutter and thrombocytopenia could not be calculated.	
Risk of bias across studies	22	There was symmetrical distribution in funnel plots of continuous variables (**Figure4 A**) and RRs (**Figure 4 B**). 	9
Additional analysis	23	Because only 2 studies were used to perform meta-analysis of treatment effect, we recalculated overall SMD of $CD8^+$ T cell counts (I^2=0, overall SMD=-3.59, 95%CI=-4.37~-2.80, *p*=0.737, **Table 4**), lesion volume (I^2=0, overall SMD=-0.17, 95%CI=-0.75~0.42, *p*=0.917, **Table 4**) and mBI scores (I^2=10.6%, overall SMD=2.43, 95%CI=1.59~3.26, *p*=0.290, **Table 4**) after fixed effects model was switched to random effects model. Pooled RRs and their 95%CIs of fever (I^2=0, pooled RR=0.93, 95%CI=0.37-2.32, *p*=0.723, **Table 4**), suspected lung infection(I^2=0, pooled RR=0.90, 95%CI=0.33-2.43, *p*=0.723, **Table 4**) and all of events with at least one adverse event (I^2=0, pooled RR=0.82, 95%CI=0.36-1.87, *p*=0.969, **Table 4**) were analyzed with the same method above. In addition, for sensitivity analysis, the pooled effect of the remaining studies were recalculated after omitting the study with the highest quality because more than 2 studies were used to perform meta-analysis of treatment safety. We considered that the study might be assessed to have higher quality for its larger number of included patients in studies with the same assessment in Cochrane Collaboration's Tool of Assessing Risk of Bias. Finally, after omitting the study with the highest quality, we recalculated pool RRs and their 95%CIs of fever (Lilong Zhu, et al omitted, I^2=0, pooled RR=0.78, 95%CI=0.23-2.68, *p*=0.723, **Table 4**), suspected lung infection (Lilong Zhu, et al omitted, I^2=0, pooled RR=0.78, 95%CI=0.23-2.68, *p*=0.723, **Table 4**) and all of events with at least one adverse event (I^2=0, pooled RR=0.84, 95%CI=0.33-2.18, *p*=0.969, **Table 4**).	9

DISCUSSION			
Summary of evidence	24	There were many clinic application of Fingolimod appeared due to its original medicine indication of reducing the migration of lymphocytes to central nervous system, such as Alzheimer's disease, Susac syndrome[2][8]. Although treatments mechanisms of AIS or ICH via Fingolimod might be different, absorption of infarction lesion in AIS or hematoma in ICH both involved cytophagy via microglias in brain tissue or immunocyte in peripheral blood across injured brain-blood barrier [18][25]. Edema around lesion has probable results of microcirculation obstruction, injured brain-blood barrier and inflammatory response caused by lymphocyte infiltration in stroke patients, which may strongly affect recovery of neurological function and prognosis due to its space occupying and toxic effect. Thus, fingolimod might reduce secondary damage after stroke via its pharmacological action. In our meta-analysis of RCTs, although we did not find that fingolimod could accelerate reduction of lesion volume (I^2=0, overall SMD=-0.17, 95%CI=-0.75~0.42, *p*=0.917, **Figure 3**) via reducing lymphocyte counts in peripheral blood (I^2=0, overall SMD=-3.59, 95%CI=-4.37~-2.80, *p*=0.737, **Figure 3**), there was effective improvement in ADL in stroke patients (I^2=10.6%, overall SMD=2.42, 95%CI=1.63~3.21, *p*=0.290, **Figure 3**), which might be the evidence that fingolimod might cause reduction of secondary damage via reducing lymphocyte infiltration in brain effectively. With regard to safety of oral fingolimod in stroke patients, infectious diseases might be the main adverse events. Especially, patients with hemiplegic paralysis may be aggravated to severe hypostatic pneumonia. In our meta-analysis, fever (I^2=0, pooled RR=0.93, 95%CI=0.97-2.32, *p*=0.864, **Figure 4**) and suspected lung infection (I^2=0, pooled RR=0.90, 95%CI=0.33-2.43, *p*=0.876, **Figure 4**) were both considered as infectious diseases. We did not find that fingolimod might increase risk of infection. In other adverse events, there were some rare severe adverse events in fingolimod groups. Risk of cerebral hernia was not increased after the medication (RR=0.35, 95%CI=0.06~1.90, **Table 3**). The reason might be the large lesion volume at baseline. In the clinical trial by Tian et al[21], fingolimod was applied based on delayed alteplase administration. Hemorrhage of digestive tract must be the potential risk during thrombolytic therapy. However, we found that the risk of digestive tract hemorrhage was not increased in fingolimod group (RR=0.42, 95%CI=0.04~4.30, **Table 3**). Sphingosine-1-phosphate can regulate calcium ion movement in cells, which can affect myocardial contractility[11]. Therefore, sphingosine-1-phosphate receptors can be bound to fingolimod-phosphate, which might result in arhythmia, such as bradycardia, atrial flutter. Likewise, sphingosine-1-phosphate can be produced from platelets and participate in platelet activation, which might result in thrombocytopenia in fingolimod group[23].	11

		Therefore, the application of fingolimod should be cautious in patients with history of cardiac disease and blood platelet disorder. In general, according to pooled RR of at least one adverse event (I^2=0, pooled RR=0.82, 95%CI=0.36-1.87, *p*=0.995, **Figure 4**), fingolimod might be safe for stroke patients.	
Limitations	25	Only four RCTs were included in the meta-analysis finally. Furthermore, two of them were completed by the team led by the same professor. The studies included was insufficient to perform a meta-analysis with high quality due to insufficient number of patients. Although publication bias was not found, the outcome might be influenced by tendentiousness of articles with the same first authors. As some results were only shown in figures in some articles, these results were not extracted, which may lead to missing data at other time points and some variables, such as counts of other types of lymphocytes, edema around lesion, and NIHSS scores. In addition, there was potential influence of non-original data because data of continuous variables used in meta-analysis were processed via mathematical transformation owing to statistical difference before meta-analysis. Although a few stroke patients used it as a therapeutic medication, we believed that our research would bring about a new direction of stroke medication.	11
Conclusions	26	Fingolimod might improve neurological function in stroke patients via reducing lymphocyte infiltration in brain effectively, however, we did not find the evidence that it could promote infarction lesion or hematoma absorption. In general, there was safety of oral fingolimod (0.5mg/d, 3 consecutive days) in stroke patients except for some rare severe adverse events.	12
FUNDING			
Funding	27	This work was founded by the National Key R&D Program of China (No. 2018YFC1312600 and 2018YFC1312601) and the Project of Science and Technology Department of Qinghai Province (No. 2020-ZJ-774).	13

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