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Systematic Review

# Immunotherapy of experimental and human stroke with agents approved for multiple sclerosis: a systematic review

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***Abstract***

**Background:** 'Thromboinflammation' describes a novel concept in stroke pathophysiology that has opened up the possibility of immunotherapeutic approaches which could become promising strategies for targeted stroke therapies in the future.

**Methods:** We reviewed current evidence for agents approved for multiple sclerosis in preclinical and clinical stroke studies. A systematic review was performed in accordance with the PRISMA statement, searching MEDLINE, the Cochrane Central Register of Controlled Trials, and reference lists of articles published until 16 October 2017.

**Results:** The review included 52 of 629 identified studies, consisting of 5 clinical and 47 preclinical trials. Most of the studies showed beneficial effects of the evaluated immunotherapeutic drugs in terms of reduction in morphological lesion size and improvement in functional outcome. Nevertheless, the significance of these findings is limited due to the high degree of heterogeneity.

**Conclusions:** Immunotherapy of stroke might be effective and could become a promising treatment strategy, but larger clinical trials with standardized interventions and outcome measures are needed.

***Keywords:*** immunotherapy, inflammation, intracranial haemorrhage, ischaemic stroke, multiple sclerosis, systematic review, thromboinflammation

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## Introduction

The concept of 'thromboinflammation' describes the pathophysiological link between thrombus formation, that is, mechanisms related to platelet aggregation and plasmatic coagulation, and inflammation in the development of ischaemic stroke (IS).[1]

With the help of murine stroke models and transgenic mouse strains, it has been demonstrated by independent groups that immunological processes involving the innate or adaptive immune system modulate IS pathophysiology. Histopathological findings demonstrating immune cell infiltration in the ischaemic brain were reported as early as the 1990s, but the pathophysiological relevance remained unclear.[2] Yilmaz and coworkers were the first to show that *Rag1*$^{-/-}$ mice, that is, mice lacking lymphocytes, develop smaller cerebral infarctions compared with wild-type animals.[3] This observation paved the way for rodent stroke studies analysing immunomodulating therapies with agents that have already been approved for the treatment of patients with multiple sclerosis. As the majority of rodent studies had positive outcomes, in the next step, the first clinical trials in stroke patients were started.

The aim of our study was to systematically assess the published preclinical and clinical studies that

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analysed an immunotherapeutic compound approved for multiple sclerosis in stroke.

## Methods

We conducted a systematic review and followed the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines.[4]

Prior to systematically reviewing the literature, the following eligibility criteria were defined: (a) clinical studies: randomized controlled trials (RCTs) or prospective studies with or without a control group if they analysed a compound approved for multiple sclerosis in patients aged 18 years or older with stroke [cerebral ischaemia or spontaneous intracranial haemorrhage (ICH)] or transient ischaemic attack; (b) animal studies that analysed a compound approved for multiple sclerosis in an experimental stroke model. Publications about spinal cord ischaemia and subarachnoid haemorrhages have been excluded due to particular pathophysiological mechanisms.

The main outcome measures that have been considered were mainly stroke volume and functional deficits. In part, additional outcomes, such as brain oedema, local brain inflammation or systemic cytokine levels, have been assessed. Due to space restrictions, it has been necessary to limit Tables 1–6 to principal content. Because of the pronounced heterogeneity of the study design, stroke model, intervention and outcome variables, it was not possible to calculate a meaningful meta-analysis for any of the outcome variables.

## Literature search and data extraction

A literature search was conducted on 16 October 2017 including MEDLINE (*via* PubMed) and the Cochrane Central Register of Controlled Trials. In addition, the reference lists of the included studies were reviewed to identify further studies. We continued the literature search until no further publications were identified. Four reviewers (ZM, MD, VP and PK) independently screened each title and abstract. Studies published until 16 October 12017 were considered. In the case of disagreement regarding study eligibility, a consensus meeting was arranged.

The databases were searched combining extensive search strings with the following Boolean operators: (mitoxantrone OR azathioprine OR 'glatiramer acetate' OR glatiramer OR 'interferon beta' OR 'Peginterferon beta-1a' OR 'pegylated interferon' OR FTY720 OR fingolimod OR natalizumab OR 'α4 integrin' OR daclizumab OR ocrelizumab OR cladribine OR teriflunomide OR 'dimethyl fumarate' OR fumarate OR alemtuzumab) AND (stroke OR 'ischemic stroke' OR 'hemorrhagic stroke' OR 'experimental stroke' OR 'cerebral ischemia' OR ICH OR 'brain hemorrhage' OR 'cerebral hemorrhage' OR 'cerebral infarction' OR 'ischemia-reperfusion'). The compounds [mitoxantrone, azathioprine, glatiramer acetate, interferon β (IFN-β), fingolimod, natalizumab, daclizumab, ocrelizumab, alemtuzumab, cladribine, teriflunomide, dimethyl fumarate (DMF)] were chosen based on their approval for treatment of patients with multiple sclerosis in the European Union. FTY720 is used as a synonym for fingolimod. Mouse CD49d-specific antibodies and selective anti-α4-antibodies equate to natalizumab in humans. Monomethyl fumarate, as the main metabolite of DMF, has been evaluated in preclinical stroke studies,[5] but is not approved for multiple sclerosis and, therefore, not part of this review. At the time of the literature search, ocrelizumab has not been approved in the European Union (EU). As approval was expected in the EU soon, we decided to include ocrelizumab in the literature search.

Extracted data included species, stroke model, intervention and major outcome in the rodent studies (Tables 1–5), as well as study design, population, stroke type, intervention, major end points and major results in the clinical trials (Table 6).[6–57]

## Results

The database literature search identified 624 papers. Five additional publications were found after screening of the reference lists. Of these 629 publications, 552 papers were excluded after abstract review with regard to inappropriate content. The 77 remaining articles were reviewed on a full-text basis. Further, 25 of them were excluded due to the study design or other violation of inclusion criteria. Finally, 52 studies met our eligibility criteria and were included in the review (Figure 1, Tables 1–6).

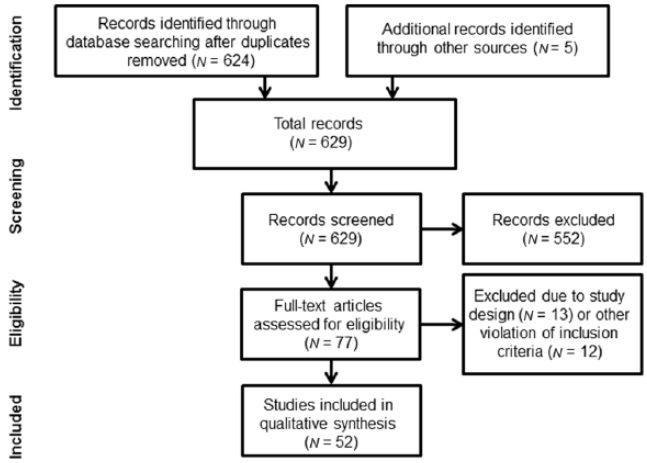


**Figure 1.** Preferred reporting items for systematic reviews and meta-analyses (PRISMA) flow diagram.

### *Description of included studies*

Of the 52 included studies, 47 were animal studies (Tables 1–5)[6–52] and 5 were clinical trials (Table 6).[53–57] Study characteristics and interventions are summarized in Tables 1–6.

### *Description of preclinical studies analysing glatiramer acetate*

We identified four studies that met our inclusion criteria.[6–9] In all of them, a transient middle cerebral artery occlusion (MCAO) was performed and in one, an additional permanent MCAO[7] was performed. Two studies used mice,[7,8] the other, two rats.[6,9] Glatiramer acetate application (dose, route, time point), read-out times, as well as outcomes differed between the studies. Two of the studies showed stroke volume reduction;[6,9] the other two did not.[7,8] A more detailed synopsis can be found in Table 1.

### *Description of preclinical studies analysing interferon β*

Six studies met our inclusion criteria.[10–15] Different stroke models have been used including transient MCAO,[11–14] permanent MCAO,[13] photothrombotic stroke[15] and a clot embolus model.[10] Two studies used mice,[14,15] three used rats,[11–13] and one used rabbits.[10] IFN-β (dose, route, time point), read-out times, as well as outcomes differed between the studies, with four studies showing a reduction in stroke volume[10–12,14] and one not.[13] Cruz and colleagues provide evidence that the anti-inflammatory and stroke-protective effect of IFN-β is lost in mice lacking interferon regulatory factor 2 binding protein 2 (IRF2BP2).[15] A more detailed synopsis can be found in Table 2.

### *Description of preclinical studies analysing fingolimod*

We identified 23 studies that met our inclusion criteria.[16–38] Sixteen studies analysed ischaemic stroke using transient[16–23,26,28,30,32–34] or permanent MCAO,[22,23] a thromboembolic stroke model[27] or photothrombotic stroke.[29] Seven studies investigated ICH.[24,25,31,35–38] A broad spectrum of mice, as well as Sprague–Dawley rats have been used throughout the studies. Fingolimod (FTY720) treatment varied between the studies regarding dose (0.24–3 mg/kg),[17,23,32] application route and time. The majority of studies evaluating IS described FTY720-related reduction in stroke volumes.[16–21,23,26,27,30,32,33] A more detailed synopsis can be found in Table 3.

### *Description of preclinical studies analysing natalizumab*

We identified eight studies that used different MCAO models[39–43,45,46] or an ICH model[44] and analysed different rat strains[39–41] or mice.[42–46] Five of the IS studies described a reduction in stroke volume associated with antibody-mediated α4 integrin blockade,[39–41,42,46] Langhauser and colleagues did not.[43] Llovera and colleagues found the type of MCAO model used (transient *versus* permanent) to be crucial for stroke volume reduction.[45] Hammond and coworkers evaluated α4 integrin blockade in an ICH model and presented evidence of improvement in

**Table 1.** Characteristics of preclinical studies analysing glatiramer acetate.

| Author | Species | Stroke model | Intervention | Main outcome |
|---|---|---|---|---|
| Ibarra[6] | Sprague–Dawley rats (age: n.a.; sex: male) | Transient MCAO (120 min) | Injection of 200 µg Cop-I and CFA 30 min after reperfusion *versus* saline and CFA only | Reduction in stroke volume and functional deficit on day 7 ($p < 0.05$) but not on day 1 ($p > 0.05$) |
| Poittevin[7] | C57BL/6 mice (age: 10–12 weeks; sex: male) | Transient (45 min) and permanent MCAO | Glatiramer acetate 2 mg/200 µl NaCl 0.9% s.c. *versus* vehicle | Permanent MCAO at day 3, no difference in infarct volume or brain oedema ($p > 0.05$); transient MCAO no change at day 3 ($p > 0.05$), no difference in functional outcome at day 7 ($p > 0.05$) |
| Kraft[8] | C57BL/6 mice (age: 6–8 weeks; sex: male) | Transient MCAO (60 min) | Glatiramer acetate 3.5 mg/kg i.v. 30 min before ischaemia *versus* vehicle | No difference in infarct volume ($p > 0.05$) and neurological outcome ($p > 0.05$) |
| Cruz[9] | Sprague–Dawley rats (age: 9 weeks; sex: male) | Transient MCAO (90 min) | Glatiramer acetate 200 µg in saline emulsified in CFA containing 5 mg/ml of *Mycobacterium tuberculosis* H37Ra in a total volume of 150 µl *versus* saline and CFA only | Improvement in neurological recovery after MCAO not at day 1 ($p > 0.05$), but at day 7 ($p < 0.05$) and between days 14 and 60 ($p < 0.01$); reduced stroke volume ($p < 0.05$) 60 days after stroke |

CFA, complete Freund's adjuvant; COP-I, copolymer I; i.v., intravenously; MCAO, middle cerebral artery occlusion; n.a., not applicable; NaCl, sodium chloride; s.c., subcutaneously.

functional outcome.[44] A more detailed synopsis can be found in Table 4.

### *Description of preclinical studies analysing dimethyl fumarate*

We detected six studies that performed an experimental ICH by injection of collagenase[47] or autologous blood,[47,48] or used transient MCAO as a model of IS.[49–52] The intervention (dose, timing and route of administration of DMF) differed between the studies. All studies were positive in at least one outcome variable, including function,[47,48,50–52] stroke volume[50,51] and brain oedema.[49] A more detailed synopsis can be found in Table 5.

### *Description of clinical studies*

In total, five clinical trials have been identified that met our inclusion criteria.[53–57] Of these, only the study of Elkins and colleagues is a double-blinded RCT;[53] the others are single-blinded studies.[54–57] Elkins and coworkers evaluated natalizumab 300 mg intravenously in patients with acute and first IS ($n = 161$). Despite promising data in most of the preclinical studies (see above), the primary end point remained negative.[53] In contrast, all of the studies that analysed fingolimod in IS[55,57] or ICH[54,56] ($n = 23$–$47$) reached their end points, including functional outcome[54,55,57] and reduced infarct volume increase.[55,57] A more detailed synopsis can be found in Table 6.

## Discussion

In this systematic review, we found that immunotherapy in preclinical IS and ICH improved clinical and paraclinical outcome variables in most of the studies. As a limitation, the preclinical trials are very heterogeneous in design and used different stroke models, different occlusion times of the MCAO model, different doses of the immunotherapeutic drug, distinct time points of treatment and different application routes. Therefore, the comparability of the studies is very low and calculation of a meta-analysis regarding major outcome variables is not possible. The heterogeneity of the studies can also contribute to discrepant results in preclinical trials, which can be paradigmatically seen in studies regarding the

**Table 2.** Characteristics of preclinical studies analysing interferon β.

| Author | Species | Stroke model | Intervention | Main outcome |
|---|---|---|---|---|
| Liu[10] | New Zealand white rabbits (age: n.a.; sex: n.a.) | Clot embolus surgically injected into MCA | IFN-β pretreatment with $10^7$ U s.c. 4 h before clot placement and $0.5 \times 10^7$ U within 30 min after; IFN-β post-treatment with $10^7$ U s.c. immediately after clot placement and $0.5 \times 10^7$ U 4 h later; control group: no IFN-β application | Reduced infarct volume (pretreatment $p = 0.003$; post-treatment $p = 0.004$) |
| Veldhuis[11] | Fischer rats (age: n.a.; sex: male) | Transient MCAO (60 min) | Recombinant rat IFN-β 500,000 U s.c. 2 days prior to surgery, or at reperfusion, or 4 h after stroke onset, or 6 h after stroke onset *versus* control (saline) | Infarct volume smaller for IFN-β on day 1 ($p < 0.01$) *versus* control; on day 1, greater improvement in pretreated group compared with groups treated after stroke ($p < 0.05$); from day 7 onwards, no difference between the IFN-β groups ($p > 0.05$) |
| Veldhuis[12] | Fischer rats (age: 8–12 weeks; sex: male) | Transient MCAO by using a microclip on the MCA through a small cranial burr hole, reperfusion after 60 min | IFN-β 500,000 U (8 µg) s.c. once daily until 7 days after reperfusion *versus* vehicle; treatment began 2 days before MCAO, on reperfusion, 4 h after stroke onset or 6 h after stroke onset | Reduction in lesion volume in all IFN-β treatment strategies on days 1, 7 and 21 ($p < 0.05$ to $p < 0.001$) |
| Maier[13] | Sprague–Dawley rats (age: n.a.; sex: male) | Transient MCAO (60 min) or permanent MCAO | Rat IFN-β 8 or 16 µg i.v. once daily for 3 or 7 days, or PEG-IFN-β i.v. or s.c. for 1 day | IFN-β and PEG-IFN-β failed to mitigate stroke volume and functional deficits on day 7 ($p > 0.05$) |
| Kuo[14] | C57BL/6 and *Ifnar1*$^{tm1Agt}$/Mmjax (*Ifnar1*$^{-/-}$ mice) (age: 8–12 weeks; sex: male) | Transient MCAO (40 min) | Recombinant murine IFN-β 10,000 U i.v. 3 h before MCAO induction or 3 h after reperfusion | Pre- and post-treatment with IFN-β reduced infarct volume ($p = 0.001$) and functional deficit ($p < 0.05$) in C57/BL6 mice; no change in infarct volume in *Ifnar1*$^{-/-}$ mice |
| Cruz[15] | C57BL/6 mice *versus* C57BL/6 mice with LysMCre/IRF2BP2flox (ablation of IRF2BP2) (age: 2 months; sex: male) | Photothrombotic stroke | Mouse recombinant IFN-β 10,000 U in 100 µl saline, 30 min after photothrombosis for all animals | Similar infarct volume in wild-type and transgenic mice 1 day after stroke; lesion volumes reduced in control *versus* knock-out mice on day 4 ($p < 0.05$); worse functional outcome in transgenic mice *versus* control animals ($p < 0.05$) |

IFN-β, interferon β; IRF2BP2, interferon regulatory factor 2 binding protein 2; i.v., intravenously; MCA, middle cerebral artery; MCAO, MCA occlusion; n.a., not applicable; PEG, pegylated; s.c., subcutaneously.

**Table 3.** Characteristics of preclinical studies analysing fingolimod.

| Author | Species | Stroke model | Intervention | Main outcome |
|---|---|---|---|---|
| Czech[16] | C57BL/6J mice (age: 10 weeks; sex: male) | Transient MCAO (90 min) | FTY720 1 mg/kg i.p. after initiation of anaesthesia | Reduction in stroke volume ($p < 0.05$) and functional deficits ($p < 0.01$) |
| Wacker[17] | Swiss-Webster ND4 mice (age: n.a.; sex: male) | Transient MCAO (60 min) | FTY720 0.24 mg/kg or 1 mg/kg i.p. 30 min before hypoxic preconditioning; for mice not subjected to HPC, FTY720 treatment 48 h before MCAO | Reduction in infarct volume and functional deficits with 1 mg/kg FTY720 ($p < 0.05$), not 0.24 mg/kg ($p > 0.05$); even stronger protection from ischaemic stroke in combination with HPC ($p < 0.05$) |
| Shichita[18] | C57BL/6 and other mouse strains (age: 9–17 weeks; sex: male) | Transient MCAO (60 min) | FTY720 1 mg/kg 5 min before reperfusion and once daily for 3 days *versus* $H_2O$ | Reduction in infarct volume ($p < 0.01$) |
| Hasegawa[19] | Sprague–Dawley rats (age: n.a.; sex: male) | Transient MCAO (120 min) | FTY720 0.25 mg/kg or 1 mg/kg i.p. immediately after reperfusion *versus* vehicle; in other groups SEW2871 (selective S1P1 agonist) and VPC23019 (S1P1, S1P3 and S1P4 antagonist) | Reduction in infarct volume and functional deficits on days 1 and 3 ($p < 0.05$) |
| Pfeilschifter[20] | C57BL/6J mice (age: 10 weeks; sex: male) | Transient MCAO (90 min, 180 min) | FTY720 1 mg/kg i.p. 2 h after vessel occlusion *versus* vehicle | Smaller lesion size on day 1 after 3 h MCAO ($p = 0.001$); better neurological performance ($p = 0.005$); smaller lesion size after 90 min MCAO ($p = 0.013$), no improvement in functional outcome ($p = 0.81$) |
| Pfeilschifter[21] | C57BL/6, *SphK1*$^{-/-}$ and *SphK2*$^{-/-}$ mice (age: 10–12 weeks; sex: n.a.) | Transient MCAO (90 min, 180 min) | FTY720 1 mg/kg i.p. 2 h after vessel occlusion *versus* vehicle | Reduction in stroke volume at day 1 ($p = 0.001$ and 0.013, respectively) |
| Liesz[22] | C57BL/6 mice (age: 8–10 weeks; sex: male) | Permanent MCAO, transient MCAO (60 min) | FTY720 1 mg/kg p.o. starting at 48 h before or at 3 h after ischaemia induction *versus* PBS; single dose of FTY720; FTY720 1 mg/kg i.p. once daily beginning 48 h before MCAO | No difference ($p > 0.05$) in infarct volume and functional outcome |
| Wei[23] | C57BL/6 mice, Sprague–Dawley rats (age: n.a.; sex: male) | Transient MCAO in mice (90 min) and rats (2 h); permanent MCAO in mice | FTY720 (1 mg/kg or 0.5 mg/kg) i.p. 30 min after reperfusion *versus* saline; FTY720 (1 mg/kg) *versus* saline 1 h before distal MCAO; 3 mg/kg FTY720 2, 24 and 48 h after reperfusion; FTY720 (1 mg/mg, i.p.) 30 min after reperfusion in rats; 1 mg/kg FTY720 2 or 4 h after occlusion in permanent model | Reduced infarct volume on day 2 in all experimental settings ($p < 0.05$ to $p < 0.001$); improved neurological function for the 1 mg/kg group ($p < 0.05$), not in the 0.5 mg/kg group ($p > 0.05$) on day 2, and in group given 3 mg/kg twice after reperfusion on days 1, 3, 7, 10 and 14 ($p < 0.05$ to $p < 0.001$) |

**Table 3.** (Continued)

| Author | Species | Stroke model | Intervention | Main outcome |
|---|---|---|---|---|
| Rolland[24] | CD-1 mice (age: 8 weeks; sex: n.a.) | ICH induction by intrastriatal collagenase injection *versus* needle insertion only (sham operation) | FTY720 1 mg/kg i.p. 1 h after ICH induction *versus* vehicle | Reduced brain oedema ($p < 0.05$), better functional outcome on days 1 and 3 ($p < 0.05$) |
| Rolland[25] | CD-1 mice and Sprague–Dawley rats (age: n.a.; sex: male) | Experimental ICH (collagenase or autologous blood injection in striatum) | FTY720 1 mg/kg i.p. single dose 1 h after or daily dose 1, 24 and 48 h after ICH *versus* vehicle | Less brain oedema in FTY720-treated mice *versus* vehicle group ($p < 0.05$); better neurological function ($p < 0.05$) |
| Kraft[26] | C57BL/6 and *Rag1*$^{-/-}$ mice (age: 6–8 weeks; sex: male) | Transient MCAO (60 min, 90 min) | FTY720 1 mg/kg i.p. immediately before reperfusion *versus* vehicle | Reduction in stroke volume at day 1 ($p = 0.048$); improved neurological function ($p = 0.02$ to $p = 0.03$) |
| Campos[27] | C57BL/6 mice (age: n.a.; sex: male) | Thromboembolic stroke model using mouse-α-thrombin dissolved in 18% glycerol/saline | 1. MCAO not treated with rt-PA (permanent occlusion); fingolimod 0.5 mg/kg i.p. *versus* saline 45 min, 24 and 48 h after occlusion<br>2. MCAO + early rt-PA; rt-PA i.v. 30 min after thrombin injection (transient occlusion); fingolimod *versus* saline 30 min (together with rt-PA), 24 and 48 h after occlusion<br>3. MCAO + delayed rt-PA, rt-PA i.v. 3 h after thrombin injection (transient occlusion); fingolimod *versus* saline 3 h (together with rt-PA), 24 and 48 h after occlusion | In absence of rt-PA, fingolimod reduced stroke volumes ($p < 0.05$) and improved functional outcome ($p < 0.05$); early rt-PA and fingolimod applications had no impact on stroke volume ($p > 0.05$) but improved functional outcome ($p < 0.05$); late rt-PA and fingolimod applications reduced stroke volume ($p < 0.05$) and improved functional outcome ($p < 0.05$) |
| Cai[28] | C57BL/6 mice (age: 10–12 weeks; sex: n.a.) | Transient MCAO (180 min) | FTY720 1 mg/kg i.p. *versus* vehicle *versus* rt-PA 10 mg/kg i.v. *versus* rt-PA 10 mg/kg i.v. + FTY720 1 mg/kg i.p.; all directly before reperfusion | Higher mortality in FTY720 + rt-PA group (61%) *versus* vehicle (33%), FTY720 (39%) and rt-PA only (44%) |
| Brunkhorst[29] | C57BL/6J mice (age: 6–12 weeks; sex: male) | Photothrombotic stroke | FTY720 1 mg/kg i.p. twice daily for 5 days, beginning 3 days after photothrombotic stroke *versus* saline | Improvement in functional outcome on day 7 ($p = 0.013$ to $p = 0.003$) and day 31 ($p = 0.02$ to $p = 0.03$) |

*(Continued)*

**Table 3.** (Continued)

| Author | Species | Stroke model | Intervention | Main outcome |
|---|---|---|---|---|
| Hasegawa[30] | Sprague–Dawley rats (age: n.a.; sex: male) | Transient MCAO (120 min) | FTY720 0.25 mg/kg in DMSO *versus* DMSO i.p. directly after reperfusion | Infarct volume reduction on day 1 in FTY720 group ($p = 0.05$) |
| Lu[31] | CD-1 mice (age: n.a.; sex: male) | Experimental ICH (collagenase injection in basal ganglia) | FTY720 0.5 mg/kg i.p. 30 min after surgery *versus* vehicle and once daily in following 2 days | Reduction in brain oedema and haematoma volume after 72 h ($p < 0.05$). After 3, 7 and 14 days, reduced oedema and brain atrophy ($p < 0.05$) |
| Moon[32] | ICR mice (age: 7 weeks; sex: male) | Transient MCAO (60 min, 90 min) | FTY720 3 mg/kg i.p. immediately after reperfusion (90 min MCAO) or 30 min prior to 60 min MCAO *versus* vehicle | Reduction in stroke volume ($p < 0.05$) |
| Schuhmann[33] | C57BL/6 mice (age: 6–8 weeks; sex: male) | Transient MCAO (30 min) | FTY720 1 mg/kg i.p before ischaemia + after 2 days *versus* vehicle | Smaller lesion size on day 1 ($p < 0.05$); functional improvement ($p < 0.05$) |
| Nazari[34] | Sprague–Dawley rats (age: n.a.; sex: male) | Transient MCAO (60 min) | FTY720 0.5 mg/kg i.p. 24 h before vessel occlusion *versus* vehicle plus once daily every 2 days | Reduced brain oedema ($p < 0.01$) and neurological deficit score ($p < 0.05$) after 24 h, 3 and 7 days |
| Sun[35] | C57BL/6J and *Rag2*$^{-/-}$ mice (for the FTY720 part only C57BL/6J mice were used) (age: 7–8 weeks; sex: male) | Experimental ICH (injection of autologous blood) | FTY720 1 mg/kg 30 min after ICH induction *versus* vehicle *versus* RP101075 (selective S1PR1 agonist) *versus* RP101075 + W146 (S1PR1 antagonist) | Reduction in functional deficits on days 1 and 3 ($p < 0.01$); infarct volumes have not been published for the FTY720 group |
| Schlunk[36] | CD-1 mice (age: 12–16 weeks; sex: male) | Experimental ICH (injection of collagenase type VII-S) | FTY720 1 mg/kg i.p. 1 h after ICH induction *versus* vehicle | No change in mortality, functional outcome, haematoma volume and oedema ($p > 0.05$) |
| Rolland[37] | Sprague–Dawley rats (age: n.a.; sex: male/female) | Germinal matrix haemorrhage in rat pups | FTY720 0.25 mg/kg or 1.0 mg/kg i.p. 1, 24 and 48 h after surgical intervention *versus* DMSO in saline | Better functional outcome ($p < 0.05$) and increased total brain surface area ($p < 0.05$) in both dosages |
| Zhang[38] | C57BL/6 and BALB/c nude mice (age: 8–12 weeks; sex: male) | Experimental ICH (autologous blood injection in striatum) | FTY720 1 mg/kg i.p. 1, 24 and 48 h after ICH *versus* vehicle | Lower BBB leakage and CD4+/CD8+ cells in nude and FTY720 treated wild-type mice *versus* vehicle group ($p < 0.05$) |

BBB, blood–brain barrier; DMSO, dimethyl sulphoxide; FTY720, fingolimod; HPC, hypoxic preconditioning; ICH, intracerebral haemorrhage; i.p., intraperitoneally; i.v., intravenously; MCAO, middle cerebral artery occlusion; n.a., not applicable; PBS, phosphate-buffered saline; p.o., per os; rt-PA, recombinant-tissue plasminogen activator.

**Table 4.** Characteristics of preclinical studies analysing natalizumab.

| Author | Species | Stroke model | Intervention | Main outcome |
|---|---|---|---|---|
| Becker[39] | Lewis rats (age: n.a.; sex: male) | Transient MCAO (180 min) | TA-2 (selective anti-α4 antibody) 2.5 mg/kg i.p. 2 h after vessel occlusion *versus* isotype control | Improved functional outcome on day 1 ($p = 0.006$), day 2 ($p = 0.011$); smaller infarct volume ($p = 0.012$) |
| Relton[40] | Wistar rats (age and sex: n.a.) | Transient MCAO (90 min) | TA-2 (selective anti-α4 antibody) 2.5 mg/kg *versus* isotype control i.v. immediately after reperfusion | Reduced total ($p < 0.05$) and subcortical stroke volume ($p < 0.001$), functional improvement after 24 h ($p < 0.01$) |
| Relton[41] | SHR (hypertensive) or Sprague–Dawley rats (age: n.a.; sex: male) | Transient MCAO (60 min) | TA-2 (selective anti-α4 antibody) 2.5 mg/kg *versus* isotype control i.v. 24 h before induction of cerebral ischaemia | Reduced total ($p < 0.05$) and subcortical stroke volume ($p < 0.01$) in hypertensive SHR rats; reduced total and subcortical stroke volume ($p < 0.001$) in normotensive Sprague–Dawley rats |
| Liesz[42] | C57BL/6J mice (age: 10–12 weeks; sex: male) | Transient (30 or 60 min) or permanent MCAO | CD49d-specific monoclonal antibody 300 µg i.p. 24 h before or 3 h after induction of ischaemia *versus* isotype control | Reduced infarct volumes at day 7 ($p < 0.001$), not at day 1 ($p > 0.05$), for permanent MCAO and 30 min (but not 60 min) transient MCAO; improved functional outcome on days 3 and 7 ($p < 0.05$) |
| Langhauser[43] | C57BL/6 mice (age: 6–8 weeks; sex: male) | Transient MCAO (30 min) or permanent MCAO (coagulation model) | CD49d-specific monoclonal antibody 300 µg i.p. 24 h before or 3 h after induction of ischaemia *versus* isotype control | Independent of prophylactic or therapeutic treatment and stroke model: no change in stroke volume and functional scores on days 1 and 7 ($p > 0.05$); no change in survival ($p > 0.05$) |
| Hammond[44] | C57BL/6J mice (age: 8–12 weeks; sex: male) | Experimental ICH (injection of autologous blood) | Anti-α4 integrin antibody (clone R1-2) 300 µg i.p. 2–6 h before ICH | Improved functional outcome ($p < 0.01$) |
| Llovera[45] | C57BL/6J mice (age: 8–10 weeks; sex: male) | Transient MCAO (60 min) or permanent MCAO (coagulation model) | CD49d-specific monoclonal antibody 300 µg i.p. 3 h after induction of ischaemia *versus* isotype control | Reduced stroke volume in permanent MCAO (day 7, $p < 0.05$), not for transient MCAO (day 4, $p > 0.05$); no impact on functional outcome ($p > 0.05$) |
| Neumann[46] | LysM-eGFP and CX3CR1-eGFP mice (age: 8–10 weeks; sex: male) | Permanent MCAO (coagulation model) or transient MCAO (45 min) | CD49d-specific monoclonal antibody 150 µg i.v. at beginning of reperfusion (transient MCAO) and 24 h later *versus* isotype control | Reduced stroke volume and improved functional outcome ($p < 0.05$) |

ICH, intracranial haemorrhage; i.p., intraperitoneally; i.v., intravenously; MCAO, middle cerebral artery occlusion; n.a., not applicable; SHR, spontaneously hypertensive rat.

role of regulatory T cells in IS.[58] Very often, the methodological quality of preclinical studies is low compared with clinical trials, and blinding and randomization procedures are not common in every laboratory, potentially leading to biased results. Moreover, only one preclinical study

**Table 5.** Characteristics of preclinical studies analysing dimethyl fumarate.

| Author | Species | Stroke model | Intervention | Main outcome |
|---|---|---|---|---|
| Iniaghe[47] | CD-1 mice (age: n.a.; sex: male) | Experimental ICH (injection of collagenase or autologous blood) | DMF 10 or 100 mg/kg i.p. 1 h after ICH *versus* vehicle; further experimental groups, including siRNA or MAFG siRNA | Low-dose DMF (10 mg/kg) did not improve functional outcome ($p > 0.05$), high dose (100 mg/kg) reduced functional deficits at days 1 and 3 ($p < 0.05$); no impact on haematoma volume ($p > 0.05$) |
| Zhao[48] | Sprague–Dawley rats, *Nrf2*+/+ and *Nrf2*$^{-/-}$ mice (age: n.a.; sex: male) | Experimental ICH (injection of autologous blood) | Rats: DMF 15 mg/kg i.p. 2 h after ICH and then twice daily on days 1–3 *versus* vehicle; mice: DMF 15 mg/kg i.p. 24 h after ICH and then at days 2 and 3 *versus* vehicle | Amelioration of neurological deficit in rats at days 1 and 3 after ICH, in wild-type but not *Nrf*$^{-/-}$ mice ($p < 0.05$) |
| Kunze[49] | C57BL/6 and *Nrf2*$^{-/-}$ mice (age: 8–10 weeks; sex: male) | Transient MCAO (60 min) | DMF 15 mg/kg twice daily for 1, 2 or 3 consecutive days *versus* vehicle; alternatively, DMF in 0.08% Methocel™ 15 mg/kg twice daily via oral gavage for 1, 2 or 3 consecutive days *versus* vehicle | Lower BBB leakage and brain oedema ($p < 0.01$) |
| Lin[50] | Sprague–Dawley rats (age: n.a.; sex: male) | Transient MCAO (120 min) | DMF 25 or 50 mg/kg p.o. 2–3 h after transient MCAO and twice daily afterwards | Reduced infarct volume and improved neurobehavioural deficits 24 h, 72–84 h, 7 days and 14 days after MCAO ($p < 0.05$) |
| Yao[51] | C57BL/6 and *Nrf2*$^{-/-}$ mice (age: 8–10 weeks; sex: n.a.) | Transient MCAO (60 min) | DMF 30 or 45 mg/kg twice daily p.o. for 7 days, first dose given 15 min before reperfusion; *Nrf2*$^{-/-}$ mice were treated with 45 mg/kg only; control groups received PBS | Reduced infarct volumes in the 30 mg/kg ($p < 0.05$) and the 45 mg/kg DMF group ($p < 0.01$) on days 3 and 7 in C57/BL6 mice, not in *Nrf2*$^{-/-}$ mice ($p > 0.05$); functional improvement on days 3 and 7 in both DMF groups ($p < 0.05$ to $p < 0.01$), not on day 1 ($p > 0.05$) |
| Safari[52] | Sprague–Dawley rats (age: n.a.; sex: male) | Transient MCAO (60 min) | DMF 15 mg/kg diluted in 200 µl 0.08% Methocel™/$H_2O$ twice daily p.o. on days 0–14 (first application immediately after MCAO) *versus* vehicle *versus* sham treatment | Functional improvement on days 10 and 14 ($p < 0.05$) |

BBB, blood–brain barrier; DMF, dimethyl fumarate; ICH, intracerebral haemorrhage; i.p., intraperitoneally; i.v., intravenously; MCAO, middle cerebral artery occlusion; n.a., not applicable; PBS, phosphate-buffered saline; p.o., per os; MAFG, musculo-aponeurotic fibrosarcoma-G; siRNA, small interfering ribonucleic acid.

identified in our review analysed female animals that definitely does not represent the typical stroke population.[37] Methodological limitations might be one of the reasons for translational roadblocks, that is, difficulties in confirming positive preclinical results in clinical trials. Standardization of animal studies,[59] adherence to the Animal Research: Reporting of *In Vivo* Experiments guidelines (available at: https://www.nc3rs.org.uk/arrive-guidelines) and multicentre animal RCTs can improve data quality.[45]

Encouraged by positive preclinical trials, the first clinical trials evaluating natalizumab and fingolimod in stroke patients have been conducted. From a methodological perspective, the RCT by Elkins and coworkers is the best of these studies, but remained negative regarding the primary end point, with slight treatment-associated benefits on functional outcomes (ACTION trial).[53] In contrast, the trials analysing fingolimod were not double blinded and much smaller, but positive regarding major outcome variables.[54–57] The latter trials included mainly Asian patients; therefore, generalizability of data might be limited. In summary, the main limitation of the clinical trials is the heterogeneity of the included studies, the restricted data quality and generalizability, as well as the, in part, very low numbers of patients per study. Study heterogeneity comprises mainly population (IS *versus* ICH) and outcome variables.

**Table 6.** Characteristics of clinical studies.

| Author | Study design | Population | Stroke type | Intervention | Major end points | Major results |
|---|---|---|---|---|---|---|
| **Natalizumab** | | | | | | |
| Elkins[53] | RCT | Intervention = 79 (mean age 70 ± 14 years; sex: 51% male), control = 82 (mean age 72 ± 12 years; sex: 59% male) | First ischaemic stroke | Natalizumab 300 mg i.v. *versus* placebo up to 9 h after stroke onset | Primary end points: change in infarct volume from baseline to day 5; secondary end points: change in infarct volume 24 h to days 5 and 30, functional scores, others | No difference between natalizumab and control group regarding primary end point ($p > 0.05$); improvement in some of the functional scores ($p < 0.05$) |
| **Fingolimod** | | | | | | |
| Fu[54] | Prospective two-arm, evaluator-blinded study | Intervention = 11 (mean age 61 ± 12 years; sex: 36% male), control = 12 (mean age 58 ± 9 years; sex: 91% male) | Primary supratentorial basal-ganglia ICH, volume 5–30 ml, onset less than 72 h prior to admission, GCS ⩾ 6 | Fingolimod 0.5 mg p.o. once daily for 3 consecutive days, max. 1 h after baseline CT scan, max. 72 h after symptom onset | GCS and NIHSS on day 7, 14, 30, and 90; haematoma volume and perihaematomal oedema volume on days 7, 14 and 90 | Lower NIHSS scores at 7, 14 and 30 days ($p = 0.03$ to $p < 0.001$), lower oedema volume on day 7 ($p = 0.04$) |
| Fu[55] | Single-centre, open-label, parallel-group, evaluator-blinded, pilot trial | Intervention = 11 (mean age 62 ± 8 years; sex: 73% male), control = 12 (mean age 55 ± 11 years; sex: 82% male) | Acute ischaemic stroke in anterior circulation, NIHSS ⩾ 5, age ⩾ 18 years, symptom onset to admission 4.5–72 h | Standard treatment according to AHA guidelines + fingolimod 0.5 mg p.o. once daily for 3 consecutive days beginning within 1 h after baseline MRI and no later than 72 h after symptom onset *versus* standard treatment only | NIHSS, mRS, mBI and lesion volume (MRI) at different time points until day 90 | Reduced NIHSS at day 30 ($p = 0.049$) and day 90 ($p = 0.019$); reduced infarct volume increase until day 7 ($p = 0.0003$) |
| Li[56] | Prospective two-arm, evaluator-blinded study | Intervention = 11, control = 12 (age and sex distribution: n.a.) | ICH patients with matched clinical characteristics, haematoma location, and volume | Fingolimod 0.5 mg p.o. once daily for 3 days, the first dose given within 1 h after baseline CT + standard of care for ICH *versus* standard management only | Changes of lymphocyte subsets, serum cytokines and impact on vascular permeability | Significant reduction in various immune cells and cytokines ($p < 0.05$ to $p < 0.001$) |
| Zhu[57] | Randomized, open-label, evaluator-blinded, multicentre pilot trial | Intervention = 22 (mean age 60 ± 3 years; sex: 59% male), control = 25 (mean age 59 ± 2 years; sex: 68% male) | First-ever hemispheric ischaemic stroke, age 18–80 years and NIHSS > 5 | Alteplase (0.9 mg/kg) *versus* alteplase + fingolimod 0.5 mg p.o. once daily for 3 consecutive days with the first dose being given before alteplase administration | Primary end points: changes in lesion volume from baseline (DWI) to day 1 (FLAIR), the haemorrhage volume (GRE) at day 1 and extent of clinical improvement at day 1 (NIHSS); secondary outcomes: lesion volume growth from days 1 to 7, recovery at day 90 | Better outcome regarding all primary end points ($p < 0.05$) in the fingolimod group; reduced lesion volume growth ($p < 0.01$); decreased NIHSS scores from days 1 to 7 ($p < 0.01$) and good recovery (mRS at day 90, $p = 0.01$) |

AHA, American Heart Association; CT, computed tomography; DWI, diffusion-weighted imaging; FLAIR, fluid-attenuated inversion recovery; GCS, Glasgow Coma Scale; GRE, gradient echo sequences; ICH, intracerebral haemorrhage; i.v., intravenously; max., maximum; mBI, modified Barthel Index; MRI, magnetic resonance imaging; mRS, modified Rankin Scale; NIHSS, National Institutes of Health Stroke Scale; p.o., per os; RCT, randomized controlled trial.

The identified studies analysed immunomodulatory treatment with INF-β, glatiramer acetate, fingolimod, natalizumab and DMF. Until now, it is only incompletely understood what the stroke-specific mechanisms of these agents are. Therefore, the following aspects known from multiple sclerosis treatment might be the most relevant effects, but also other mechanisms might play an important role. INF-β inhibits IFN-γ, induces interleukin 10 expression and reduces the transmigration of lymphocytes and monocytes into the central nervous system (CNS). Glatiramer acetate (among other mechanisms) induces protective TH2 cells that secrete immunomodulating cytokines like interleukin-4, -6 and -10. Fingolimod is a sphingosine-1-phosphate-analogon that inhibits the efflux of lymphocytes out of lymph nodes leading to a profound lymphopenia and thus reduced CNS infiltration. Moreover, fingolimod seems to reduce thromboinflammation and improves cerebral blood flow.[26] The monoclonal antibody natalizumab blocks the adhesion molecule α4-integrin that is relevant for the infiltration of immune cells over the blood–brain barrier into the CNS. Finally, DMF has an antioxidant effect and activates the nuclear factor (erythroid-derived 2)-related factor 2 (Nrf2) pathway.

In conclusion, immunotherapy in stroke instrumentalizes the concept of thromboinflammation and could become a novel treatment option in the future. Despite translational limitations, the available clinical data are promising. Nevertheless, given the heterogeneity and low number of clinical studies, it is too early to reliably judge the novel strategy of immunotherapy in general. Therefore, further well-designed trials are urgently needed and are on the way (e.g. ACTION 2 and FAMTAIS[60]) [ClinicalTrials.gov identifiers: NCT02730455 and NCT02956200].

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