Received: 2 February 2021 | Accepted: 31 March 2021

DOI: 10.1111/jth.15336

REVIEW ARTICLE

Thrombolytic strategies for ischemic stroke in the thrombectomy era

Maxime Gauberti[1,2] | Sara Martinez de Lizarrondo[1] | Denis Vivien[1,3]

[1]Normandie Univ, UNICAEN, INSERM, PhIND "Physiopathology and Imaging of Neurological Disorders", Institut Blood and Brain @ Caen-Normandie, Cyceron, Caen, France

[2]CHU Caen, Department of Neuroradiology, CHU de Caen Côte de Nacre, Caen, France

[3]CHU Caen, Clinical Research Department, CHU de Caen Côte de Nacre, Caen, France

Correspondence
Denis Vivien, INSERM U1237 "Physiopathology and Imaging for Neurological Disorders (PhIND)", GIP Cyceron, Bd Henri Becquerel, BP5229, 14074 Caen, France.
Email: vivien@cyceron.fr

Abstract

Twenty-five years ago, intravenous thrombolysis has revolutionized the care of patients with acute ischemic stroke. Since 2015, randomized clinical trials have demonstrated that mechanical thrombectomy improves functional outcome in stroke patients over intravenous thrombolysis alone. More recently, three randomized clinical trials have suggested that mechanical thrombectomy alone is noninferior to a combined strategy with both intravenous thrombolysis and mechanical thrombectomy. In the present review, we will present the last clinical and preclinical studies on the use of thrombolysis in stroke patients in the modern thrombectomy era. At the cost of a potential increased risk of hemorrhagic transformation, thrombolysis may promote arterial recanalization before thrombectomy, improve the rate of successful recanalization after thrombectomy, and restore microcirculation patency downstream of the main thrombus. Besides, new thrombolytic strategies targeting tissue-type plasminogen activator resistant thrombi are being developed, which could strengthen the beneficial effects of thrombolysis without carrying additional pro-hemorrhagic effects. For instance, tenecteplase has shown improved rate of recanalization compared with tissue-type plasminogen activator (alteplase). Beyond fibrinolysis, DNA- and von Willebrand factor-targeted thrombolytic strategies have shown promising results in experimental models of ischemic stroke. New combined strategies, improved thrombolytics, and dedicated clinical trials in selected patients are eagerly awaited to further improve functional outcome in stroke.

KEYWORDS
fibrinolysis, ischemia/reperfusion, ischemic stroke, thrombectomy, tissue-type plasminogen activator

1 | INTRODUCTION

Each year, more than 2.7 million people die from ischemic stroke and 51.9 million years of healthy life is lost because of ischemic stroke-related death and disabilities (World Stroke Organization). In most cases, ischemic stroke is caused by embolization of a thrombus from an atherosclerotic large vessel or from the heart into the intracranial circulation.[1] Prompt recanalization can alleviate ischemic damages and is currently the aim of acute stroke management, but the best way to achieve complete reperfusion remains controversial. There are two proven reperfusion strategies: intravenous fibrinolysis with a recombinant form of tissue-type plasminogen activator (tPA,

Manuscript handled by: Ton Lisman

Final decision: Ton Lisman, 31 March 2021

alteplase) and mechanical thrombectomy.[2] According to current guidelines,[3] intravenous alteplase is the first line therapy and should be initiated within 4.5 h of symptom onset. Mechanical thrombectomy is indicated for large artery occlusion in the anterior circulation in all patients within 6 h of symptom onset and in patients selected by advanced imaging between 6 and 24 h after symptom onset. Of note, most ischemic stroke patients are not eligible to mechanical thrombectomy because they do not present large artery occlusion when they arrive at the hospital. Therefore, ischemic stroke patients can receive either alteplase alone, mechanical thrombectomy alone, or a combined treatment starting by intravenous alteplase and followed by mechanical thrombectomy.

With the development of mechanical thrombectomy, the role of pharmacological thrombolysis has been questioned, especially because it might increase the risk of hemorrhagic side effects.[4] Yet, even if mechanical thrombectomy achieves recanalization rates exceeding 80% in large vessel stroke, there is still room for improvement: some patients with distal occlusion or tortuous anatomy are not eligible for endovascular procedures, delay between first medical contact and actual reperfusion by thrombectomy can be as long as several hours, and distal microthrombosis is not treatable by endovascular means. The aims of this review are first to summary the current state-of-the-art of reperfusion strategies and second to present recent evidence supporting the use of clinically available or experimental thrombolytic strategies to improve brain reperfusion when combined with thrombectomy.

2 | FROM STROKE PATHOPHYSIOLOGY TO CURRENT REPERFUSION STRATEGIES

Acute ischemic stroke is a time-sensitive condition. Downstream of the arterial thrombus, collateral blood flow can maintain cellular integrity for several hours in a brain region termed the ischemic penumbra. Over time and in the absence of reperfusion, this ischemic penumbra is recruited into the irreversibly injured ischemic core.[2] On average, 2 million neurons die per minute in the hypoperfused brain. Reperfusion is the most effective strategy to improve neurological outcome in ischemic stroke patients. In 1995, the National Institute of Neurological Disorders and Stroke rt-PA Stroke Study Group published the first randomized clinical trial demonstrating the safety and clinical efficacy of intravenous administration of alteplase in the first 3 h after symptom onset.[5] Two other fibrinolytic agents, desmoteplase and streptokinase, failed in clinical trials.[6] Since the pivotal NINDS study, further trials have demonstrated benefit when alteplase is administered up to 4.5 h after stroke onset in all patients, up to 9 h after stroke onset in patients with salvageable tissue and in patients with unknown onset time presenting a favorable profile on magnetic resonance imaging.[3] The rate of symptomatic intracranial hemorrhage is higher with alteplase, but this increase does not negate its overall benefit.[6,7]

Still, the rate of recanalization within 2 h after alteplase administration remains low. A review of the Calgary Stroke program including 1341 patients, reported an average recanalization rate of 4.4% for internal carotid artery occlusion, 4.0% for basilar artery occlusion, and 32.8% for proximal middle cerebral artery occlusion.[8] Prolonged time to treatment, poor collaterals, and thrombus size greater than 8 mm are major predictive factors for tPA treatment failure.[6] This low recanalization rate for proximal occlusion and large thrombus prompted the development of mechanical recanalization strategies. In 2015, five randomized clinical trials demonstrated the efficacy of mechanical thrombectomy in stroke caused by large vessel occlusion in the anterior circulation when performed in the first 6 h after stroke onset.[9] Since then, other trials have extended the indications of mechanical thrombectomy up to 24 h after stroke onset in patients with salvageable tissue identified on imaging.[10,11] In the most recent clinical trials, recanalization rates reach 80% for substantial reperfusion at the end of the procedure for occlusion of the internal carotid artery or proximal middle cerebral artery. There was no significant difference between the mechanical thrombectomy and control groups for rates of symptomatic intracranial hemorrhage in a pooled patient-level meta-analysis.[9]

Current guidelines advocate the use of intravenous alteplase as first-line therapy.[3] Because the benefit of alteplase is time dependent, patients should be treated without delay even if mechanical thrombectomy is being considered. Mechanical thrombectomy is indicated for patients with acute ischemic stroke due to a large artery occlusion, regardless of whether they have received intravenous alteplase, and should also be performed as fast as possible.[12] Thus, thrombolysis and mechanical thrombectomy are currently considered independently, setting aside the potential beneficial or deleterious interactions between the two treatments.

3 | RANDOMIZED CLINICAL TRIALS ON THROMBECTOMY WITH OR WITHOUT INTRAVENOUS THROMBOLYSIS

There are six main randomized clinical trials comparing the effects of direct thrombectomy (dMT) vs. intravenous thrombolysis followed by thrombectomy (IVT+MT) in patients with acute ischemic stroke and proximal artery occlusion within 4.5 h from onset: Direct Intraarterial Thrombectomy in Order to Revascularize Acute Ischemic Stroke Patients with Large Vessel Occlusion Efficiently in Chinese Tertiary Hospitals: a Multicenter Randomized Clinical Trial (DIRECT-MT), endovascular therapy with vs. without intravenous tissue plasminogen (SKIP) study, Multicenter Randomized Clinical Trial of Endovascular Treatment for Acute Ischemic Stroke in the Netherlands, Solitaire With the Intention for Thrombectomy Plus Intravenous t-PA Versus DIRECT Solitaire stent study, A Randomized Controlled Trial of DIRECT Endovascular Clot Retrieval Versus Standard Bridging Thrombolysis With Endovascular Clot Retrieval, and Direct Endovascular Thrombectomy vs. Combined IVT and Endovascular Thrombectomy for Patients With Acute Large Vessel Occlusion in the Anterior Circulatio (DEVT). To date, only the results of DIRECT-MT, DEVT, and SKIP have been released.

The DIRECT-MT study was conducted in 41 centers in China.[13] They randomized 656 patients with proximal occlusion of the anterior circulation to receive either dMT (n = 327) or IVT+MT (n = 329) at the acute phase of stroke. Primary outcome was the score on the modified Rankin scale assessed at 90 days and was analyzed for noninferiority. dMT was noninferior to IVT+MT (adjusted common odds ratio 1.07 [0.81-1.40], p = .04 for noninferiority). However, it is worth mentioning that the noninferiority margin was set at 20% in the DIRECT-MT trial, which might be larger than the expected benefit from thrombolysis in this population. Interestingly, dMT was associated with a statistically significant lower rate of successful reperfusion before thrombectomy (2.4% vs. 7.0%) and a trend for a lower rate of overall successful reperfusion (79.4% vs. 84.5%). The number of intracranial hemorrhages was numerically lower in the dMT group (4.3% vs. 6.1% for symptomatic hemorrhages and 33.3% vs. 36.2% for asymptomatic hemorrhages) but it did not reach statistical significance.

The randomized study SKIP was conducted in Japan and compared the modified Rankin scale assessed at 90 days between acute ischemic stroke patients treated with dMT or with IVT+MT (204 patients).[14] There was no statistically significant difference between the two groups for the primary outcome (59% achieved good outcome with thrombectomy alone vs. 57% with the combination approach). Statistical significance for noninferiority was not achieved. Importantly, the rate of any intracranial hemorrhage within 36 h was significantly lower for dMT (34% vs. 50%; p = .02), despite the use of a lower dose of alteplase in Japan than in most other countries (0.6 mg/kg vs. 0.9 mg/kg). The rates of symptomatic intracranial hemorrhage were similar. No heterogeneity across subgroup such as age, sex, or ASPECTS at admission was found.

The DEVT trial was conducted in China at 33 stroke centers and included 234 patients.[15] The primary endpoint was the proportion of patients achieving functional independence at 90 days (modified Rankin score 0-2) and was analyzed for noninferiority. A total of 54.3% of patients in the dMT group and 46.6% in the IVT+MT group met the primary outcome (p = .003 for noninferiority). No significant difference was detected in the rate of symptomatic hemorrhages, but the rate of any intracranial hemorrhage was numerically higher in the IVT+MT group (32.5% vs. 21.7%). Notably, and in contrast to DIRECT-MT, there were more puncture access complication in the IVT+MT group (5.1% vs. 0.9%).

In addition to these randomized studies, many observational studies, systematic reviews, and meta-analyses of nonrandomized studies have been published[4,16–20] with different and sometimes opposite conclusions on the added benefit of intravenous thrombolysis in patients treated by thrombectomy. It should be acknowledged that nonrandomized studies comparing dMT with IVT+MT are subject to biases because most of the dMT patients are not eligible for IVT because of presentation beyond the alteplase time window, anticoagulation, or other reasons that can themselves influence stroke outcome.

Overall, data from randomized trials suggest that there is no significant extra benefit of intravenous thrombolysis in patients treated by mechanical thrombectomy. Moreover, IVT+MT may carry an additional risk of hemorrhagic transformation compared with dMT, although most of these appear asymptomatic. Several factors are important to consider when trying to translate the results of these trials to clinical practice. First, these trials were performed according to a "mothership" design: patients were already in a thrombectomy-capable center when they were randomized. Thus, the delay between intravenous thrombolysis and thrombectomy were short (median of 29 min and 97.2% of patients had delays shorter than 60 min in the DIRECT-MT trial). It is tempting to speculate that the benefit of intravenous thrombolysis will be larger in patients who have a delay of a few hours before thrombectomy, for instance to allow transfer to a thrombectomy capable center. In line with this hypothesis, in the cardiology field, intravenous thrombolysis is recommended before endovascular treatment for ST-elevation myocardial infarction patients who have an anticipated delay longer than 90 min to reach an angiography suite. Second, depending on the local organization, start of intravenous thrombolysis might significantly delay thrombectomy. In the DIRECT-MT trial, the median time from randomization to arterial puncture was only 5 min higher in patients treated with IVT+MT than in patients treated with dMT. In real-life settings with possible larger delays, delaying thrombectomy may blunt the benefit of intravenous thrombolysis. In contrast, the distorted workflow in these trials might have artificially prolonged onset to IVT delay, for instance by requiring advanced imaging before administering IVT, thereby shortening the available time for thrombolysis to induce reperfusion. Third, there is continuous progress in endovascular techniques, and it is likely that the rate of complete reperfusion at the end of endovascular procedure will increase.[21] This is susceptible to weaken the extra benefit expected from intravenous thrombolysis.

Pending publication of the other randomized trials, current guidelines recommend the use of the combined approach in patients eligible to intravenous thrombolysis. Interestingly, the higher reperfusion rate in patients who received intravenous thrombolysis in the DIRECT-MT study supports the hypothesis that pharmacological thrombolysis has the potential to improve reperfusion status over thrombectomy alone. In the next sections, we will review the limitations of current reperfusion strategies and how pharmacological thrombolysis might still play a role to improve stroke outcome.

4 | LIMITATIONS OF CURRENT REPERFUSION STRATEGIES

There is still room for improvement for reperfusion strategies, even in patients eligible for mechanical thrombectomy. First, generalization of mechanical thrombectomy at the acute phase poses a challenging problem for stroke care organization. It requires a trained team of interventional neuroradiologists, an angiography suite and several devices to navigate in the affected artery and ultimately remove the clot. When patients present to a stroke center that is unable to perform mechanical thrombectomy, transfer to a secondary center

significantly prolongs the delay before reperfusion. In the recent Endovascular Therapy Following Imaging Evaluation for Ischemic Stroke 3 trial for instance, median time from imaging to arterial puncture was 59 min and median time from arterial puncture to actual reperfusion was 38 min.[11] Even if ultimately reperfusion is achieved in most patients, this 1.5-h delay can jeopardize the benefit of the reperfusion procedure.

Second, complete reperfusion at the end of the endovascular procedure is achieved only in a subset of patients. In a recent observational study by the HERMES collaboration, complete or near-complete reperfusion (when at least 90% of the impacted territory is reperfused) was achieved in only 32% of ischemic stroke patients (reperfusion >50% of the impacted territory was achieved in 76%).[22] This lack of complete reperfusion in most patients is worrisome because it is now well established that the better the reperfusion grade, the better the clinical outcome.[23] The main explanations for this lack of complete reperfusion are the inability to access the intracranial circulation (especially because of the tortuosity of the extracranial vessels), the inability to remove the main thrombus, the presence of inaccessible distal thrombi (either spontaneous or iatrogenic due to manipulation of the main thrombus), the risk of emboli in a new vascular territory, and the deleterious effect of the mechanic stress exerted by endovascular manipulation on cerebral arteries (dissection, spasm).

In the first five randomized clinical trials of mechanical thrombectomy that included patients treated with and without intravenous thrombolysis, successful reperfusion was achieved in 71% of the patients.[9] At that time, successful reperfusion was defined as a modified thrombolysis in cerebral infarction (mTICI) score of 2B or 3, corresponding to reperfusion of at least 50% of the affected vascular territory.[9] Recent data showed that patients with mTICI 2B scores had significantly worse outcome than patients with mTICI 2C and mTICI 3 scores,[22–24] corresponding respectively to patients with near-complete perfusion except for slow flow in a few distal cortical vessels or presence of small distal cortical emboli after mechanical thrombectomy (mTICI 2C) and patients with complete reperfusion (mTICI 3). Therefore, it is now commonly accepted that achieving mTICI 2C or 3 reperfusion should be the aim of reperfusion procedures. Moreover, complete reperfusion with a single pass of the thrombectomy device is an independent factor for favorable outcome and should also be aimed for in mechanical thrombectomy.[25] In the latest studies, mTICI 2C-3 is achieved in 54.5% to 79.5% of patients eligible for mechanical thrombectomy. Thus, there is still room for improvement to increase the rate of complete reperfusion.

Third, reperfusion strategies are associated with an increased risk of hemorrhagic transformation. Hemorrhagic transformation occurs in 4.4% of patients with a higher incidence within the first 24 h and a mortality reaching almost 50%. Importantly, the addition of mechanical thrombectomy to patients receiving intravenous thrombolysis is not associated with a significantly increased risk of hemorrhagic transformation.[9] Exploratory data, however, suggest that mechanical thrombectomy could favor hemorrhagic transformations in patient with large infarct at baseline imaging.[26] In addition, it is still unclear if intravenous thrombolysis significantly increases the risk of hemorrhagic transformation in patients treated by mechanical thrombectomy as observed in the SKIP and DEVT trials.

In this context, the expected advantage of adding pharmacological thrombolysis to mechanical thrombectomy would be to achieve earlier and more complete recanalization, especially when patient transfer to a comprehensive stroke center delays the start of the thrombectomy procedure, and hopefully without increasing the risk of hemorrhagic transformation. The lack of significant beneficial effects of intravenous thrombolysis in the DIRECT-MT, DEVT, and SKIP trials suggest however that refinement of existing pharmacological thrombolytic strategies will be necessary to significantly improve functional outcome over thrombectomy alone.

5 | THROMBOLYSIS TO ACHIEVE EARLY REPERFUSION

Early reperfusion is defined as reperfusion occurring before mechanical thrombectomy (Figure 1). According to a large meta-analysis including 1561 patients from 13 studies,[27] early reperfusion in patients eligible for mechanical thrombectomy occurs in one of 10 cases. Usually, these patients present a remarkably high rate of favorable outcome.[28] The main predictors for early reperfusion are the use of intravenous thrombolysis, the delay between thrombolysis and evaluation of vessel patency (larger delays are associated with higher reperfusion rates), thrombus length, and the occlusion site (distal occlusion is associated with a higher reperfusion rate).[29–31] Tandem occlusions, involving occlusion of both an extracranial and an intracranial artery (typically middle cerebral artery occlusion with proximal internal carotid artery occlusion), are associated with lower early reperfusion rate.[32] Notably, functional outcome in the DIRECT-MT study was not superior in patients treated by intravenous thrombolysis although early reperfusion was achieved more frequently.

A potential drawback of thrombolysis before thrombectomy is the risk of thrombus fragmentation and migration. This is observed by comparing the location of the thrombus on the first imaging (usually computed tomography or a magnetic resonance imaging) to the digital subtraction angiography acquired immediately before mechanical thrombectomy. In the MR-CLEAN registry including 1349 patients, the incidence of thrombus migration was 22%. Intravenous thrombolysis was associated with more thrombus migration (OR = 2.01 [1.29-3.11]) and thrombus migration was associated with a lower chance of complete reperfusion (OR = 0.57 [0.42-0.78]).[33] Even if it might be associated with worse reperfusion at the end of the endovascular procedure, thrombus migration after intravenous thrombolysis was associated with improved functional outcome in the two previously mentioned studies.[33] In the DEVT trial, thrombus migration occurred in 17.7% of the patients in the dMT group and in 23.9% in the IVT+MT group. In line with these results, a recent study including 314 patients found that 24.9% experienced thrombus migration after intravenous thrombolysis.[34] Importantly,

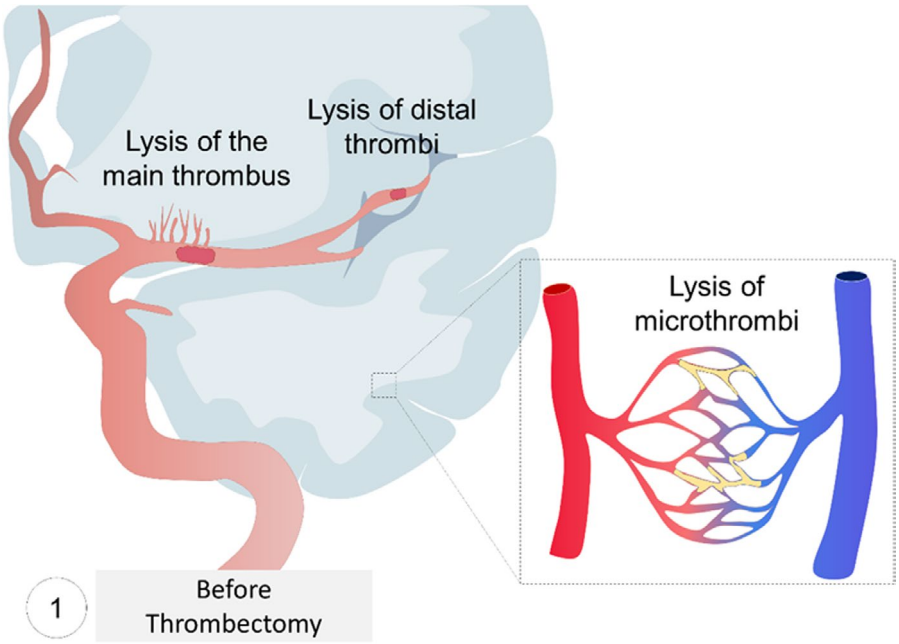


FIGURE 1 Schematic representation of the target of pharmacological thrombolysis before thrombectomy

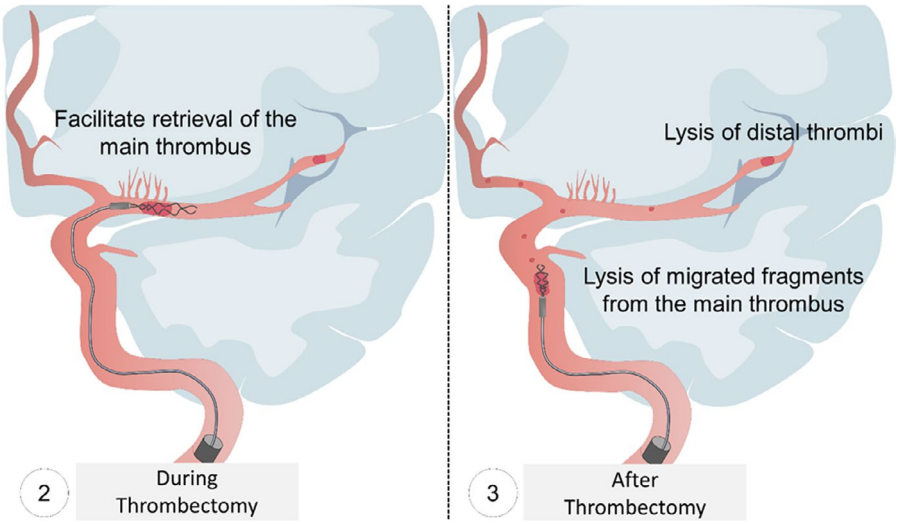


FIGURE 2 Schematic representation of the target of pharmacological thrombolysis during and after thrombectomy

in 41.3% of these patients, mechanical thrombectomy was not performed because of the inaccessibility of the thrombus. Only two of 229 patients who had received alteplase had complete recanalization without visible distal emboli. As underlined by these studies, thrombus migration can place the thrombus in a vessel too small for subsequent mechanical thrombectomy and also be associated with increased thrombus fragility and difficulty for complete retrieval.[35] Histological analysis of migrated thrombi showed that they tend to contain higher amounts of neutrophils and lymphocytes.[36,37]

Overall, thrombolysis using alteplase allows early reperfusion in one of 10 patients eligible for mechanical thrombectomy, especially when there is a large delay between thrombolysis and mechanical thrombectomy and when the occlusion is distal. This comes with a risk of thrombus migration, which is associated with a lower chance of complete reperfusion after subsequent mechanical thrombectomy.

6 | THROMBOLYSIS TO ACHIEVE COMPLETE REPERFUSION

Whether intravenous thrombolysis improves the rate of complete reperfusion at the end of the thrombectomy procedure remains unproven. The rational is that intravenous thrombolysis may both facilitate retrieval of the main thrombus by the endovascular device and promote lysis of distal thrombi inaccessible for mechanical thrombectomy (Figure 2). Regarding the effect on the main thrombus, discrepant results have been reported. A study-level meta-analysis including 5279 patients found no evidence that intravenous thrombolysis increases the rate of successful reperfusion after mechanical thrombectomy.[18] The authors explained previous discrepant results by the fact that some studies compared patients eligible with intravenous thrombolysis to ineligible patients, therefore

creating a selection bias. Moreover, in a study including only patients with complete reperfusion after mechanical thrombectomy, the use of intravenous thrombolysis was not associated with the number of thrombectomy passes.[25] In contrast, time from onset to reperfusion was a major predictor of complete reperfusion after only a single pass of the thrombectomy device. This parameter should be adequately adjusted in observational studies and might have constituted a bias in previous studies. None of the randomized trial demonstrated a statistically significant higher rate of successful reperfusion (TICI 2b or better) in IVT+MT vs. dMT (DIRECT-MT: 84.5% vs. 79.4%; DEVT: 87.2% vs. 88.5%; SKIP: 93.2% vs. 90.1%). Even if further evidence is required, especially in patients with large delay between intravenous thrombolysis and thrombectomy, the latest studies do not support a substantial effect of intravenous thrombolysis to facilitate thrombus retrieval during mechanical thrombectomy.

Because 6% to 26% of patients experience multifocal emboli with distal or remote occlusions,[4,38,39] intravenous thrombolysis may promote recanalization of vessels beyond the site of primary artery occlusion (Figure 1). It is also increasingly recognized that mechanical thrombectomy can fragmentate the main thrombus during retrieval, leading to secondary small or medium vessel occlusion.[40] The consequences of these iatrogenic distal occlusions could be mitigated by pharmacological thrombolysis. In line with this hypothesis, in the Endovascular Treatment for Small Core and Anterior Circulation Proximal Occlusion With Emphasis on Minimizing CT to Recanalization Times trial, the use of IVT was associated with a 68% reduction in the odds of infarct in a new previously unaffected territory.[41] Other subgroup analyses of randomized clinical trials will provide further answers to this question. Preclinical studies also suggest that thrombolysis may improve microvascular patency downstream of the main thrombus. Even in the absence of early recanalization of the main artery, thrombolysis could maintain a perfusion high enough to prolong the viability of the penumbra and thereby increase the benefit of large artery recanalization.[42] In rats, after transient monofilament middle cerebral artery occlusion, leukocytes and platelets accumulate in postcapillary venules. Intravenous thrombolysis using alteplase significantly increases percentage of perfused microvessels and reduces infarct size, at least in part by plasma fibrinogen depletion.[42,43] Although appealing, this hypothesis has not been demonstrated in human stroke and the DIRECT-MT, DEVT, and SKIP trials did not show additional benefit of intravenous thrombolysis over mechanical thrombectomy alone. Moreover, a recent clinical study failed to prove the existence of persistent distal microthrombosis in patients after mechanical thrombectomy.[44]

An alternative use of pharmacological thrombolysis in the context of mechanical thrombectomy is to perform intra-arterial injection of a fibrinolytic agent during or after the thrombectomy procedure to improve the quality of the reperfusion.[45] In a recent study in which the investigators used urokinase (a plasminogen activator) after a failed or incomplete mechanical thrombectomy in 100 patients, adjunctive treatment with intra-arterial urokinase during or after mechanical thrombectomy was safe and improved angiographic reperfusion.[46] In another observational cohort including 78 patients in whom use of the solitaire stent retriever failed to achieve successful reperfusion, intra-arterial fibrinolysis nonsignificantly improved the rate of revascularization success (61.2% vs. 46.6%, p = .13) at the cost of a numerically higher rate of symptomatic intracranial hemorrhage (13.9% vs. 6.8%, p = .29).[47] A recent meta-analysis of six observational cohort studies and three observational datasets including 2797 patients (405 with additional intra-arterial fibrinolytics) did not find an increased risk of symptomatic intracranial hemorrhage nor excess mortality after intra-arterial injection of a fibrinolytic agent.[48] Although included studies were heterogeneous, some studies found improved reperfusion. Recently, results from the Intra-arterial Fibrinolytics in Thrombectomy registry have been published.[49] Of 5612 patients screened, 311 received additional intra-arterial fibrinolysis during or after mechanical thrombectomy, mostly because of insufficient recanalization at the end of the procedure. In patients with available angiographic follow-up, 116 of 228 patients (50.9%) showed angiographic reperfusion improvement after intra-arterial fibrinolysis, which was associated with favorable outcome. Other case series reporting the use of intra-arterial fibrinolysis at various timepoints during or after mechanical thrombectomy have been published with positive effects on reperfusion rates, but additional randomized trials are mandatory to confirm these findings.[46,50,51]

7 | NEW FIBRINOLYTIC AGENTS

The data presented here suggest that using alteplase, the effect-size of additional thrombolysis in patients treated by mechanical thrombectomy will probably remain limited. Other clinically available fibrinolytics could be of interest in this indication. Accordingly, recent studies suggest that the choice of the thrombolytic agent influences the rate of early reperfusion. The Extending the time for Thrombolysis in Emergency Neurological Deficits-Intra-Arterial using Tenecteplase (EXTEND-IA TNK) trial showed twofold higher early recanalization rate before mechanical thrombectomy following intravenous thrombolysis with tenecteplase 0.25 mg/kg, compared with alteplase 0.9 mg/kg.[52,53] Tenecteplase is a recombinant plasminogen activator that has increased resistance to plasminogen activator inhibitor 1 (PAI-1), improved fibrin specificity, and enhanced half-life (Figure 3). Like alteplase, tenecteplase is a 527 amino acid glycoprotein. Tenecteplase has modifications at three sites of the protein structure: substitution of threonine 103 with asparagine, substitution of asparagine 117 with glutamine, both within the kringle 1 domain, and a tetra-alanine substitution at amino acids 296 to 299 in the protease domain. The enhanced half-life allows administration of the full tenecteplase dose as a bolus, whereas using alteplase, 10% of the total dose is administered as a bolus and 90% is infused over 60 min. In addition to its better fibrinolytic properties, the simpler route of administration of tenecteplase could translate in shorter delays between thrombolysis and mechanical thrombectomy. In the EXTEND-IA TNK trial, symptomatic intracranial hemorrhage occurred in 1% of the patients in each group, suggesting

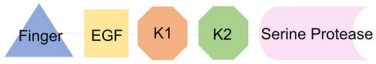
	Plasminogen Activator	Structure	Inhibitors	Other names	Administration route	Half-life	FS
Eucaryotic	Alteplase		PAI-1, PA1-2, Lysine analogues (TXA, EACA…), Protease Nexin 1, C1-inhibitor	tPA Actilyse	Bolus and infusion	3-4 minutes	++
Mutant	Tenecteplase		PAI-1, PA1-2, Lysine analogues	TNKase Metalyse	Single Bolus	20-24 minutes	+++

FIGURE 3 Comparison of alteplase and tenecteplase structures. EACA, ε-aminocaproic acid; EGF, epithelial growth factor; Finger, finger domain; FN, fibronectin domain; FS, fibrin specificity; K, Kringle domain; PAI, plasminogen-activator inhibitor; tPA, tissue-type plasminogen activator; TXA, tranexamic acid. Star indicates mutation/substitution. For the serine protease domain, the double star indicates a four amino acid substitution in the serine protease domain: Lys296Ala, Hys297Ala, Arg298Ala, Arg299Ala

that tenecteplase does not significantly increase the risk of hemorrhagic transformation compared to alteplase. Overall, tenecteplase resulted in a better functional outcome than alteplase at 90 days. Doses of 0.25 and 0.4 mg/kg have been tested in different trials, but no advantage of the higher dose has been demonstrated.[53,54] Current guidelines for stroke include intravenous tenecteplase at the 0.25 mg/kg dose for large-vessel occlusions as an alternative to alteplase. The results of ongoing randomized phase 3 trials are awaited to define the role of tenecteplase in thrombectomized patients. In contrast to alteplase and tenecteplase, reteplase, desmoteplase, streptokinase, and staphylokinase (all plasminogen activators) have not been investigated in the context of mechanical thrombectomy.

New recombinant fibrinolytic agents with potentially better efficacy and safety profiles are currently under development, such as opt-tPA.[55] Moreover, nanomedicine has made tremendous progresses over the past few years in the design of safer and more effective fibrinolytic agents.[56,57] Nanomedicine-based vectorization of plasminogen activators aims at protecting the fibrinolytic agent from enzymatic degradation and proteolytic inhibition, at optimizing its biodistribution to the thrombus by targeting strategies and thereby, at diminishing its adverse effect on hemostasis. These approaches have been recently and extensively reviewed elsewhere.[58] To date, no clinical trial of these promising fibrinolytic agents has been performed. Importantly, as a class effect, all plasminogen activators are susceptible to induce activation of the contact phase, including bradykinin generation, a mechanism suspected to play a role in angioedema and brain oedema formation after fibrinolysis, although higher fibrin specificity might mitigate this side effect.[59–61]

An alternative to the administration of a recombinant plasminogen activator is the injection of drugs targeting the inhibitors of the fibrinolytic pathway.[62] Recently, new profibrinolytic drugs targeting thrombin activatable fibrinolysis inhibitor (TAFI) have been developed. In its active form, TAFI inhibits the fibrinolytic pathway by removing tPA and plasminogen binding sites on the thrombus. Interestingly, the concentration of TAFI in cerebrospinal fluid during ischemic stroke is associated with stroke progression, worse outcome, and blood–brain barrier dysfunction.[63] In murine models of ischemic stroke, inhibition of TAFI with pharmacological inhibitors reduces microvascular thrombosis,[64] but failed to improve ischemic lesion size.[65] In contrast, the use of a heterodimer diabody targeting both TAFI and PAI-1 reduces brain damage in several rodent models of stroke.[66,67] Moreover, TAFI inhibition using exogenous administration of matrix metalloproteinase-10 (MMP-10) effectively reduces infarct size in experimental stroke by enhancing fibrinolysis, alone[68] or in combination with tPA.[69] The use of TAFI inhibitors should be used however with cautions according to experimental studies showing that TAFI deficiency results in increased brain damage in a model of thrombolysis after ischemic stroke, an effect associated with neuronal degeneration and cell-derived microparticle production.[70] Inhibitors of a2-antiplasmin, the major inhibitor of plasmin, have also been shown to promote fibrinolysis.[71]

8 | THROMBOLYSIS BEYOND FIBRINOLYSIS

Histological analyses of retrieved thrombi during mechanical thrombectomy for acute ischemic stroke have revealed that these thrombi are highly heterogeneous.[72,73] This heterogeneity probably arises from unique conditions of thrombus formation in each patient, depending for instance on the local rheological conditions. Intracranial thrombi are made of different combinations of platelets, red blood cells, fibrin, von Willebrand factor (VWF), and DNA (Figure 4). Notably, most thrombi display a core-shell structure, with an outer shell that impairs fibrinolysis.[74] Immunohistological analyses revealed that this outer shell is enriched in fibrin, platelets, VWF, and DNA originating from neutrophil extracellular traps. Therefore, alone or in combination with fibrinolysis, new thrombolytics targeting non-fibrin components of intracranial thrombi have the potential to increase the efficacy of pharmacological thrombolysis. Accordingly, recent clinical and experimental data point toward VWF and DNA as two promising targets for thrombolysis of intracranial thrombi.

VWF is a circulating multimeric protein made of several monomers linked together by disulfide bonds. It is implicated in platelet adhesion and subsequent aggregation, especially in conditions of high shear rates.[75] Accordingly, inhibition of the interaction between platelet GpIbα receptor and VWF in experimental models of occlusive thrombosis restores vessel patency and improves stroke outcome.[75,76] Several inhibitors of GpIbα-VWF interactions, targeting either GpIbα or the A1 domain of VWF, are under clinical

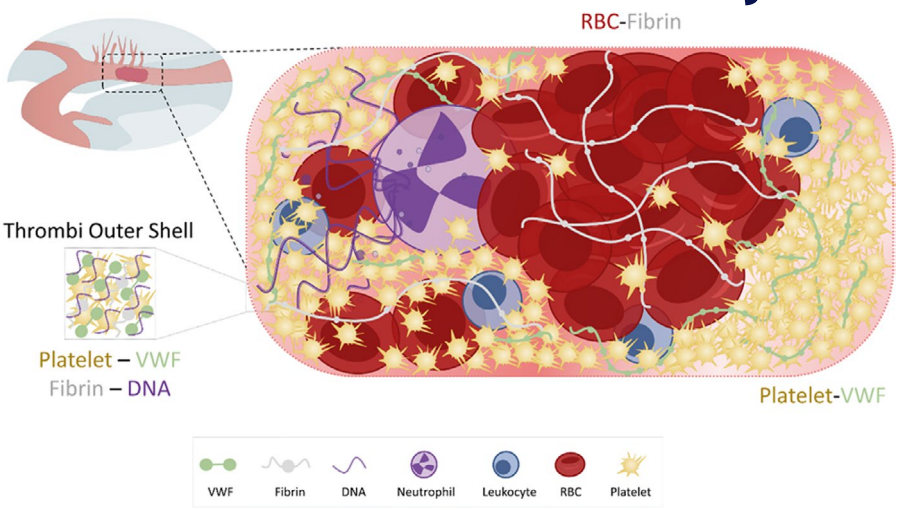


FIGURE 4 Schematic representation of the inner structure of intracranial thrombi according to histological analyses. RBC, red blood cells; VWF, von Willebrand factor

investigation,[77] including one already approved for the treatment of thrombotic thrombocytopenic purpura (caplacizumab, a divalent nanobody).[78] To date, there are no clinical data in stroke on these inhibitors. Several molecules have also been shown to cleave VWF, such as a disintegrin and metalloprotease with thrombospondin type 1 repeats 13 (ADAMTS-13) and N-acetylcysteine. ADAMTS-13 is the endogenous protease responsible for the degradation of VWF multimers. In an experimental model of stroke with tPA-resistant thrombus, intravenous injection of recombinant ADAMTS-13 promotes thrombus dissolution and improves outcome.[79] A similar effect has been observed using a variant of ADAMTS-13 displaying an enhanced proteolytic activity.[80] Importantly, no significant hemorrhagic transformation was observed using ADAMTS-13 and a preclinical study even showed that ADAMTS-13 reduces tPA-induced hemorrhage after stroke.[81] As a cheaper and already clinically available alternative, N-acetylcysteine is also able to reduces the size and activity of VWF by reducing the disulfide bonds inside VWF multimers.[82] In three experimental models of stroke in mice, N-acetylcysteine was shown to restore vessel patency, reduce ischemic lesion size, and improve neurological outcome.[83] The dose administered to observe this thrombolytic effect (400 mg/kg) was only slightly superior to the dose of N-acetylcysteine used to treat acetaminophen poisoning in humans (300 mg/kg). Importantly, N-acetylcysteine neither induced hemorrhagic transformation nor worsened preexisting brain hemorrhage in murine models. Moreover, in a randomized controlled clinical trial in patients with myocardial infarction, intravenous N-acetylcysteine administered with low-dose intravenous nitroglycerin was associated with reduced infarct size and doubled myocardial salvage.[84] Clinical trials in stroke patients are awaited.

Histological analyses of intracranial thrombi also revealed large amount of extracellular DNA, especially in the thrombus shell.[74] Remarkably, the presence of citrullinated histone H3, a hallmark of neutrophil extracellular traps, is observed in most thrombi, suggesting that this extracellular DNA comes from the nucleus of neutrophils. In a study involving 68 thrombi, citrullinated histone H3-positive area represented up to 13.45% of total thrombus area.[85] Interestingly, this extracellular DNA seems to inhibit alteplase induced fibrinolysis as evidenced by at least three studies showing that *ex vivo* lysis of patient thrombi was faster when adding DNase 1 to tPA.[85–87] Moreover, in a mouse photothrombotic stroke model, administration of DNase 1 recanalized the occluded vessel, whereas tPA was ineffective.[86] Altogether, these data suggest that DNase could be used as a thrombolytic agent in acute ischemic stroke. It is in theory possible to administer a cocktail of thrombolytics including a fibrinolytic, a VWF-degrading molecules and DNase for instance, to maximize the reperfusion rate. The role of antithrombotic agents such as anti-P2Y12, anti-GpIIbIIIa, or anti-GpIbα also remains to be investigated in the context of mechanical thrombectomy.

9 | CONCLUSION

At the cost of a potential increased risk of hemorrhagic transformation, pharmacological thrombolysis may promote arterial recanalization before thrombectomy, improve the rate of successful recanalization after thrombectomy, and restore microcirculation patency downstream of the main thrombus. However, the available results of the first randomized clinical trials suggest that current pharmacological thrombolysis using alteplase does not improve clinical outcome in thrombectomized patients and does not increase the rate of successful reperfusion. New thrombolytic strategies targeting alteplase-resistant thrombi are being developed such as tenecteplase, ADAMTS-13, N-acetylcysteine, or DNase. These new thrombolytic agents could strengthen the beneficial effects of thrombolysis without carrying additional pro-hemorrhagic effects. New combined strategies, improved thrombolytics, and dedicated clinical trials in selected patients are eagerly awaited to further improve functional outcome in stroke.

ACKNOWLEDGMENTS

D. V. holds patents but does not receive royalties on Optimised tPA (licensed US9249406 B2), and on the TAFIa/PAI-1 diabody (licensed GB1404879).

CONFLICT OF INTEREST

All other authors declare no competing interests.

AUTHOR CONTRIBUTIONS

All authors contributed equally to this manuscript.

TWITTER

Denis Vivien @Vivienslab1

REFERENCES

1. Campbell BCV, Khatri P. Stroke. *Lancet*. 2020;396:129-142.
2. Powers WJ. Acute ischemic stroke. *N Engl J Med*. 2020;383:252-260.
3. Powers WJ, Rabinstein AA, Ackerson T, et al. Guidelines for the early management of patients with acute ischemic stroke: 2019 update to the 2018 guidelines for the early management of acute ischemic stroke: a guideline for healthcare professionals from the American Heart Association/American Stroke Association. *Stroke*. 2019;50:e344-e418.
4. Siegler JE, Jovin TG. Thrombolysis before thrombectomy in acute large vessel occlusion: a risk/benefit assessment and review of the evidence. *Curr Treat Options Neurol*. 2020;22:22.
5. National Institute of Neurological D, Stroke rt PASSG. Tissue plasminogen activator for acute ischemic stroke. *N Engl J Med*. 1995;333:1581-1587.
6. Thiebaut AM, Gauberti M, Ali C, et al. The role of plasminogen activators in stroke treatment: fibrinolysis and beyond. *Lancet Neurol*. 2018;17:1121-1132.
7. Sandercock P, Wardlaw JM, Lindley RI, et al. The benefits and harms of intravenous thrombolysis with recombinant tissue plasminogen activator within 6 h of acute ischaemic stroke (the third international stroke trial [IST-3]): a randomised controlled trial. *Lancet*. 2012;379:2352-2363.
8. Bhatia R, Hill MD, Shobha N, et al. Low rates of acute recanalization with intravenous recombinant tissue plasminogen activator in ischemic stroke: real-world experience and a call for action. *Stroke*. 2010;41:2254-2258.
9. Goyal M, Menon BK, van Zwam WH, et al. Endovascular thrombectomy after large-vessel ischaemic stroke: a meta-analysis of individual patient data from five randomised trials. *Lancet*. 2016;387:1723-1731.
10. Nogueira RG, Jadhav AP, Haussen DC, et al. Thrombectomy 6 to 24 hours after stroke with a mismatch between deficit and infarct. *N Engl J Med*. 2018;378:11-21.
11. Albers GW, Marks MP, Kemp S, et al. Thrombectomy for stroke at 6 to 16 hours with selection by perfusion imaging. *N Engl J Med*. 2018;378:708-718.
12. Zhu F, Gauberti M, Marnat G, et al. Time from I.V. thrombolysis to thrombectomy and outcome in acute ischemic stroke. *Ann Neurol*. 2021;89:511–519. https://doi.org/10.1002/ana.25978
13. Yang P, Zhang Y, Zhang L, et al. Endovascular thrombectomy with or without intravenous alteplase in acute stroke. *N Engl J Med*. 2020;382:1981-1993.
14. Suzuki K, Matsumaru Y, Takeuchi M, et al. Effect of mechanical thrombectomy without vs with intravenous thrombolysis on functional outcome among patients with acute ischemic stroke: the SKIP Randomized Clinical Trial. *JAMA*. 2021;325:244-253.
15. Zi W, Qiu Z, Li F, et al. Effect of endovascular treatment alone vs intravenous alteplase plus endovascular treatment on functional independence in patients with acute ischemic stroke: the DEVT Randomized Clinical Trial. *JAMA*. 2021;325:234-243.
16. Fan L, Zang L, Liu X, Wang J, Qiu J, Wang Y. Outcomes of mechanical thrombectomy with pre-intravenous thrombolysis: a systematic review and meta-analysis. *J Neurol*. 2020. https://doi.org/10.1007/s00415-020-09778-4. Online ahead of print.
17. Fischer U, Kaesmacher J, Mendes Pereira V, et al. Direct mechanical thrombectomy versus combined intravenous and mechanical thrombectomy in large-artery anterior circulation stroke: a topical review. *Stroke*. 2017;48:2912-2918.
18. Katsanos AH, Malhotra K, Goyal N, et al. Intravenous thrombolysis prior to mechanical thrombectomy in large vessel occlusions. *Ann Neurol*. 2019;86:395-406.
19. Mistry EA, Mistry AM, Nakawah MO, et al. Mechanical thrombectomy outcomes with and without intravenous thrombolysis in stroke patients: a meta-analysis. *Stroke*. 2017;48:2450-2456.
20. Hassan AE, Ringheanu VM, Preston L, Tekle W, Qureshi AI. IV tPA is associated with increase in rates of intracerebral hemorrhage and length of stay in patients with acute stroke treated with endovascular treatment within 4.5 hours: should we bypass IV tPA in large vessel occlusion? *J Neurointerv Surg*. 2021;13:114–118. https://doi.org/10.1136/neurintsurg-2020-016045
21. Ospel JM, McTaggart R, Kashani N, Psychogios M, Almekhlafi M, Goyal M. Evolution of stroke thrombectomy techniques to optimize first-pass complete reperfusion. *Semin Intervent Radiol*. 2020;37:119-131.
22. Liebeskind DS, Bracard S, Guillemin F, et al. eTICI reperfusion: defining success in endovascular stroke therapy. *J Neurointerv Surg*. 2019;11:433-438.
23. Dargazanli C, Fahed R, Blanc R, et al. Modified thrombolysis in cerebral infarction 2C/Thrombolysis in cerebral infarction 3 reperfusion should be the aim of mechanical thrombectomy: insights from the ASTER trial (contact aspiration versus stent retriever for successful revascularization). *Stroke*. 2018;49:1189-1196.
24. Tung EL, McTaggart RA, Baird GL, et al. Rethinking thrombolysis in cerebral infarction 2b: which thrombolysis in cerebral infarction scales best define near complete recanalization in the modern thrombectomy era? *Stroke*. 2017;48:2488-2493.
25. Nikoubashman O, Dekeyzer S, Riabikin A, et al. True first-pass effect. *Stroke*. 2019;50:2140-2146.
26. Román LS, Menon BK, Blasco J, et al. Imaging features and safety and efficacy of endovascular stroke treatment: a meta-analysis of individual patient-level data. *Lancet Neurol*. 2018;17:895-904.
27. Tsivgoulis G, Katsanos AH, Schellinger PD, et al. Successful reperfusion with intravenous thrombolysis preceding mechanical thrombectomy in large-vessel occlusions. *Stroke*. 2018;49:232-235.
28. Serna Candel C, Aguilar Pérez M, Hellstern V, AIMatter M, Bäzner H, Henkes H. Recanalization of emergent large intracranial vessel occlusion through intravenous thrombolysis: frequency, clinical outcome, and reperfusion pattern. *Cerebrovasc Dis*. 2019;48:115-123.
29. Seners P, Turc G, Naggara O, et al. Post-thrombolysis recanalization in stroke referrals for thrombectomy: incidence, predictors, and prediction scores. *Stroke*. 2018;49:2975-2982.
30. Seners P, Turc G, Maïer B, Mas JL, Oppenheim C, Baron JC. Incidence and predictors of early recanalization after intravenous thrombolysis: a systematic review and meta-analysis. *Stroke*. 2016;47:2409-2412.
31. Seners P, Delepierre J, Turc G, et al. Thrombus length predicts lack of post-thrombolysis early recanalization in minor stroke with large vessel occlusion. *Stroke*. 2019;50:761-764.
32. Saqqur M, Uchino K, Demchuk AM, et al. Site of arterial occlusion identified by transcranial Doppler predicts the response to intravenous thrombolysis for stroke. *Stroke*. 2007;38:948-954.
33. Alves HC, Treurniet KM, Jansen IGH, et al. Thrombus migration paradox in patients with acute ischemic stroke. *Stroke*. 2019;50:3156-3163.
34. Flint AC, Avins AL, Eaton A, et al. Risk of distal embolization from tPA (tissue-type plasminogen activator) administration prior to endovascular stroke treatment. *Stroke*. 2020;51:2697-2704.
35. Kaesmacher J, Maegerlein C, Kaesmacher M, et al. Thrombus migration in the middle cerebral artery: incidence, imaging signs, and impact on success of endovascular thrombectomy. *J Am Heart Assoc*. 2017;6:e005149.

36. Kaesmacher J, Boeckh-Behrens T, Simon S, et al. Risk of thrombus fragmentation during endovascular stroke treatment. *AJNR Am J Neuroradiol*. 2017;38:991-998.
37. Goebel J, Gaida BJ, Wanke I, et al. Is histologic thrombus composition in acute stroke linked to stroke etiology or to interventional parameters? *AJNR Am J Neuroradiol*. 2020;41:650-657.
38. Bracard S, Ducrocq X, Mas JL, et al. Mechanical thrombectomy after intravenous alteplase versus alteplase alone after stroke (THRACE): a randomised controlled trial. *Lancet Neurol*. 2016;15:1138-1147.
39. Berkhemer OA, Fransen PS, Beumer D, et al. A randomized trial of intraarterial treatment for acute ischemic stroke. *N Engl J Med*. 2015;372:11-20.
40. Goyal M, Kappelhof M, McDonough R, Ospel JM. Secondary medium vessel occlusions: when clots move north. *Stroke*. 2021;52:1147-1153.
41. Ganesh A, Al-Ajlan FS, Sabiq F, et al. Infarct in a new territory after treatment administration in the ESCAPE Randomized Controlled Trial (endovascular treatment for small core and anterior circulation proximal occlusion with emphasis on minimizing CT to recanalization times). *Stroke*. 2016;47:2993-2998.
42. Desilles JP, Loyau S, Syvannarath V, et al. Alteplase reduces downstream microvascular thrombosis and improves the benefit of large artery recanalization in stroke. *Stroke*. 2015;46:3241-3248.
43. Desilles JP, Syvannarath V, Di Meglio L, et al. Downstream microvascular thrombosis in cortical venules is an early response to proximal cerebral arterial occlusion. *J Am Heart Assoc*. 2018;7:e007804.
44. Schiphorst AT, Charron S, Hassen WB, et al. Tissue no-reflow despite full recanalization following thrombectomy for anterior circulation stroke with proximal occlusion: a clinical study. *J Cereb Blood Flow Metab*. 2020;41:253-266.
45. Castonguay AC, Jumaa MA, Zaidat OO, et al. Insights into intra-arterial thrombolysis in the modern era of mechanical thrombectomy. *Front Neurol*. 2019;10:1195.
46. Kaesmacher J, Bellwald S, Dobrocky T, et al. Safety and efficacy of intra-arterial urokinase after failed, unsuccessful, or incomplete mechanical thrombectomy in anterior circulation large-vessel occlusion stroke. *JAMA Neurol*. 2020;77:318-326.
47. Zaidi SF, Castonguay AC, Jumaa MA, et al. Intraarterial thrombolysis as rescue therapy for large vessel occlusions. *Stroke*. 2019;50:1003-1006.
48. Kaesmacher J, Meinel TR, Kurmann C, et al. Safety and efficacy of intra-arterial fibrinolytics as adjunct to mechanical thrombectomy: a systematic review and meta-analysis of observational data. *J Neurointerv Surg*. 2021. https://doi.org/10.1136/neurintsurg-2020-016680. Online ahead of print.
49. Kaesmacher J, Abdullayev N, Maamari B, et al. Safety and angiographic efficacy of intra-arterial fibrinolytics as adjunct to mechanical thrombectomy: results from the INFINITY registry. *J Stroke*. 2021;23:91-102.
50. Heiferman DM, Li DD, Pecoraro NC, Smolenski AM, Tsimpas A, Ashley WW Jr. Intra-arterial alteplase thrombolysis during mechanical thrombectomy for acute ischemic stroke. *J Stroke Cerebrovasc Dis*. 2017;26:3004-3008.
51. Yi TY, Chen WH, Wu YM, Zhang MF, Lin DL, Lin XH. Adjuvant intra-arterial rt-PA injection at the initially deployed solitaire stent enhances the efficacy of mechanical thrombectomy in acute ischemic stroke. *J Neurol Sci*. 2018;386:69-73.
52. Campbell BCV, Mitchell PJ, Churilov L, et al. Tenecteplase versus alteplase before thrombectomy for ischemic stroke. *N Engl J Med*. 2018;378:1573-1582.
53. Campbell BCV, Mitchell PJ, Churilov L, et al. Effect of intravenous tenecteplase dose on cerebral reperfusion before thrombectomy in patients with large vessel occlusion ischemic stroke: the EXTEND-IA TNK Part 2 Randomized Clinical Trial. *JAMA*. 2020;323:1257-1265.
54. Warach SJ, Dula AN, Milling TJ Jr. Tenecteplase thrombolysis for acute ischemic stroke. *Stroke*. 2020;51:3440-3451.
55. Goulay R, Naveau M, Gaberel T, Vivien D, Parcq J. Optimized tPA: a non-neurotoxic fibrinolytic agent for the drainage of intracerebral hemorrhages. *J Cereb Blood Flow Metab*. 2018;38:1180-1189.
56. Bonnard T, Gauberti M, Martinez de Lizarrondo S, Campos F, Vivien D. Recent advances in nanomedicine for ischemic and hemorrhagic stroke. *Stroke*. 2019;50:1318-1324.
57. Disharoon D, Marr DWM, Neeves KB. Engineered microparticles and nanoparticles for fibrinolysis. *J Thromb Haemost*. 2019;17:2004-2015.
58. Zenych A, Fournier L, Chauvierre C. Nanomedicine progress in thrombolytic therapy. *Biomaterials*. 2020;258:120297.
59. Marcos-Contreras OA, Martinez de Lizarrondo S, Bardou I, et al. Hyperfibrinolysis increases blood-brain barrier permeability by a plasmin- and bradykinin-dependent mechanism. *Blood*. 2016;128:2423-2434.
60. Gauberti M, Potzeha F, Vivien D, Martinez de Lizarrondo S. Impact of bradykinin generation during thrombolysis in ischemic stroke. *Front Med*. 2018;5:195.
61. Simao F, Ustunkaya T, Clermont AC, Feener EP. Plasma kallikrein mediates brain hemorrhage and edema caused by tissue plasminogen activator therapy in mice after stroke. *Blood*. 2017;129:2280-2290.
62. Henderson SJ, Weitz JI, Kim PY. Fibrinolysis: strategies to enhance the treatment of acute ischemic stroke. *J Thromb Haemost*. 2018;16:1932-1940.
63. Mertens JC, Leenaerts D, Brouns R, et al. Procarboxypeptidase U (proCPU, TAFI, proCPB2) in cerebrospinal fluid during ischemic stroke is associated with stroke progression, outcome and blood-brain barrier dysfunction. *J Thromb Haemost*. 2018;16:342-348.
64. Mertens JC, Boisseau W, Leenaerts D, et al. Selective inhibition of carboxypeptidase U may reduce microvascular thrombosis in rat experimental stroke. *J Thromb Haemost*. 2020;18:3325-3335.
65. Durand A, Chauveau F, Cho TH, et al. Effects of a TAFI-inhibitor combined with a suboptimal dose of rtPA in a murine thromboembolic model of stroke. *Cerebrovasc Dis*. 2014;38:268-275.
66. Denorme F, Wyseure T, Peeters M, et al. Inhibition of thrombin-activatable fibrinolysis inhibitor and plasminogen activator inhibitor-1 reduces ischemic brain damage in mice. *Stroke*. 2016;47:2419-2422.
67. Wyseure T, Rubio M, Denorme F, et al. Innovative thrombolytic strategy using a heterodimer diabody against TAFI and PAI-1 in mouse models of thrombosis and stroke. *Blood*. 2015;125:1325-1332.
68. Orbe J, Barrenetxe J, Rodriguez JA, et al. Matrix metalloproteinase-10 effectively reduces infarct size in experimental stroke by enhancing fibrinolysis via a thrombin-activatable fibrinolysis inhibitor-mediated mechanism. *Circulation*. 2011;124:2909-2919.
69. Roncal C, Martinez de Lizarrondo S, Salicio A, et al. New thrombolytic strategy providing neuroprotection in experimental ischemic stroke: MMP10 alone or in combination with tissue-type plasminogen activator. *Cardiovasc Res*. 2017;113:1219-1229.
70. Orbe J, Alexandru N, Roncal C, et al. Lack of TAFI increases brain damage and microparticle generation after thrombolytic therapy in ischemic stroke. *Thromb Res*. 2015;136:445-450.
71. Reed GL, Houng AK, Singh S, Wang D. α2-Antiplasmin: new insights and opportunities for ischemic stroke. *Semin Thromb Hemost*. 2017;43:191-199.
72. Sporns PB, Hanning U, Schwindt W, et al. Ischemic stroke: what does the histological composition tell us about the origin of the thrombus? *Stroke*. 2017;48:2206-2210.
73. De Meyer SF, Andersson T, Baxter B, et al. Analyses of thrombi in acute ischemic stroke: a consensus statement on current knowledge and future directions. *Int J Stroke*. 2017;12:606-614.
74. Di Meglio L, Desilles JP, Ollivier V, et al. Acute ischemic stroke thrombi have an outer shell that impairs fibrinolysis. *Neurology*. 2019;93:e1686-e1698.

75. Le Behot A, Gauberti M, Martinez De Lizarrondo S, et al. GpIbα-VWF blockade restores vessel patency by dissolving platelet aggregates formed under very high shear rate in mice. *Blood*. 2014;123:3354-3363.
76. Momi S, Tantucci M, Van Roy M, Ulrichts H, Ricci G, Gresele P. Reperfusion of cerebral artery thrombosis by the GPIb-VWF blockade with the nanobody ALX-0081 reduces brain infarct size in guinea pigs. *Blood*. 2013;121:5088-5097.
77. Prasannan N, Scully M. Novel antiplatelet strategies targeting VWF and GPIb. *Platelets*. 2021;32:42-46.
78. Knoebl P, Cataland S, Peyvandi F, et al. Efficacy and safety of open-label caplacizumab in patients with exacerbations of acquired thrombotic thrombocytopenic purpura in the HERCULES study. *J Thromb Haemost*. 2020;18:479-484.
79. Denorme F, Langhauser F, Desender L, et al. ADAMTS13-mediated thrombolysis of t-PA-resistant occlusions in ischemic stroke in mice. *Blood*. 2016;127:2337-2345.
80. South K, Denorme F, Salles C II, De Meyer SF, Lane DA. Enhanced activity of an ADAMTS-13 variant (R568K/F592Y/R660K/Y661F/Y665F) against platelet agglutination in vitro and in a murine model of acute ischemic stroke. *J Thromb Haemost*. 2018;16:2289-2299.
81. Wang L, Fan W, Cai P, et al. Recombinant ADAMTS13 reduces tissue plasminogen activator-induced hemorrhage after stroke in mice. *Ann Neurol*. 2013;73:189-198.
82. Chen J, Reheman A, Gushiken FC, et al. N-acetylcysteine reduces the size and activity of von Willebrand factor in human plasma and mice. *J Clin Invest*. 2011;121:593-603.
83. Martinez de Lizarrondo S, Gakuba C, Herbig BA, et al. Potent thrombolytic effect of N-acetylcysteine on arterial thrombi. *Circulation*. 2017;136:646-660.
84. Pasupathy S, Tavella R, Grover S, et al. Early use of N-acetylcysteine with nitrate therapy in patients undergoing primary percutaneous coronary intervention for ST-segment-elevation myocardial infarction reduces myocardial infarct size (the NACIAM Trial [N-acetylcysteine in Acute Myocardial Infarction]). *Circulation*. 2017;136:894-903.
85. Laridan E, Denorme F, Desender L, et al. Neutrophil extracellular traps in ischemic stroke thrombi. *Ann Neurol*. 2017;82:223-232.
86. Peña-Martínez C, Durán-Laforet V, García-Culebras A, et al. Pharmacological modulation of neutrophil extracellular traps reverses thrombotic stroke tPA (tissue-type plasminogen activator) resistance. *Stroke*. 2019;50:3228-3237.
87. Ducroux C, Di Meglio L, Loyau S, et al. Thrombus neutrophil extracellular traps content impair tPA-induced thrombolysis in acute ischemic stroke. *Stroke*. 2018;49:754-757.

How to cite this article: Gauberti M, Martinez de Lizarrondo S, Vivien D. Thrombolytic strategies for ischemic stroke in the thrombectomy era. *J Thromb Haemost*. 2021;19:1618–1628. https://doi.org/10.1111/jth.15336