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STATE OF THE ART

Thrombus composition and thrombolysis resistance in stroke

Benoit Ho-Tin-Noé PhD¹ | Jean-Philippe Desilles MD, PhD^{1,2} |
Mikael Mazighi MD, PhD^{1,2}

¹Université Paris Cité, Inserm, Optimisation Thérapeutique en Neuropsychopharmacologie, Paris, France

²Interventional Neuroradiology Department and Biological Resources Center, Rothschild Foundation Hospital, Paris, France

Correspondence

Benoit Ho-Tin-Noé, PhD Inserm UMR-S 1144, Optimisation Thérapeutique en Neuropsychopharmacologie, Faculté de Pharmacie, Université Paris Cité 4 Avenue de l'Observatoire, 75006 Paris, France.
Email: benoit.ho-tin-noe@inserm.fr

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Abstract

A State of the Art lecture titled “*Thrombus Composition and Thrombolysis Resistance in Stroke*” was presented at the ISTH Congress in 2022. Intravenous thrombolysis (IVT) remains the only pharmacologic option to re-establish cerebral perfusion at the acute phase of ischemic stroke. IVT is based on the administration of recombinant tissue plasminogen activator with the objective of dissolving fibrin, the major fibrillar protein component of thrombi. Almost 30 years on from its introduction, although the clinical benefits of IVT have been clearly demonstrated, IVT still suffers from a relatively low efficacy, with a rate of successful early recanalization below 50% overall. Analyses of thrombectomy-recovered acute ischemic stroke (AIS) thrombi have shown that apart from occlusion site, thrombus length, and collateral status, AIS thrombus structure and composition are also important modulators of IVT efficacy. In this article, after a brief presentation of IVT principle and current knowledge on IVT resistance, we review recent findings on how compaction and structural alterations of fibrin together with nonfibrin thrombus components such as neutrophil extracellular traps and von Willebrand factor interfere with IVT in AIS. We further discuss how these new insights could soon result in the development of original adjuvant therapies for improved IVT in AIS. Finally, we summarize relevant new data presented during the 2022 ISTH Congress.

KEYWORDS

acute ischemic stroke, fibrinolysis, neutrophil extracellular traps, thrombolysis, thrombolysis resistance, thrombus composition

Essentials

- Thrombi causing the occlusion of a large intracranial artery are often resistant to tissue plasminogen activator (t-PA).
- Analyses of thrombi recovered by mechanical thrombectomy have helped understand t-PA resistance.
- Resistance to t-PA involves structural alterations of fibrin and nonfibrin fibrillar components.
- Pharmacologic targeting of nonfibrin thrombus components may help overcome tPA resistance.

1 | INTRODUCTION

Before the introduction of intravenous thrombolysis (IVT) with recombinant tissue plasminogen activator (rt-PA) for acute ischemic

stroke (AIS) in the 1990s [1–5] and its approval by the United States Food and Drug Administration in 1996, stroke management had been focused on poststroke secondary prevention and rehabilitation. By opening the way to emergency interventions at the acute phase of

ischemic stroke, IVT has revolutionized stroke care. The rationale and objective of IVT are straight forward: to re-establish cerebral perfusion by dissolving the thrombus at the origin of the intracranial arterial occlusion and brain ischemia, via the administration of a drug targeting one of the thrombus cement elements, fibrin. Almost 30 years on from its introduction, although the benefits of IVT have been clearly demonstrated (ie, an increase in the proportion of patients with better neurologic and functional outcome) [1,6], IVT has suffered several significant limitations that have hindered its efficacy and broader use. These limitations include a narrow therapeutic window (within 4.5 hours after stroke onset) [7], a moderate rate of early recanalization efficacy (30%-40% overall and below 10% toward the most proximal occlusions) [4,8,9], and an associated increased risk of hemorrhagic complications [1,10].

The more recent development of mechanical thrombectomy (MT) as an alternative or adjunct recanalization therapy for AIS due to large vessel occlusion (LVO) has partly overcome the limitations of IVT. MT offers a widened therapeutic window (6 hours), achieves tremendous rates of successful recanalization (over 75%), and presents no inherent risk of hemorrhage [11–17]. Nonetheless, MT has limitations that prevent its widespread use and positioning as a universal and ultimate recanalization therapy for AIS. MT is indicated only for patients with AIS due to LVO in the anterior circulation (20%-30% of AIS) [18–20], and it requires access to specialized stroke centers with trained neurointerventionalists. Therefore, despite the progress brought by MT, there is still a significant potential and critical need for improvement of recanalization therapy, which remains the therapeutic cornerstone for improved functional outcomes and reduced mortality in AIS [21].

Interestingly, by enabling the collection of AIS thrombi, MT has provided the unique opportunity to study their composition, structure, and mechanical properties and to determine whether and how these parameters relate to each other and to various clinical variables, including etiology, time from onset, imaging, outcome, and response to recanalization therapies [22–31]. With respect to the latter aspect of treatment response, recent analyses of MT-retrieved thrombi have provided new information on the mechanisms underlying IVT resistance in AIS, which could be instrumental in designing more effective IVT strategies that would benefit a wider patient population.

In this review, after a brief overview of the fundamental basis of IVT and notion of IVT resistance, we present recent advances in the understanding of the impact of AIS thrombus composition and structure on IVT efficacy and discuss promising therapeutic leads derived from these insights for improved IVT in AIS.

2 | BASICS OF INTRAVENOUS THROMBOLYSIS IN ACUTE ISCHEMIC STROKE

IVT is based on the administration of rt-PA, a serine protease whose proteolytic action allows the conversion of the single-chain proenzyme plasminogen into the active 2-chain plasmin, an enzyme with

broad specificity but that is primarily responsible for fibrin degradation. Thrombolysis thus relies on promoting a natural process, fibrinolysis, with the intent of targeting a single but major component of the thrombus fibrillar network, fibrin.

For optimal conversion of plasminogen into plasmin by t-PA, both plasminogen and t-PA must be bound to the fibrin surface (Figure 1). Fibrin indeed provides a template for the formation of a ternary activation complex between t-PA, plasminogen, and fibrin [32–34], before being subsequently degraded by plasmin. Assembly of this ternary activation complex is made possible by the fact that plasminogen and t-PA possess specific binding domains for fibrin: the plasminogen kringle 1 and 4 domains and the t-PA finger and kringle 2 domains. Binding of plasminogen and t-PA to fibrin not only concentrates them and brings them in proximity, but it also induces conformational changes in both plasminogen [35–37] and t-PA [38,39] that further promote their interaction (Figure 1). Fibrin thus acts as a cofactor for t-PA and improves considerably its catalytic efficiency (k_{cat}/K_m) toward plasminogen, which is much lesser in the presence of fibrinogen and poor in the fluid phase [33,34,40]. Because of these mechanistic features, at physiological blood concentration of plasminogen (1.5–2 μ M), t-PA-mediated plasminogen activation occurs preferentially on fibrin surfaces. This reduces the risk of systemic plasminogen activation and uncontrolled proteolysis of fibrinogen and other circulating proteins by plasmin. Another mechanism that helps confine plasmin activity to the thrombus microenvironment is the instantaneous inhibition of free plasmin by the fast-reacting serpin α 2-antiplasmin, against which fibrin-bound plasmin is protected [4].

Importantly, the cofactor effect of fibrin toward plasminogen activation has long been known to be specific for t-PA and to not apply to other plasminogen activators such as urokinase and streptokinase [40]. The fibrin-selective character of t-PA has made of its recombinant form, rt-PA, the thrombolytic agent of choice for AIS. As compared with t-PA, urokinase and streptokinase display a less-favorable fibrinolytic/fibrinogenolytic activity ratio, and therefore an increased theoretical risk of systemic plasminogen activation, fibrinogenolysis, and α 2-antiplasmin exhaustion [41,42], all of which undesired side effects can favor intracranial hemorrhage, the most devastating complication of AIS [43].

In AIS, rt-PA is classically administered as an initial 10% intravenous bolus over 1 minute followed by the continuous intravenous infusion of the remainder over 60 minutes for a total dose of 0.9 mg/kg (maximum dose, 90 mg; Figure 1) [44]. This high dose administration protocol is necessary to allow a continuous supply of active rt-PA to the thrombus for over an hour, because rt-PA is rapidly inactivated by endogenous plasminogen activator inhibitor 1 (PAI-1) and has a short initial plasma half-life (<6 minutes), and a terminal elimination half-life of 26 to 72 minutes [45–47]. Delays between bolus and infusion initiation may occur, which might affect rt-PA concentration and efficacy [44,48,49]. Tenecteplase, a genetically modified variant of rt-PA with increased fibrin specificity and plasma half-life (22 minutes), as well as a reduced susceptibility to PAI-1 [47], is emerging as a more convenient alternative to rt-PA. Because of its improved pharmacokinetics and activity compared with rt-PA, tenecteplase can

Intravenous Thrombolysis for Acute Ischemic Stroke

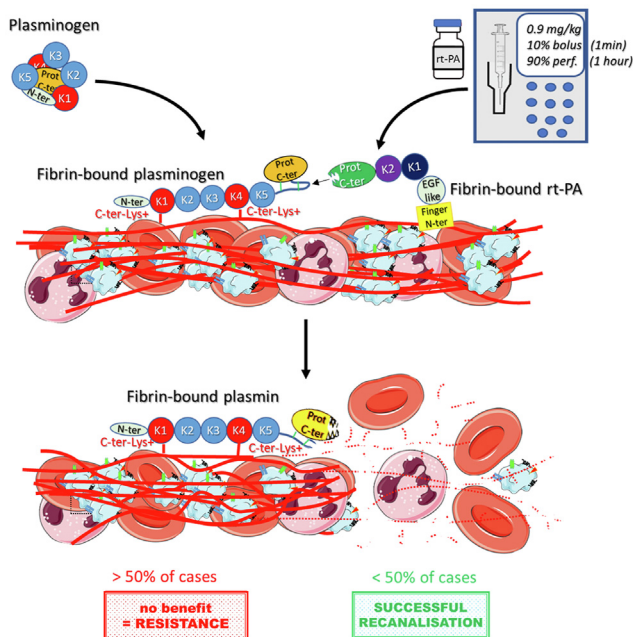


FIGURE 1 Schematic representation of the principle of intravenous thrombolysis for the treatment of acute ischemic stroke. C-ter, C-terminal; EGF-like, epidermal growth factor (EGF)-like domain; Lys, lysine; N-ter, N-terminal; perf, perfusion; Prot, protease domain; rt-PA, recombinant tissue plasminogen activator.

be administered as a single bolus and does not require intravenous infusion [50]. Several regimens of tenecteplase have been tested, mainly 0.1, 0.25, and 0.4 mg/kg. Results from clinical trials have indicated that, besides its obvious practical advantages over rt-PA, tenecteplase at 0.25 mg/kg may be equally safe and as or more effective as rt-PA (0.9 mg/kg). Safety concerns have, however, been raised for the higher dose of 0.4 mg/kg [51–60].

3 | RESISTANCE TO INTRAVENOUS THROMBOLYSIS IN ACUTE ISCHEMIC STROKE

Considering the natural specificity of rt-PA for fibrin, as well as its administration protocol that helps compensate for its short half-life and inhibition by circulating PAI-1, rt-PA appears as an ideal thrombolytic drug, which raises the question of why it fails in more than 50% of the cases. To address this question, it is important to define the concept of IVT failure or resistance in AIS. Surprisingly, to date, there is no clear definition of IVT resistance in AIS. For treating myocardial infarction (MI), IVT is considered a failure if it has not produced significant recanalization of the occluded artery (thrombolysis in myocardial infarction score ≥ 2 , assessed by angiography) at 90 minutes after fibrinolytic administration (Table). Clinical evaluation criteria for successful thrombolysis in MI further include the resolution of clinical signs (ie, chest pain) and that of ST-segment elevation

TABLE The thrombolysis in myocardial infarction (TIMI) and revised thrombolysis in cerebral infarction (TICI) scales are intended to standardize the grading of *angiographic* assessment of perfusion following arterial recanalization therapy.

TIMI flow scale	
Grade 0	No perfusion = no antegrade flow beyond occlusion site
Grade 1	Penetration but no perfusion: the contrast material passes beyond the occlusion site but with minimal filling of the distal territory
Grade 2	Partial perfusion: the contrast material passes beyond the occlusion site and opacifies the distal coronary bed, incomplete or sluggish distal branch filling
Grade 3	Complete perfusion: full perfusion with antegrade filling of all distal branches, normal flow
Revised TICI flow scale	
Grade 0	No perfusion = no antegrade flow beyond occlusion site
Grade 1	Penetration but no perfusion: the contrast material passes beyond the occlusion site but with minimal filling of the distal territory
Grade 2a	Partial filling < 50% territory
Grade 2b	Partial filling \geq 50% territory
Grade 2c	Near complete reperfusion except slow flow or few distal cortical emboli
Grade 3	Complete perfusion: full perfusion with filling of all distal branches, normal flow

In the treatment of myocardial infarction, thrombolysis is considered a success if the TIMI flow grade reaches 2 within 90 minutes of fibrinolytic administration. In the case of acute ischemic stroke, early recanalization has been defined as a TICI score $\geq 2b$ being reached within 3 hours of thrombolysis initiation.

by more than 50%, within 60 to 90 minutes of fibrinolytic administration [61–63]. There are no well-established time, clinical, or recanalization score criteria for evaluating IVT success in AIS.

The expression “Time is brain” was introduced in 1993 by Camilo R. Gomez to emphasize the rapidity of human nervous tissue loss as stroke progresses and stresses the urgent need for initiation of therapeutic interventions in AIS [64–66]. In line with this notion, for IVT to be considered successful in AIS, it must not only achieve arterial recanalization but must also do so rapidly. In 2 recent studies by Seners et al. [67–69], an early recanalization was defined as a thrombolysis in cerebral infarction score $\geq 2b$ being reached within 3 hours of IVT initiation (Table). Therefore, similar to that in MI, the notion of time and rapidity of action are central when assessing IVT success in AIS. With this in mind, it appears that clinical failure of IVT does not necessarily mean that rt-PA has failed to exert its biological action, ie, to promote the degradation of the fibrin network. Failed IVT may also occur if fibrinolysis is too slow or not sufficient in itself to cause early recanalization. The fact that imaging data have identified the larger and most proximal AIS thrombi, which are often the same, as those being the less responsive to IVT [8,67,69], supports the idea that IVT failure results from the inability to complete fibrinolysis in a

timely manner. As described below, various endogenous fibrinolysis inhibition mechanisms likely contribute to the slowdown of fibrinolysis in AIS. Moreover, thrombolysis experiments in perfusion chambers have shown that thrombi can persist and even grow due to platelet accumulation during, and despite, effective fibrinolysis [70–73]. The latter data stress how nonfibrin components can contribute to resistance to fibrinolysis-based thrombolysis by providing alternative thrombus frameworks. Analyses of MT-retrieved AIS thrombi have also shown that they contain nonfibrin scaffolding components that could lessen the efficacy of IVT.

Imaging studies have shown that better collaterals were independently associated with a higher rate of early recanalization and better functional outcome post-IVT [74–76]. The improved recanalization efficacy of IVT in individuals with good collaterals has been interpreted as a result of improved delivery of rt-PA to the thrombus [74]. Nonetheless, whereas rt-PA delivery issues may indeed represent a first obstacle to IVT efficacy, comparison of AIS thrombi composition and structure according to IVT status has indicated that rt-PA does reach AIS thrombi. In fact, results from several recent histologic and scanning electron microscopy studies are all converging to show that IVT induces changes in AIS thrombus structure. In particular, AIS thrombi from patients having received IVT prior to MT were slightly smaller, and displayed thinner and loosened fibrin fibers, compared with thrombi from patients treated directly by MT [77–80].

In a recent study, we assessed the individual response of 16 AIS thrombi to *ex vivo* thrombolysis triggered by direct incubation of AIS thrombi with high doses of rt-PA and plasminogen. Our results showed that even in those conditions where delivery issues do not apply, the majority of AIS thrombi were fully or partially resistant to lysis, with rt-PA and plasminogen causing no or only partial reduction in thrombus weight within 1 hour of treatment [81]. In comparable experimental settings (ie, direct incubation of AIS thrombi in plasma supplemented with rt-PA), Laridan et al. [82] reported a mean decrease in AIS thrombus weight of approximately 40% after 2 hours of incubation with rt-PA. Very similar results were obtained in another study by Marder et al. [83] in which AIS thrombus area instead of thrombus weight was used as a readout for assessing thrombus lysis. Thus, it appears from these results that IVT resistance goes well beyond the sole matter of rt-PA delivery to the thrombus.

4 | COMPONENTS OF ACUTE ISCHEMIC STROKE THROMBI

In 2006, long before MT became the standard of care for AIS due to LVO, the first histologic analysis of human AIS thrombi was published by the group of Jeffrey Saver [84]. The observations reported in this seminal study still hold true: despite a marked variability in shape, size, as well as in the distribution of the thrombus cellular and fibrillar components, all AIS shared the same basic components. These components included red blood cells (RBCs), fibrin, platelets, and nucleated cells. Calcifications and cholesterol crystals were absent. Since then, the most notable new findings regarding human AIS thrombus

composition have been the identification of von Willebrand factor (VWF) as a constitutive component of the thrombus fibrillar framework [85–87], and that of neutrophils as the main population of nucleated cells in AIS thrombi [81,82,88–90]. In addition, neutrophil extracellular traps (NETs) are now also recognized as substantial components of human AIS thrombi [25,81,82,88–93]. Notably, the scarcity of calcifications and cholesterol crystals in AIS thrombi was recently confirmed in a large retrospective multicenter study [94]. Although some recent studies have suggested that AIS thrombus composition is dynamic, changes associated with thrombus aging have been shown to affect the respective proportions but not the nature of the various thrombus components [89,95]. Taken together, these data show that, as varied as they are, AIS thrombi have a common framework made of platelets, RBCs, fibrin, VWF, neutrophils, and NETs, with the distribution and proportions of these components representing their major distinguishing factors.

5 | THE STRUCTURAL ORGANIZATION OF ACUTE ISCHEMIC STROKE THROMBI

Another common feature of human AIS thrombi pertains to their structural organization. There are converging data from several groups indicating that AIS thrombi comprise 2 major types of regions with different susceptibilities to rt-PA-mediated thrombolysis: RBC-rich areas and platelet-rich or composite areas [25,77,86–88,92,96]. RBC-rich areas contain an abundance of RBCs trapped in a mesh of sparse thin fibrin fibers [86,87,93,96], with some RBCs displaying the shape of polyhydrocytes [77,87,93], which was previously shown to be indicative of clot retraction and compaction [97–99]. Platelet-rich or composite areas are mainly composed of platelets and contain leukocytes, mostly neutrophils but no or few RBCs [77,86,87,93,96]. Their fibrillar network contains VWF and NETs, alongside densely matted and compressed fibrin [77,86,87,93,96]. The inner core of AIS thrombi can vary from a homogeneous RBC-rich area to the other extreme of an almost exclusively platelet-rich area with little RBCs. Yet, in the majority of cases, the inner core is heterogeneous and presents a mixture of juxtaposed RBC- and platelet-rich areas [86]. The outer layer of AIS thrombi consists mostly of a platelet-rich area of various thicknesses, covered by a crust of dense fibrin [87,93,96,100], whose hermetic aspect [87,93,96] closely resembles that of the fibrin biofilm covering hemostatic blood clots [101]. The formation and assembly of the platelet-rich peripheral shell of AIS thrombi likely involve several nonexclusive mechanisms. First, we have shown that platelets were crucial for its formation [87]. The presence of platelets at the periphery of AIS thrombi could result from clot contraction, which has been shown to cause a redistribution of platelets to the periphery [97,102]. It could also be the mere reflection of platelet margination in the arterial circulation [103]. Fibrin compression might be due to the combined mechanical action of contracting platelets and hemodynamic pressure [104–106]. Besides mechanical forces, several posttranslational modifications alter fibrin morphology and organization. These modifications include factor XIII-

mediated transglutamination [107,108], ferric iron-induced oxidation [109], nitrotyrosination [108,110], carbamylation [111], and even exposure to platelet factor 4 [112]. Concerning IVT resistance, the peripheral compaction and densification of AIS thrombus components are likely to impair the perfusion and diffusion of rt-PA to the thrombus core [113]. Furthermore, posttranslational modifications of fibrin such as carbamylation have been shown to reduce the cofactor effect of fibrin toward t-PA-mediated plasminogen activation [111]. In accordance with these data, we have shown that, as suggested by their histologic features [86], the shell and platelet-rich regions of AIS thrombi display a decreased susceptibility to rt-PA, as compared with RBC-rich regions [87]. This indicates that the components of these regions contribute to IVT resistance and may, therefore, represent targets for improving the thrombolytic efficacy of rt-PA (Figure 2) [24,25,92,93].

6 | PLATELETS AND THROMBOLYSIS RESISTANCE

As the name implies, platelets are central components of platelet-rich regions. Platelets not only participate in clot compaction but also contain and release the 2 major direct inhibitors of t-PA, PAI-1 and protease nexin 1 [114], which were found in abundance in platelet-rich, rt-PA-resistant regions of AIS thrombi [87]. Although PAI-1 is a short-lived inhibitor of rt-PA in the circulation, recent results have shown that a functional pool of PAI-1 remains anchored to the platelet membrane following platelet activation, promoting local fibrinolysis resistance [115]. Platelets may also interfere with fibrinolysis via promotion of coagulation (Figure 2). Procoagulant platelets are highly activated platelets that stimulate thrombin generation by expressing phosphatidylserine and P-selectin at their surface [116]. Coagulation was previously shown to interfere directly with rt-PA-mediated thrombolysis in mice [117]. Interference of coagulation with IVT is also illustrated by the fact that in the treatment of MI, the best recanalization efficacy of thrombolysis was found when combining fibrinolytics, including rt-PA, with anticoagulation [62,118,119].

7 | VON WILLEBRAND FACTOR AND THROMBOLYSIS RESISTANCE

There are contradictory data regarding the impact of VWF on t-PA-mediated fibrinolysis, with reports of either impaired or enhanced fibrinolysis in the presence of VWF [120,121]. Yet, irrespective of a possible modulatory effect toward fibrinolysis and fibrin structure [121], VWF contributes to IVT resistance by providing a secondary fibrillar support frame to AIS thrombi that may help to compensate partly for the loss of fibrin. Recent results indeed indicate that VWF in AIS thrombi is not affected by rt-PA treatment [91]. Interestingly, several experimental studies in mice have shown that drugs targeting VWF exert a thrombolytic effect toward rt-PA-resistant thrombi (Figure 2) [86,122,123–125].

8 | NEUTROPHILS AND THROMBOLYSIS RESISTANCE

There is strong and converging evidence that the antithrombotic effect of neutrophils depends on the release of NETs. In 2010, Fuchs et al. [126] showed that NETs could provide a fibrinolysis-resistant scaffold sufficient to support clot formation despite rt-PA-mediated fibrin degradation. Mangold et al. [127] later showed that DNase 1 could accelerate rt-PA-mediated lysis of human coronary thrombi, which contained NETs. Remarkably, the results by Mangold et al. [127] brought the proof of concept that DNase 1 could be used not only as an antithrombotic but also as an adjuvant for thrombolytic therapy. Simon De Meyer's and our groups have found that NETs are constitutive components of AIS thrombi, and confirmed that the potential of DNase 1 as an adjuvant to rt-PA for thrombolytic therapy also applies to these thrombi [82,90]. Notably, whereas drugs targeting VWF have standalone thrombolytic activity in mice [85,122,123–125], DNase 1 has no major lytic effect on human AIS thrombi in the absence of rt-PA [90]. This suggests that, in contrast to fibrin and VWF, extracellular DNA plays only a minor role in the structural stability of AIS thrombi. We have recently shown that the thrombolytic benefits brought about by degradation of extracellular DNA in human AIS thrombi come from enhancement of rt-PA-mediated fibrinolysis [81], thus indicating that NETs in AIS thrombi are antifibrinolytic. Several antifibrinolytic activities of NETs have been described. Direct inhibition of fibrinolysis by NETs can occur via degradation of plasminogen by NETs-associated neutrophil elastase [128] and via retention of PAI-1 in the thrombus [91]. As for platelets, another mechanism by which NETs may interfere with fibrinolysis is through the promotion of coagulation. Neutrophils and NET components have well-known procoagulant properties due to the ability of extracellular DNA to ignite the contact phase by providing a negatively charged surface, and to that of neutrophil elastase to degrade tissue factor pathway inhibitor and thrombomodulin (Figure 2) [129,130].

9 | POTENTIAL DETERMINANTS OF THROMBUS COMPOSITION AND STRUCTURE

Considering the importance of thrombus composition and structure in IVT resistance, identifying factors that influence these parameters may help better predict response to IVT. The determinants are most likely multifactorial, encompassing stroke etiology (eg, cardioembolic vs large artery atherosclerosis) [131,132] and underlying conditions (eg, cancer) [133], risk factors, comorbidities, and inflammation, all of which factors can be interrelated. With respect to etiology, there is converging evidence that AIS thrombi from cardioembolic origin have higher leukocyte, DNA, and NET content as compared with AIS thrombi linked to large artery atherosclerosis [82,131,134,135]. Whether the higher DNA and NET content of AIS thrombi from cardioembolic origin translates into higher resistance to IVT remains to be determined. Several risk factors for ischemic stroke are known to

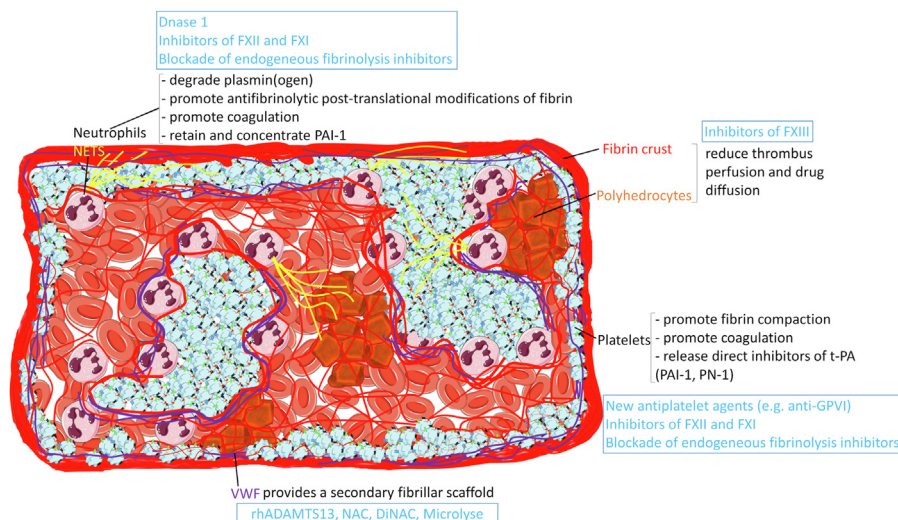


FIGURE 2 The mechanisms of fibrinolysis resistance in acute ischemic stroke thrombi and the pharmacologic options to antagonize them. F, factor; GPVI, glycoprotein VI; NAC, N-acetylcysteine; DiNAC, N,N'-diacetyl-L-cystine; PAI-1, plasminogen activator inhibitor 1; PN-1, protease nexin 1; t-PA, tissue plasminogen activator; VWF, von Willebrand factor.

affect fibrin structure and susceptibility to thrombolysis. For instance, diabetes has been shown to induce the formation of denser, less-porous clots, with increased resistance to fibrinolysis [136–138]. Likewise, a higher concentration of fibrinogen also leads to increased fibrin density and resistance to fibrinolysis [139,140], and data from patients with MI have shown an independent link between plasma clot permeability and resistance to fibrinolysis and intracoronary thrombus fibrin content [141]. Recently, lipoprotein(a), which possesses anti-fibrinolytic properties by competing with plasminogen for binding to fibrin [142,143], was confirmed to be a risk factor for AIS [144]. Infections and inflammation may also impact AIS thrombus composition and susceptibility to thrombolysis. Analyses of blood clots and thrombi from COVID-19 patients have indicated that COVID-19 was associated with compact fibrin, increased resistance to fibrinolysis, and possibly higher NET content, including in AIS thrombi [82,145–147].

10 | TOWARD NEW ADJUVANT THERAPIES FOR IMPROVED INTRAVENOUS THROMBOLYSIS IN ACUTE ISCHEMIC STROKE

As discussed above, DNase 1 has largely proven its efficacy as an adjuvant to rt-PA in *ex vivo* thrombolysis assays using human AIS thrombi [81,82,90]. Moreover, it was previously shown to be well tolerated when administered intravenously to patients with lupus [148]. Its clinical formulation, Dornase alfa (Pulmozyme), has entered 2 phase 2 trials (EXTEND-IA DNase, NCT05203224 and NETS-target, and NCT04785066) aiming at evaluating its efficacy in patients who had an AIS treated with IVT and eligible for MT. Notably, however, because Pulmozyme is initially intended for inhalation, its current formulation and conditioning are not optimal for intravenous administration and limit the dosage amount that can be administered to patients who had an AIS.

In contrast to DNase 1, drugs targeting VWF present the originality of having proved their thrombolytic potential irrespective of rt-PA use, at least in mice [85,122,124,125]. Moreover, these drugs are already in clinical use, which could ease their repurposing toward a new indication such as AIS. These drugs include N-acetylcysteine (NAC, the active ingredient of HIDONAC) [122] and recombinant human disintegrin and metalloprotease with thrombospondin type 1 repeats 13 (rhADAMTS-13, the recombinant form of the endogenous protease responsible for the degradation of VWF multimers) [85,125]. Interestingly, recent data have suggested that the disulfide dimer of NAC, N,N'-diacetyl-L-cystine, may have an increased thrombolytic potential toward rt-PA-resistant thrombi, as compared with NAC [124]. To date, although the thrombolytic action of NAC, N,N'-diacetyl-L-cystine, and rhADAMTS-13 has been shown in various experimental models of thrombosis [85,122,124,125], their effect on AIS thrombus degradation in *ex vivo* thrombolysis assays remains to be assessed.

Conventional anticoagulation with heparin or direct oral anticoagulants on top of rt-PA cannot be envisioned in AIS due to the obvious risk of precipitating hemorrhagic transformation [149]. New drugs targeting factors XI and XII could provide adjuvant anticoagulant therapies well suited for AIS treatment because of their increased safety profile. Moreover, these selective inhibitors of the coagulation contact pathway appear particularly relevant for targeting NET-dependent coagulation [150].

Antiplatelet drugs also present an inherent risk of hemorrhagic complications that has limited their use in AIS [151]. The development of new antiplatelet agents targeting noncanonical pathways of platelet activation has opened the way for a reconsideration of antiplatelet strategies in AIS. Interestingly, after successfully completing a phase 1b/2a trial (ACTIMIS study, NCT03803007), glenzocimab, a humanized Fab fragment to platelet glycoprotein VI that blocks collagen-dependent platelet activation without compromising hemostasis [152,153], has entered an adaptive phase 2/3 study for AIS (ACTISAVE study, NCT05070260).

11 | ISTH CONGRESS REPORT

The 2022 ISTH Congress was an opportunity to learn new insights on the mechanisms of fibrinolysis resistance and to discover new therapeutic leads for improving thrombolytic therapies.

Ollivier et al. [154] showed that carbamylation occurred in AIS thrombi in relation to the presence of neutrophils. Consistent with predictions from *in vitro* studies, fibrin carbamylation in AIS thrombi had a negative impact on the response to both IVT and MT, by increasing resistance to fibrinolysis and thrombus stiffness.

Kadaba Ramanujam et al. [155] showed that fibrinogen concentration was a critical determinant of fibrin structure and clot resistance to rupture, as increasing fibrinogen concentration resulted in fewer, thicker fibrin fibers, and increased clot mechanical toughness. Gauer et al. [156] reported that the interaction between fibrin and platelet glycoprotein VI stimulated the development of platelet procoagulant activity, thereby increasing fibrin fiber density and reducing clot porosity.

By combining a mathematical and experimental approach, Risman et al. [114] strengthened the notion that densification of the fibrin network at the clot periphery acts as a temporary barrier for t-PA diffusion into the clot, thus impairing external fibrinolysis of contracted clots.

Solomonjanahary et al. [157] provided quantitative data showing that IVT was associated with a significant increase in t-PA content of AIS thrombi, thus supporting the notion that IVT resistance does not result from rt-PA delivery issues. Instead, they showed that the thrombolytic action of IVT-derived rt-PA could be triggered by degrading extracellular DNA with DNase 1, thus identifying NETs in AIS thrombi as being responsible for blocking the activity of intravenously-administered rt-PA.

A presentation by Marc van Moorsel was dedicated to a new thrombolytic agent, microlyse, a fusion protein consisting of a high-affinity single variable domain on a heavy chain antibody (VHH or nanobody) to VWF combined with the protease domain of urokinase-type plasminogen activator [158]. Interestingly, the superiority of microlyse over rt-PA in reducing ischemic injury following FeCl₃-induced cerebral thrombosis in mice was shown.

Finally, Mathews et al. [159] showed that CPI, a natural carboxypeptidase inhibitor, accelerated rt-PA-mediated thrombolysis in mice, when given prior to thrombolytic therapy initiation.

12 | FUTURE DIRECTIONS

The analysis of AIS thrombus composition in the last years has already led to the identification of several promising targets for the improvement of IVT. Future directions in the field of IVT in the setting of AIS should focus on translating these findings into clinical practice. Several drugs with known safety profiles are readily available to target the nonfibrin AIS thrombus components of interest. This should facilitate the design of prospective randomized trials, as illustrated by the start of the EXTEND-IA DNase and NETS-target trials in 2022.

13 | CONCLUSION

The analysis of AIS thrombus composition has been a fast-growing field of research since the development of MT as an alternative or complementary therapy to IVT for achieving early recanalization in patients with occlusion of proximal large vessels. In addition to its tremendous recanalization efficacy, MT may thus prove instrumental in helping design the future of IVT, which still holds a notable potential, either as a standalone or bridging therapy, as access to MT remains limited in many places.

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AUTHOR CONTRIBUTIONS

B.H.T.N., J.P.D., and M.M. wrote the manuscript.

RELATIONSHIP DISCLOSURE

The authors have submitted a patent application entitled "Methods and pharmaceutical compositions for the treatment of acute ischemic stroke" (PCT/EP2018/062588).

REFERENCES

- [1] The National Institute of Neurological Disorders and Stroke rt-PA Stroke Study Group. Tissue plasminogen activator for acute ischemic stroke. *N Engl J Med.* 1995;333:1581–7.
- [2] Haley EC, Levy DE, Brott TG, Sheppard GL, Wong MCW, Kongable GL, et al. Urgent therapy for stroke. Part II. Pilot study of tissue plasminogen activator administered 91–180 minutes from onset. *Stroke.* 1992;23:641–5.
- [3] Brott TG, Haley EC, Levy DE, Barsan W, Broderick J, Sheppard GL, et al. Urgent therapy for stroke. Part I. Pilot study of tissue plasminogen activator administered within 90 minutes. *Stroke.* 1992;23:632–40.
- [4] del Zoppo GJ, Poeck K, Pessin MS, Wolpert SM, Furlan AJ, Ferbert A, et al. Recombinant tissue plasminogen activator in acute thrombotic and embolic stroke. *Ann Neurol.* 1992;32:78–86.
- [5] von Kummer R, Hacke W. Safety and efficacy of intravenous tissue plasminogen activator and heparin in acute middle cerebral artery stroke. *Stroke.* 1992;23:646–52.
- [6] Mishra NK, Lyden P, Grotta JC, Lees KR, VISTA Collaborators. Thrombolysis is associated with consistent functional improvement across baseline stroke severity: a comparison of outcomes in patients from the virtual international stroke trials archive (VISTA). *Stroke.* 2010;41:2612–7.
- [7] Hacke W, Kaste M, Bluhmki E, Brozman M, Dávalos A, Guidetti D, et al. Thrombolysis with alteplase 3 to 4.5 hours after acute ischemic stroke. *N Engl J Med.* 2008;359:1317–29.
- [8] Bhatia R, Hill MD, Shobha N, Menon B, Bal S, Kochar P, 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;41:2254–8.
- [9] Lee KY, Han SW, Kim SH, Nam HS, Ahn SW, Kim DJ, et al. Early recanalization after intravenous administration of recombinant

- tissue plasminogen activator as assessed by pre- and post-thrombolytic angiography in acute ischemic stroke patients. *Stroke*. 2007;38:192–3.
- [10] Saqqur M, Tsvigoulis G, Molina CA, Demchuk AM, Siddiqui M, Alvarez-Sabín J, et al. Symptomatic intracerebral hemorrhage and recanalization after IV rt-PA: a multicenter study. *Neurology*. 2008;71:1304–12.
 - [11] Nogueira RG, Jadhav AP, Haussen DC, Bonafe A, Budzik RF, Bhuva P, et al. Thrombectomy 6 to 24 hours after stroke with a mismatch between deficit and infarct. *N Engl J Med*. 2018;378:11–21.
 - [12] Kaesmacher J, Kaesmacher M, Maegerlein C, Zimmer C, Gersing AS, Wunderlich S, et al. Hemorrhagic transformations after thrombectomy: risk factors and clinical relevance. *Cerebrovasc Dis*. 2017;43:294–304.
 - [13] Campbell BCV, Mitchell PJ, Kleinig TJ, Dewey HM, Churilov L, Yassi N, et al. Endovascular therapy for ischemic stroke with perfusion-imaging selection. *N Engl J Med*. 2015;372:1009–18.
 - [14] Berkhemer OA, Fransen PSS, Beumer D, van den Berg LA, Lingsma HF, Yoo AJ, et al. A randomized trial of intraarterial treatment for acute ischemic stroke. *N Engl J Med*. 2015;372:11–20.
 - [15] Zivelonghi C, Tamburin S. Mechanical thrombectomy for acute ischemic stroke: the therapeutic window is larger but still “time is brain”. *Funct Neurol*. 2018;33:5–6.
 - [16] Hui W, Wu C, Zhao W, Sun H, Hao J, Liang H, et al. Efficacy and safety of recanalization therapy for acute ischemic stroke with large vessel occlusion: a systematic review. *Stroke*. 2020;51:2026–35.
 - [17] Haussen DC, Al-Bayati AR, Mohammaden MH, Sheth SA, Salazar-Marioni S, Linfante I, et al. The Society of Vascular and Interventional Neurology (SVIN) Mechanical Thrombectomy Registry: methods and primary results. *Stroke Vasc Interv Neurol*. 2022;2:e000234.
 - [18] Duloquin G, Graber M, Garnier L, Crespy V, Comby PO, Baptiste L, et al. Incidence of acute ischemic stroke with visible arterial occlusion: a population-based study (Dijon Stroke Registry). *Stroke*. 2020;51:2122–30.
 - [19] Lakomkin N, Dhamoon M, Carroll K, Singh IP, Tuhim S, Lee J, et al. Prevalence of large vessel occlusion in patients presenting with acute ischemic stroke: a 10-year systematic review of the literature. *J Neurointerv Surg*. 2019;11:241–5.
 - [20] Waqas M, Rai AT, Vakharia K, Chin F, Siddiqui AH. Effect of definition and methods on estimates of prevalence of large vessel occlusion in acute ischemic stroke: a systematic review and meta-analysis. *J Neurointerv Surg*. 2020;12:260–5.
 - [21] Rha JH, Saver JL. The impact of recanalization on ischemic stroke outcome: a meta-analysis. *Stroke*. 2007;38:967–73.
 - [22] Brinjikji W, Abbasi M, Mereuta OM, Fitzgerald S, Larco JA, Dai D, et al. Histological composition of retrieved emboli in acute ischemic stroke is independent of pre-thrombectomy alteplase use. *J Stroke Cerebrovasc Dis*. 2022;31:106376.
 - [23] Jolugbo P, Ariens RAS. Thrombus composition and efficacy of thrombolysis and thrombectomy in acute ischemic stroke. *Stroke*. 2021;52:1131–42.
 - [24] Staessens S, François O, Brinjikji W, Doyle KM, Vanacker P, Andersson T, De Meyer SF. Studying stroke thrombus composition after thrombectomy: what can we learn? *Stroke*. 2021;52:3718–27.
 - [25] Desilles JP, Di Meglio L, Delvoye F, Maïer B, Piotin M, Ho-Tin-Noé B, Mazighi M. Composition and organization of acute ischemic stroke thrombus: a wealth of information for future thrombolytic strategies. *Front Neurol*. 2022;13:870331.
 - [26] Rossi R, Mereuta OM, Barbachan e Silva B, Molina Gil S, Douglas A, Pandit A, et al. Potential biomarkers of acute ischemic stroke etiology revealed by mass spectrometry-based proteomic characterization of formalin-fixed paraffin-embedded blood clots. *Front Neurol*. 2022;13:854846.
 - [27] Gilbert A, Detraz L, Alexandre PL, Serfaty JM, Desal H, Toquet C, Bourcier R. Magnetic resonance imaging quantitative T2* mapping to predict the red blood cell content of in vivo thrombi retrieved from patients with large vessel occlusions in acute ischemic stroke. *Interv Neuroradiol*. 2022;28:523–30.
 - [28] Di Meglio L, Derraz I, Solonomenjanahary M, Daly D, Chodraui Filho S, Ben Maacha M, et al. Two-layered susceptibility vessel sign is associated with biochemically quantified thrombus red blood cell content. *Eur J Neurol*. 2020;27:1264–71.
 - [29] Joundi RA, Menon BK. Thrombus composition, imaging, and outcome prediction in acute ischemic stroke. *Neurology*. 2021;97:S68–78.
 - [30] Boodt N, Snouckaert Van Schauburg PRW, Hund HM, Fereidoonzehad B, McGarry JP, Akyildiz AC, et al. Mechanical characterization of thrombi retrieved with endovascular thrombectomy in patients with acute ischemic stroke. *Stroke*. 2021;52:2510–7.
 - [31] Shimizu H, Hatakeyama K, Saito K, Shobatake R, Takahashi N, Deguchi J, et al. Age and composition of the thrombus retrieved by mechanical thrombectomy from patients with acute ischemic stroke are associated with revascularization and clinical outcomes. *Thromb Res*. 2022;219:60–9.
 - [32] Zamarron C, Lijnen HR, Collen D. Kinetics of the activation of plasminogen by natural and recombinant tissue-type plasminogen activator. *J Biol Chem*. 1984;259:2080–3.
 - [33] Hoylaerts M, Rijken DC, Lijnen HR, Collen D. Kinetics of the activation of plasminogen by human tissue plasminogen activator. Role of fibrin. *J Biol Chem*. 1982;257:2912–9.
 - [34] Rånby M. Studies on the kinetics of plasminogen activation by tissue plasminogen activator. *Biochim Biophys Acta*. 1982;704:461–9.
 - [35] Xue Y, Bodin C, Olsson K. Crystal structure of the native plasminogen reveals an activation-resistant compact conformation. *J Thromb Haemost*. 2012;10:1385–96.
 - [36] Ho-Tin-Noé B, Rojas G, Vranckx R, Lijnen HR, Anglés-Cano E. Functional hierarchy of plasminogen kringles 1 and 4 in fibrinolysis and plasmin-induced cell detachment and apoptosis. *FEBS J*. 2005;272:3387–400.
 - [37] Markus G. Conformational changes in plasminogen, their effect on activation, and the agents that modulate activation rates – a review. *Fibrinolysis*. 1996;10:75–85.
 - [38] Horrevoets AJG, Smilde A, De Vries C, Pannekoek H. The specific roles of finger and kringle 2 domains of tissue-type plasminogen activator during in vitro fibrinolysis. *J Biol Chem*. 1994;269:12639–44.
 - [39] Kim PY, Tieu LD, Stafford AR, Fredenburgh JC, Weitz JI. A high affinity interaction of plasminogen with fibrin is not essential for efficient activation by tissue-type plasminogen activator. *J Biol Chem*. 2012;287:4652–61.
 - [40] Camiolo SM, Thorsen S, Astrup T. Fibrinogenolysis and fibrinolysis with tissue plasminogen activator, urokinase, streptokinase-activated human globulin, and plasmin. *Proc Soc Exp Biol Med*. 1971;138:277–80.
 - [41] Samama M, Nguyen G, Desnoyers P, Lourenco DM, Fretault J, Horellou MH, et al. Comparison of thrombolytic, fibrinolytic, and fibrinogenolytic properties of tissue plasminogen activator, streptokinase, single-chain urokinase, high molecular weight and low molecular weight urokinase in human plasma in vitro. *Fundam Clin Pharmacol*. 1988;2:509–23.
 - [42] Matsuo O, Rijken DC, Collen D. Comparison of the relative fibrinogenolytic, fibrinolytic and thrombolytic properties of tissue plasminogen activator and urokinase in vitro. *Thromb Haemost*. 1981;45:225–9.

- [43] Charbonnier G, Bonnet L, Biondi A, Moulin T. Intracranial bleeding after reperfusion therapy in acute ischemic stroke. *Front Neurol*. 2020;11:629920.
- [44] Mohedano AMI, García Pastor A, De Lorenzo Pinto A, Gil Núñez AC. Bolus-to-infusion time in stroke patients treated with alteplase on the CT table: a clinical concern. *Cerebrovasc Dis*. 2019;48:96–8.
- [45] Rijken DC, Otter M, Kuiper J, van Berkel TJC. Receptor-mediated endocytosis of tissue-type plasminogen activator (t-PA) by liver cells. *Thromb Res Suppl*. 1990;10:63–71.
- [46] Frontera JA, Lewin JJ, Rabinstein AA, Aisiku IP, Alexandrov AW, Cook AM, et al. Guideline for reversal of antithrombotics in intracranial hemorrhage: a statement for healthcare professionals from the neurocritical care society and society of critical care medicine. *Neurocrit Care*. 2016;24:6–46.
- [47] Tanswell P, Tebbe U, Neuhaus KL, Gfásle-Schwarz L, Wojcik J, Seifried E. Pharmacokinetics and fibrin specificity of alteplase during accelerated infusions in acute myocardial infarction. *J Am Coll Cardiol*. 1992;19:1071–5.
- [48] Smith C, Al-Nuaimi Y, Wainwright J, Sherrington C, Singh A, Kallungal J, et al. The influence of bolus to infusion delays on plasma tissue plasminogen activator levels. *Int J Stroke*. 2014;9:939–42.
- [49] Acheampong P, May MT, Ford GA, Dixit AK. Bolus-infusion delays of alteplase during thrombolysis in acute ischaemic stroke and functional outcome at 3 months. *Stroke Res Treat*. 2014;2014:358640.
- [50] Menon BK, Buck BH, Singh N, Deschaintre Y, Almekhlafi MA, Coutts SB, et al. Intravenous tenecteplase compared with alteplase for acute ischaemic stroke in Canada (AcT): a pragmatic, multi-centre, open-label, registry-linked, randomised, controlled, non-inferiority trial. *Lancet*. 2022;400:161–9.
- [51] Parsons M, Spratt N, Bivard A, Campbell B, Chung K, Miteff F, et al. A randomized trial of tenecteplase versus alteplase for acute ischemic stroke. *N Engl J Med*. 2012;366:1099–107.
- [52] Kvistad CE, Novotny V, Kurz MW, Rønning OM, Thommessen B, Carlsson M, et al. Safety and outcomes of tenecteplase in moderate and severe ischemic stroke: results from NOR-TEST. *Stroke*. 2019;50:1279–81.
- [53] Menon BK, Singh N, Sylaja PN. Tenecteplase use in patients with acute ischaemic stroke. *Lancet*. 2023;401:618–9.
- [54] Zitek T, Ataya R, Brea I. Using tenecteplase for acute ischemic stroke: what is the hold up? *West J Emerg Med*. 2020;21:199–202.
- [55] Warach SJ, Dula AN, Milling TJ. Tenecteplase thrombolysis for acute ischemic stroke. *Stroke*. 2020;51:3440–51.
- [56] Katsanos AH, Psychogios K, Turc G, Sacco S, De Sousa DA, De Marchis GM, et al. Off-label use of tenecteplase for the treatment of acute ischemic stroke: a systematic review and meta-analysis. *JAMA Netw Open*. 2022;5:e224506.
- [57] Bivard A, Huang X, Levi CR, Spratt N, Campbell BCV, Cheripelli BK, et al. Tenecteplase in ischemic stroke offers improved recanalization: analysis of 2 trials. *Neurology*. 2017;89:62–7.
- [58] Huang X, Cheripelli BK, Lloyd SM, Kalladka D, Moreton FC, Siddiqui A, et al. Alteplase versus tenecteplase for thrombolysis after ischaemic stroke (ATTEST): a phase 2, randomised, open-label, blinded endpoint study. *Lancet Neurol*. 2015;14:368–76.
- [59] Kvistad CE, Naess H, Helleberg BH, Idicula T, Hagberg G, Nordby LM, et al. Tenecteplase versus alteplase for the management of acute ischaemic stroke in Norway (NOR-TEST 2, part A): a phase 3, randomised, open-label, blinded endpoint, non-inferiority trial. *Lancet Neurol*. 2022;21:511–9.
- [60] Putaala J, Saver JL, Nour M, Kleindorfer D, McDermott M, Kaste M. Should tenecteplase be given in clinical practice for acute ischemic stroke thrombolysis? *Stroke*. 2021;52:3075–80.
- [61] Van De Werf F, Bax J, Betriu A, Blomstrom-Lundqvist C, Crea F, Falk V, et al. Management of acute myocardial infarction in patients presenting with persistent ST-segment elevation: The Task Force on the management of ST-segment elevation acute myocardial infarction of the European Society of Cardiology. *Eur Heart J*. 2008;29:2909–45.
- [62] White HD, Van de Werf FJJ. Thrombolysis for acute myocardial infarction. *Circulation*. 1998;97:1632–46.
- [63] Ibanez B, James S, Agewall S, Antunes MJ, Bucciarelli-Ducci C, Bueno H, et al. 2017 ESC Guidelines for the management of acute myocardial infarction in patients presenting with ST-segment elevation: the Task Force on the Management of ST-Segment Elevation Acute Myocardial Infarction of the European Society of Cardiology. *Eur Heart J*. 2018;39:119–77.
- [64] Lyden P. Thrombolytic therapy for acute stroke—not a moment to lose. *N Engl J Med*. 2008;359:1393–5.
- [65] Saver JL. Time is brain –quantified. *Stroke*. 2006;37:263–6.
- [66] Gomez CR. Editorial: time is brain. *J Stroke Cerebrovasc Dis*. 1993;3:1–2.
- [67] Seners P, Turc G, Lion S, Cottier JP, Cho TH, Arquizan C, et al. Relationships between brain perfusion and early recanalization after intravenous thrombolysis for acute stroke with large vessel occlusion. *J Cereb Blood Flow Metab*. 2020;40:667–77.
- [68] Seners P, Turc G, Maier B, Mas JL, Oppenheim C, Baron JC. Incidence and predictors of early recanalization after intravenous thrombolysis: a systematic review and meta-analysis. *Stroke*. 2016;47:2409–12.
- [69] Seners P, Deleplere J, Turc G, Henon H, Piotin M, Arquizan C, et al. Thrombus length predicts lack of post-thrombolysis early recanalization in minor stroke with large vessel occlusion. *Stroke*. 2019;50:761–4.
- [70] Parise P, Agnelli G. Thrombus resistance to lysis and reocclusion after thrombolysis: the role of platelets. *Blood Coagul Fibrinolysis*. 1991;2:749–58.
- [71] Loyau S, Ho-Tin-Noé B, Bourrienne MC, Boulaftali Y, Jandrot-Perrus M. Microfluidic modeling of thrombolysis effect of anti-platelet and anticoagulant agents on tpa (tissue-type plasminogen activator)-induced fibrinolysis. *Arterioscler Thromb Vasc Biol*. 2018;38:2626–37.
- [72] Whyte CS, Swieringa F, Mastenbroek TG, Lionikiene AS, Lancé MD, Van Der Meijden PEJ, et al. Plasminogen associates with phosphatidylserine-exposing platelets and contributes to thrombus lysis under flow. *Blood*. 2015;125:2568–78.
- [73] Li R, Elmongy H, Sims C, Diamond SL. Ex vivo recapitulation of trauma-induced coagulopathy and preliminary assessment of trauma patient platelet function under flow using microfluidic technology. *J Trauma Acute Care Surg*. 2016;80:440–9.
- [74] Seners P, Roca P, Legrand L, Turc G, Cottier JP, Cho TH, et al. Better collaterals are independently associated with post-thrombolysis recanalization before thrombectomy. *Stroke*. 2019;50:867–72.
- [75] Tan BYQ, Wan-Yee K, Paliwal P, Gopinathan A, Nadarajah M, Ting E, et al. Good intracranial collaterals trump poor ASPECTS (Alberta Stroke Program Early CT Score) for intravenous thrombolysis in anterior circulation acute ischemic stroke. *Stroke*. 2016;47:2292–8.
- [76] Leng X, Lan L, Liu L, Leung TW, Wong KS. Good collateral circulation predicts favorable outcomes in intravenous thrombolysis: a systematic review and meta-analysis. *Eur J Neurol*. 2016;23:1738–49.
- [77] Mereuta OM, Fitzgerald S, Christensen TA, Jaspersen AL, Dai D, Abbasi M, et al. High-resolution scanning electron microscopy for the analysis of three-dimensional ultrastructure of clots in acute ischemic stroke. *J Neurointerv Surg*. 2021;13:906–11.
- [78] Rossi R, Fitzgerald S, Molina S, Mereuta OM, Douglas A, Pandit A, et al. The administration of rtPA before mechanical thrombectomy in acute ischemic stroke patients is associated with a significant

- reduction of the retrieved clot area but it does not influence revascularization outcome. *J Thromb Thrombolysis*. 2021;51:545–51.
- [79] Rossi R, Molina S, Mereuta OM, Douglas A, Fitzgerald S, Tierney C, et al. Does prior administration of rtPA influence acute ischemic stroke clot composition? Findings from the analysis of clots retrieved with mechanical thrombectomy from the RESTORE registry. *J Neurol*. 2022;269:1913–20.
- [80] Krajičková D, Krajina A, Šteiner I, Vyšata O, Herzig R, Lojik M, et al. Fibrin clot architecture in acute ischemic stroke treated with mechanical thrombectomy with stent-retrievers –cohort study. *Circ J*. 2018;82:866–73.
- [81] Desilles JP, Solonomenjanahary M, Consoli A, Ollivier V, Faille D, Bourrienne MC, et al. Impact of COVID-19 on thrombus composition and response to thrombolysis: insights from a monocentric cohort population of COVID-19 patients with acute ischemic stroke. *J Thromb Haemost*. 2022;20:919–28.
- [82] Laridan E, Denorme F, Desender L, François O, Andersson T, Deckmyn H, et al. Neutrophil extracellular traps in ischemic stroke thrombi. *Ann Neurol*. 2017;82:223–32.
- [83] Marder VJ, Blinc A, Gruber T, Tratar G, Sabovic M, Starkman S, et al. Comparison of plasmin with recombinant tissue-type plasminogen activator in lysis of cerebral thromboemboli retrieved from patients with acute ischemic stroke. *Stroke*. 2011;42:2222–8.
- [84] Marder VJ, Chute DJ, Starkman S, Abolian AM, Kidwell C, Liebeskind D, et al. Analysis of thrombi retrieved from cerebral arteries of patients with acute ischemic stroke. *Stroke*. 2006;37:2086–93.
- [85] Denorme F, Langhauser F, Desender L, Vandenbulcke A, Rottensteiner H, Plaimauer B, et al. ADAMTS13-mediated thrombolysis of t-PA-resistant occlusions in ischemic stroke in mice. *Blood*. 2016;127:2337–45.
- [86] Staessens S, Denorme F, François O, Desender L, Dewaele T, Vanacker P, et al. Structural analysis of ischemic stroke thrombi: histological indications for therapy resistance. *Haematologica*. 2020;105:498–507.
- [87] Di Meglio L, Desilles JP, Ollivier V, Nomenjanahary MS, Di Meglio S, Deschildre C, et al. Acute ischemic stroke thrombi have an outer shell that impairs fibrinolysis. *Neurology*. 2019;93:e1686–98.
- [88] Essig F, Kollikowski AM, Pham M, Solymosi L, Stoll G, Haeusler KG, et al. Immunohistological analysis of neutrophils and neutrophil extracellular traps in human thromboemboli causing acute ischemic stroke. *Int J Mol Sci*. 2020;21:1–13.
- [89] Chen X, Wang L, Jiang M, Lin L, Ba Z, Tian H, et al. Leukocytes in cerebral thrombus respond to large-vessel occlusion in a time-dependent manner and the association of NETs with collateral flow. *Front Immunol*. 2022;13:834562.
- [90] Ducroux C, Di Meglio L, Loyau S, Delbosc S, Boisseau W, Deschildre C, et al. Thrombus neutrophil extracellular traps content impair tPA-induced thrombolysis in acute ischemic stroke. *Stroke*. 2018;49:754–7.
- [91] Zhang S, Cao Y, Du J, Liu H, Chen X, Li M, et al. Neutrophil extracellular traps contribute to tissue plasminogen activator resistance in acute ischemic stroke. *FASEB J*. 2021;35:e21835.
- [92] Xu RG, Ariëns RAS. Insights into the composition of stroke thrombi: heterogeneity and distinct clot areas impact treatment. *Haematologica*. 2020;105:257–9.
- [93] Brinjikji W, Madalina Mereuta O, Dai D, Kallmes DF, Savastano L, Liu Y, et al. Mechanisms of fibrinolysis resistance and potential targets for thrombolysis in acute ischaemic stroke: lessons from retrieved stroke emboli. *Stroke Vasc Neurol*. 2021;6:658–67.
- [94] Aspegren O, Staessens S, Vandelanotte S, Desender L, Cordonnier C, Puy L, et al. Unusual histopathological findings in mechanically removed stroke thrombi—a multicenter experience. *Front Neurol*. 2022;13:846293.
- [95] Abbasi M, Arturo Larco J, Mereuta MO, Liu Y, Fitzgerald S, Dai D, et al. Diverse thrombus composition in thrombectomy stroke patients with longer time to recanalization. *Thromb Res*. 2022;209:99–104.
- [96] Di Meglio L, Desilles JP, Mazighi M, Ho-Tin-Noé B. Thrombolysis-resistant intracranial clot. *Neurology*. 2018;90:1075.
- [97] Cines DB, Lebedeva T, Nagaswami C, Hayes V, Masefski W, Litvinov RI, et al. Clot contraction: compression of erythrocytes into tightly packed polyhedra and redistribution of platelets and fibrin. *Blood*. 2014;123:1596–603.
- [98] Tutwiler V, Mukhitov AR, Peshkova AD, Le Minh G, Khismatullin RR, Vicksman J, et al. Shape changes of erythrocytes during blood clot contraction and the structure of polyhedrocytes. *Sci Rep*. 2018;8:17907.
- [99] Weisel JW, Lebedeva T, Nagaswami C, Hayes VM, Masefski W, Litvinov RI, et al. Polyhedrocytes: compressed polyhedral erythrocytes in contracted blood clots and thrombi. *Blood*. 2013;122:452.
- [100] Ahn SH, Hong R, Choo IS, Heo JH, Nam HS, Kang HG, et al. Histologic features of acute thrombi retrieved from stroke patients during mechanical reperfusion therapy. *Int J Stroke*. 2016;11:1036–44.
- [101] Macrae FL, Duval C, Papareddy P, Baker SR, Yuldasheva N, Kearney KJ, et al. A fibrin biofilm covers blood clots and protects from microbial invasion. *J Clin Invest*. 2018;128:3356–68.
- [102] Kovalenko TA, Giraud MN, Eckly A, Ribba AS, Proamer F, Fraboulet S, et al. Asymmetrical forces dictate the distribution and morphology of platelets in blood clots. *Cells*. 2021;10:1–17.
- [103] Oh D, li S, Takagi S. Numerical study of particle margination in a square channel flow with red blood cells. *Fluids*. 2022;7:96.
- [104] Litvinov RI, Weisel JW. Fibrin mechanical properties and their structural origins. *Matrix Biol*. 2017;60–61:110–23.
- [105] Kim OV, Litvinov RI, Alber MS, Weisel JW. Quantitative structural mechanobiology of platelet-driven blood clot contraction. *Nat Commun*. 2017;8:1274.
- [106] Freiherr von Seckendorff A, Delvoye F, Levant P, Solonomenjanahary M, Ollivier V, Bourrienne MC, et al. Modeling large vessel occlusion stroke for the evaluation of endovascular therapy according to thrombus composition. *Front Neurol*. 2021;12:815814.
- [107] Hethershaw EL, Cilia La Corte AL, Duval C, Ali M, Grant PJ, Ariëns RAS, Philippou H. The effect of blood coagulation factor XIII on fibrin clot structure and fibrinolysis. *J Thromb Haemost*. 2014;12:197–205.
- [108] Poole LG, Kopec AK, Groeneveld DJ, Pant A, Baker KS, Cline-Fedewa HM, et al. Factor XIII cross-links fibrin(ogen) independent of fibrin polymerization in experimental acute liver injury. *Blood*. 2021;137:2520–31.
- [109] Pretorius E, Vermeulen N, Bester J, Lipinski B. Novel use of scanning electron microscopy for detection of iron-induced morphological changes in human blood. *Microsc Res Tech*. 2013;76:268–71.
- [110] Ill-Raga G, Palomer E, Ramos-Fernández E, Guix FX, Bosch-Morató M, Guivernau B, et al. Fibrinogen nitrotyrosination after ischemic stroke impairs thrombolysis and promotes neuronal death. *Biochim Biophys Acta*. 2015;1852:421–8.
- [111] Binder V, Bergum B, Jaisson S, Gillery P, Scavenius C, Spriet E, et al. Impact of fibrinogen carbamylation on fibrin clot formation and stability. *Thromb Haemost*. 2017;117:899–910.
- [112] Amelot AA, Tagzirt M, Ducouret G, Kuen RL, Le Bonniec BF. Platelet factor 4 (CXCL4) seals blood clots by altering the structure of fibrin. *J Biol Chem*. 2007;282:710–20.
- [113] Risman RA, Abdelhamid A, Weisel JW, Bannish BE, Tutwiler V. Effects of clot contraction on clot degradation: a mathematical and experimental approach. *Biophys J*. 2022;121:3271–85.

- [114] Boulaftali Y, Ho-Tin-Noé B, Pena A, Loyau S, Venisse L, François D, et al. Platelet protease nexin-1, a serpin that strongly influences fibrinolysis and thrombolysis. *Circulation*. 2011;123:1326–34.
- [115] Morrow GB, Whyte CS, Mutch NJ. Functional plasminogen activator inhibitor 1 is retained on the activated platelet membrane following platelet activation. *Haematologica*. 2020;105:2824–33.
- [116] Denorme F, Campbell RA. Procoagulant platelets: novel players in thromboinflammation. *Am J Physiol Cell Physiol*. 2022;323:C951–8.
- [117] Boulaftali Y, Lamrani L, Rouzaud M-C, Loyau S, Jandrot-Perrus M, Bouton M-C, Ho-Tin-Noé B. The mouse dorsal skinfold chamber as a model for the study of thrombolysis by intravital microscopy. *Thromb Haemost*. 2012;107:962–71.
- [118] Collen D, Lijnen HR. Molecular basis of fibrinolysis, as relevant for thrombolytic therapy. *Thromb Haemost*. 1995;74:167–71.
- [119] GUSTO Investigators. An international randomized trial comparing four thrombolytic strategies for acute myocardial infarction. *N Engl J Med*. 1993;329:673–82.
- [120] Tanka-Salamon A, Kolev K, Machovich R, Komorowicz E. Proteolytic resistance conferred to fibrinogen by von Willebrand factor. *Thromb Haemost*. 2010;103:291–8.
- [121] Marchi R, Rojas H. Effect of von Willebrand factor on clot structure and lysis. *Blood Coagul Fibrinolysis*. 2015;26:533–6.
- [122] De Lizarrondo SM, Gakuba C, Herbig BA, Repessé Y, Ali C, Denis CV, et al. Potent thrombolytic effect of N-acetylcysteine on arterial thrombi. *Circulation*. 2017;136:646–60.
- [123] van Moorsel MVA, de Maat S, Vercruyse K, van Leeuwen EM, Jacqmarcq C, Bonnard T, et al. VWF-targeted thrombolysis to overcome rh-tPA resistance in experimental murine stroke models. *Blood*. 2022;140:2844–8.
- [124] Kim D, Shea SM, Ku DN. Lysis of arterial thrombi by perfusion of N, N'-diacetyl-L-cystine (DiNAC). *PLOS ONE*. 2021;16:e0247496.
- [125] Crescente M, Thomas GM, Demers M, Voorhees JR, Wong SL, Ho-Tin-Noé B, Wagner DD. ADAMTS13 exerts a thrombolytic effect in microcirculation. *Thromb Haemost*. 2012;108:527–32.
- [126] Fuchs TA, Brill A, Duerschmied D, Schatzberg D, Monestier M, Myers DD, et al. Extracellular DNA traps promote thrombosis. *Proc Natl Acad Sci U S A*. 2010;107:15880–5.
- [127] Mangold A, Alias S, Scherz T, Hofbauer M, Jakowitsch J, Panzenböck A, et al. Coronary neutrophil extracellular trap burden and deoxyribonuclease activity in ST-elevation acute coronary syndrome are predictors of ST-segment resolution and infarct size. *Circ Res*. 2015;116:1182–92.
- [128] da Cruz DB, Helms J, Aquino LR, Stiel L, Cougourdan L, Broussard C, et al. DNA-bound elastase of neutrophil extracellular traps degrades plasminogen, reduces plasmin formation, and decreases fibrinolysis: proof of concept in septic shock plasma. *FASEB J*. 2019;33:14270–80.
- [129] Massberg S, Grahl L, Von Bruehl ML, Manukyan D, Pfeiler S, Goosmann C, et al. Reciprocal coupling of coagulation and innate immunity via neutrophil serine proteases. *Nat Med*. 2010;16:887–96.
- [130] von Brühl ML, Stark K, Steinhart A, Chandraratne S, Konrad I, Lorenz M, et al. Monocytes, neutrophils, and platelets cooperate to initiate and propagate venous thrombosis in mice *in vivo*. *J Exp Med*. 2012;209:819–35.
- [131] Abbasi M, Fitzgerald S, Ayers-Ringler J, Espina V, Mueller C, Rucker S, et al. Proteomic analysis of cardioembolic and large artery atherosclerotic clots using reverse phase protein array technology reveals key cellular interactions within clot microenvironments. *Cureus*. 2021;13:e13499.
- [132] Roessler FC, Kalms N, Jann F, Kemmling A, Ribbat-Idel J, Stellmacher F, et al. First approach to distinguish between cardiac and arteriosclerotic emboli of individual stroke patients applying the histological THROMBEX-classification rule. *Sci Rep*. 2021;11:8433.
- [133] Bang OY, Chung JW, Lee MJ, Seo WK, Kim GM, Ahn MJ, et al. Cancer-related stroke: an emerging subtype of ischemic stroke with unique pathomechanisms. *J Stroke*. 2020;22:1–10.
- [134] Di Meglio L, Desilles JP, Solonomenjanahary M, Labreuche J, Ollivier V, Dupont S, et al. DNA content in ischemic stroke thrombi can help identify cardioembolic strokes among strokes of undetermined cause. *Stroke*. 2020;51:2810–6.
- [135] Boeckh-Behrens T, Kleine JF, Zimmer C, Neff F, Scheipl F, Pelisek J, et al. Thrombus histology suggests cardioembolic cause in cryptogenic stroke. *Stroke*. 2016;47:1864–71.
- [136] Konieczynska M, Fil K, Bazanek M, Undas A. Prolonged duration of type 2 diabetes is associated with increased thrombin generation, prothrombotic fibrin clot phenotype and impaired fibrinolysis. *Thromb Haemost*. 2014;111:685–93.
- [137] Dunn EJ, Ariëns RAS, Grant PJ. The influence of type 2 diabetes on fibrin structure and function. *Diabetologia*. 2005;48:1198–206.
- [138] de Vries JJ, Hoppenbrouwers T, Martinez-Torres C, Majied R, Özcan B, van Hoek M van, et al. Effects of diabetes mellitus on fibrin clot structure and mechanics in a model of acute neutrophil extracellular traps (NETs) formation. *Int J Mol Sci*. 2020;21:1–15.
- [139] Rothwell PM, Howard SC, Power DA, Gutnikov SA, Algra A, Van Gijn J, et al. Fibrinogen concentration and risk of ischemic stroke and acute coronary events in 5113 patients with transient ischemic attack and minor ischemic stroke. *Stroke*. 2004;35:2300–5.
- [140] Machlus KR, Cardenas JC, Church FC, Wolberg AS. Causal relationship between hyperfibrinogenemia, thrombosis, and resistance to thrombolysis in mice. *Blood*. 2011;117:4953–63.
- [141] Zalewski J, Bogaert J, Sadowski M, Woznicka O, Doulaptsis K, Ntoumpanaki M, et al. Plasma fibrin clot phenotype independently affects intracoronary thrombus ultrastructure in patients with acute myocardial infarction. *Thromb Haemost*. 2015;113:1258–69.
- [142] Anglés-Cano E, Hervio L, Rouy D, Fournier C, Chapman JM, Laplaud M, Koschinsky ML. Effects of lipoprotein(a) on the binding of plasminogen to fibrin and its activation by fibrin-bound tissue-type plasminogen activator. *Chem Phys Lipids*. 1994;67–68:369–80.
- [143] Rouy D, Grailhe P, Nigon F, Chapman J, Anglés-Cano E. Lipoprotein(a) impairs generation of plasmin by fibrin-bound tissue-type plasminogen activator. In vitro studies in a plasma milieu. *Arterioscler Thromb*. 1991;11:629–38.
- [144] Arora P, Kalra R, Callas PW, Alexander KS, Zakai NA, Wadley V, et al. Lipoprotein(a) and risk of ischemic stroke in the REGARDS study. *Arterioscler Thromb Vasc Biol*. 2019;39:810–8.
- [145] Wygrecka M, Birnhuber A, Seeliger B, Michalick L, Pak O, Schultz AS, et al. Altered fibrin clot structure and dysregulated fibrinolysis contribute to thrombosis risk in severe COVID-19. *Blood Adv*. 2022;6:1074–87.
- [146] Genchi A, Semerano A, Schwarz G, Dell'Acqua B, Gullotta GS, Sampaolo M, et al. Neutrophils predominate the immune signature of cerebral thrombi in COVID-19 stroke patients. *Acta Neuropathol Commun*. 2022;10:14.
- [147] Boeckh-Behrens T, Golkowski D, Ikenberg B, Schlegel J, Protzer U, Schulz C, et al. COVID-19-associated large vessel stroke in a 28-year-old patient: NETs and platelets possible key players in acute thrombus formation. *Clin Neuroradiol*. 2021;31:511–4.
- [148] Davis JC, Manzi S, Yarboro C, Rairie J, McInnes I, Averthelyi D, et al. Recombinant human DNase I (rhDNase) in patients with lupus nephritis. *Lupus*. 1999;8:68–76.
- [149] Basso C, Goldstein E, Dai X, Rana M, Shu L, Chen C, et al. Acute ischemic stroke on anti-Xa inhibitors: pharmacokinetics and outcomes. *J Stroke Cerebrovasc Dis*. 2022;31:106612.
- [150] Kalinin DV. Factor XII(a) inhibitors: a review of the patent literature. *Expert Opin Ther Pat*. 2021;31:1155–76.

- [151] Adams HP, Effron MB, Torner J, Dávalos A, Frayne J, Teal P, et al. Emergency administration of abciximab for treatment of patients with acute ischemic stroke: results of an international phase III trial: abciximab in emergency treatment of stroke trial (AbESTT-II). *Stroke*. 2008;39:87–99.
- [152] Jadoui S, Le Chapelain O, Ollivier V, Mostefa-Kara A, Di Meglio L, Dupont S, et al. Glenzocimab does not impact glycoprotein VI-dependent inflammatory haemostasis. *Haematologica*. 2021;106:2000–3.
- [153] Wichaiyo S, Parichatikanond W, Rattanavipanon W. Glenzocimab: a GPVI (glycoprotein VI)-targeted potential antiplatelet agent for the treatment of acute ischemic stroke. *Stroke*. 2022;53:3506–13.
- [154] Ollivier V, Freiherr Von Seckendorff A, Solonomenjanahary M, Bourrienne M, Desilles J, Mazighi M, et al. Carbamylation in acute ischemic stroke thrombi impairs t-PA-mediated thrombolysis and increases thrombus frailty [abstract]. *Res Pract Thromb Haemost*. 2022;6(Suppl 1):OC 46. d.
- [155] Kadaba Ramanujam R, Garyfallogiannis K, Yu T, Nagaswami C, Litvinov R, Weisel J, et al. Fracture mechanics of human blood clots: fibrinogen concentration is a critical determinant of clot toughness [abstract]. *Res Pract Thromb Haemost*. 2022;6(Suppl 1):VPB0962.
- [156] Sandrin Gauer J, Duval C, Xu R, Macrae F, Tiede C, Tomlinson D, et al. Increased platelet procoagulant activity driven by fibrin-GPVI interaction alters clot structure [abstract]. *Res Pract Thromb Haemost*. 2022;6(Suppl 1):PB0383.
- [157] Di Meglio L, Solonomenjanahary M, Bourrienne M, Ollivier V, Mazighi M, Ho-Tin-Noé B, et al. DNase 1 unveils the presence and unlocks the thrombolytic potential of intravenously administered t-PA in large vessel occlusion acute ischemic stroke [abstract]. *Res Pract Thromb Haemost*. 2022;6(Suppl 1):VPB0647.
- [158] van Moorsel M, Maas C, De Maat S. von Willebrand factor-targeting thrombolysis to overcome tissue plasminogen activator resistance in vivo [abstract]. *Res Pract Thromb Haemost*. 2022;6(Suppl 1):OC 46, 4.
- [159] Mathews N, Suzuki Y, Honkura N, Sano H, Urano T. Early systemic administration of a natural carboxypeptidase inhibitor potentiates tPA-mediated thrombolysis: evidence from real-time intravital imaging analysis of microthrombi in mice [abstract]. *Res Pract Thromb Haemost*. 2022;6(Suppl 1):PBO630.