Stroke

ORIGINAL CONTRIBUTION

Parabiosis Discriminates the Circulating, Endothelial, and Parenchymal Contributions of Endogenous Tissue-Type Plasminogen Activator to Stroke

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BACKGROUND: Intravenous injection of alteplase, a recombinant tPA (tissue-type plasminogen activator) as a thrombolytic agent has revolutionized ischemic stroke management. However, tPA is a more complex enzyme than expected, being for instance able to promote thrombolysis, but at the same time, also able to influence neuronal survival and to affect the integrity of the blood-brain barrier. Accordingly, the respective impact of endogenous tPA expressed/present in the brain parenchyma versus in the circulation during stroke remains debated.

METHODS: To address this issue, we used mice with constitutive deletion of tPA (tPA^{Null} [tPA-deficient mice]) or conditional deletion of endothelial tPA (VECad [vascular endothelial-Cadherin-Cre-recombinase]-$Cre^{\Delta tPA}$). We also developed parabioses between tPA^{Null} and wild-type mice (tPA^{WT}), anticipating that a tPA^{WT} donor would restore levels of tPA to normal ones, in the circulation but not in the brain parenchyma of a tPA^{Null} recipient. Stroke outcomes were investigated by magnetic resonance imaging in a thrombo-embolic or a thrombotic stroke model, induced by local thrombin injection or $FeCl_3$ application on the endothelium, respectively.

RESULTS: First, our data show that endothelial tPA, released into the circulation after stroke onset, plays an overall beneficial role following thrombo-embolic stroke. Accordingly, after 24 hours, tPA^{Null}/tPA^{Null} parabionts displayed less spontaneous recanalization and reperfusion and larger infarcts compared with tPA^{WT}/tPA^{WT} littermates. However, when associated to tPA^{WT} littermates, tPA^{Null} mice had similar perfusion deficits, but less severe brain infarcts. In the thrombotic stroke model, homo- and hetero-typic parabionts did not differ in the extent of brain damages and did not differentially recanalize and reperfuse.

CONCLUSIONS: Together, our data reveal that during thromboembolic stroke, endogenous circulating tPA from endothelial cells sustains a spontaneous recanalization and reperfusion of the tissue, thus, limiting the extension of ischemic lesions. In this context, the impact of endogenous parenchymal tPA is limited.

GRAPHIC ABSTRACT: A graphic abstract is available for this article.

Key Words: embolic stroke ■ endothelium ■ magnetic resonance imaging ■ parabiosis ■ stroke

The prompt re-establishment of blood perfusion remains the only therapeutic approach that has proven efficient in reducing brain lesions and functional deficits at the acute stage of ischemic stroke. Reperfusion is achievable via pharmacological dissolution (thrombolysis) or mechanical removal (endovascular thrombectomy). Thrombolysis with alteplase has revolutionized the treatment of ischemic stroke[1] and has paved the way to the

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Nonstandard Abbreviations and Acronyms

CBF	cerebral blood flow
NMDAR	N-methyl-D-aspartate receptors
PAI-1	type 1 plasminogen activator inhibitor
TAFI	thrombin-activatable fibrinolysis inhibitor
tPA	tissue-type plasminogen activator
tPA^{Null}	tissue-type plasminogen activator deficient
tPA^{WT}	tissue-type plasminogen activator wild type

development of innovative clot-busting drugs, including tenecteplase.[2] Despite a favorable risk/benefit ratio, the overall clinical profit of alteplase may be reduced because of a low rate of recanalization, a risk of intracerebral bleeding,[3] and side effects on/above the neurovascular unit.[4]

tPA (tissue-type plasminogen activator), is a natural fibrinolytic protease, that activates plasminogen into plasmin, leading to the degradation of fibrin clots. Endogenous levels of active tPA during ischemic stroke are not sufficient to promote fibrinolysis, explaining why alteplase administration (a recombinant form of tPA) is required.[5] Endothelial cells and hepatocytes[6,7] release tPA in the bloodstream, especially in response to hypoxia/ischemia or high shear stress conditions,[8] but also constitutively.[9] tPA released by endothelial cells in the bloodstream can access the brain parenchyma by an LRP-1 (low density lipoprotein receptor-related protein-1) mechanism.[10] Still, it remains unknown whether endothelial cells can directly release tPA to the brain parenchyma. tPA is also expressed in key brain structures (eg, hippocampus, cortex, amygdala), there, originating from several cell types including neurons and activated microglia.[11–13]

tPA can interact with various extracellular or membrane substrates or receptors. This diversity of interactions is why tPA is considered a critical mediator of brain pathophysiology throughout life.[14] Among its various roles, tPA is known to influence neuronal fate,[15–17] neuroinflammation,[18,19] learning and memory processes,[20,21] anxiety,[22] blood-brain barrier homeostasis.[23,24] Importantly, some of these functions do not depend on plasmin production and do not even on tPA's enzymatic activity.

Beyond its vascular fibrinolytic role, circulating tPA can also play a part in some of the central nervous system dysfunctions mentioned above. These effects on the brain can occur through direct actions on the vessel wall. For instance, tPA is involved in the regulation of physiological neurovascular coupling, which connects neuronal activity to cerebral blood flow (CBF) by acting on endothelial NMDAR (N-methyl-D-aspartate) .[25,26] Conversely, under pathological conditions, the partnership between circulating tPA and endothelial NMDAR promotes blood-brain barrier leakage and inflammation.[24,27] In addition to these direct effects on brain vasculature, tPA can also exit the bloodstream[28,29] and contribute to tPA within the brain parenchyma, leading to increased neuronal activity and potential neuronal loss.[17,30]

Preclinical studies investigating the role and effect of tPA during ischemic stroke have yielded controversial findings, with some reporting beneficial outcomes[31–33] and others indicating deleterious effects.[34–38] However, this literature can be criticized because the results are primarily obtained from mice in which tPA is invalidated throughout the organism, or by increasing tPA expression to irrelevant levels (such as in genetically modified mice, through hydrodynamic transfection, or via intravenous injection of alteplase, etc). Furthermore, concerns have been raised about potential genetic contamination and its impact on the specificity of some phenotypic features in the historical tPA-deficient mouse line.[39]

Thus, to date, elucidating the true contribution of endogenous vascular tPA to brain physiopathology remains challenging. Here, we aimed at developing an original tool that allows for the discrimination of the effects of endogenous vascular tPA on cerebral physiopathology. To achieve this, we established heterochronic parabioses between tPA wild-type (tPA^{WT}) and tPA-deficient (tPA^{Null}) mice, with the assumption that blood circulation sharing would restore circulating levels of tPA in the deficient mouse without affecting the levels in other compartments of this animal.

METHODS

The data that support the findings of this study are available from the corresponding author upon reasonable request. Our study was approved by the national animal care and local ethical committee CENOMEXA ([Comité d'éthique Normandie en Matière d'expérimentation Animale]; #19978, #19435). Detailed Materials and Methods are available in the Supplemental Materials. The design of the study is described in panel A of each figure. Briefly, all animals were submitted to an occlusion of the middle cerebral artery and imaged by laser Doppler flowmetry monitoring until 40 minutes after middle cerebral artery occlusion and magnetic resonance imaging at 24 hours after middle cerebral artery occlusion, to evaluate ischemic lesion volumes, brain edema, the occurrence of hemorrhagic transformation and quantification of tissular perfusion. Statistical significance was set at $P<0.05$.

RESULTS

Endogenous tPA Is Beneficial in a Model of Thrombo-Embolic Stroke

To study the effect of endogenous circulating tPA in relevant preclinical models, we first compared a new tPA^{Null},[40] and its tPA^{WT} in a model of ischemic stroke in which the occlusive clot is sensitive to thrombolysis[33,41] (Figure 1A). In this model of clots induced by local injection of

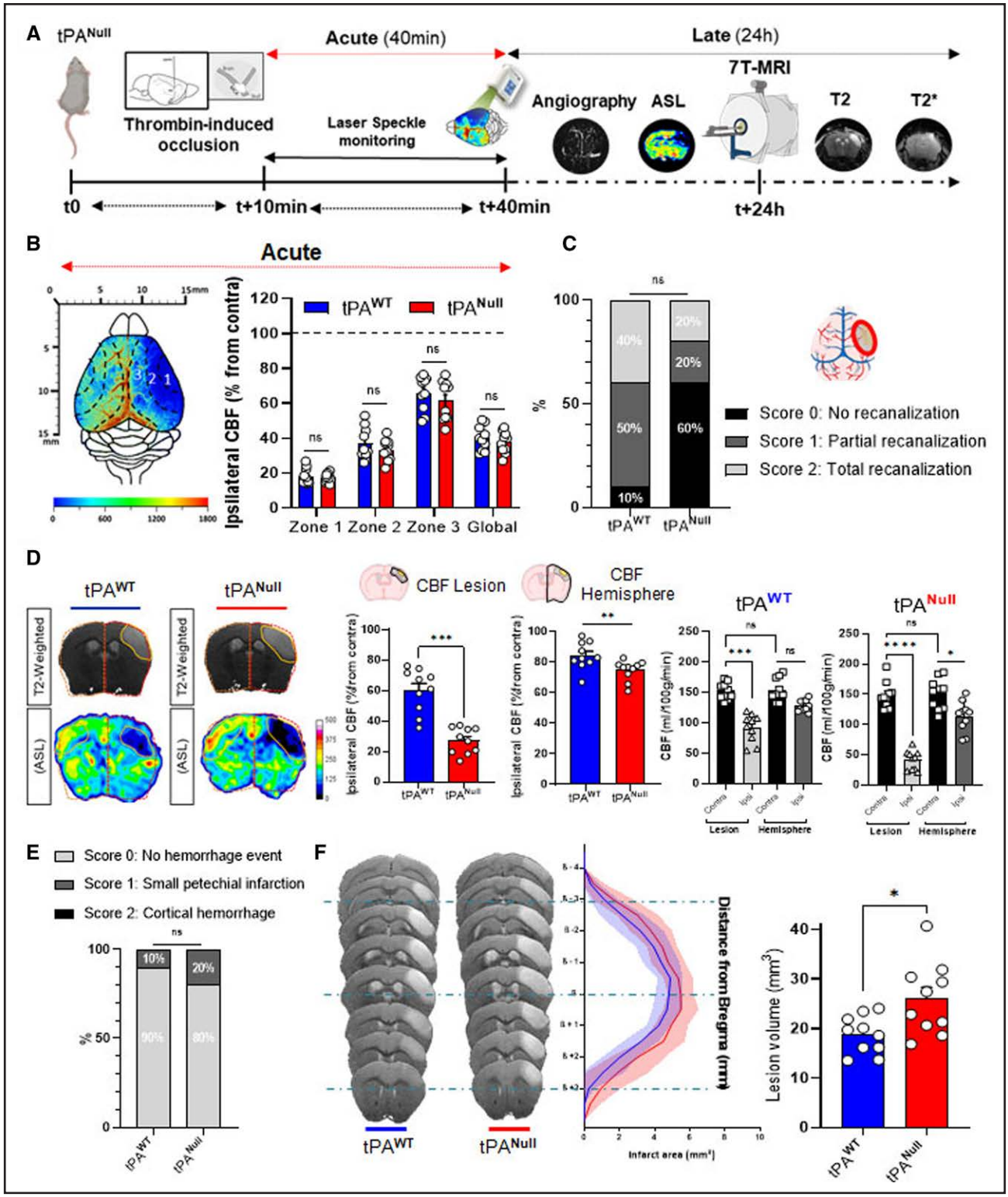

Figure 1. Endogenous tPA (tissue-type plasminogen activator) is beneficial in a thrombo-embolic stroke model.
A, Schematic representation of the experimental timeline for laser Doppler flowmetry monitoring of cerebral blood flow (CBF) up to 40 min after occlusion of the middle cerebral artery (MCAo) and for 24-h magnetic resonance imaging (MRI) follow-up. **B**, Colormaps and corresponding quantification showing the CBF decrease in the ispilateral hemisphere, during stroke induction in tPA^{WT} (tPA wild-type) or tPA^{Null} (tPA-deficient) mice, using laser Doppler flowmetry. Measurements were made either in the full ipsilateral hemisphere (global) or in 3 areas (zone 1, ischemic core; zone 2, penumbra; and zone 3, oligemia) and compared with contralateral mirror regions of interest (ROIs). Circles represent individual values (mean±SEM, n=10 per group). Kruskal–Wallis test. **C**, Proportions of recanalization scores 24 h after stroke onset in tPA^{WT} or tPA^{Null} mice (mean±SEM, n=10 per group), Fisher exact test. **D**, Representative arterial spin labeling (ASL) MRI images and quantification of (*Continued*)

thrombin, the impact of tPA deficiency is a global reflect of the vascular and parenchymal effects of the endogenous protease. The drop in CBF achieved at the initiation of stroke (measured by Laser Doppler Speckle Flowmetry) was identical in both genotypes (Figure 1B; 37.62% in tPA^{Null} versus 39.94% in tPA^{WT}, n=10 per group; P=0.6842). The analysis of angiographic scores showed that 24 hours later, while full or partial recanalization of the MCA has occurred in 90% of tPA^{WT} mice, the majority of tPA^{Null} mice showed no recanalization (Figure 1C). In line with this, arterial spin labeling maps of CBF (Figure 1D), showed that the ischemic area remained largely hypoperfused compared with its contralateral homolog in tPA^{Null} mice (27.28% versus contralateral). This hypoperfusion was less severe in tPA^{WT} animals (60.31% versus contralateral; Figure 1D). Consistent with these vascular aspects, the infarcted lesion was 38.79% bigger in tPA^{Null} versus tPA^{WT} mice (n=10 per group, 26.01 versus 18.74 mm^3; P=0.0232; Figure 1F). Regarding bleeding, tPA^{WT} and tPA^{Null} mice were not different, as animals exhibited no hemorrhage or minor petechiae, but never significant hemorrhagic transformation (Figure 1E). Altogether, these findings demonstrate the overall benefit of endogenous tPA in a thrombo-embolic stroke model.

We sought to determine if, in response to clot formation, endothelial cells release enough tPA to promote spontaneous reperfusion and recanalization in this stroke model. We found that mice with a selective depletion of tPA from endothelial origin[26] (Figure 2A) recapitulated the phenotype of tPA^{Null} mice. First, the extent of CBF loss achieved at the initiation of stroke was identical in both the VECad (vascular endothelial-Cadherin-Cre-recombinase)-$Cre^{\Delta tPA}$ and VECad-Cre^{WT} (Figure 2B; 44.13% versus 47.76%, P=0.5132, n=10 per group). Twenty-four hours later, while all VECad-Cre^{WT} animals had totally or partially recanalized, 40% of VECad-$Cre^{\Delta tPA}$ mice had no recanalization (Figure 2C). The extent of residual hypoperfusion in the lesioned area was worse in the VECad-$Cre^{\Delta tPA}$ (Figure 2D), and the infarcted lesion was 18.95% bigger in VECad-$Cre^{\Delta tPA}$ versus VECad-Cre^{WT} mice (n=10 per group, 25.32 versus 21.27 mm^3, Figure 2F; P=0.0147). In parallel, T2* magnetic resonance imaging revealed that the proportion of VECad-Cre^{WT} and VECad-$Cre^{\Delta tPA}$ mice displaying bleeding was low, with no hemorrhagic event in most animals, and 20% of petechiae in both groups (Figure 2E). Altogether, these findings demonstrate the overall benefit of endogenous tPA released by endothelial cells, promoting spontaneous recanalization and reperfusion, as well as brain protection in a thrombo-embolic model of stroke.

Parabiosis Unmasks the Differential Effects of Endogenous Circulating Basal Levels of tPA, tPA Released From Endothelial Cells and Parenchymal tPA After Stroke

We then sought to determine the respective contribution of vascular and parenchymal tPA to the above-described phenotype after thrombin-induced stroke. We thus developed parabiosis with the idea that via the shared circulation, a tPA^{WT} mouse could restore circulating levels of a tPA^{Null} mouse to physiological levels, without influencing other compartments. A complete demonstration of this efficient chimerism is provided in Figure S2.

We constituted 3 experimental groups of parabionts: tPA^{Null*}/tPA^{Null}, tPA^{WT*}/tPA^{WT}, and tPA^{Null*}/tPA^{WT} in which 1 animal (the 1 with an asterisk) was subjected to thrombin-induced stroke (Figure 3A). The drop in CBF achieved at the initiation of stroke was identical in the 3 groups (Figure 3B), suggesting that clot formation was homogeneous. After 24 hours, the quantification of CBF by arterial spin labeling in the lesioned area showed that the residual hypoperfusion was more severe in the absence of tPA throughout the body (tPA^{Null*}/tPA^{Null} versus tPA^{WT*}/tPA^{WT} parabioses). Despite the sharing of circulation, the tPA^{WT} of tPA^{Null*}/tPA^{WT} pairs did not rescue spontaneous reperfusion in their tPA^{Null} partners (Figure 3C through 3E). These data suggest that the basal levels of endogenous vascular tPA are not sufficient to promote reperfusion of the tissues and must be accompanied by a local release of endothelial tPA t the site of occlusion to promote recanalization and reperfusion of the tissue.

As observed in nonparabiontic animals (Figure 1), the infarcted lesion was bigger in tPA^{Null} versus tPA^{WT} mice of the homotypic pairs (+69.4%, n=10 per group, 30.16 versus 17.80 mm^3; P<0.0001, Figure 3F). In heterotypic tPA^{Null*}/tPA^{WT} pairs, the tPA^{WT} parabiont partly protected the tPA^{Null} against stroke-induced infarction (21% smaller lesions in the tPA^{Null} when linked to a tPA^{WT} versus to a tPA^{Null} mouse). Together, these data show that in the thrombo-embolic stroke model, endogenous vascular tPA is nevertheless an important player in the evolution of stroke damages, with an overall prevailing beneficial effect.

We finally constituted another series of 3 experimental groups: tPA^{Null*}/tPA^{Null}, tPA^{WT*}/tPA^{WT}, and tPA^{Null*}/tPA^{WT} in which 1 animal (the one with an asterisk) was subjected to the ferric chloride-induced thrombotic stroke model, which is not sensitive to alteplase-driven thrombolysis[39] (Figure 4A). The drop in CBF achieved at the initiation of stroke was identical in the 3 groups (Figure 4B),

Figure 1 Continued. tissular perfusion in the full hemisphere and lesion site 24 h after stroke onset. Each point represents individual values (mean±SEM, n=10 per group). **E**, Quantification of hemorrhagic scores 24 h after stroke onset in tPA^{WT} or tPA^{Null} mice (mean±SEM, n=10 per group), Fisher exact test. **F**, Representative T2-weighted MRI images of the ischemic lesion (**left**), edema distribution around the bregma (**middle**) and quantification of lesion volumes (**right**) 24 h after stroke onset in tPA^{WT} and tPA^{Null} mice (mean±SEM, n=10 per group). *P<0.05, Mann-Whitney U test, **P<0.005, ***P<0.001 Mann-Whitney U test (**middle**). **P<0.005, ***P<0.001, Friedman test (**right**).

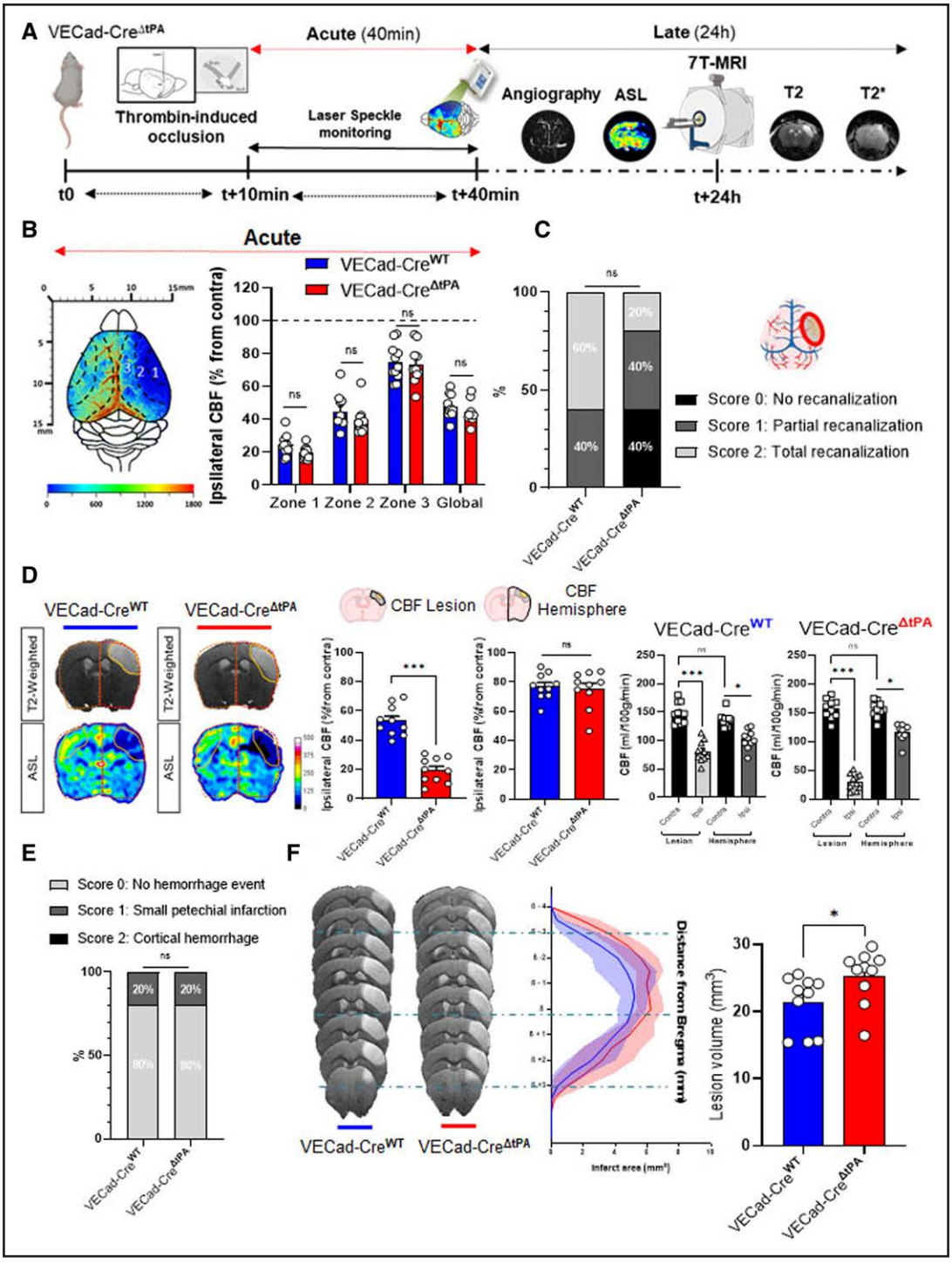

Figure 2. Endothelial tPA (tissue-type plasminogen activator) is beneficial in a thrombo-embolic stroke model.
A, Schematic representation of the experimental timeline for laser Doppler flowmetry monitoring of cerebral blood flow (CBF) and for magnetic resonance imaging (MRI) follow-up after occlusion of the middle cerebral artery (MCAo). **B**, Colormaps and corresponding quantification showing the CBF decrease in ispilateral hemisphere, during stroke induction in VECad (vascular endothelial-Cadherin-Cre-recombinase)-Cre^{WT} or VECad-Cre$^{\Delta tPA}$ mice, using laser Doppler flowmetry. Circles represent values for each mouse (mean±SEM, n=10 per group). Kruskal-Wallis test. **C**, Proportions of recanalization scores 24 h after stroke onset in VECad-Cre^{WT} or VECad-Cre$^{\Delta tPA}$ mice (mean±SEM, n=10 per group), Fisher exact test. **D**, Representative arterial spin labeling (ASL) MRI images and quantification of tissular perfusion in the full hemisphere and lesion site 24 h after stroke onset in VECad-Cre^{WT} and VECad-Cre$^{\Delta tPA}$ mice. Each point represents individual values (mean±SEM, n=10 per group). **E**, Quantification of hemorrhage score at 24 h after stroke onset in VECad-Cre^{WT} or VECad-Cre$^{\Delta tPA}$ mice (mean±SEM, n=10 per group), Fisher exact test. **F**, Representative T2-weighted MRI images of the ischemic lesion (**left**), edema distribution around the bregma (**middle**) and quantification of lesion volume (**right**) at 24 h after stroke in VECad-Cre^{WT} and VECad-Cre$^{\Delta tPA}$ mice (mean±SEM, n=10 per group). *P<0.05, Mann-Whitney U test, **P<0.005, ***P<0.001 Mann-Whitney U test (**middle**). **P<0.005, ***P<0.001, Friedman test (**right**).

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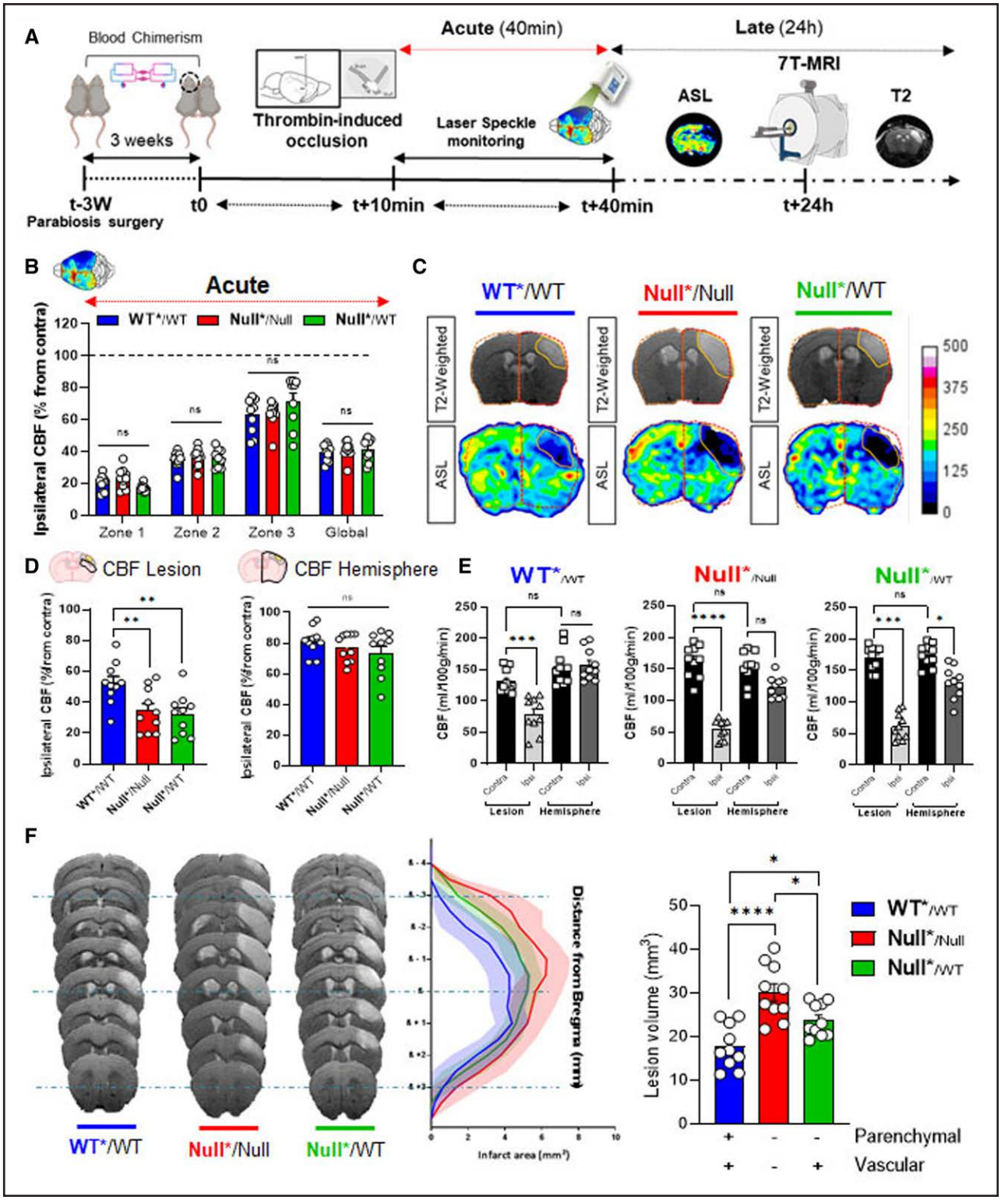

Figure 3. Rescue for circulating tPA (tissue-type plasminogen activator) levels is beneficial in a thrombo-embolic stroke model. **A**, Schematic representation of the experimental timeline for laser Doppler flowmetry monitoring of cerebral blood flow (CBF) and for magnetic resonance imaging (MRI) follow-up after occlusion of the middle cerebral artery (MCAo). **B**, Quantification showing the CBF decrease in ipsilateral hemisphere, during stroke induction in homo-typic (**WT***/WT; **Null***/Null) and hetero-typic (**Null***/WT) groups (the ischemic mouse is mentioned in bold with *), using laser Doppler flowmetry measurement. Circles represent values for each mouse (mean±SEM, n=10 per group). Kruskal-Wallis test. **C**, Representative ASL MRI images at 24 h after stroke, in homo-typic (**WT***/WT; **Null***/Null) and hetero-typic (**Null***/WT) groups. **D**, Quantification of tissular perfusion in the lesion site between groups at 24 h after stroke onset. Each point represents values for each mouse (mean±SEM, n=10 per group). **E**, Quantification of tissular perfusion in the lesion site compared with contralateral (*Continued*)

suggesting that clot formation was homogeneous. In contrast to the findings in the thrombo-embolic stroke model, after 24 hours, whatever the experimental group, spontaneous reperfusion in the lesioned area was minor (Figure 4C through 4E). Importantly, the stroke lesions were similar in the 3 groups: 20.40 mm^3 in the tPA^{WT*}/tPA^{WT}, 23.52 mm^3 in the tPA^{Null*}/tPA^{Null}, and 22.70 mm^3 in the tPA^{Null*}/tPA^{WT} (Figure 4F). Together, these data confirm that $FeCl_3$ produces alteplase-resistant clots, and that in this context, endogenous tPA has no impact at all, neither in the blood nor in the parenchyma.

DISCUSSION

Although the utility of alteplase for ischemic stroke treatment is not questioned,[2,42] several limits exist, some being associated to the access/eligibility to the drug, others to the drug itself.[43] Endogenous tPA may well exert deleterious effects during stroke but this remains controversial.

Our aim here was to address deeper this issue, and taking into account vascular versus parenchymal endogenous tPA. Our findings are summarized in Figure S4. We demonstrate thanks to constitutive knockout, conditional knockout mice, and parabioses, that circulating levels of tPA allow brain protection, especially the one locally released from endothelial cells, provided that the occlusive clot is fibrin-rich.

Dissociating parenchymal versus circulating actions of tPA (potentially produced and released by hepatocytes and endothelial cells)[7,26] was to date not possible. Recently, Torrente et al[44] used an interesting approach targeting the 2 main inhibitors of tPA: neuroserpin, which is only expressed in the brain, and the PAI-1 (type 1 plasminogen activator inhibitor), which is mainly expressed in the circulation. Their findings suggest that NSP deficiency enhances tPA activity in the parenchyma which increases blood-brain barrier permeability and worsens stroke outcomes, while PAI-1 deficiency enhances fibrinolysis and improves recovery. Here, we used another strategy to dissect the parenchymal versus the circulating effects of tPA: parabiosis, with the idea that when paired to a tPA^{Null} mouse, a tPA^{WT} mouse should allow rescuing levels of tPA to physiological ranges only in the circulation of the deficient mouse. Of note, while our ELISA assays confirm this rescue of tPA levels, it would have been elegant to provide additional support by blocking the effect of tPA with an inhibitor such as PAI-1.

Our main findings are that in a model of thrombo-embolic stroke, which is sensitive to alteplase, endogenous circulating tPA can, with time, allow the recanalization of the occluded artery, as well as the reperfusion in the ischemic tissue, thus, avoiding expansion of the infarct. More precisely, our study shows that tPA locally released from endothelial cells is critical to promote tissue recanalization and reperfusion. Nevertheless, we found that although the poststroke CBF defect in the ischemic lesion of tPA^{Null} mice was not compensated by pairing with tPA^{WT} mice, the brain infarct was efficiently reduced. These data suggest that the basal levels of vascular tPA may play a role to limit re-occlusion or thromboinflammation. A similar apparent discrepancy was also observed in endothelial tPA-deleted mice. However, in these animals, the lack of endothelial tPA significantly reduced the recanalization rate, showing that this endothelial release of tPA can locally destroy the blood clot. A potential explanation of why poststroke CBF and brain lesions do not systematically match may be that circulating levels of active tPA must reach a threshold to restore tissue reperfusion, maybe acting on microthombosis. Endothelial cells alone may not allow reaching this threshold, and tPA released in the circulation from hepatocytes could be required for a full recovery of vascular patency and of CBF.[26] It is also important not to exclude a potential protective contribution from parenchymal tPA during thromboembolic stroke (but not after thrombotic stroke).

We did not observe any effect of genotypes on CBF drops at stroke initiation. In the same model of stroke and in the absence of any treatment, it has been shown by laser doppler[33,36] and by ultrafast ultrasound imaging,[45] that no reperfusion has yet occurred after 2 hours in the ischemic area. Thus, the spontaneous reperfusion observed 24 hours later may begin at later stages than in these first 2 hours of stroke. Another interesting finding is that, in the same model, a diabody specific for TAFI (thrombin-activatable fibrinolysis inhibitor) and PAI-1 administered early after stroke onset (20 minutes) caused a 2-fold decrease in brain infarct size 24 hours later, and was even more efficient than administration of exogenous tPA at the same time point.[46] This indicates that endogenous PAI-1 prevents the early action of tPA but that later on, active levels of tPA are then sufficient to allow partial recanalization and reperfusion, as well as brain protection.

No study, before the present one, has ever tested tPA-deficient mice in a stroke model involving a thromboembolic blood clot. But an interesting meta-analysis[47] of the impact of mechanical stroke models in tPA knockout mice concluded that due to a huge heterogeneity (ie, both smaller and bigger lesions reported), endogenous

Figure 3 Continued. hemisphere in **WT***/WT, **Null***/Null, **Null***/WT groups, at 24 h after stroke onset. Each point represents values for each mouse (mean±SEM, n=10 per group). *P<0.05, **P<0.01, 1 way ANOVA test, ***P<0.001, ****P<0.001, Friedman test. **F**, Representative T2-weighted MRI brain images (**left**), edema distribution around the bregma (**middle**) and corresponding quantification of lesion volume (**right**) at 24 h after stroke onset in homo-typic (**WT***/WT; **Null***/Null) and hetero-typic (**Null***/WT) groups (mean±SEM, n=10 per group). *P<0.0001, one way ANOVA test.

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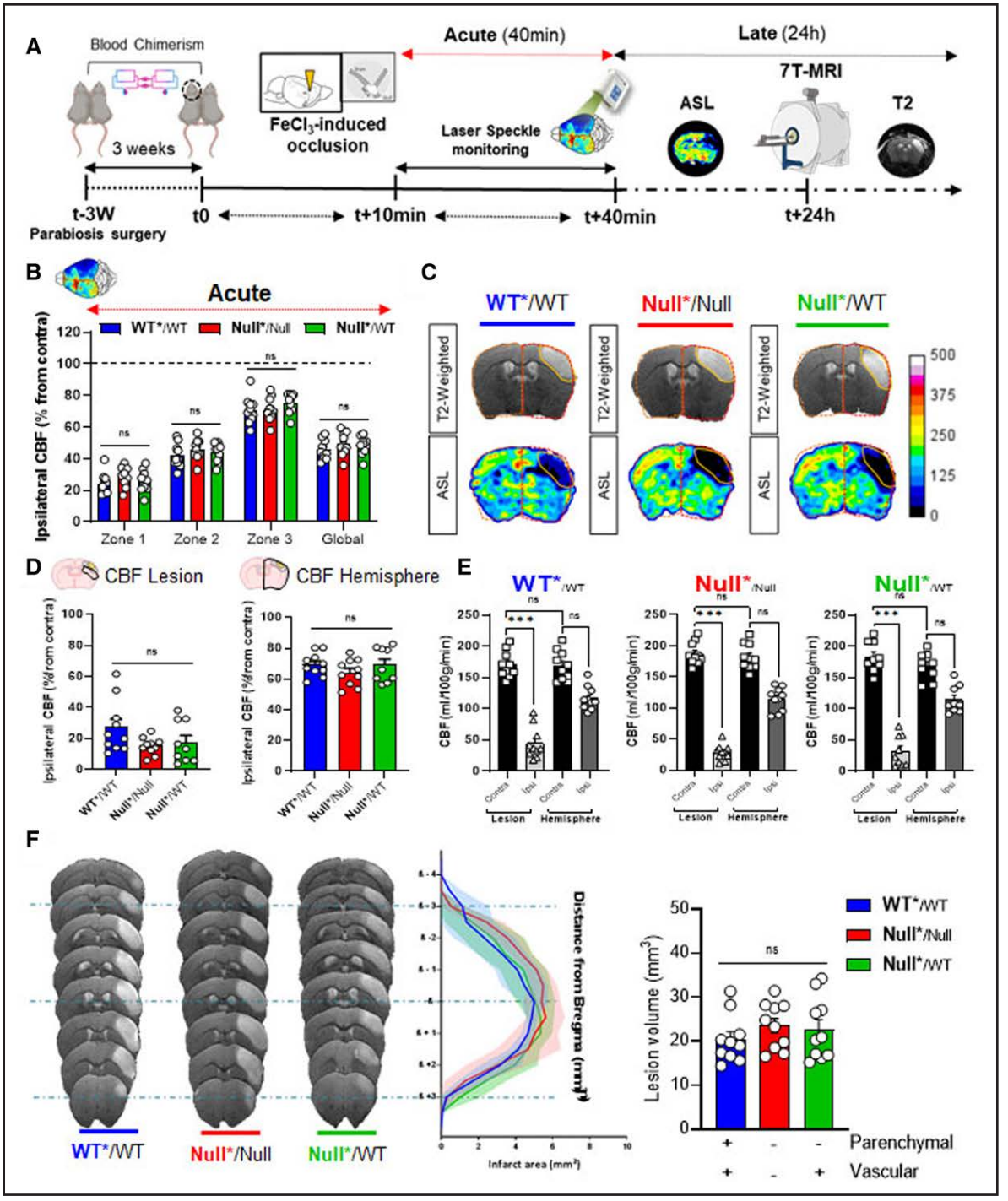

Figure 4. Rescue of circulating tPA (tissue-type plasminogen activator) levels has no effect in a thrombotic stroke model. **A**, Schematic representation of the experimental timeline for laser Doppler flowmetry monitoring of cerebral blood flow (CBF) and for magnetic resonance imaging (MRI) follow-up after occlusion of the middle cerebral artery (MCAo). **B**, Quantification showing the CBF decrease in ispilateral hemisphere, during stroke induction in homo-typic (**WT***/WT; **Null***/Null) and hetero-typic (**Null***/WT) groups (the ischemic mouse is mentioned in bold with *), using laser Doppler flowmetry. Circles represent values for each mouse (mean±SEM, n=10 per group). Kruskal-Wallis test. **C**, Representative arterial spin labeling (ASL) MRI images at 24 h after stroke, in homo-typic (**WT***/WT; **Null***/Null) and hetero-typic (**Null***/WT) groups. **D**, Quantification of tissular perfusion in the lesion site between groups at 24 h after stroke onset. Each point represents values for each mouse (mean±SEM, n=10 per group). One way ANOVA test. **E**, Quantification of tissular perfusion in the lesion site compared with contralateral hemisphere in **WT***/WT, **Null***/Null, **Null***/WT groups, 24 h after stroke onset. Each point represents values for each mouse (mean±SEM, n=10 per group). **F**. Representative T2-weighted MRI brain images (**left**), edema distribution around the bregma (**middle**) and corresponding quantification of lesion volumes (**right**) 24 h after stroke onset in homo-typic (**WT***/WT; **Null*/**Null) and hetero-typic (**Null*/**WT) groups (mean±SEM, n=10 per group). One way ANOVA test. *P<0.05, ***P<0.001, ****P<0.001, Friedman test.

tPA has no effect on brain infarction.[31,34,48,49] Overall, this highlights the importance of choosing relevant stroke models: tPA (endogenous or exogenous) may be beneficial via its thrombolytic activity, in stroke models with secondary brain thrombosis or thromboembolism[31–33]; otherwise, or if thrombolysis is performed outside of its therapeutic window, the neurotoxic tPA effects may prevail.[34–38]

ARTICLE INFORMATION

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Drs Ali, Vivien, and Furon designed the study. Dr Furon, Dr Lebrun, Dr Yétim, Dr Levard, P. Marie, Dr Orset, Martinez de Lizarrondo performed the experiments. Dr Furon performed data acquisition and analysis. Drs Ali, Furon, and Levard wrote the article. All the Authors reviewed the article. The authors are grateful to Mikaël Naveau and Quentin Gérard for their technical advices.

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Disclosures

None.

Supplemental Material

Supplemental Materials and Methods
Supplemental Results
Figures S1–S4
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REFERENCES

1. NINDS. Tissue plasminogen activator for acute ischemic stroke. *N Engl J Med*. 1995;333:1581–1587. doi: 10.1056/NEJM199512143332401
2. Gauberti M, Martinez de Lizarrondo S, Vivien D. Thrombolytic strategies for ischemic stroke in the thrombectomy era. *J Thromb Haemost*. 2021;19:1618–1628. doi: 10.1111/jth.15336
3. Tang G, Cao Z, Luo Y, Wu S, Sun X. Prognosis associated with asymptomatic intracranial hemorrhage after acute ischemic stroke: a systematic review and meta-analysis. *J Neurol*. 2022;269:3470–3481. doi: 10.1007/s00415-022-11046-6
4. Cheng J-W, Zhang X-J, Cheng L-S, Li G-Y, Zhang L-J, Ji K-X, Zhao Q, Bai Y. Low-dose tissue plasminogen activator in acute ischemic stroke: a systematic review and meta-analysis. *J. Stroke Cerebrovasc. Dis*. 2018;27:381–390. doi: 10.1016/j.jstrokecerebrovasdis.2017.09.014
5. Collen D, Lijnen H. Basic and clinical aspects of fibrinolysis and thrombolysis. *Blood*. 1991;78:3114–3124. doi: 10.1182/blood.v78.12.3114.3114
6. Levin EG, Santell L, Osborn KG. The expression of endothelial tissue plasminogen activator in vivo: a function defined by vessel size and anatomic location. *J. Cell Sci*. 1997;110:139–148. doi: 10.1242/jcs.110.2.139
7. Zheng Z, Nayak L, Wang W, Arif Yurdagul AY, Wang X, Cai B, Lapping S, Ozcan L, Ramakrishnan R, Pestell RG, et al. An ATF6-tPA pathway in hepatocytes contributes to systemic fibrinolysis and is repressed by DACH1. *Blood*. 2019;133:743–753. doi: 10.1182/blood-2018-07-864843
8. Huber D, Cramer EM, Kaufmann JE, Meda P, Massé JM, Kruithof EKO, Vischer UM. Tissue-type plasminogen activator (t-PA) is stored in Weibel-Palade bodies in human endothelial cells both in vitro and in vivo. *Blood*. 2002;99:3637–3645. doi: 10.1182/blood.v99.10.3637
9. Knipe L, Meli A, Hewlett L, Bierings R, Dempster J, Skehel P, Hannah MJ, Carter T. A revised model for the secretion of tPA and cytokines from cultured endothelial cells. *Blood*. 2010;116:2183–2191. doi: 10.1182/blood-2010-03-276170
10. Klecker PH, Fritzen L, Mazura AD, Weggen S, Pietrzik CU. Antibody-mediated inhibition of tissue-type plasminogen activator binding to the low-density lipoprotein receptor-related protein 1 as a potential beneficial modulator for stroke therapy. *J Cell Biochem*. 2023;124:1040–1049. doi: 10.1002/jcb.30431
11. Louessard M, Lacroix A, Martineau M, Mondielli G, Montagne A, Lesept F, Lambolez B, Cauli B, Mothet JP, Vivien D, et al. Tissue plasminogen activator expression is restricted to subsets of excitatory pyramidal glutamatergic neurons. *Mol Neurobiol*. 2016;53:5000–5012. doi: 10.1007/s12035-015-9432-7
12. Siao CJ, Fernandez SR, Tsirka SE. Cell type-specific roles for tissue plasminogen activator released by neurons or microglia after excitotoxic injury. *J Neurosci*. 2003;23:3234–3242. doi: 10.1523/JNEUROSCI.23-08-03234.2003
13. Lépine M, Douceau S, Devienne G, Prunotto P, Lenoir S, Regnauld C, Pouettre E, Piquet J, Lebouvier L, Hommet Y, et al. Parvalbumin interneuron-derived tissue-type plasminogen activator shapes perineuronal net structure. *BMC Biol*. 2022;20:1–15. doi: 10.1186/s12915-022-01419-8
14. Thiebaut AM, Gauberti M, Ali C, Martinez De Lizarrondo S, Vivien D, Yepes M, Roussel BD. The role of plasminogen activators in stroke treatment: fibrinolysis and beyond. *Lancet Neurol*. 2018;17:1121–1132. doi: 10.1016/S1474-4422(18)30323-5
15. Tsirka SE, Gualandris A, Amaral DG, Strickland S. Excitotoxin-induced neuronal degeneration and seizure are mediated by tissue plasminogen activator. *Nature*. 1995;377:340–344. doi: 10.1038/377340a0
16. Liot G, Roussel BD, Lebeurrier N, Benchenane K, López-Atalaya JP, Vivien D, Ali C. Tissue-type plasminogen activator rescues neurones from serum deprivation-induced apoptosis through a mechanism independent of its proteolytic activity. *J Neurochem*. 2006;98:1458–1464. doi: 10.1111/j.1471-4159.2006.03982.x
17. Nicole O, Docagne F, Ali C, Margaill I, Carmeliet P, MacKenzie ET, Vivien D, Buisson A. The proteolytic activity of tissue-plasminogen activator enhances NMDA receptor-mediated signaling. *Nat Med*. 2001;7:59–64. doi: 10.1038/83358
18. Tsirka SE, Rogove AD, Strickland S. Origin of patterning in neural tubes SIR. *Nature*. 1996;384:123–124. doi: 10.1038/384123b0
19. Zhang C, An J, Haile WB, Echeverry R, Strickland DK, Yepes M. Microglial low-density lipoprotein receptor-related protein 1 mediates the effect of tissue-type plasminogen activator on matrix metalloproteinase-9 activity in the ischemic brain. *J Cereb Blood Flow Metab*. 2009;29:1946–1954. doi: 10.1038/jcbfm.2009.174
20. Benchenane K, López-Atalaya JP, Fernández-Monreal M, Touzani O, Vivien D. Equivocal roles of tissue-type plasminogen activator in stroke-induced injury. *Trends Neurosci*. 2004;27:155–160. doi: 10.1016/j.tins.2003.12.011
21. Hébert M, Lesept F, Vivien D, Macrez R. The story of an exceptional serine protease, tissue-type plasminogen activator (tPA). *Rev Neurol (Paris)*. 2016;172:186–197. doi: 10.1016/j.neurol.2015.10.002
22. Matys T, Pawlak R, Matys E, Pavlides C, McEwen BS, Strickland S. Tissue plasminogen activator promotes the effects of corticotropin- releasing factor on the amygdala and anxiety-like behavior. *Proc Natl Acad Sci USA*. 2004;101:16345–16350. doi: 10.1073/pnas.0407355101
23. Yepes M, Sandkvist M, Moore EG, Bugge TH, Strickland DK, Lawrence DA. Tissue-type plasminogen activator induces opening of the blood-brain barrier via the LDL receptor-related protein. *J Clin Invest*. 2003;112:1533–1540. doi: 10.1172/JCI19212
24. Macrez R, Ortega MC, Bardou I, Mehra A, Fournier A, Van Der Pol SMA, Haelewyn B, Maubert E, Lesept F, Chevilley A, et al. Neuroendothelial NMDA receptors as therapeutic targets in experimental autoimmune encephalomyelitis. *Brain*. 2016;139:2406–2419. doi: 10.1093/brain/aww172
25. Anfray A, Drieu A, Hingot V, Hommet Y, Yetim M, Rubio M, Deffieux T, Tanter M, Orset C, Vivien D. Circulating tPA contributes to neurovascular coupling by a mechanism involving the endothelial NMDA receptors. *J Cereb Blood Flow Metab*. 2020;40:2038–2054. doi: 10.1177/0271678X19883599
26. Furon J, Yetim M, Pouettre E, Martinez de Lizarrondo S, Maubert E, Hommet Y, Lebouvier L, Zheng Z, Ali C, Vivien D. Blood tissue Plasminogen Activator (tPA) of liver origin contributes to neurovascular coupling involving brain endothelial N-Methyl-D-Aspartate (NMDA) receptors. *Fluids Barriers CNS*. 2023;20:1–17. doi: 10.1186/s12987-023-00411-w
27. Mehra A, Guérit S, Macrez R, Gosselet F, Sevin E, Lebas H, Maubert E, de Vries HE, Bardou I, Vivien D, et al. Nonionotropic action of endothelial NMDA receptors on blood–brain barrier permeability via Rho/

ROCK-mediated phosphorylation of myosin. *J Neurosci*. 2020;40:1778–1787. doi: 10.1523/JNEUROSCI.0969-19.2019
28. Benchenane K, Berezowski V, Ali C, Fernández-Monreal M, López-Atalaya JP, Brillault J, Chuquet J, Nouvelot A, MacKenzie ET, Bu G, et al. Tissue-type plasminogen activator crosses the intact blood-brain barrier by low-density lipoprotein receptor–related protein-mediated transcytosis. *Circulation*. 2005;111:2241–2249. doi: 10.1161/01.CIR.0000163542.48611.A2
29. Zuba V, Furon J, Bellemain-Sagnard M, Martinez de Lazarrondo S, Lebouvier L, Rubio M, Hommet Y, Gauberti M, Vivien D, Ali C. The choroid plexus: a door between the blood and the brain for tissue-type plasminogen activator. *Fluids Barriers CNS*. 2022;19:1–17. doi: 10.1186/s12987-022-00378-0
30. Anfray A, Brodin C, Drieu A, Potzeha F, Dalarun B, Agin V, Vivien D, Orset C. Single- and two- chain tissue type plasminogen activator treatments differentially influence cerebral recovery after stroke. *Exp Neurol*. 2021;338:113606–113610. doi: 10.1016/j.expneurol.2021.113606
31. Tabrizi P, Wang L, Seeds N, McComb JG, Yamada S, Griffin JH, Carmeliet P, Weiss MH, Zlokovic BV. Tissue plasminogen activator (tPA) deficiency exacerbates cerebrovascular fibrin deposition and brain injury in a murine stroke model studies in tPA-deficient mice and wild-type mice on a matched genetic background. *Arterioscler Thromb Vasc Biol*. 1999;19:2801–2806. doi: 10.1161/01.atv.19.11.2801
32. Jiang ZW, Gong QZ, Di X, Zhu J, Lyeth BG. Dicyclomine, an M1 muscarinic antagonist, reduces infarct volume in a rat subdural hematoma model. *Brain Res*. 2000;852:37–44. doi: 10.1016/s0006-8993(99)02230-1
33. Orset C, Macrez R, Young AR, Panthou D, Angles-Cano E, Maubert E, Agin V, Vivien D. Mouse model of in situ thromboembolic stroke and reperfusion. *Stroke*. 2007;38:2771–2778. doi: 10.1161/STROKEAHA.107.487520
34. Wang YF, Tsirka SE, Strickland S, Stieg PE, Soriano SG, Lipton SA. Tissue plasminogen activator (tPA) increases neuronal damage after focal cerebral ischemia in wild-type and tPA-deficient mice. *Nat Med*. 1998;4:228–231. doi: 10.1038/nm0298-228
35. Liu D, Cheng T, Guo H, Fernández JA, Griffin JH, Song X, Zlokovic BV. Tissue plasminogen activator neurovascular toxicity is controlled by activated protein C. *Nat Med*. 2004;10:1379–1383. doi: 10.1038/nm1122
36. El Amki M, Lerouet D, Coqueran B, Curis E, Orset C, Vivien D, Plotkine M, Marchand-Leroux C, Margaill I. Experimental modeling of recombinant tissue plasminogen activator effects after ischemic stroke. *Exp Neurol*. 2012;238:138–144. doi: 10.1016/j.expneurol.2012.08.005
37. Orset C, Haelewyn B, Allan SM, Ansar S, Campos F, Cho TH, Durand A, Amki M El Fatar M, Grudzenski S, et al. Stroke: a retrospective pooled analysis. *Stroke*. 2016;47:1312–1318. doi: 10.1161/STROKEAHA.116.012238
38. Zhao XJ, Larkin TM, Lauver MA, Ahmad S, Ducruet AF. Tissue plasminogen activator mediates deleterious complement cascade activation in stroke. *PLoS One*. 2017;12:e0180822–e0180815. doi: 10.1371/journal.pone.0180822
39. Szabo R, Samson AL, Lawrence DA, Medcalf RL, Bugge TH. Passenger mutations and aberrant gene expression in congenic tissue plasminogen activator-deficient mouse strains. *J Thromb Haemost*. 2016;14:1618–1628. doi: 10.1111/jth.13338
40. Douceau S, Lemarchand E, Hommet Y, Lebouvier L, Joséphine C, Bemelmans AP, Maubert E, Agin V, Vivien D. PKCδ-positive GABAergic neurons in the central amygdala exhibit tissue-type plasminogen activator: role in the control of anxiety. *Mol Psychiatry*. 2022;27:2197–2205. doi: 10.1038/s41380-022-01455-4
41. Martinez de Lizarrondo S, Gakuba C, Herbig BA, Repessé Y, Ali C, Denis CV, Lenting PJ, Touzé E, Diamond SL, Viven D, et al. Potent thrombolytic effect of N-acetylcysteine on arterial thrombi. *Circulation*. 2017;136:646–660. doi: 10.1161/CIRCULATIONAHA.117.027290
42. Powers WJ, Rabinstein AA, Ackerson T, Adeoye OM, Bambakidis NC, Becker K, Biller J, Brown M, Demaerschalk BM, Hoh B, 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. doi: 10.1161/STR.0000000000000211
43. Sandercock P, Wardlaw JM, Lindley RI, Dennis M, Cohen G, Murray G, Innes K, Venables G, Czlonkowska A, Kobayashi A, et al; IST-3 collaborative group. 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. doi: 10.1016/S0140-6736(12)60768-5
44. Torrente D, Su EJ, Fredriksson L, Warnock M, Bushart D, Mann KM, Emal CD, Lawrence DA. Compartmentalized actions of the plasminogen activator inhibitors, PAI-1 and Nsp, in ischemic stroke. *Transl Stroke Res*. 2022;13:801–815. doi: 10.1007/s12975-022-00992-y
45. Hingot V, Brodin C, Lebrun F, Heiles B, Chagnot A, Yetim M, Gauberti M, Orset C, Tanter M, Couture O, et al. Early ultrafast ultrasound imaging of cerebral perfusion correlates with ischemic stroke outcomes and responses to treatment in mice. *Theranostics*. 2020;10:7480–7491. doi: 10.7150/thno.44233
46. Wyseure T, Rubio M, Denorme F, De Lizarrondo SM, Peeters M, Gils A, De Meyer SF, Vivien D, Declerck PJ. Innovative thrombolytic strategy using a heterodimer diabody against TAFI and PAI-1 in mouse models of thrombosis and stroke. *Blood*. 2015;125:1325–1332. doi: 10.1182/blood-2014-07-588319
47. Dong MX, Hu QC, Shen P, Pan JX, Wei YD, Liu YY, Ren YF, Liang ZH, Wang HY, Zhao LB, et al. Recombinant tissue plasminogen activator induces neurological side effects independent on thrombolysis in mechanical animal models of focal cerebral infarction: a systematic review and meta-analysis. *PLoS One*. 2016;11:e0158848–e0158829. doi: 10.1371/journal.pone.0158848
48. Nagai N, De Mol M, Lijnen HR, Carmeliet P, Collen D. Role of plasminogen system components in focal cerebral ischemic infarction: a gene targeting and gene transfer study in mice. *Circulation*. 1999;99:2440–2444. doi: 10.1161/01.cir.99.18.2440
49. Roussel BD, MacRez R, Jullienne A, Agin V, Maubert E, Dauphinot L, Potier MC, Plawinski L, Castel H, Hommet Y, et al. Age and albumin D site-binding protein control tissue plasminogen activator levels: neurotoxic impact. *Brain*. 2009;132:2219–2230. doi: 10.1093/brain/awp162
50. Percie du Sert N, Hurst V, Ahluwalia A, Alam S, Avey MT, Baker M, Browne WJ, Clark A, Cuthill IC, Dirnagl U, et al. The ARRIVE guidelines 20: updated guidelines for reporting animal research. *Br J Pharmacol*. 2020;177:3617–3624. doi: 10.1111/bph.15193
51. Kamran P, Sereti KI, Zhao P, Ali SR, Weissman IL, Ardehali R. Parabiosis in mice: a detailed protocol. *J. Vis. Exp*. 2013;1:5. doi: 10.3791/50556
52. Liu X, Bai X, Li M, Li H, Zhang Y, Yang B. Detecting establishment of shared blood supply in parabiotic mice by caudal vein glucose injection. *J. Vis. Exp*. 2020;2020:1–6. doi: 10.3791/60411
53. van Moorsel MVA, de Maat S, Vercruysse K, van Leeuwen EM, Jacqmarcq C, Bonnard T, Vivien D, van der Worp HB, Dijkhuizen RM, Maas C. VWF-targeted thrombolysis to overcome rh-tPA resistance in experimental murine ischemic stroke models. *Blood*. 2022;140:2844–2848. doi: 10.1182/blood.2022016342
54. Fugate JE, Klunder AM, Kallmes DF. What is meant by "TICI?". *Am. J Neuroradiol*. 2013;34:1792–1797. doi: 10.3174/ajnr.A3496