Review: Clinical Trial Outcomes

CLINICAL INVESTIGATION

Clinical investigation of desmoteplase in acute ischemic stroke: rationale and progress

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Mònica Millán[1] & Antoni Dávalos[†2]

[1]Stroke Unit, Neurosciences Department, Carretera del Canyet, s/n, Badalona 08160 (Barcelona), Spain
[2]Neuroscience Department, Carretera del Canyet, s/n, Badalona 08160 (Barcelona), Spain
[†]Author for correspondence:
Tel.: +34 934 978 911
Fax: +34 934 978 742
E-mail: adavalos.germanstrias@gencat.cat

The thrombolytic agent desmoteplase produced by recombinant biotechnology has its natural place in the saliva of the vampire *Desmodus rotundus*. The structure of desmoteplase is similar to tissue plasminogen activator, but it does not contain the lysine-binding Kringle 2 domain. Accordingly, desmoteplase has higher fibrin specificity, absence of neurotoxicity and a better profile in terms of plasma half-life compared with tissue plasminogen activator in experimental stroke models. The Phase II trials DIAS and DEDAS indicated that intravenous desmoteplase administered within 3–9 h after stroke onset at doses up to 125 µg/kg was associated with a low rate of symptomatic intracranial hemorrhage and a high rate of reperfusion that correlated with clinical benefit. The subsequent DIAS-2 study did not show the same benefit, probably owing to the inclusion of a substantial number of patients with mild strokes and small mismatch volumes associated with no vessel occlusion. A *post hoc* analysis of the DIAS-2 data showed a clinical benefit of desmoteplase in those patients with proximal arterial occlusion on baseline angiography that lead to the planning and development of the ongoing DIAS-3 and DIAS-4 studies, whose results will determine whether desmoteplase could be used as a treatment for acute ischemic stroke.

Keywords: acute stroke thrombolysis • desmoteplase • fibrin specificity • multimodal MRI • neurotoxicity

Although stroke treatment has changed completely in the last 15 years, stroke remains an important public health concern, as it is the third leading cause of death in the USA, Canada, Europe and Japan and the primary cause of adult disability in these developed countries [1].

Intravenous (iv.) thrombolysis with tissue plasminogen activator (tPA) is approved within 3 h in patients suffering acute ischemic stroke and it is the standard of care in current clinical practice. However, tPA has been shown to be effective up to 4.5 h after stroke onset [2–5]. Despite the use of iv. tPA in different countries and continents since 1996, iv. thrombolysis has several limitations, such as a short time window for administration, a low rate of arterial recanalization in some arteries, a substantial risk of intracranial hemorrhage and numerous exclusion criteria or contraindications which lead to a low proportion of treated patients [6]. Nearly half of patients treated with iv. tPA, despite being seen early and having signs of a salvageable brain, do not achieve a good response to tPA. Although neurotoxicity of tPA has never been demonstrated in patients with ischemic stroke, animal models suggest that this relative inefficiency of iv. tPA could be related in part to neurotoxic actions [7]. Experimental data show that tPA may have pleiotropic effects in the brain, including cleavage of the *N*-methyl D-aspartate (NMDA) NR1 subunit, amplification of intracellular Ca^{++} conductance, and activation of other extracellular proteases from the matrix metalloproteinase

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family. These effects may increase excitotoxicity and blood–brain barrier (BBB) disruption, and worsen brain injury, edema and cerebral hemorrhage [8,9].

All these facts have contributed in recent years to the development and study of new thrombolytic agents, new routes of administration, longer time windows of treatment and different mechanical devices to locally remove the thrombus to overcome the main troubles and limitations of tPA treatment [10]. Desmoteplase is a novel thrombolytic agent found in the saliva of the vampire bat *Desmodus rotundus* (Figure 1) and produced by recombinant biotechnology, and may have a good profile in terms of plasma half-life, neurotoxicity and safety. Desmoteplase has been widely studied in *in vitro* and *in vivo* experimental stroke models and clinical investigation began in 2001. Two ongoing large clinical trials started in 2009 will determine whether desmoteplase is a safe and effective treatment for patients with acute ischemic stroke. This article provides a comprehensive review of the investigational program of desmoteplase in ischemic stroke.

Basic mechanisms of action & properties of desmoteplase & desmoteplase preclinical review

Plasminogen activators are enzymes found in all vertebrate species investigated so far. Vampire bats are the only mammalian species feeding exclusively on blood and their saliva contains very potent plasminogen activators for the rapid lysis of fresh blood clots, a fact originally described in 1966 [11]. Four salivary plasminogen activators have been identified in the vampire bat *D. rotundus* (DSPAs) composed of various conserved domains known from related proteins (DSPAα1, α2, β and γ) and coded by four different genes [12]. The characteristics that distinguish DSPAs from other plasminogen activators is their strict requirement for fibrin as a cofactor. In a plasminogen activation assay, all four DSPAs were almost inactive in the absence of fibrin but strongly stimulated by fibrin addition [13]. Biochemical and pharmacological analysis indicated that DSPAα1 exhibited the most favorable profile since the ratio of plasminogen activation in the presence of fibrin versus fibrinogen (fibrin selectivity) of DSPAα1, α2, β and γ was found to be 12,900, 6500, 250 and 90, respectively, and only 72 for tPA [13,14]. Therefore, DSPAα1 (desmoteplase) was chosen for further studies.

Figure 1. Desmoteplase is a thrombolytic agent found in the saliva of the vampire bat *Desmodus rotundus*.

Experimental models have studied the thrombolytic properties of desmoteplase compared with tPA [15–22]. In animal arterial thrombosis models, the incidence of reperfusion and maximal reperfusion flows were equivalent (80 vs 78%) or superior (85 vs 56%) to tPA after an equimolar dose of desmoteplase, but the time to reach maximal flow for desmoteplase was approximately one half that of tPA (26 vs 45 min) [15–18]. The efficacy of thrombolysis was dose-dependent, achieving lesser arterial residual thrombus mass in the highest doses of plasminogen activators [15,16]. Furthermore, in contrast to the same dose of tPA, the restoration of flow by desmoteplase was accomplished without fibrinogenolysis (plasma fibrinogen concentrations decreased maximally by 14% in desmoteplase and 69% in tPA from control values) and with only a small decrease in the plasma plasminogen and α2-antiplasmin levels [15–17]. This fibrin selectivity and the lack of activation by cofactors such as β-amyloid and prion proteins have been related to a lower incidence of bleeding [19,20]; however, these are controversial experimental data [21] and additional studies are required to broadly demonstrate this advantageous consequence.

Another important differential feature between both plasminogen activators comes from their diverse pharmacokinetics. The clearance of desmoteplase is best described by a biphasic elimination profile that exhibited a dominant slow β Phase, whereas the elimination profile of tPA is much steeper than that of desmoteplase and can adequately be described as a monophasic profile [15,22]. Approximately 80% of the desmoteplase is cleared by the relative slow β elimination phase [15]. Hence, the clearance rate of desmoteplase from plasma after iv. bolus administration is approximately fourfold slower than that of tPA [15]. This property would allow

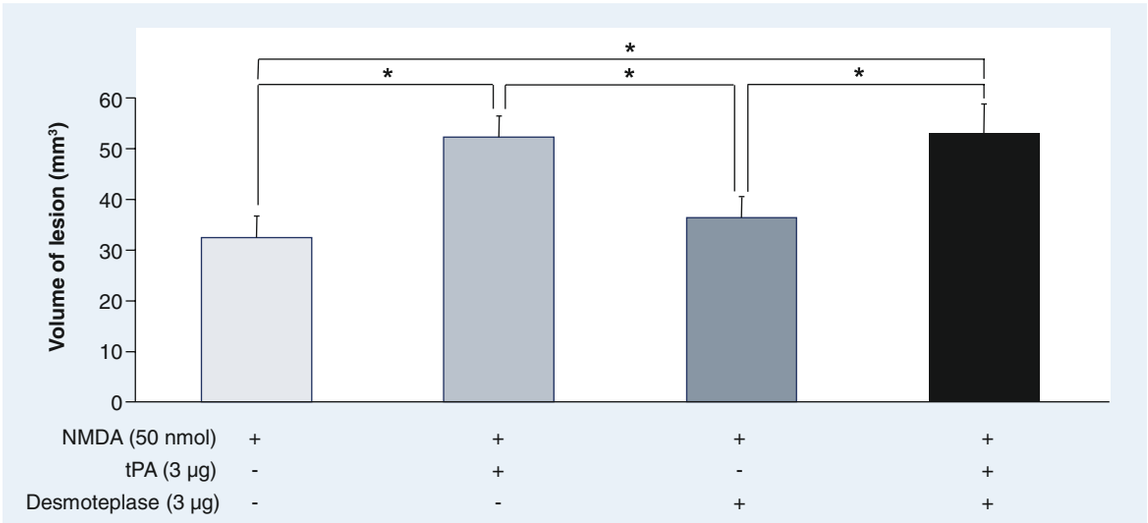

Figure 2. Desmoteplase is not neurotoxic *in vivo*. Effects of intrastructure injection of tPA and/or desmoteplase on the extent of neuronal death induced by striatal administration of NMDA.
*p < 0.001.
NMDA: *N*-methyl D-aspartate; tPA: Tissue plasminogen activator.
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maintaining more stable plasma desmoteplase concentrations after a single iv. bolus which might reduce vessel reocclusion, but may also increase unwanted effects such as bleeding complications. No differences have been found between desmoteplase and tPA in the bleeding time and platelet count of aspirin-pretreated animals [15].

Apart from its thrombolytic and pharmacokinetic properties, *in vitro* and *in vivo* animal models have demonstrated that desmoteplase has no neurotoxic effects on the brain [23–26]. The essential structural difference between desmoteplase and tPA lies in the absence of the lysine binding site Kringle 2 Domain in DSPAα1 [27]. The Kringle 2 Domain plays a critical role in the interaction of tPA with the amino-terminal domain of the NR1 subunit of the NMDA glutamate receptor, the critical step for potentiating excitotoxic neuronal death. Infusion of desmoteplase into the striatum of rodents did not cause any further increase in lesion size by NMDA treatment, whereas co-injection of the same concentration of tPA with NMDA increased the lesion area compared with that produced by NMDA alone (Figure 2) [23,24] . Moreover, in knockout tPA mice, desmoteplase did not promote microglial activation [23]. A comparable effect was observed when desmoteplase was iv. injected, since NMDA-mediated cell death did not increase whereas excitotoxic injury was enhanced by iv. tPA [24,25]. Importantly, the toxic effects of tPA were blocked by iv. co-administration of desmoteplase (Figure 3) [24,25]. The mechanistic basis for this blocking effect of desmoteplase is presently uncertain, but may be caused by competition of desmoteplase with tPA for low-density lipoprotein receptor-related protein (LRP) binding at the BBB, preventing tPA access across BBB to the brain parenchyma [24,26,28,29]. Moreover, under oxygen glucose deprivation conditions, desmoteplase appears to cross the BBB at a lower rate than tPA [24]. *In vitro* competition experiments showed that desmoteplase could display a higher affinity for LRP than tPA that would explain the differences between these two thrombolytic agents in the BBB passage [24].

In conclusion, experimental data suggest that desmoteplase might display some advantages over tPA: a higher fibrin selectivity with equal to superior thrombolytic potency, longer plasma half-life, no neurotoxic effects and attenuation of excitotoxic damage. Whether these properties may lead to a safer profile of this thrombolytic agent in patients with ischemic stroke must be confirmed in current clinical research.

Design & results of clinical trials with desmoteplase (DIAS, DEDAS & DIAS-2)

Clinical studies with desmoteplase were started in the setting of myocardial infarction. A Phase II study was conducted to evaluate the efficacy, safety and tolerability of desmoteplase as a thrombolytic agent in the treatment of patients with acute myocardial infarction. The study was designed as a nonrandomized, open-label, prospective dose finding study (0.5 or 0.75 g/kg of desmoteplase). A total of 26 patients were enrolled. Recanalization was achieved in 65%, laboratory data confirmed the high fibrin specificity demonstrated by

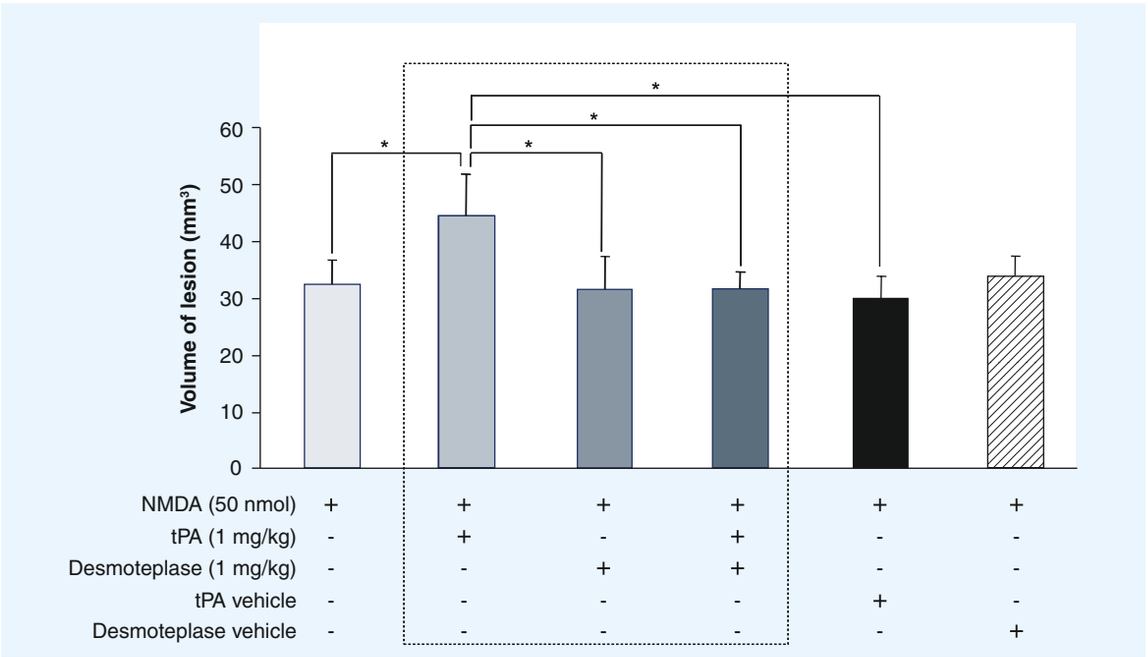

Figure 3. Desmoteplase prevents the passage of tPA across the blood–brain barrier *in vivo*. Effects of intravenous injection of tPA and/or desmoteplase and their vehicles on the extent of neuronal death induced by the striatal administration of NMDA.
*p < 0.01.
NMDA: *N*-methyl D-aspartate; tPA: Tissue plasminogen activator.
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normal levels of fibrinogen and the safety profile was comparable to other thrombolytics [14]. After the favorable pharmacotoxicological and pharmacokinetic profile of desmoteplase, a Phase II/III study in acute stroke appeared promising.

The DIAS study was initiated in 2001. DIAS was a placebo-controlled, double-blind, randomized, dose-finding Phase II trial carried out in two parts in 44 centers in 12 countries [30]. The first 47 patients were randomized to fixed doses of desmoteplase (25, 37.5 or 50 mg) or placebo but the design was subsequently changed due to a high rate (26.7%) of symptomatic intracranial hemorrhage (sICH) in this part. Part 2 used lower weight-adjusted doses escalating through 62.5, 90 and 125 µg/kg in 57 patients. The main inclusion criteria were ages 18 through to 85 years, National Institute of Health Stroke Scale (NIHSS) 4 through 20, stroke onset within 3–9 h and at least 20% perfusion/diffusion weighted imaging (PWI/DWI) mismatch (evaluated by visual inspection) involving hemispheric gray matter and a DWI lesion less than one third of the middle cerebral artery (MCA) territory on baseline screening multimodal MRI. In Part 2, one of 15 desmoteplase-treated patients in the 90 µg/kg group had sICH (6.7%) whereas the rate of asymptomatic ICH was similar in placebo-treated (27.3%) and desmoteplase-treated patients (31.1%). No differences were found in other major systemic bleeding between groups. Early reperfusion at 4–8 h after treatment on the follow-up PWI images was observed in 20, 23.1, 46.7 and 71.4% in the placebo and 62.5, 90 and 125 µg/kg dose groups, respectively. Moreover, there was a dose-dependent response on the primary favorable outcome (combined end point of modified Rankin Scale (mRS) 0–2, Barthel index of 75–100 and improvement of NIHSS ≥8 or scoring 0–1) at 90 days: the rate was 13.3, 46.7 and 60% for each desmoteplase dose compared with 18.2% in the placebo group (Table 1). Importantly, reperfusion was associated with higher frequency of favorable clinical outcome (52.5 vs 24.6%; p = 0.0028). These findings suggested a clinical benefit of 90 and 125 µg/kg doses of desmoteplase in patients selected according to PWI/DWI mismatch within 3–9 h time window.

The DEDAS study further evaluated safety and efficacy of iv. desmoteplase in 37 patients with PWI/DWI mismatch 3–9 h after stroke onset [31]. The DEDAS study was developed mainly in the US and its methods and procedures were largely identical to DIAS, but only 90 and 125 µg/kg doses were evaluated. As in

the DIAS study, no sICH were observed. Reperfusion was achieved in 37.5% of the placebo group, 18.2% of 90 µg/kg desmoteplase group and in 53.3% of 125 µg/kg desmoteplase group. Good clinical outcome at 90 days occurred in 25, 28.6 and 60%, correspondingly (Table 1). Hence, in DEDAS study, desmoteplase was safe and the dose of desmoteplase 125 µg/kg but not 90 µg/kg, appeared to improve clinical outcome.

The encouraging results of the DIAS and DEDAS studies encouraged clinical investigators to design and develop a new prospective, randomized, double-blind, placebo-controlled, Phase III trial to confirm prior results and validate desmoteplase efficacy. DIAS-2 was started in 2005 and its results were published in 2009 [32]. Patients with acute ischemic stroke and tissue at risk seen on either MRI or CT imaging were randomly assigned (1:1:1) to desmoteplase 90 or 125 µg/kg or placebo within 3–9 h after symptom onset. The primary efficacy end point was good clinical outcome at 90 days following DIAS and DEDAS definition. Of a total of 193 randomized patients from 12 countries in Europe, Asia, Australia and North America, 186 received treatment. In total 66% of patients were randomized by MRI screening. Vessel occlusion (thrombolysis in myocardial infarction [TIMI] 0–1) at baseline was found in only 30% of patients and, accordingly, mean core lesion volume and mismatch volume (10.6 and 52.5 cm^3, respectively) were small in the total series. Tissue reperfusion data were not collected in DIAS-2. Favorable clinical outcome at 90 days was observed in 46, 47 and 36% for placebo, desmoteplase 90 and 125 µg/kg groups (Table 1). The frequency sICH was 0, 3.5 and 4.5%, whereas mortality rate was 6, 11 and 21%. Most of the deaths in the highest dose group occurred after day 7, and were due to stroke or to reasons unrelated to the study drug. No differences were found in the proportion of serious adverse events between groups. In summary, the DIAS-2 study found a high response rate in the placebo group and did not show a benefit of desmoteplase given 3–9 h after the onset of stroke.

The disappointing and unexpected results of the DIAS-2 study led to exploration of the reasons for the lack of clinical efficacy in contrast to the previous studies in a *post hoc* analysis. Despite having similar selection criteria, DIAS-2 differed from the DIAS and DEDAS studies in several aspects (Table 2):

- Baseline strokes were less severe across all study groups in DIAS-2 (median admission NIHSS 9 vs 12);
- Mild severity of stroke was associated with smaller core lesion and mismatch volumes;
- The rate of baseline proximal vessel occlusion was surprisingly low (30 vs 57%);
- The sample size was too small for a Phase III trial aimed to achieve a treatment effect.

These characteristics may explain the high response rate in the placebo group in the DIAS-2 trial and possibly reduced any outcome difference between the demosteplase and placebo group. However, a lack of efficacy of desmoteplase itself cannot be ruled out.

To conclude, although desmoteplase could be a thrombolytic agent with several theoretical advantages over tPA, its clinical efficacy has not been proven in patients with ischemic stroke within 3–9 h after stroke onset.

Rationale for DIAS-3 & DIAS-4 trials

The DIAS, DEDAS and DIAS-2 conflicting results prompted investigators to further research and elucidate the complex relationship between site and degree of vascular occlusion, collateral blood flow, tissue perfusion, mismatch imaging and clinical outcomes [33–37]. This section shows some of these controversies, which justify the development of new clinical trials.

Table 1. Baseline characteristics, safety variables and primary efficacy end points in the DIAS, DEDAS & DIAS-2 studies.

	DIAS (Part 2)			DEDAS			DIAS-2		
	90 µg/kg (n = 15)	125 µg/kg (n = 15)	Placebo (n = 11)	90 µg/kg (n = 14)	125 µg/kg (n = 15)	Placebo (n = 8)	90 µg/kg (n = 57)	125 µg/kg (n = 66)	Placebo (n = 63)
Age	69	70	70	74	72	71	71	73	73
NIHSS	12	12	8	10	9	12	9	9	9
Core lesion volume (cm^3)	53.3	49.7	29.7	25.3	20.7	35.1	7.9	11.3	12.3
Reperfusion (%)	46.7	71.4	20	18.2	53.3	37.5	N/A	N/A	N/A
sICH (%)	6.7	0	0	0	0	0	3.5	4.5	0
Mortality (%)	6.6	6.6	9.1	7.1	6.7	12.5	11	21	6
Good outcome (%)	46.7	60	18.2	28.6	60	25	47	36	46

Data are proportions or median values.
N/A: Data not available; NIHSS: National Institute of Health stroke scale; sICH: Symptomatic intracranial hemorrhage.

Table 2. Comparison of baseline characteristics between DIAS/DEDAS studies and DIAS-2 study.

Characteristics	Desmoteplase 90 µg/kg		Desmoteplase 125 µg/kg		Placebo		DIAS/DEDAS vs DIAS-2 (p-value)
	DIAS/DEDAS (n = 29)	DIAS-2 (n = 57)	DIAS/DEDAS (n = 30)	DIAS-2 (n = 66)	DIAS/DEDAS (n = 35)	DIAS-2 (n = 63)	
NIHSS	11	9	11	9	12	9	0.057
Core lesion volume (cm^3)	27.6	7.9	22.1	11.3	23.5	12.3	<0.0001
Mismatch volume (cm^3)	113.9	51.9	129.9	66.2	99.2	48.8	0.008
Mismatch volume (%)	310	510	240	530	270	480	0.002
Vessel occlusion TIMI 0–1 (%)	54	26	59	36	59	27	0.0001
Vessel occlusion TIMI 2–3 (%)	48	74	41	64	42	73	<0.0001
Time since stroke onset 3–6 h (%)	21	33	63	36	66	41	0.03
Time since stroke onset 6–9 h (%)	79	67	37	63	34	59	0.03

Data are proportions or median values. Patients with available TIMI assessment (DIAS/DEDAS vs DIAS-2): 90 µg/kg (n = 28 vs 54); 125 µg/kg (n = 27 vs 62); placebo (n = 34 vs 63).
NIHSS: National Institute of Health stroke scale; TIMI: Thrombolysis in myocardial infarction.

■ Reperfusion therapies based on the mismatch concept or baseline arterial occlusion as criteria for patient selection

Imaging technology has rapidly advanced in the past two decades, and multimodal MRI or CT perfusion (CTP) may theoretically select patients based on a pathophysiological concept, since these techniques may provide a way to identify salvageable brain at risk of infarction in longer time windows than 3 h [37]. In recent years, several controlled trials (DEFUSE, EPITHET, DIAS, DEDAS and DIAS-2) have investigated the efficacy of iv. thrombolysis beyond 3 h in patients with PWI/DWI MRI or CTP mismatch [30–32,38,39]. A meta-analysis of these studies showed that thrombolysis was associated with a higher rate of recanalization and that patients who underwent reperfusion/recanalization compared with those who did not have improved outcomes (OR: 5.4; 95% CI: 3–9.1). However, favorable clinical outcome was not significantly improved (OR: 1.3; 95% CI: 0.8–2) by delayed thrombolysis in mismatch patients compared with placebo [35]. Heterogeneity in neuroimaging selection criteria and in primary end-points in these studies might explain in part the lack of benefit of thrombolysis based on the penumbral paradigm (Table 3).

DWI/PWI mismatch in the DIAS, DEDAS and DIAS-2 studies was defined qualitatively at each site as an arbitrary increase of 20% in PWI lesion volume, estimated with the relative mean transit time map and compared with the standard DWI lesion volume by visual judgment [30–32]. However, *post hoc* analysis of DEFUSE and EPITHET studies suggest that the relative mean transit map could overestimates the volume of tissue at risk [40–43] and that a higher mismatch (2:1) ratio seems to be a better predictor of clinical outcome and a more realistic target for therapy [34]. According to this, a mismatch volume higher than 100 cc was associated with a substantial clinical improvement in DIAS-2 study [Dávalos A. Unpublished Data]. A further point of concern was the inconsistency of the results on mismatch volume, core estimation and infarct volume at day 30 between the CTP and MRI assessment of penumbral tissue. Hence, the DIAS-2 patient selection might not have followed an appropriate mismatch threshold and included patients with limited penumbral tissue and a narrow chance of improvement. Mismatch concept refinement with new parameters or an automated online analysis of mismatch should facilitate a rapid assessment of patients for delayed thrombolysis in future trials of acute stroke therapy.

A simpler paradigm to identify patients with salvageable brain in clinical routine, is to assess vessel patency and core lesion volume [34]. MRA/DWI mismatch (Lansberg Stroke 2008) or CT angiography/CT mismatch (occlusion and either normal CT or ASPECTS >7) might be used as surrogates to the mismatch volume avoiding mismatch quantifications in the acute setting. In *post hoc* analysis of patients with arterial occlusion (TIMI score of 0–1) in DIAS-2, desmoteplase showed an absolute difference in treatment effect of 18% for the low dose (90 µg/kg) and of 9% for the high dose (125 µg/kg) compared with the placebo group. In contrast, no treatment effect was seen in patients with TIMI score 2–3 (Figure 4) [32]. As nearly a half of the occluded vessels recanalyze spontaneously within 6 h [44,45], the rationale for later administration of a thrombolytic drug should be based on patients with documented occluded arteries.

Table 3. Comparison of designs of the mismatch-based thrombolysis studies.

Study	Design	Drugs and doses	Inclusion	Onset (h)	Primary end point at 90 days	Ref.
DEFUSE	Phase II, open-label study, all patients received tPA regardless of MRI results	tPA 0.9 mg/kg	>20% mismatch (≥10 ml) MRI	3–6	NIHSS (8↓) or mRS, ≤1 reperfusion	[38]
EPITHET	Phase II, randomized-controlled trial, patients treated without reference to MRI findings	tPA vs placebo 0.9 mg/kg	>20% mismatch (≥10 ml) MRI	3–6	Attenuation infarct growth	[39]
DIAS	Phase II, dose-finding, only patients with mismatch treated	Desmoteplase vs placebo Part 1: fixed: 25, 37.5, 50 mg; Part 2: 62.5, 90, 125 µg/kg	≥20% mismatch MRI	3–9	NIHSS (8↓) + mRS ≤2 + BI 75–100, reperfusion	[30]
DEDAS	Phase II, dose-efficacy, safety, similar design to DIAS	Desmoteplase vs placebo 90, 125 µg/kg	≥20% mismatch MRI	3–9	NIHSS (8↓) + mRS ≤2 + BI 75–100, reperfusion	[31]
DIAS-2	Phase III, randomized-controlled trial, confirmatory	Desmoteplase vs placebo 90, 125 µg/kg	≥20% mismatch MRI or CT	3–9	NIHSS (8↓) + mRS ≤2 + BI 75–100	[32]
DIAS-3	Phase III, randomized-controlled trial, confirmatory	Desmoteplase vs placebo 90 µg/kg	Angiogram: occlusion/high-grade stenosis	3–9	mRS ≤2	[101]

mRS: Modified Rankin Scale; NIHSS: National Institute of Health stroke scale; tPA: Tissue plasminogen activator.

■ Recanalization or reperfusion as targets for thrombolytic therapies

Arterial recanalization and subsequent reperfusion have extensively demonstrated the ability to restore the brain function when performed shortly after acute ischemic stroke. Recanalization (restoring the patency of the occluded artery) and reperfusion (restoring the downstream capillary blood flow) are strongly correlated [38,39,46,47]. However, futile recanalization can occur as a result of rapid recruitment of ischemic

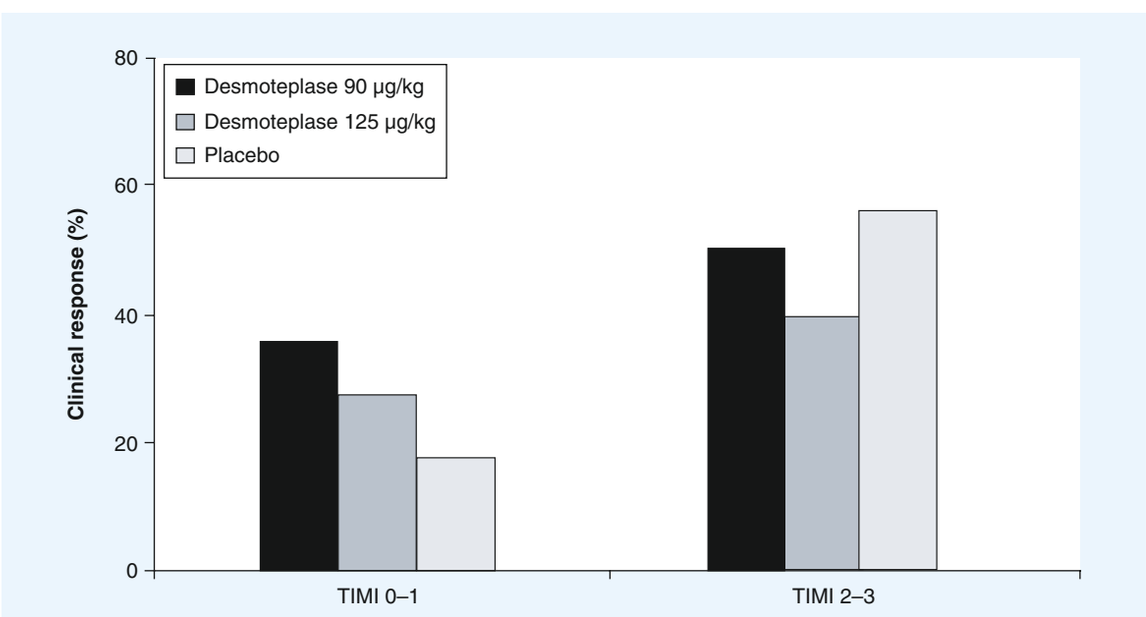

Figure 4. DIAS-2 *post hoc* analysis shows a clinical response according to the thrombolysis in myocardial infarction subgroups. Clinical response was defined as a composite of National Institutes of Health Stroke Scale, modified Rankin Scale and Barthel Index.
TIMI: Thrombolysis in myocardial infarction.

Table 4. Comparison of designs of the current thrombolysis clinical trials DIAS 3/DIAS 4 and EXTEND.

Clinical trials	Design	Drugs and doses	Onset (h)	Clinical inclusion criteria	Neuroimaging inclusion criteria	Primary outcome	Secondary outcomes
DIAS-3/4 (estimated enrollment: 400/400 patients)	Randomized, double-blind, parallel-group, placebo-controlled, Phase III	Desmoteplase 90 µg/kg vs placebo Single bolus	3–9	18–85 years, NIHSS: 4–24	Angiogram MRI or CT scan: - Occlusion/high-grade stenosis MCA, ACA or PCA - No extensive early infarction on MRI or CT scan in any affected area (>1/3) - No penumbral mismatch criteria is needed	mRS: 0–2 at 90 days	Mortality at 90 days, recanalization at 24 h, reperfusion at 24 h (patients with baseline multimodal MRI)
EXTEND (estimated enrollment: 400 patients)	Randomized, double-blind, parallel-group, placebo-controlled, Phase III	tPA 0.9 mg/kg vs placebo 1 h infusion	3–9, wake-up stroke	≥18 years, NIHSS: 4–26	Multimodal MRI: - Penumbral mismatch criteria: T_{max} >6 s delay; PWI lesion volume:DWI lesion volume ratio >1.2; DWI volume ≤70 ml; PWI/DWI difference >10 ml - No extensive early infarction on DWI (>1/3 of territory) - Arterial occlusion is not needed - Automated online penumbra selection	mRS: 0–1 at 90 days	Categorical shift in mRS at 90 days, change in ≥8 NIHSS points or reaching ≤1 on this scale at 90 days, mortality at 90 days, sICH at 24 h, recanalization at 24 h, reperfusion at 24 h, infarct growth at 24 h

ACA: Anterior cerebral artery; DWI: Diffusion Weighted Imaging; MCA: Middle cerebral artery; mRS: Modified Rankin Scale; NIHSS: National Institute of Health stroke scale; PCA: Posterior cerebral artery; PWI: Perfusion Weighted Imaging; sICH: Symptomatic intracranial hemorrhage; tPA: Tissue plasminogen activator.

tissue before recanalization, re-occlusion or reperfusion injury [48]. Furthermore, arterial recanalization does not necessarily lead to brain tissue reperfusion due to multiple downstream embolization or blockage of microcirculation due to nonreflow phenomenon [49]. As an example, nearly 30% of patients who recanalized in the EPITHET study did not achieve a brain reperfusion [46]. Recent retrospective data support that reperfusion is a more accurate predictor of follow-up infarct volume and improved clinical outcome than recanalization since collateral flow may be a contributor to reperfusion in the absence of recanalization [49,50]. Future prospective studies should focus on early estimation of post-treatment recanalization/reperfusion as surrogate efficacy measurements in revascularization therapies.

DIAS-3 & DIAS-4 studies development

Post hoc analysis of DIAS-2 study and recent aforementioned reports provided the basis for the current ongoing clinical Phase III program. DIAS-3 and DIAS-4 are randomized, placebo-controlled, double-blind, Phase III studies both planned to enroll 402 patients (201 per arm) with acute ischemic stroke. The treatment (90 µg/kg of iv. desmoteplase or placebo in a single bolus over 1–2 min) can be initiated within 3–9 h after the onset of stroke symptoms in patients with ages between 18–85 years and NIHSS between 4 and 24. The main inclusion criteria, which is the main differential characteristic of patient selection from DIAS/DEDAS/DIAS-2 trials, is the presence of vessel occlusion or high-grade stenosis seen on MRI or CT-angiography in proximal cerebral arteries (M1, M2, ACA, PCA) and the main exclusion criteria is extensive infarct core of higher than 1/3 of MCA territory or ½ of ACA and PCA territory on DWI. The primary end point is mRS Score 0–2 at 90 days. Several secondary efficacy end points will be evaluated such as arterial recanalization at 12–24 h and the efficacy of desmoteplase in patients with baseline small core lesion volume or in the subgroup of patients screened with MRI who undergo PWI and DWI sequences at baseline applying different thresholds of PWI/DWI mismatch volume. DIAS-3 (Europe and Asia) and DIAS-4 (USA, Canada, South America, Europe and South Africa) were started in 2009 and 275 patients have been included by June 2011 [101,102]. The DIAS-3 and DIAS-4 trials will provide additional insights in the scientific and clinical value of vessel occlusion and mismatch as imaging biomarkers for acute stroke.

Future perspective & future clinical trials.

New Phase III trials with thrombolytic drugs in extended windows and based on the mismatch selection

paradigm in patients with ischemic stroke are expected in the next 5 years. To overcome prior methodological difficulties, new trials should improve study organization and sample size calculation to better predict the treatment benefit and risk, optimize mismatch calculation by an automated penumbral selection, consider a dual neuroimaging selection target including vessel status and DWI/PWI mismatch and evaluate recanalization and reperfusion as primary end points, although considering short and long term clinical outcomes as the main primary end point.

Two Phase III clinical trials are following this methodology. The EXTEND trial is a randomized, controlled, double blind, Phase III study in which iv. tPA versus placebo is given in patients with acute ischemic stroke within 3–9 h or wake-up stroke selected by multimodal MRI [103]. The study uses an automated online estimation of penumbra by the RAPID program. Penumbra is defined with a T_{max} >6 s delay, PWI lesion volume to DWI lesion volume ratio >1.2, DWI lesion volume ≤70 ml and a PWI/DWI absolute difference >10 ml. The primary end point is no disability (mRS 0–1) at 3 months. Safety and efficacy secondary end points include sICH, mortality, recanalization, reperfusion and infarct growth. The estimated enrollment is 400 patients. This study has currently been recruiting patients since June 2010 and its results will probably be reported in close proximity to the DIAS-3/4 studies. A twin clinical trial, the ECASS-4, is in progress and hopefully will start soon. Table 4 shows a comparison of the design and intended analysis between DIAS-3/4 and EXTEND trials.

Forthcoming clinical trials with desmoteplase and tPA may help to expand the time window for reperfusion therapies in patients with acute ischemic stroke and ischemic penumbra. Taking into account the potential advantages of desmoteplase over tPA in experimental studies, desmoteplase should be compared to iv. tPA in the 0–4.5 h window in a clinical trial with a simpler diagnostic paradigm if the ongoing trials show safety and efficacy of desmoteplase from 3–9 h after stroke onset.

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Executive summary

- Desmoteplase is a recombinant plasminogen activator derived from *Desmodus rotundus* salivary plasminogen activator α 1 (DSPAα1).
- Desmoteplase offers pharmacological advantages in comparison with tPA in experimental studies: high fibrin specificity, lack of intrinsic neurotoxicity and longer half-life.
- The essential structural difference between desmoteplase and tPA lies in the absence of the lysine binding site Kringle 2 Domain in DSPAα1 which plays a critical role in the interaction of tPA with the NR1 subunit of the *N*-methyl D-aspartate glutamate receptor, the critical step for potentiating excitotoxic neuronal death.
- The Phase II DIAS and DEDAS studies reported that desmoteplase at doses of 90 and 125 μg/kg had acceptable safety profiles, higher reperfusion rates and clinical efficacy up to 9 h compared with placebo in patients with acute ischemic stroke selected after perfusion/diffusion weighted imaging (PWI/DWI) mismatch on baseline MRI.
- The Phase III DIAS-2 study with similar criteria selection did not confirm the DEDAS/DIAS results and did not show any benefit for either of the two doses of desmoteplase given 3–9 h after the onset of stroke symptoms.
- The failure in detecting any benefit in DIAS-2 study might be explained in part for methodological factors such as the inclusion of a substantial amount of patients with mild strokes, small core lesions and mismatch volumes, low rate of arterial occlusions and a too small sample size.
- A *post hoc* analysis of DIAS-2 study showed a treatment effect of desmoteplase in patients with arterial occlusion or high-grade stenosis, whereas no treatment effect was seen in patients with partial or no arterial occlusion.
- EPITHET data analysis suggest that a PWI/DWI mismatch of 20% could include a limited penumbral tissue with a narrow chance of improvement, whereas a 2:1 mismatch ratio seems to be a good predictor of clinical outcome and a more realistic target for therapy.
- The randomized Phase III clinical trials DIAS 3 and DIAS 4 are currently under way in patients with ischemic stroke within 3–9 h and proven vessel occlusion or high-grade stenosis on MRI or CT angiography. PWI/DWI parameters will be additionally analyzed in a sub-study.
- Forthcoming clinical trial using extended thrombolysis based on vessel occlusion and mismatch paradigm should confirm desmoteplase as a safe and effective treatment for patients with acute ischemic stroke.

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