PROTOCOL TITLE

DEFUSE 3:
Endovascular Therapy Following Imaging Evaluation for Ischemic Stroke 3

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DEFUSE 3

AGREEMENT ON THE PROTOCOL

By signing below I confirm that:

1) I have read this protocol and it contains all necessary details for conducting this study

   AND

2) I agree to conduct the trial in compliance with this protocol and to adhere to all regulations that govern the conduct of the study.

_____ _____

Principal Investigator's Signature **Date**

_____

Principal Investigator's Name

_____

Site Name:

TABLE OF CONTENTS

1. SUMMARY OF TRIAL

DEFUSE 3 is a prospective, randomized, multi-center, Phase III, adaptive, blinded endpoint, controlled trial. A maximum of 476 patients will be randomized and treated between 6 and 16 hours of symptom onset. Subjects will be randomized 1:1 to endovascular therapy plus medical management or medical management alone. *Only the devices listed in this protocol are approved for use in DEFUSE 3. The choice of device or devices employed is at the discretion of the clinical investigator.*

2. SCIENTIFIC BACKGROUND

Although stroke is the number one cause of adult disability in the United States[1], treatment options for stroke are limited. The only FDA approved treatment for stroke is administration of intravenous (iv) tissue plasminogen activator (tPA) within 3 hours after symptom onset. Nationwide, only about 4 percent of stroke patients receive this therapy.[2] The main reason for this low treatment rate is that most patients present to the hospital outside the time-window for tPA.[3, 4] Even when administered, tPA is often not effective because it either fails to recanalize the occluded artery[5-7] or because the brain is already irreversibly injured.[8] As a result, it is estimated that only 12-25% of treated patients benefit from tPA.[9] Thus, in order to improve outcomes from stroke, we need better treatments that are available to a greater proportion of stroke patients.

2.1 State of the science on endovascular stroke therapy for acute stroke

Endovascular stroke therapy, the removal of blood clots with mechanical devices or thrombolytic drugs administered intra-arterially, is the most promising new treatment for patients who "fail" treatment with iv tPA or are not eligible for iv tPA. The main advantage of endovascular therapy is that it has a high rate of recanalization.[10, 11] Blood flow can be restored with a success rate of up to 82% with modern thrombectomy devices and 66% for intra-arterial thrombolysis.[10, 12, 13] This is approximately twice as effective as iv tPA which has a recanalization rate of 10-50% depending on the location of the blood clot.[6, 7] Despite higher rates of recanalization with endovascular therapy, two recent randomized controlled trials of endovascular therapy, IMS III and MR RESCUE, have failed to demonstrate a clinical benefit. Patient and treatment related factors likely both contributed to the neutral results of these trials (see below for details).

Patient-related factors: A central consideration in the optimization of patient selection for acute stroke therapies is the concept of the ischemic penumbra. Ischemic penumbra is defined as ischemic tissue that is potentially salvageable and is distinguished from the ischemic core that has already sustained irreversible injury. Clearly, the target of acute stroke therapies is salvage of the ischemic penumbra, preventing infarct growth and, most importantly, improved functional outcome. Acute stroke trials should therefore ideally be limited to patients with an ischemic penumbra. MRI-based studies, such as DEFUSE 1 and 2, indicate that MRI can be used to identify these patients.[8, 14, 15 16, 17]

Treatment-related factors: Recent studies have emphasized the importance of recanalization rates, demonstrating the influence on patient outcome of highly effective endovascular procedures that lead to complete or near-complete reperfusion.[18, 19] Currently, the most common metric for rating the quality of reperfusion is the modified Thrombolysis

In Cerebral Infarction (mTICI) scale, and a clear relationship exists between the degree of reperfusion on the mTICI scale and patient outcome.[20-22] Patients with >50% reperfusion (mTICI 2B-3) are much more likely to have a good outcome than patients with <50% reperfusion.

IMS III, the largest endovascular trial to date, did not use advanced imaging criteria to select patients.[23] Instead it used relatively strict time-criteria, anticipating that this would yield a high proportion of patients with a substantial penumbra. However, several categories of patients who likely did not have substantial penumbra were enrolled. Nearly one third of the patients did not have a vessel occlusion at angiography and 23% had distal MCA occlusions; both of these subgroups are unlikely to have substantial penumbral tissue. In addition, 42% of patients had some evidence of irreversible tissue injury (ASPECTS <8) on their baseline CT and 14% had evidence of extensive irreversible injury (ASPECTS <5).[24] Finally, the endovascular devices that were available during IMS III had relatively low rates of early reperfusion; mTICI 2B-3 was only 40% in IMS III.

MR RESCUE, a stroke trial that aimed to demonstrate benefit of endovascular therapy in patients with a penumbra based on MRI, had neutral results.[25] Several factors likely contributed to this. First, the rate of endovascular reperfusion was extremely low. Only 8 patients (24%) in the MR RESCUE penumbral group achieved TICI 2B-3 reperfusion during endovascular therapy. Second, patients in the penumbral group in MR RESCUE had larger baseline infarct core lesions (median volume 36 ml; IQR 24–51 ml) than the Target Mismatch patients in DEFUSE 2 (median volume 13 ml; IQR 5–26 ml). The combination of low rates of endovascular reperfusion and relatively large core lesions, both strong predictors of poor clinical outcome, likely explains the lack of a treatment effect in "penumbral patients" in MR RESCUE. Moreover, with only 8 endovascular patients in the penumbral cohort achieving TICI 2B-3 reperfusion, MR RESCUE was substantially underpowered.

New Generation Trials: Recently, a series of positive randomized studies of endovascular therapy with treatment initiated within 6 hours of stroke onset in the vast majority of patients were reported. This has prompted new guidelines endorsing endovascular therapy up to 6 hours after symptom onset. The American Heart Association is now calling for late window studies using advanced imaging for patient selection: ""Further randomized, controlled trials should be done to determine whether advanced imaging paradigms using CT perfusion and MRI perfusion, CTA, and diffusion imaging, including measures of infarct core, collateral flow status, and penumbra, are beneficial for selecting patients for acute reperfusion therapy who are beyond 6 hours from symptom onset. (New recommendation, 2015 AHA Guidelines).

DEFUSE 3 will address this new mandate. Enrollment is limited to patients with salvageable tissue (Target Mismatch patients) who are likely to respond favorably to endovascular reperfusion in the 6-16 hour window. Use of the latest generation thrombectomy devices, coupled with strict qualification and oversight criteria for the neuro-interventionalists, will result in high rates of reperfusion. Based on the compelling preliminary data from DEFUSE 2, the trial is adequately powered to demonstrate a clear treatment effect.

2.2. Prior studies and rationale for development

This protocol aims to shift the selection of patients for reperfusion therapy from a relatively arbitrary decision based on poorly validated clinical characteristics to an objective decision based on scientific evidence. Many factors affect the evolution of the ischemic penumbra into the ischemic core, and the rate of progression of irreversible injury is highly variable between individuals. This variability is likely mediated by the adequacy of collateral blood flow as well as the metabolic milieu of individual stroke patients. The individuality of penumbral evolution among stroke patients implies that identifying the extent of the ischemic core and penumbra is useful for making treatment decisions. Currently diffusion-weighted imaging (DWI) / perfusion-weighted imaging (PWI) magnetic resonance imaging (MRI) affords the best opportunity for approximating the ischemic core and penumbra in real time clinical practice.[27]

The DWI lesion provides a dependable estimation of the ischemic core and only very rarely shows permanent reversal following early reperfusion.[28, 29] PWI identifies hypoperfused ischemic tissue. Regions defined as abnormal on PWI that do not demonstrate a DWI abnormality, often referred to as the DWI/PWI mismatch, can estimate the ischemic penumbra.[30] It is critical that PWI utilizes an appropriately validated threshold parameter that excludes ischemic tissue with modest blood flow reduction (i.e. benign oligemia), because this tissue is unlikely to infarct even if reperfusion does not occur. Which PWI parameter is optimal, as well as what threshold to use to define critical hypoperfusion, has been the focus of multiple research efforts.[31] Prior work from our group and others supports the use of Tmax, thresholded at >6 seconds, as the optimal PWI parameter to identify ischemic tissue destined to become infarcted if timely reperfusion does not occur.[32] This Tmax threshold correlates well with the penumbral range of cerebral blood flow decline as determined by both positron emission tomography and Xenon CT.[33, 34]

Using a difference between the volume of the baseline PWI Tmax lesion and the DWI volume to identify mismatch, the DEFUSE and EPITHET studies found that most patients with a PWI/DWI mismatch responded favorably if reperfusion occurred following iv tPA treatment in the 3- to 6-hour time window. However, despite having a mismatch, patients with very large baseline DWI lesions (large core infarct volumes) had highly unfavorable outcomes following reperfusion. Patients with this MRI pattern, referred to as the Malignant profile, had a significantly higher rate of both parenchymal hemorrhage and severe disability/death if reperfusion occurred.[35] Mismatch patients who do not have the Malignant profile have been designated as having a Target Mismatch, and these patents respond extremely favorably to reperfusion following iv tPA therapy. In a pooled analysis of DEFUSE and EPITHET, Target Mismatch profile patients who experienced reperfusion had a 5-fold increase in favorable clinical response at 90 days and significantly less infarct growth when compared to those who did not reperfuse.[36] No association between reperfusion and favorable outcomes, or a reduction in infarct growth, was apparent for patients without the mismatch profile.

The DEFUSE 2 study utilized an automated mismatch analysis program (RAPID) to prospectively establish MRI profiles in a consecutive cohort of patients who then underwent endovascular therapy. DEFUSE 2 confirmed the concepts demonstrated in DEFUSE and EPITHET; Target Mismatch patients who achieve early reperfusion therapy have less infarct growth and more favorable clinical outcomes (8·8,95% CI 2·7–29·0).[37] No association between reperfusion and favorable outcomes or infarct growth was present in patients

without Target Mismatch. Furthermore, the positive association between reperfusion, favorable clinical response, and attenuation of infarct growth did not diminish in DEFUSE 2 patients with Target Mismatch who were treated relatively late (6-12 hours after symptom onset, see **Figure 1**).

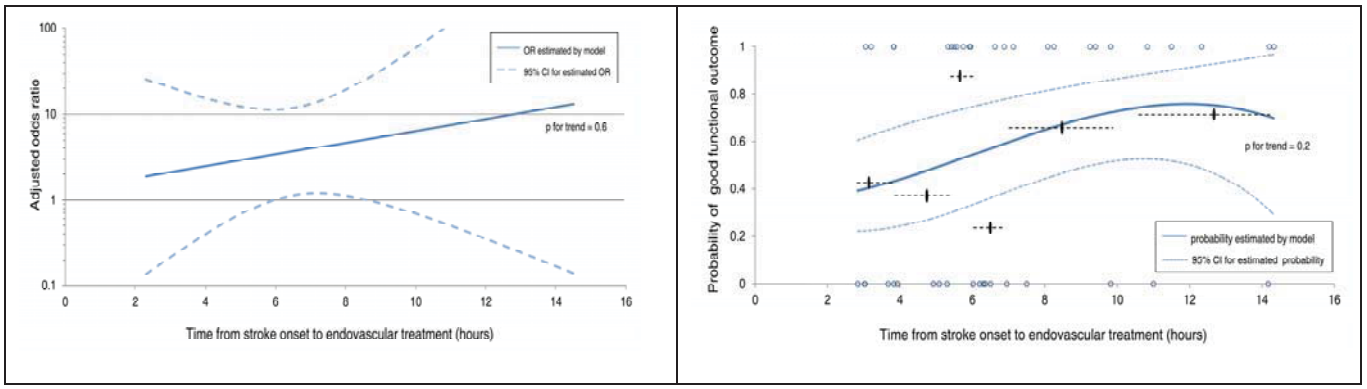

Figure 1: The effect of time on the association between reperfusion and good functional outcome (graph on the left) and the effect of time to treatment on the probability of good functional outcome patients with reperfusion (graph on the right) in Target mismatch patients. 95% CIs are indicated by dashed lines. Estimates are based on multivariate logistic regression, adjusted for age and baseline DWI volume. There is no significant effect of time in either model.[38]

This finding contrasts sharply with prior studies that did not use penumbral imaging to select patients and suggests that imaging findings may be of equal, or potentially even greater, importance than time from symptom onset for identification of optimal patients who might benefit from reperfusion therapy.

How could Target Mismatch patients who are treated late have outcomes that are as favorable as those of earlier treated patients? At later time points, the Target Mismatch profile identifies patients in whom the infarct is evolving at a relatively slow rate; a DWI lesion that is still considerably smaller than the PWI lesion reflects good collateral circulation. These collaterals typically allow prolonged, but not permanent, survival of the hypoperfused mismatch region. Evidence that the mismatch region is still at considerable risk for infarct expansion, even at later time points, was provided by the DEFUSE 2 finding that Target Mismatch patients imaged between 6 and 12 hours from symptom onset consistently demonstrated substantial infarct growth if reperfusion was not achieved.[38] Patients with slowly evolving infarct cores are ideal candidates for later time window reperfusion therapy, particularly endovascular therapies. One of the drawbacks of the endovascular approach is that the time between hospital arrival and achievement of endovascular reperfusion is typically at least 90 to 120 minutes. For patients with rapidly growing infarct cores (such as patients with the Malignant profile), substantial growth of the infarct core has been reported despite endovascular reperfusion. Therefore, removing this population, which represents about 20% of eligible patients, from a randomized endovascular trial, has important advantages.

DEFUSE 2 confirmed that early DWI lesions are an excellent surrogate for the ischemic core. Despite endovascular therapy, only 2 patients had a final infarct that was smaller than the baseline DWI lesion and the size and location of the early DWI lesion was a reliable predictor of the final infarct volume in patients with complete reperfusion.[39, 40] In DEFUSE 2, younger age and smaller DWI volume were significant independent predictors of favorable outcome. Subgroup analysis of EPITHET identified DWI lesion size ≤25 ml as a strong predictor of a

favorable response to reperfusion.[37, 41] These findings suggest that certain subgroups, in particular individuals with Target mismatch and small DWI lesions are most likely to benefit from reperfusion. The adaptive design of DEFUSE 3 (see below) has the potential to focus patient enrollment on a subgroup of patients (e.g. those with smaller DWI lesion volumes and/or shorter times from symptom onset to randomization) who respond most favorably to endovascular therapy. This will allow the study to identify the largest population that has a statistically reliable benefit of endovascular therapy.

New data (presented at the International Stroke Conference, February 2015) suggest that CT Perfusion studies, processed with the same software (RAPID) used in the studies described above, can identify the ischemic core with accuracy similar to MRI (Cereda, et al ISC 2015) and select patients who respond to endovascular reperfusion therapy in early time windows (Campbell, et al NEJM 2015, EXTEND-IA study, Saver, et al NEJM 2015, SWIFT PRIME study). Therefore, DEFUSE 3 will allow patient selection with both MRI and CT Perfusion.

3. INVESTIGATIONAL PLAN

3.1. Purpose

DEFUSE 3 is a prospective randomized Phase III multicenter controlled trial of patients with acute ischemic anterior circulation strokes due to large artery occlusion treated between 6-16 hours of stroke onset with endovascular thrombectomy therapy vs. control. The primary endpoint, the modified Rankin Scale, will be assessed at 3 months. The patients' participation in the study concludes at that time (3 months from stroke onset). The study will randomize up to 476 patients over 4 years. The purpose of DEFUSE 3 is to assess the safety and efficacy of thrombectomy in carefully selected patients in an extended time window. Only the devices listed in this protocol will be used. Selection of the specific device (or devices) is determined by the individual endovascular therapist.

3.2. Protocol Design

DEFUSE 3 is a prospective randomized Phase III multicenter controlled trial of patients with acute ischemic anterior circulation strokes due to large artery occlusion treated between 6-16 hours of stroke onset. Patients who meet the inclusion criteria will undergo either CT Perfusion/CTA or MR DWI/PWI/MRA studies prior to randomization. Patients who have evidence of an ICA or MCA M1 occlusion and a Target Mismatch Profile will be randomized in a 1:1 ratio to treatment with endovascular therapy (using one or more DEFUSE 3 approved thrombectomy devices) plus standard medical therapy versus standard medical therapy alone. Patients who are consented, but not randomized, will receive standard therapy according to local guidelines. Baseline data, and information about early stroke therapies, will be captured for this group of patients.

Randomization of a maximum of 476 patients is planned. At the first interim analysis when 200 subjects complete follow-up, if the overall analysis crosses the futility boundary, a novel adaptive design will identify, if it exists, a subgroup with the best prospect for showing benefit from endovascular treatment, based on baseline ischemic core lesion volumes and the time to treatment. The second interim analyses will be conducted at 340 patients at which time the study may stop for efficacy/futility, or the inclusion criteria may be adjusted in the case of futility.

Approximately 45 sites will be chosen. Individual site selection will be based on a number of factors including endovascular volume, MRI and/or CT perfusion access, number of competing trials, clinical trial experience, and the diversity of their patient population. If a site does not consent a patient within 4 months of activation, it will be placed on probation. If no patient consent occurs in the next 2 months, the site will be replaced with a "back-up" site.

3.3. Enrollment Criteria

3.3.1. Clinical Inclusion Criteria:

1. Signs and symptoms consistent with the diagnosis of an acute anterior circulation ischemic stroke
2. Age 18-90 years
3. Baseline NIHSSS is ≥ 6 and remains ≥6 immediately prior to randomization
4. Endovascular treatment can be initiated (femoral puncture) between 6 and 16 hours of stroke onset. Stroke onset is defined as the time the patient was last known to be at their neurologic baseline (wake-up strokes are eligible if they meet the above time limits).
5. modified Rankin Scale less than or equal to 2 prior to qualifying stroke (functionally independent for all ADLs)
6. Patient/Legally Authorized Representative has signed the Informed Consent form.

3.3.2. Clinical Exclusion Criteria:

1. Other serious, advanced, or terminal illness (investigator judgment) or life expectancy is less than 6 months.
2. Pre-existing medical, neurological or psychiatric disease that would confound the neurological or functional evaluations
3. Pregnant
4. Unable to undergo a contrast brain perfusion scan with either MRI or CT
5. Known allergy to iodine that precludes an endovascular procedure
6. Treated with tPA >4.5 hours after time last known well
7. Treated with tPA 3-4.5 hours after last known well AND any of the following: age >80, current anticoagulant use, history of diabetes AND prior stroke, NIHSS >25
8. Known hereditary or acquired hemorrhagic diathesis, coagulation factor deficiency; recent oral anticoagulant therapy with INR > 3 (recent use of one of the new oral anticoagulants is not an exclusion if estimated GFR > 30 ml/min).
9. Seizures at stroke onset if it precludes obtaining an accurate baseline NIHSS
10. Baseline blood glucose of <50mg/dL (2.78 mmol) or >400mg/dL (22.20 mmol)
11. Baseline platelet count < 50,000/uL
12. Severe, sustained hypertension (Systolic Blood Pressure >185 mmHg or Diastolic Blood Pressure >110 mmHg)
13. Current participation in another investigational drug or device study
14. Presumed septic embolus; suspicion of bacterial endocarditis
15. Clot retrieval attempted using a neurothrombectomy device prior to 6 hours from symptom onset
16. Any other condition that, in the opinion of the investigator, precludes an endovascular procedure or poses a significant hazard to the subject if an endovascular procedure was performed.

3.3.3. Neuroimaging Inclusion Criteria:

1. ICA or MCA-M1 occlusion (carotid occlusions can be cervical or intracranial; with or without tandem MCA lesions) by MRA or CTA

AND

2. Target Mismatch Profile on CT perfusion or MRI (ischemic core volume is < 70 ml, mismatch ratio is ≥ 1.8 and mismatch volume* is ≥ 15 ml)

Notes: The mismatch volume is determined by the RAPID software in real time based on the difference between the ischemic core lesion volume and the Tmax>6s lesion volume. If both a CT perfusion and a multimodal MRI scan are performed prior to enrollment, the later of the 2 scans is assessed to determine eligibility. Only an intracranial MRA is required for patients screened with MRA; cervical MRA is not required. Cervical and intracranial CTA are typically obtained simultaneously in patients screened with CTA, but only the intracranial CTA is required for enrollment.

Alternative neuroimaging inclusion criteria (if perfusion imaging or CTA/MRA is technically inadequate):

A) If CTA (or MRA) is technically inadequate:

Tmax>6s perfusion deficit consistent with an ICA or MCA-M1 occlusion

AND

Target Mismatch Profile (ischemic core volume is < 70 ml, mismatch ratio is ≥1.8 and mismatch volume is ≥15 ml as determined by RAPID software)

B) If MRP is technically inadequate:

ICA or MCA-M1 occlusion (carotid occlusions can be cervical or intracranial; with or without tandem MCA lesions) by MRA (or CTA, if MRA is technically inadequate and a CTA was performed within 60 minutes prior to the MRI)

AND

DWI lesion volume < 25 ml

C) If CTP is technically inadequate:

Patient can be screened with MRI and randomized if neuroimaging criteria are met.

3.3.4. Neuroimaging Exclusion Criteria:

1. ASPECT score <6 on non-contrast CT (if patient is enrolled based on CT perfusion criteria)
2. Evidence of intracranial tumor (except small meningioma) acute intracranial hemorrhage, neoplasm, or arteriovenous malformation
3. Significant mass effect with midline shift
4. Evidence of internal carotid artery dissection that is flow limiting or aortic dissection
5. Intracranial stent implanted in the same vascular territory that precludes the safe deployment/removal of the neurothrombectomy device
6. Acute symptomatic arterial occlusions in more than one vascular territory confirmed on CTA/MRA (e.g., bilateral MCA occlusions, or an MCA and a basilar artery occlusion).

3.4. Enrollment and Randomization

3.4.1 Enrollment: All patients who meet the clinical criteria listed above are eligible for

enrollment. This includes both patients who are directly admitted to the study site and patients who are transferred from an outside hospital. The time of enrollment is the time when the informed consent is signed. After obtaining consent the RAPID output from a CT perfusion or multimodal MRI scan will be assessed. If the patient is confirmed to meet the neuroimaging eligibility criteria listed above and not have any of the neuroimaging exclusion criteria, then the patient will be randomized. In general, patients will be consented prior to obtaining the RAPID output maps. In some situations the CT perfusion/ multimodal MRI may have been performed as part of standard care *prior* to the patient being assessed for study eligibility. Patients who are consented but do not meet the imaging criteria will not be randomized.

Determination of Target Mismatch and Large Artery Occlusion: At the conclusion of the MRI or CT Perfusion scan, the technologist sends the sequences from the console to RAPID with a single mouse click for automated processing. The RAPID software was developed based on data from DEFUSE 1 and was prospectively validated in DEFUSE 2. The system provides fully automated processing of brain images.

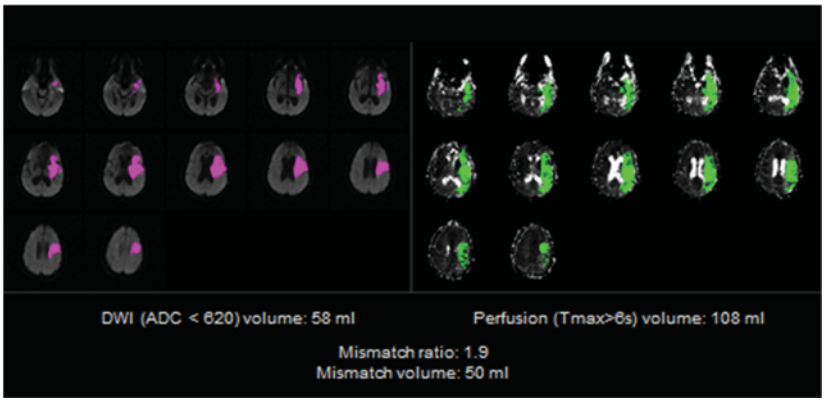

Figure 2. The RAPID mismatch summary allows investigators to quickly, accurately and easily determine if the patient meets the imaging criteria for enrollment. The patient above meets the Target Mismatch criteria: core volume is < 70 ml, mismatch ratio is ≥ 1.8 and mismatch volume is ≥ 15 ml.

The RAPID output maps, which identify the volume and location of ischemic core and perfusion lesions, are emailed to investigators (protected health information is automatically removed) and auto-sent to PACS as well as to a secure email system for viewing within 5 minutes of completion of the scan. Immediately after the images are available, the investigator will review the results of the RAPID mismatch map (**Figure 2**) and the MRA/CTA to determine if the patient meets the imaging criteria (listed above). If a patient has undergone multiple imaging evaluations (both MRI and CT or multiple CTs or MRIs), the most recent imaging study will be used to determine if the patient meets the imaging criteria. The accuracy of the software for identifying the size and location of perfusion and diffusion lesions has been established by extensive validation and testing on blood flow phantoms; the software received FDA 510K clearance for clinical use in 2013. The agreement between local investigators and the Imaging Core Lab for identification of the mismatch profile in DEFUSE 2 was 97%, κ 0·92; 95% CI 0·83–1·

Baseline data will be captured for all consented patients, including for patients who do not meet the imaging criteria. Patients who do not meet the imaging eligibility criteria will receive standard care per local hospital practice.

3.4.2 Randomization using a Dynamic Stratification Algorithm: Once a consented patient is determined to meet all Neuroimaging criteria, the patient ***will immediately be***

randomized on the WebDCU™ website. A dynamic stratification system will ensure well-balanced subgroups. The randomization algorithm, which will be programmed into the data capture system, will employ biased-coin minimization and the variance method with stratification weights.[44] The strategy is to balance treatment assignment along the marginal distribution of each stratification factor. The stratification factors used will be: 1) age, 2) core lesion volume, 3) time from symptom onset to enrollment, 4) baseline NIHSS, and 5) study site. When a new patient is enrolled, the site will enter the stratification factor values into the eCRF (electronic case report form) on WebDCU™. The dynamic randomization algorithm will determine an imbalance measure for each treatment group. The treatment group associated with the smallest imbalance measure will receive the largest probability of assignment in the biased-coin randomization. The biased-coin acceptance region and stratification weights are specified in the Randomization Plan. The superior balancing characteristics of dynamic randomization over blocked randomization have been well established.

Patients will be assigned to either endovascular therapy plus medical therapy or to medical therapy alone (1:1 randomization). Crossover from medical to endovascular therapy is strictly prohibited; endovascular to medical therapy crossover is defined as a patient who is assigned to endovascular therapy but does not have a conventional angiogram performed. Endovascular to medical therapy crossover is only allowed if an endovascular contraindication arises after randomization. Sites will be closely monitored for crossovers (see site monitoring plan below).

3.5 Acute Treatment

3.5.1 Endovascular Therapy: In patients randomized to endovascular therapy, the goal for femoral artery puncture will be within 45 minutes of randomization; femoral artery puncture must occur within 90 minutes of the completion of the qualifying imaging. Patients will be treated with thrombectomy devices (stent-retrievers) and/or suction thrombectomy systems currently cleared by the FDA for thrombus removal in patients experiencing an acute stroke within 8 hours of symptom onset following the published instructions for use for these devices. These devices will be used up to 16 hours following symptom onset in DEFUSE 3 based on an FDA IDE. The devices which will be used are the Trevo Retriever, the Solitaire Revascularization Device, Covidien MindFrame Capture Revascularization Device and the Penumbra thrombectomy system.

Standard medical therapy, based on current AHA guidelines, will also be provided for all patients. Individual investigators may use any of these devices or any combination of these devices to remove thrombus from the ICA, MCA M1 segment or, if needed, from M2 segments of the intracranial circulation. These are all approved anatomic locations for these devices. The use of thrombectomy devices should be performed in accordance with the indications for use. If there is a severe stenosis of the common carotid artery or the proximal internal carotid artery, investigators may also use other FDA devices approved for angioplasty or FDA devices approved for stenting of the carotid artery as deemed appropriate. The use of adjuvant intra-arterial (IA) thrombolytic medication is not currently approved by the FDA for stroke treatment and cannot be used in DEFUSE 3.

Sites will use local protocols for femoral access, sedation, heparin infusion, monitoring, etc. Sites will perform a cervical injection of the involved carotid circulation as a baseline

angiogram. At the conclusion of the procedure, a post-treatment angiogram as a cervical injection of the involved carotid circulation will also be obtained. Imaging will cover the full region of the normal circulation in AP and lateral projections at 2-3 films per second through the entire venous phase. All brain imaging from stroke onset through hospital discharge, including the baseline MRI and CT, as well as angiographic images obtained for the diagnostic and therapeutic portions of the procedure, will be transmitted to the core lab.

3.5.2 Medical Therapy: Patients randomized to medical therapy will receive standard medical therapy based on current AHA guidelines. Based on the time window for DEFUSE 3, it is anticipated that very few of the patients enrolled in DEFUSE 3 will have received iv tPA prior to randomization ("tPA failures"). For these patients, the sites' post-tPA protocol will be followed. Non-tPA treated patients randomized to medical therapy will be treated with aspirin, 325 mg on Day 1, and 81-325 mg/day (investigator's preference) on day 2-5, unless an indication for early anticoagulation is present (as determined by the patient's attending physician). All patients will receive standard DVT prevention therapy. Intravenous anticoagulants are prohibited (unless a clear indication for early anticoagulation is documented); dual antiplatelet therapy is prohibited unless carotid stenting was performed during the endovascular procedure or a clear indication for dual antiplatelet therapy is documented. Subsequent antithrombotic therapy will be determined by the patient's attending physician.

3.6 Clinical and Imaging Evaluations

Follow-up (imaging and clinical): Randomized patients will be followed clinically for 90 days and will have an MRI/MRA/MR perfusion at 24 hours (range 18-30 hours) to assess infarct volume, recanalization, hemorrhage and reperfusion (**Table 1 below**).

Table 1 Schedule of Events

	Screening	Enrollment	Baseline / Randomization	Endovascular Procedure	24 hours (+/-6 hrs)	Hospital Discharge	Day 30 (+/- 7 days)	Day 90 (+/- 14 days)
Screen Failure Log	X							
Informed Consent		X						
Subject Enrollment		X						
Inclusion and Exclusion Criteria			X					
MRI or CTP			X		X++			
Randomization			X					
Medical History			X					
Vital Signs			X					
NIH Stroke Scale			X		X	X	X	X
Modified Rankin Scale			X+			X	X	X
Baseline ASPECTS Score			X					
Baseline Labs*			X					
Endovascular Therapy				X				
24 Hour Labs					X			
Hospital Discharge						X		
Adverse Event Assessment				X	X	X	X	X
NeuroQOL								X

*Laboratory Evaluation includes CBC with Platelets, Creatinine, Glucose, INR, activated PTT, and Pregnancy test (if applicable). At 24 hour follow-up only creatinine is required. + Historical mRS at baseline, mRS/NIHSS to be performed by an NIHSS/mRS certified member of the research team who is blinded to treatment allocation at 30 and 90 days. ++ Patients will preferably undergo an MRI with MRA AND MR Perfusion at 24 hours; if an MR cannot be performed, a CT with CTA AND CTP can be substituted. For patients who are consented but not randomized, the schedule of events is limited to a summary of stroke therapies received within 24-hrs of stroke onset.

3.6.1 Assessments and follow-up visits

Baseline visit: All items in Table 1 above listed under "baseline" are to be performed prior to randomization. The MRI or CT scan should be performed with the DEFUSE 3 (baseline) protocol, which will be installed at all study sites. In addition, the inclusion/exclusion page of the case report form must be completed to determine if the patient meets the eligibility requirements for the study. If the patient is eligible and the consent form is signed by the patient or authorized representative, then the randomization procedure should occur immediately.

24 hour visit (+/- 6 hours): The items listed for this visit in Table 1 should be performed between 18 and 30 hours from the time of randomization. The only laboratory value required at the 24 hour visit is a serum creatinine. If possible, the 24 hour follow-up imaging study should be performed with multimodal MRI, rather than CT perfusion. The MRI or CT scan performed at this time should be performed with the DEFUSE 3 protocol.

Discharge visit: The items listed for this visit in Table 1 should be performed on the day of hospital discharge

30 and 90 day visits: The items listed for this visit in Table 1 should be performed on Day 30 (+/- 7) days and Day 90 (+/- 14) days. The mRS score must be performed by an mRS certified investigator who is blinded to treatment allocation at both the 30 and 90 day visits. If an in person visit is not possible, then the mRS should be performed by phone by an mRS certified investigator who is blinded to treatment allocation. If an in person visit is not possible, then the NIHSS score will be marked "not available" in the case report form.

Neurological worsening: If clinical worsening (defined as a $\geq$4 point increase on the NIHSS score) occurs prior to discharge, a CT scan or MRI should be obtained as soon as possible. Neurological worsening is a reportable adverse event.

3.6.2 Sources of Materials

Information on the clinical status of patients will be obtained from the patient's medical record. Study coordinators at the site will complete the DEFUSE 3 case report forms to collect basic demographic and medical information about the patients. Data will subsequently be entered into the StrokeNet's WebDCU electronic data capture system. Imaging data will be electronically transmitted to the coordinating center at Stanford via RAPID (all patient identifiers are removed by the software prior to exporting the data outside of the site's firewall). All study sites will complete a stroke screening log that documents all patients treated in the cath lab beyond 6 hours at their center, and reason for exclusion of patients not enrolled, in the StrokeNet's WebDCU electronic data capture system. Serious adverse events (SAEs) will be reported within 24 hours of the event in the StrokeNet's WebDCU electronic data capture system. The data collection process will include patient demographics, medical history, vital signs, laboratory assessments, NIHSS and mRS scores, and results of diagnostic studies performed to clarify stroke etiology.

3.7. Site Approval and Monitoring Plan

Site approval: Individual sites approved for participation in the study will be high-volume sites. Selected sites will have access to emergent CT perfusion and/or MR imaging 24/7. Prior to activating a site, we will verify that RAPID is functional at the site. Together with the

site's CT and/or MR technologists, we will install the DEFUSE 3 scan protocol on the local scanners and perform a dummy-run to assess image quality and train the technologists in software handling and data sending. A site will be activated for enrollment after test cases processed with RAPID have ensured good quality maps.

Monitoring for imaging quality: The Imaging Core Lab will monitor image quality throughout the study. If significant inadequacies or protocol errors are noted at a site, enrollment will be halted. Enrollment will resume after all imaging problems have been resolved and repeat dummy runs have been obtained that demonstrate adequate image quality.

Table 2. Example Imaging Sequences for DEFUSE 3 scans

Sequence	Scan Parameters (3T)	Time
MRI		**6 min**
Localizer	128X256; 28 FOV;5/5mm, GRE	24 sec
Calibration		5 sec
DWI	128x128, 24 FOV, 5/0mm, 30 slices, 1 NEX, R=2; b=0 and 1000 s/mm^2 over 3 axes, TE/TR=min/7000ms.	25 sec
GRE	256x192; 24 FOV; 5/0 mm, 30 slices, TE/TR= 25/800ms, flip 20, interleaved EPI, 16 shots	27 sec
MRA intracranial	256x192, 1 mm; 4 slabs, 26 phase-encodes; 6 overlap, 22 FOV, 0.8 rFOV, fractional echo, ZIPx2, ZIPx512, minTE, flowcomp, TR=18ms, flip=18, inferior->superior ramppulse, R=2; 19 MIPS	143 sec
PWI	128x128; 24 FOV; 5/0 mm, 17 slices, TE/TR=35ms/1800ms, R=2 using 0.1mmol/kg Gadolinium @ 4ml/sec.	108 sec
CT (example below for GE VCT; comparable protocols will be used for other scanner models)		**5-6 min**
Non-con head	2.5 – 5mm, 40 slices, 120-140kV, 265-290mA	120-180 sec
CTA	0.625mm, 0.984:1/39.37cm, 120kV, 550mA , inject and observe for 15 sec until contrast concentration in ascending aorta reaches 80HU (smart prep) then the CT gantry moves along with the bolus of the contrast material from the aortic arch up to the apex of the brain in 5sec.	90 sec
CTP	22 FOV, 40mm, 8x5mm, 1.8sec time interval, 45 cycles, 80kV, 125mA; 2 runs	90 sec

Monitoring for bias: A detailed site-monitoring plan has been developed to detect bias. This plan will protect the study from enrollment, randomization, and treatment bias. The first component involves monitoring the percentage of each site's endovascular volume (within 6-16 hrs) that is enrolled in DEFUSE 3. Sites will report their volume of endovascular stroke procedures (within 6-16 hrs) each month on a screening log. If a DEFUSE 3 eligible patient is treated with endovascular therapy outside the DEFUSE 3 study, an explanation will be required detailing why the patient was not enrolled. The second component of the plan involves tracking of patients who are consented but not randomized. These patients will require an entry in the WebDCU with an explanation why the patient was not randomized as well as documentation whether endovascular therapy was performed outside of the study. A third component involves monitoring of crossover after randomization. The Executive Committee will review the data described above for each site every 6 months. If evidence of enrollment bias is suspected, it will be investigated. If confirmed, the site will be placed on probation. If additional incidents of

suspected bias are confirmed, the site will be withdrawn. Routine monitoring of the clinical sites for source to database verification will be performed by the StrokeNet Data Management Center.

3.8. Sample Size, Adaptive Design and Statistical Analysis

DEFUSE 3 will feature a novel adaptive trial design that will allow the study to focus on a subpopulation if interim or final analyses indicate futility in the overall population.[59] The adaptive design was developed specifically for DEFUSE 3. It is based on closed testing theory and the group sequential methods for the Generalized Likelihood Ratio (GLR) statistic developed by Lai and Shih.[60] The adaptive design was chosen because there is strong preliminary data that suggests that the effect of endovascular treatment is modified by two baseline variables: core lesion size and time-to-treatment. The way the adaptive design takes advantage of these biological assumptions (when they are true) is by reallocation of future accrual to the subgroup with the best prospects for showing efficacy. Specifically, if a subgroup is chosen at an interim analysis, subsequent enrollment is limited to patients in that subgroup. As a result, this subgroup will become larger than it would have been in the absence of the adaptive design. The criterion for deciding which subgroup has the best chance of showing a benefit from endovascular therapy combines both the estimated size of the effect in the subgroup and the sample size of the subgroup. The GLR statistic (Kullback-Leibler criterion) is used to identify this subgroup because it optimally balances those two criteria. It selects the subgroup that has the best chance of showing an effect because it has an apparently large effect and is also of substantial size (note there are 5 subgroups of increasingly larger size, **figure 3**). The adaptive design employs two biologically-based assumptions to limit the inflation of sample size; a monotonicity/contiguity assumption and an a prior assumption that the effect is largest in the patients with the smallest DWI lesions and the shortest time to randomization (cell C_{11} in **figure 3**). The boundaries of the categories (cells) will be determined just prior to the 1st interim analysis based on the distribution of patients across these two dimensions (lesion volume and time-to-treatment).

		Time (hrs)	
		<10	10-16
Core lesion volume (ml)	≤20	C_{11}	C_{21}
	21-50	C_{12}	C_{22}
	51-70	C_{13}	C_{23}

Figure 3. The cohort is stratified according to core lesion volume and time to randomization. Exact boundaries of the stratification will be determined based on the distribution of patients just prior to the first interim analysis. Depending on the results of the 1st interim analysis, subsequent enrollment will continue in all 6 cells or will be limited to one of 5 sub-groups (C_{11}, C_{11+21}, $C_{11+21+12}$, $C_{11+21+12+22}$, or $C_{11+21+12+22+13}$).

Primary analysis: The primary endpoint is the distribution of scores on the modified Rankin Scale (mRS) at day 90. We will test the null hypothesis at the interim and final analysis using a normal approximation of the Wilcoxon-Mann-Whitney test (the generalized likelihood ratio [GLR] test). The primary analysis will be intention to treat, adjusted for design and not adjusted for covariates.

For each analysis, an efficacy bound will be set to control the overall (one-sided) Type I error rate at 2.5%. At each interim analysis a futility bound will be set to decide if the study should continue recruitment in the overall group, shift accrual and testing to a subgroup, or stop in its entirety. The futility boundary adapts when a subgroup is selected to the fact that the maximum analyzed sample size is a random variable that is no larger than the fixed maximum number of patients randomized (n=476). Because subgroup selection reduces the

maximum number of patients available for analysis at completion of the study, this method effectively allows an easier futility stop after subgroup selection. This setup replaces conditional power analyses with an automatic and more powerful adjustment of boundaries.

First interim analysis (n=200 randomized and completed 90 day follow-up): The null hypothesis is tested in the entire patient population:

1. If neither efficacy nor futility bound is crossed, the trial continues enrollment to the 2nd interim analysis.
2. If the efficacy bound is crossed, the trial stops and efficacy is declared in the overall population.
3. If the futility bound is crossed, the optimal subgroup is selected based on the Kullback-Leibler criterion and the null is tested in that subgroup. The futility bound is relaxed as described above, based on the expected maximum number of patients in the trial at completion (ie 476 minus the number of patients already enrolled in cells that will no longer be open for enrollment).
    3.1. If neither bound is crossed, the trial will continue with enrollment limited to the selected subgroup
    3.2. If the efficacy bound is crossed, the trial stops and efficacy is declared in the selected subgroup
    3.3. If the futility bound is crossed, the trial stops for futility.

Second interim analysis (n=340 randomized and completed 90 day follow-up): If, after the first interim analysis, the study proceeds with enrollment in the overall population (option 1 above), the testing at the 2nd interim analysis is identical to the first interim. If enrollment is limited to a selected subgroup (option 3.1), the null is tested in that subgroup:

1. If neither bound is crossed, the trial continues to the final analysis with enrollment of 136 additional patients limited to the selected subgroup
2. If the efficacy bound is crossed, the trial stops and efficacy is declared in the selected subgroup
3. If the futility bound is crossed, the trial stops for futility.

Final analysis (n=476 randomized and completed 90 day follow-up): If, after the second interim analysis, the study proceeds with enrollment in the overall population, the null is tested in the overall population:

1. If the efficacy bound is crossed, endovascular therapy is declared efficacious in the overall population.
2. If the efficacy bound is not crossed, the optimal subgroup is selected and the null is tested in that group:
    2.1. If the efficacy bound is crossed, endovascular therapy is declared efficacious in that subgroup
    2.2. If the efficacy bound is not crossed, endovascular therapy will be declared of no benefit.

If enrollment after one of the interim analyses is limited to a selected subgroup, the null will be tested in that subgroup only and efficacy or lack thereof will be declared as per options 2.1 and 2.2 above.

Power and sample size considerations: The projected overall effect of endovascular therapy is based on 1) the observed 90-day modified Rankin Scale outcomes in DEFUSE 2 of target mismatch patients treated >6hrs after symptom onset and 2) the assumption that early reperfusion will be achieved in 75% of the endovascular arm vs. 20% of the medical therapy arm.[20, 21, 61] Using these data, we projected the distributions on the mRS at 90 days in the endovascular and control arms of DEFUSE 3:

	mRS at day 90							
	0	1	2	3	4	5	6	Total
Endovascular group	18.0%	11.5%	19.6%	11.5%	16.4%	11.5%	11.5%	**100%**
Medical group	9.7%	7.9%	15.0%	17.7%	14.4%	17.7%	17.7%	**100%**

This distribution corresponds to a standardized effect of 0.36 for the primary analysis. Based on these data, the fixed sample size for a non-adaptive design requires a total of 376 patients (188/arm) to have 90% power at an alpha of 5% (Wilcoxon-Mann-Whitney test); 100 additional patients are added for the adaptive design to reach a maximum sample size of 476 for DEFUSE 3.

We ran simulations (n=5000) to compare the performance of a traditional fixed sample-size design (fixed n=476) to the adaptive design (max n=476) under various scenarios (see **Table 3**, below). For the simulations the effect size is expressed as a standardized effect, where a standardized effect of 0.3 corresponds to a conservative projected effect of endovascular therapy (anticipated effect 0.36; see above).

Imaging outcomes: We hypothesize that endovascular treatment improves radiological outcomes in stroke patients with favorable clinical and imaging characteristics. DEFUSE 2 demonstrated a substantial reduction in infarct growth among Target mismatch patients treated in the 6-12 hour time-window who achieved early reperfusion: median growth 0.5 ml (IQR: -2 – 10) with reperfusion (n=23) vs. 39 ml (IQR: 18-121) without reperfusion (n=13), p<0.001. These data have been extrapolated to DEFUSE 3 using the same assumptions described above; anticipated an early reperfusion rate of 75% in the endovascular arm vs. 20% in the medical arm. This yields a sample size of 42 per group for 90% power. Therefore, DEFUSE 3 is highly powered to demonstrate differences in lesion growth. Infarct volumes, ischemic lesion growth, and reperfusion rates at 24 hours will be compared between groups with the Mann-Whitney U test. The 24 hour endpoint is based on data demonstrating that assessment of infarct volume at 24 hours captures the effect of reperfusion therapies on infarct growth and predicts outcomes similarly to day 90 infarct volumes.[29, 62] RAPID-assessed ischemic core volume at baseline will be correlated with 24h infarct volume (DWI volume) in subjects who achieve reperfusion without PH1 or PH2 intracranial hemorrhage. Pearson's correlation coefficient will be calculated and the median absolute error (ml) will be reported. Similarly, correlation of the baseline Tmax >6 volume and the 24h infarct volume in patients without PH1 or PH2 intracranial hemorrhage who have <10% reperfusion will be performed. Correlation of RAPID predicted infarct volume (coregistered baseline ischemic core and 24h Tmax >6 volume) with the actual 24h infarct volume will also be performed.

Scenario	Standardized effect in cells C11,	C12,	C21,	C22,	C31,	C32	Average standard. effect	Adaptive Design Average No. randomized	Power	Fixed Design Number randomized	Power
#0	0	0	0	0	0	0	0	361	2.2%	476	2.5%
#1	0.3	0.3	0.3	0.3	0.3	0.3	0.3	354	80%	476	89%
#2	0.5	0.4	0.3	0	0	0	0.2	400	86%	476	55%
#3	0.5	0.5	0	0	0	0	0.17	403	87%	476	41%

Table 3. Under the null (Scenario #0), the adaptive design controls the total Type 1 error below 2.5%, stops early for futility 63% of the time, and the average number of randomizations is 361. If the effect is uniform across cells (scenario #1), the fixed-sample design is optimal, but the adaptive design results in only a small loss of power (from 89 to 80%). The adaptive design performs much better (higher power and smaller expected sample size) than the fixed sample, conventional trial when the effect size distribution across the subgroups is in accord with the biological assumptions (scenarios #2 and 3). If the effect is concentrated in two cells with small core volumes (scenario #3), the adaptive design maintains power (87%) while the conventional design collapses (41% power). The adaptive design also performs well compared to a non-adaptive, fixed sample that includes efficient multiple comparisons-adjusted testing for effect in subgroups at the end of the study. (see Lai et al[59])

Secondary analysis: Our secondary endpoint is the proportion of patients with mR S 0-2 at day 90 (indicating functional independence). The difference in the proportions of patients with mRS 0-2 between treatment arms will be assessed using logistic regression.

Subgroup analyses: Subgroup analyses of the effect of endovascular therapy on the primary and secondary endpoints will be performed. Subgroups will be defined based on the stratification variables, key demographic factors (such as race and ethnicity), tPA vs. no tPA, CTP vs. MRI selection, and witnessed vs. unwitnessed symptom onset, wake-up vs non-wake-up stroke, and TICI 0-2a vs. TICI 2b/3 results in cath lab.

Missing data/lost to follow-up (LTFU):

All effort is put forth to ensure near complete follow-up, in particular with the assessment of the primary outcome (mRS at 90 days), death (mRS=6), and stroke recurrence. If the primary outcome (mRS at 90 days) cannot be assessed in the clinic, it will instead be obtained by phone using a structured interview. If the subject's mRS cannot be obtained in clinic or by phone within the window of 60 to 120 days from randomization, then for primary analyses the day 30 mRS score will be used as the primary outcome (ie day 30 mRS carried forward). If neither the 30-day nor the 90-day mRS is available, then the mRS will be imputed. We do not expect this to alter the main study results given our estimated very low LTFU rate (<2%).

DEFUSE 3 Timetable

Year 1	Year 2	Year 3	Year 4	Year 5
Install RAPID at all sites Begin enrollment (anticipated to begin mid-year)	Pt enrollment continues for a total of 4 yrs	1st interim analysis Potential modification of enrollment criteria based on adaptive design	2nd interim analysis Potential modification of enrollment criteria based on adaptive design	Finish enrollment Data Analyses Publication of results

3.9. Risk analysis

Description and analysis of all increased risks to the research subjects:

Potential complications of MRI scan include localized twitching sensation due to the magnetic field changes during the scan, anxiety due to claustrophobia and allergic reaction to the contrast agent. The allergic reaction may include headache, nausea, rash, hives, nasal congestion, sneezing, itching or swelling. If a severe reaction occurs, swelling of the throat, chest tightness, or a marked drop in blood pressure may occur. In addition, pain, bleeding, bruising, coldness or inflammation at the injection site may occur. Precautions will be taken for early detection and rapid treatment if such reactions occur.

Potential complications of CT scan include radiation exposure and allergic reaction to CT contrast agents.

Radiation doses:
Combined scanning with comprehensive stroke imaging, which includes a noncontrast head CT scan, perfusion imaging, and CT angiography of the cervicocranial vessels starting at the aortic arch results in a dose of approximately 7-10 mSv. (AJNR 2010 31: 1003-1009). According to the National Council on Radiation Protection and Measurement, the average annual radiation dose per person in the U.S. is 620 millirem (6.2 milliSieverts).

Reactions to contrast agents:

- **Mild**
  Nausea, Vomiting**,** Headache**,** Cough**,** Nasal stuffiness, Altered taste**,** Flushing**,** Itching**,** Rash, Hives**,** Sweats**,** Swelling of eyes or face
- **Moderate**
  Mild hypotension, Tachycardia or Bradycardia, Bronchospasm, Wheezing, Dyspnea, Laryngeal edema, Generalized or diffuse erythema
- **Severe**
  Cardiopulmonary arrest, Clinically manifested arrhythmias, Profound hypotension, Convulsions, Unresponsiveness, Respiratory failure, Laryngeal edema
  The rate of major reactions (e.g., anaphylaxis, death) is very low, estimated at one in 170,000 administrations.

Potential complications of endovascular therapy include stroke; new clot in an artery; total blockage of an artery; infection and pain in the region of insertion site; lack of blood flow to the brain; rupture or puncture of an artery; significant tearing of the vessel wall; bleeding requiring blood transfusion; allergic reaction to contrast dye; abnormal low blood pressure requiring treatment; temporary closing of the artery (vessel spasm); formation of or dislodgments of clots which block the arteries (embolism). In rare circumstances, the procedure could result in death. At the puncture site in the groin, a blood clot or other blood vessel injury may occur and require blood transfusion or surgical repair. Infection may occur at the puncture site; this could cause pain and require additional medications. There is some chance of an allergic reaction to the x-ray contrast (dye) used during the angiogram procedure. Minor allergic reactions may include a rash or hives. There is also the possibility of a serious allergic reaction that could include shortness of breath and swelling, drop in

blood pressure, and even death. Patients will be closely monitored for these reactions and receive prompt treatment to reverse any allergic reactions.

Safety of endovascular therapy beyond 8 hours

Mechanical thrombectomy devices have been used beyond 8 hours of stoke onset in a number of clinical trials and registries. No safety concerns have been associated with late window therapy. In DEFUSE 2 [37], patients were treated up to 12 hours after symptom onset, and no safety concerns were identified in any time window. Based on both favorable safety data and encouraging efficacy data, Stryker Neurovascular has initiated the DAWN Study with a 24 hour treatment window. No safely issues have been identified to date in DAWN. The DAWN study is being run under an FDA IDE.

Methods to mitigate risks to subjects in the trial

Methods to mitigate risks to subjects in the trial include exclusion of subjects with bleeding disorders and selection of subjects via neuroimaging (infarct core lesions less than 70 ml) to minimize the risk of symptomatic intracranial hemorrhage. Computed Tomography (CT) scans will be performed for neurological deterioration (≥ 4 point increase in National Institutes of Health Stroke Scale (NIHSS) score) to identify new strokes, hemorrhage, or edema. Hospitals will follow their local standard of care safety procedures in order to reduce the risk of kidney dysfunction caused by contrast agents. Only investigators who are trained and experienced with use of the devices allowed within the trial are eligible to participate (see **Site Approval and Monitoring Plan**) above. The adaptive design will eliminate subgroups with an unfavorable therapeutic response.

Patients will be carefully screened for CT, MRI and endovascular treatment contraindications according to the inclusion/exclusion criteria and excluded from enrollment if any are present.

Radiation exposure: Radiation exposure during all tests will be minimized by optimizing the imaging protocols and by limiting fluoroscopy-time during the endovascular procedure. All CT sequences, including the CTP sequence, meet all FDA guidelines for radiation exposure.

Stopping rules or safety triggers for the study

Symptomatic ICH or death rates that exceed pre-specified thresholds will trigger a meeting of the DSMB to discuss the events and make a determination on the continuation of the trial. Below are the pre-specified triggers:

The DEFUSE 3 has established the following automatic stopping rules, based on identifying with 95% probability:

1) the rate of symptomatic ICH (NIHSS worsening of 4 or more points associated with ICH) in the endovascular group is exceeding 10%
2) the rate of 90 day mortality in the endovascular group is exceeding 20%.

If either threshold is crossed, the study will be automatically placed on hold until the investigators and the DSMB can conduct a review of events.

Adverse events (AEs) will be collected, recorded, and analyzed in accordance with Section 3.11 below.

Safety oversight for this study will be provided by both the DSMB and an independent Medical Safety Monitor. Please see Section 3.11 for details.

Patient Population: Four hundred and seventy six acute stroke patients meeting the pre-defined inclusion criteria will be enrolled in the trial. The mean age is anticipated to be 69 years of age. Our targeted planned enrollment breakdown is as follows:

Racial Categories	Ethnic Categories				Total
	Not hispanic or Latino		Hispanic or Latino		
	Female	Male	Female	Male	
American Indian/Alaska Native	2	2	1	1	6
Asian	13	13	1	1	28
Native Hawaiian or Other Pacific Islander	2	3	0	0	5
Black or African American	32	32	1	1	66
White	175	174	11	11	371
Racial Categories: Total of All Subjects	224	224	14	14	476

Imaging core lab: The Stanford imaging core lab has 15 years of experience with MR image storage and processing. It will perform the organization, archiving and blinded analysis of all imaging data collected in DEFUSE 3. They will be responsible for MRI and CT Perfusion image processing and artifact removal and will generate final lesion volumes for all MRI scans performed in the study.

DSA Angiograms sent to the core lab will include a baseline (pre-treatment) and a final angiogram for the territory of treatment. In addition, angiographic images from MRA or CTA will be sent to the core lab. The MRA or CTA will be used to assign a primary arterial lesion (AOL) from non-invasive imaging. The baseline DSA angiogram will also be used to assign a primary arterial occlusive lesion (AOL) and a pre-treatment mTICI score. The final angiogram will have a post-treatment mTICI score assigned.[22],[52] This scoring system defines TICI 2A as partial perfusion of < 50% of the vascular distribution of the occluded artery and 2B as partial perfusion of > 50% of the vascular distribution. The scoring system will use these previously described definitions[22]:

- Grade 0 No perfusion
- Grade 1 Antegrade reperfusion past the initial occlusion, but limited distal branch filling with little or slow distal reperfusion
- Grade 2a Antegrade reperfusion of less than half of the occluded target artery previously ischemic territory (eg, in 1 major division of the MCA and its territory)
- Grade 2b Antegrade reperfusion of more than half of the previously occluded target artery ischemic territory (eg, in 2 major divisions of the MCA and their territories)

- Grade 3 Complete antegrade reperfusion of the previously occluded target artery ischemic territory, with absence of visualized occlusion in all distal branches

Two senior neurointerventionalists will perform the angiographic analysis, blinded to the clinical data, MR imaging and CT Perfusion results, and the analysis of the other core lab reader. Any disagreements in the AOL interpretation or the mTICI scores will be adjudicated by common review of those cases and a consensus reading will be applied.

National Data Management Center: Data management and site monitoring will be performed by the StrokeNet National Data Management Center (NDMC) at Medical University of South Carolina (Director, Yuko Y. Palesch, PhD, see letter of support). The NDMC will create the database and set up the interface on the website (WebDCU™) where clinical site personnel will enter the data into the electronic CRF. Data quality assurance processes include: (1) logic and rule checks built into the database; (2) monitoring by the Data Manager at the NDMC; (3) central monitoring by the statistical programmer at the NDMC; and (4) risk-based source verification monitoring by the Clinical Research Associates. DEFUSE 3 data, including neuroimaging, will be shared in accordance with the StrokeNet data sharing policies. Anonymized neuroimaging will be stored on secure servers at the Stanford Stroke Center with nightly back-up. NINDS Common Data Elements will be used for both clinical and imaging data.

3.10. Description of devices

The following FDA approved thrombectomy devices will be included:

1) Trevo Retriever
2) Solitaire™ Revascularization Device
3) Covidien MindFrame Capture Revascularization Device
4) Penumbra thrombectomy system including the following devices and pumps:

Penumbra Aspiration Pump (1115V)	Penumbra System [026, 032, 041]
Penumbra System 054	Penumbra System Separator Flex [026, 032, 041, 054]
Penumbra System MAX	Penumbra Pump MAX
Penumbra System 110 Aspiration Tubing	Penumbra System Reperfusion Catheter ACE64 & ACE68

3.11. Monitoring procedures

The coordination of the DEFUSE 3 Trial operations will be centralized through the following:
NIH StrokeNet National Coordinating Center (NCC)/ PI: Joseph Broderick, MD
University of Cincinnati
260 Stetson Street, Suite 2300
Cincinnati, Ohio 45267-0525

Leading the NCC team will be the Project Manager, who will be assigned to coordinate the following study oversight: trial communication required training activities, site assessment and/or initiation visits, collection of trial related regulatory documents, recruitment performance tracking, site monitoring, and performance analysis. Study oversight will be handled according to the Data Monitoring Standard Operating Procedure (SOP Number ADM 19).

DEFUSE 3 will have an independent Data and Safety Monitoring Board (DSMB) appointed by the NIH to oversee study safety. Patients in both study arms will be assessed for the incidence of stroke-related mortality at 90 days, the incidence of symptomatic intracranial hemorrhage at 36 hours from symptom onset (defined as a ≥ 4 point worsening of immediate pre-deterioration NIHSS neurological status vs. post deterioration and associated with brain hemorrhage), and the incidence of significant neurologic deterioration prior to discharge (defined as ≥ 4 point worsening of the immediate pre-deterioration NIHSS neurological status vs. post deterioration and not attributed to sedation). In the endovascular arm patients will be assessed for intra-procedural complications including: intra-procedural mortality, vessel perforation, arterial dissection, access site complication requiring surgical repair or blood transfusion, embolization and device failure. SAEs will be reported within 24 hours of awareness of the event.

The DSMB will meet in person or by teleconference, on a semi-annual basis, to monitor the cumulative safety data during participant follow-up. In no instance will more than 12 months elapse between DSMB reviews of cumulative safety data after the first participant has been randomized. The DSMB will monitor the study according to the guidelines specified in the study protocol and the operating procedures established at the initial meeting, unless the DSMB determines during the course of the trial that modification of the guidelines is in the best interest of the study and its participants.

Independent Medical Safety Monitor:
In addition to the DSMB, Dr. Andrew Demchuk has been appointed as the independent Medical Safety Monitor (MSM) for DEFUSE 3. Dr. Demchuk is not involved in the study and has no conflict of interest. He will be responsible for ongoing monitoring of reports of SAEs submitted by the clinical centers in real time to ensure good clinical practice and to identify safety concerns quickly. Dr. Demchuk may suggest protocol modifications to prevent the occurrence of particular AEs, e.g., modifying the protocol to require frequent measurement of laboratory values predictive of the event or to improve expeditious identification of SAEs. In the event of unexpected SAEs or an unduly high rate of SAEs, Dr. Demchuk will promptly contact the DSMB Liaison who will notify the DSMB Chair. In the event that he is unavailable for an extended period of time (i.e., extended vacation, sabbatical, illness, etc.), a back-up MSM will be nominated by the study PI and approved by the DSMB.

Adverse Event Reporting
Consideration of adverse events will hereafter consist of adverse events, serious adverse events, and adverse device effects, including anticipated adverse device effects and unanticipated adverse device effects.

- Adverse event (AE) is defined as any untoward/undesirable clinical occurrence in a clinical investigation of a subject which does not necessarily have a causal relationship with the treatment under investigation. An Adverse Event can therefore be any unfavorable and/or unintended sign, symptom, or disease temporally associated with the use of a device product, whether or not considered related to the device product. Only abnormal laboratory values that are deemed clinically significant by the investigator will be classified as adverse events.

- Serious adverse event (SAE) is defined as any untoward/undesirable adverse experience that results in any of the following outcomes: 1) death; 2) a life-threatening adverse experience; 3) inpatient hospitalization or prolongation of existing hospitalization; 4) a permanent/persistent or significant disability/incapacity or a congenital anomaly/birth defect; 5) important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse event when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. This category includes the use of intra-arterial thrombolytics and/or intracranial stents.

- Anticipated adverse device effect (AADE) is defined as any adverse effect related to the device or procedure, which is identified in the protocol or the IFU for the device.

- Unanticipated Adverse Device Effects (UADEs) is defined as any serious adverse effect on health or safety or any life-threatening problem or death caused by, or associated with a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the investigational plan or application, or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects.

Reports of UADEs will be made to the FDA within 10 days of receiving notification of the UADE (as required in 21 CFR 812 18p (b)(1)).

Safety Monitoring

The MSM will monitor all AE reports to identify and trend all events that would require temporary discontinuation of study enrollment, to fully characterize device safety, to modify the study protocol, or to terminate the study.

Reporting Procedures for All Adverse Events

All Adverse Events, whether or not attributed to the study and/or the devices, observed by the investigator or reported by the subject, will be recorded from the time of randomization through Day 5 or discharge, whichever is earlier. All SAEs will be recorded through Day 90.

The following attributes will be assigned by the reporting investigator:

1. Description of event
2. Date of onset
3. Date of resolution (if applicable)
4. Seriousness
5. Relationship to the study device and/or procedure(s)
6. Severity
7. Action(s) taken
8. Outcome(s)

Severity is defined as a measure of the intensity of a reaction, effect or experience. The measurement(s) are described as mild, moderate, severe, life threatening or death. The event itself, however, may be of relative minor medical significance. The severity of Adverse Events is assessed according to the following index scale:

- **Mild**
  asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated
- **Moderate**
  minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental Activities of Daily Living.
- **Severe**
  medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self care Activities of Daily Living.
- **Life-threatening consequences;**
  urgent intervention indicated
- **Death** related to AE

The relationship of an AE to the study device or procedure will be graded as follows:

- Unrelated
- Unlikely
- Reasonable possibility
- Definitely

Serious Adverse Events All Serious Adverse Events including deaths will be reported to the MSM, the Central Institutional Review Board (IRB) and the FDA, as required.

4. INVESTIGATOR'S AGREEMENT

As this study will be carried out by the NIH StrokeNet, the names of the specific sites and investigators are not yet available. Investigator's agreements for key investigators at the coordinating site are included.

All investigators will be required to sign the following agreement:

INVESTIGATOR AGREEMENT
FOR THE CLINICAL INVESTIGATION OF THE DEFUSE 3 TRIAL

I_____ agree to participate as an Investigator on the DEFUSE 3 trial.

I have been provided a copy of the following Food and Drug Administration (FDA) regulations: 21 CFR Part 812, Investigational Device Exemptions; 21 CFR Part 50, Protection of Human Subjects; and 21 CFR Part 54, Financial Disclosure by Clinical Investigators.

I agree and/or certify that:

1. I will conduct the clinical investigation in accordance with this agreement, all requirements of the investigational plan, IDE regulations, other applicable regulations of the FDA, and any conditions of approval imposed by my reviewing Institutional Review Board (IRB) or FDA. I agree to abide by all of the responsibilities of Investigators addressed under 21 CFR Part 812, Subpart E and Subpart G, including but not limited to the following:

2. I will obtain written approval from the authorized IRB for the institution at which this investigation will be conducted.

3. I will ensure that Informed Consent is obtained from each subject participating in this clinical investigation in accordance with the informed consent regulation found in 21 CFR Part 50, and that a signed copy of the informed consent is available to the sponsor (sponsor-investigator) and the sponsor's (sponsor- investigator's) designated monitor.

4. I will ensure the accurate completion of protocol case report forms and, if I am not also the sponsor- investigator of the corresponding IDE application, I will submit completed protocol case report forms to the sponsor (sponsor-investigator) at the time frames specified in the Protocol and/or FDA regulations.

5. I have the appropriate, relevant qualification to conduct and to oversee the conduct of the clinical investigation as documented by the following: (***initial applicable statement***)
   ___ My relevant qualifications, including dates, location, extent and type of experience are listed in my most recent curriculum vitae (CV), which is attached to the Agreement and which will be maintained by the sponsor (sponsor-investigator) of the corresponding IDE application.
   ___ My curriculum vitae (CV) does not reflect my relevant qualifications, therefore attached to this Agreement is a statement of my relevant experience (including dates,

location(s), extent and type of experience) which will be maintained by the sponsor (sponsor-investigator) of the corresponding IDE application.

6. There are no reasons to question my ability to oversee the appropriate conduct of this clinical investigation. (***Initial applicable statement***)
   ___ I have never participated in an investigation or other research activity which was terminated (disqualified) by the FDA, IRB (or equivalent), or sponsor of a study due to non-compliance issue.
   ___ I have participated in an investigation or other research activity which was terminated (disqualified) by the FDA, IRB (or equivalent), or sponsor of a study due to non-compliance issue. The specific circumstances leading to this termination and my role in the respective problems or issues and the resolution of these problems or issues are summarized in an attachment to this Agreement.

I further certify that I have not been debarred under the Generic Drug Enforcement Act of 1992, 21 USC §§ 335a and 335b. In the event that I become debarred or receive notice of an action or threat of action with respect to my debarment during the term of this Agreement, I agree to immediately notify the sponsor (sponsor-investigator) and the authorized IRB for my study site. If I am the sponsor-investigator of the corresponding IDE application I will notify the authorized IRB and the FDA.

As required by 21 CFR Part 54, Financial Disclosure by Clinical Investigator, I will disclose sufficient and accurate financial information to the sponsor (sponsor-investigator) by completing the Certification of Financial Interest Form (attached) and if applicable, the Disclosure of Financial Interest Form (attached). I will also notify the sponsor (sponsor-investigator) if my disclosed financial information changes at any time during the clinical investigation or up to one year following the closure of the study.

Site Name and Address:
_____
_____
_____

_____ _____
Investigator Signature Date

5. EXECUTIVE COMMITTEE / KEY PARTICIPATING INVESTIGATORS

The Executive committee, composed of experts in vascular neurology, endovascular therapy and neuroimaging, will provide the overall scientific guidance for the study. The committee will typically meet monthly by phone (1 hour/month) for the full duration of the study. Responsibilities include oversight of the overall conduct of the study with regard to protocol compliance and modifications/amendments, study progress, and problem-solving. Dr. Albers will chair the executive committee.

Key Participating Investigators at Coordinating Site

Gregory W. Albers, MD Principal Investigator Stanford Stroke Center 780 Welch Rd. Suite 350 Palo Alto, CA 94305 650-723-4448 galbers@stanfordmed.org	Michael Marks, MD Co-Principal Investigator Stanford University Medical Center 300 Pasteur Dr. Stanford, CA 94305-5105 650-723-6767 mmarks@stanford.edu	Maarten Lansberg, MD, PhD Protocol Director Stanford Stroke Center 780 Welch Rd. Suite 350 Palo Alto, CA 94305 650-723-4448 lansberg@stanford.edu

6. INSTITUTIONAL REVIEW BOARD

The University of Cincinnati Institutional Review Board will serve as the National Central Institutional Review Board for all participating sites. The **Central Institutional Review Board (CIRB)** for multicenter protocols is the single IRB of record. It has regulatory responsibility for assuring the protection of the rights and welfare of research participants in accordance with Standard Operating Procedure ADM 12; Central Institutional Review Board Reporting. The National Institute of Neurological Disorders and Stroke (NINDS) selected the University of Cincinnati Institutional Review Board (IRB) to serve as the CIRB for the NIH StrokeNet (StrokeNet).

University of Cincinnati IRB Registration # 00000180 FWA #: 00003152 Expiration Date: 6/27/2016

Michael Linke, PhD, CIP
Chairman CIRB
Michael.linke@va.gov
513-304-3540

7. COSTS

All of the eligible devices that will be used in this study are currently on the market. There will be no charges beyond the typical standard of care for use of these approved devices. These devices will be used and billed according to the standard of care for each institution.

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APPENDIX

I. DEFUSE 3 Patient Informed Consent Form