Preconditioning

The Next Frontier of Neurotherapeutics

PreLIMBS II

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Editorial

Preconditioning the Brain

Moving on to the Next Frontier of Neurotherapeutics

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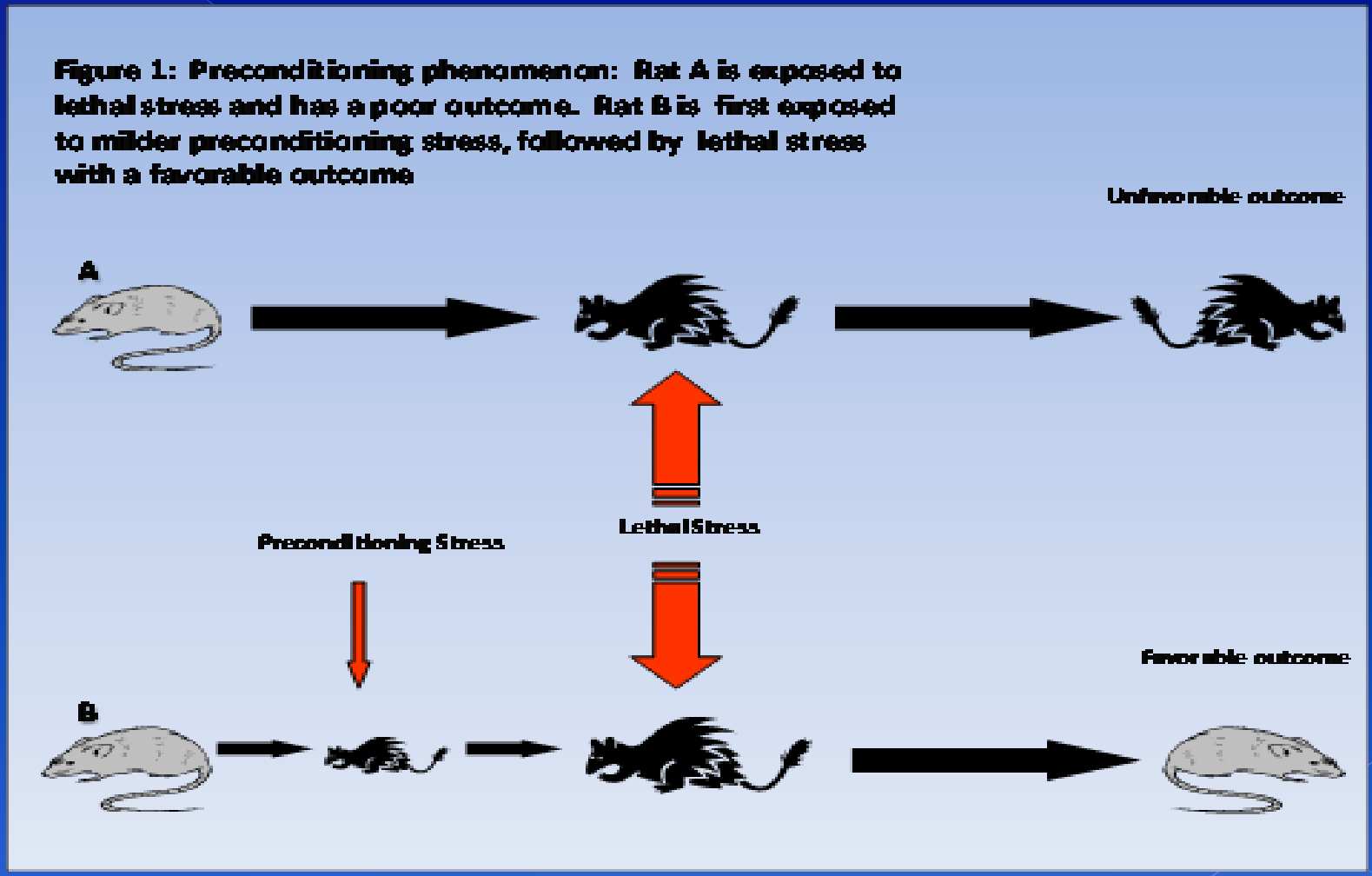
What is preconditioning?



Tolerance in Vascular Disease

Direct Preconditioning

- **Murry** (Circulation 1986)
  - Multiple temporary occlusions cause less tissue damage than one longer occlusion of same ischemia duration
  - "the whole is greater than the sum of its parts"
  - Occluded canine circumflex artery 40min
  - Half animals preconditioned with 4x 5min brief circumflex artery occlusion
  - Limited infarct size to 25% of control group

Limb Preconditioning

- **Birnbaum (Circulation 1997)**
  - 30min partial femoral artery occlusion protected myocardium in coronary artery occlusion model in rabbits.
- **Subsequently reproduced with tourniquet occlusion of the hind leg.**

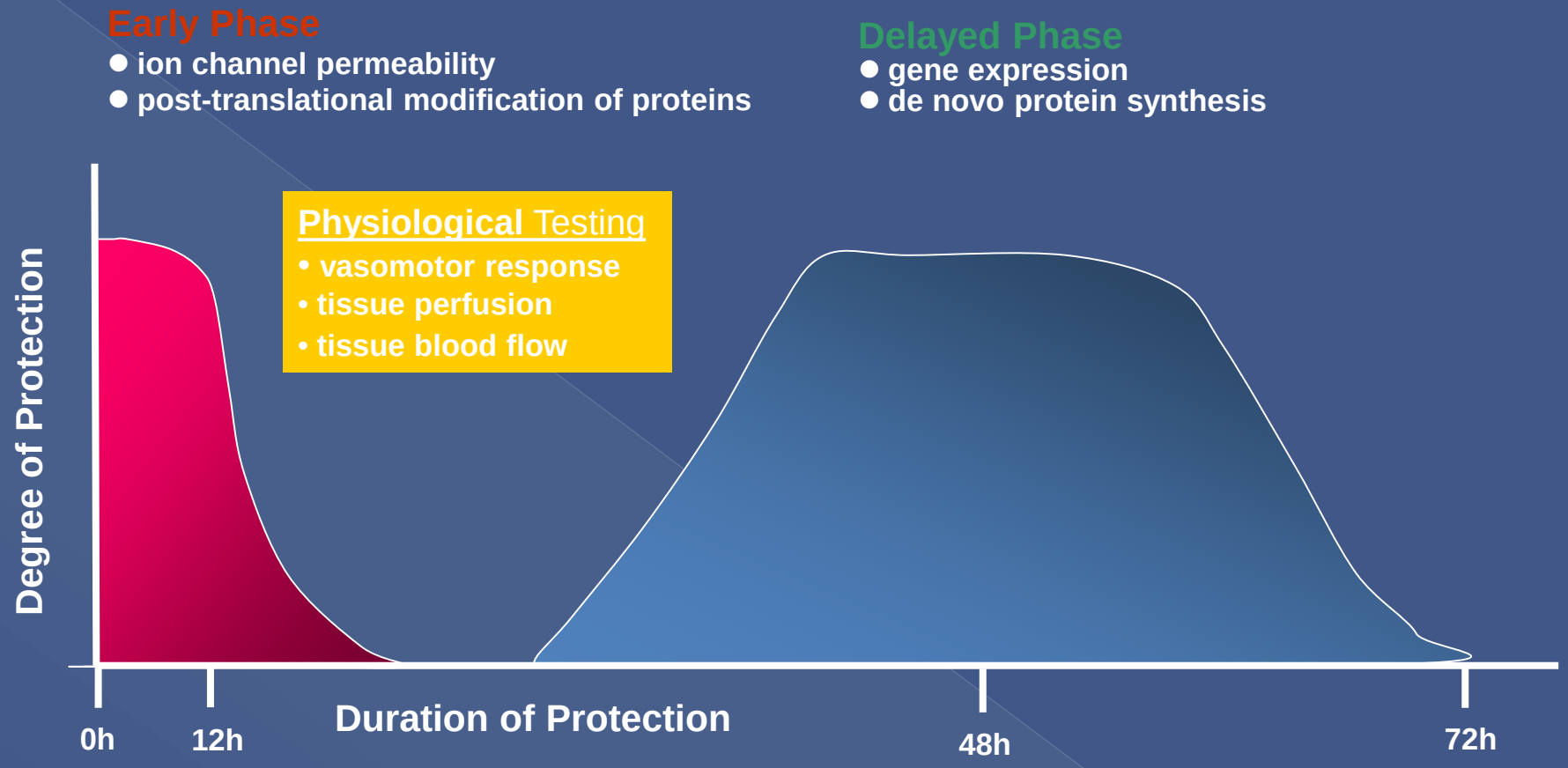
Limb Preconditioning Brain Protection
Preclinical Studies

Table 3: Preclinical studies of remote ischemic limb preconditioning in cerebral and spinal ischemia models.

Study	Stimulus	Animal	Model	Outcome	Randomized allocation	Control Group	Blinded assessments
Vlasov 2005[7]	30-min leg ischemia	Rat	Global ischemia	↑endothelial function ↓ cerebral edema	Not reported	Yes	Not reported
Jin 2006[8]	3 x 10-min leg ischemia	Rat	Global ischemia	↑pERK1/2 ↓ neuronal loss	Yes	Yes	Not reported
Dave 2006[9]	15 and 30-min leg ischemia	Rat	Global ischemia	↓ neuronal loss	Not reported	Not reported	Not reported
Gurcun 2006[10]	5-min renal ischemia	Rabbit	Spinal ischemia	↑function ↓NSE and MDA	Yes	Yes	Yes
Sun 2006[11]	3 x 10-min leg ischemia	Rat	Global ischemia	↓ neuronal loss ↑ p38 MAPK expression	Yes	Yes	Not reported
Rehni 2007[12]	15-min mesenteric artery occlusion	Mouse	Focal ischemia	↑function ↓ infarct size	Not reported	Not reported	Not reported
Zhao 2007[13]	3 x 10-min leg ischemia	Rat	Global ischemia	↑ serum, hippocampal NO and NOS expression	Yes	Yes	Not reported
Ren 2008[14]	5 and 15-min cycles of leg ischemia	Rat	Focal ischemia	↓ infarct size	Yes	Yes	Not reported
Malhotra 2011[15]	3x 10-min infra-renal aortic occlusion	Rat	Focal ischemia	↑function ↓ infarct size	Not reported	Yes	Behavioral outcomes were blinded Not reported for histological outcomes
Hahn 2011[16]	4 x 10-min leg ischemia (tourniquet)	Rat	Focal Ischemia	↑function ↓ infarct size	Yes	Yes	Not reported
Jensen 2011[17]	4x 5 min leg ischemia (Blood pressure cuff)	Pig	Neuro-circulatory arrest model	↑function ↓ neuronal loss	Yes	Yes	Yes
Xu 2011[18]	3x 10-min femoral artery occlusion	Rat	Bilateral carotid occlusion	Neurocognitive ↑ Bcl-2 levels	Yes	Yes	Not reported
Hu 2012[19]	3x 5-min leg ischemia (tourniquet)	Rat	Focal Ischemia	↑function ↓ infarct size ↓ DWI size	Yes	Yes	Blinded functional scores Not reported for histological outcomes
Wei 2012[20]	3x 15-min femoral occlusion	Rat	Focal Ischemia	↑function ↓ infarct size ↓ inflammatory and oxidative markers	Yes	Yes	Blinded functional scores Not reported for histological outcomes
Hu 2013[21]	3x 5min leg ischemia (tourniquet)	Rat	Focal ischemia	Improved cognition and histological protection	Yes	Yes	Blinded cognitive and histological outcome

pERK= extracellular signal-regulated kinases; NO=nitrous oxide; NOS=NOsynthase; MAPK= mitogen-activated protein kinase; NSE= neuron specific enolase;
MDA=malondialdehyde

Figure 1:



Ionic channel function

Post translational protein modification

Autocoid secretion (adenosine, bradykinin, nitric oxide, adrenergics)

Enchanced fibrinolysis (tPA, tPa inhibitor, platelet aggregates, D-dimer)

Modulated inflammatory response (interleukins, tumor necrosis factor alpha, superoxide dismutase, c-reactive protein, leukocyte activation)

Improved endothelial function (endothelial progenitor cells, von Willebrand factor, nitrous oxide system, bradykinin)

Reduction of oxidative stress (glutathione, malondialdehyde)

Gene expression (hypoxia inducible factor, caspases, heat shock proteins, toll-like receptor 4)

Translational Challenges

- Young animals, free of disease, free of medications.
- Older patients, with comorbidities and on medications.

Completed Studies in Brain Conditioning

- **Walsh 2010**
  - 70 patients 2 x 10min leg preconditioning cycles
  - Just prior to CEA
  - Saccadic Latency as neurological outcome
  - Deterioration noted in 32% vs. 53% in favor of conditioning group.
- **Koch 2011, Gonzalez 2013**
  - Subarachnoid hemorrhage
- **Meng 2012 and 2014**
  - Symptomatic Intracranial disease
  - Octogenarians

Completed Studies in Brain Conditioning

- Hougaard 2013
  - Stroke patients who received IV tPA
  - No evidence of effect on final infarct volume
  - But reduced the amount of tissue at risk of infarction.

Challenges for Clinical Preconditioning

- What are the optimal clinical settings for preconditioning?
- What method of preconditioning?
  - Direct preconditioning impractical.
  - Limb preconditioning.
  - Pharmacological preconditioning?

Comments and Opinions

Preconditioning the Human Brain

Proving the Principle in Subarachnoid Hemorrhage

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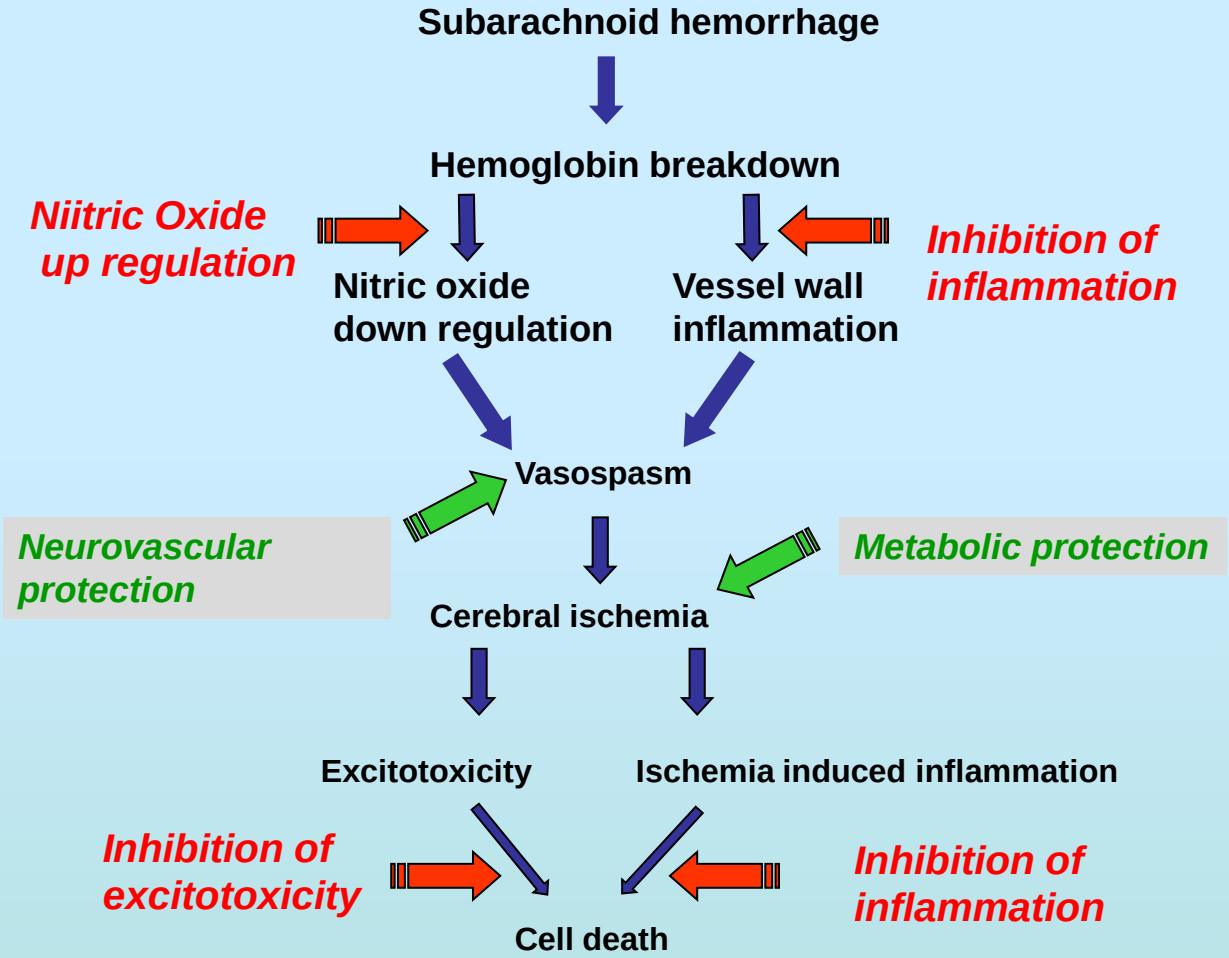
Pathophysiology of DCI and Protection conferred by Preconditioning

Figure 2: Illustration of multiple targets of protection by preconditioning in subarachnoid hemorrhage



Preconditioning with Limb Ischemia for Subarachnoid hemorrhages

PreLIMBS

Miami Experience
2008-present

Preconditioning in SAH

- Dose escalation
  - 5, 7.5 and10 min conditioning cycles
  - Cohorts of 6 subjects

Results

- Enrolled 34 subjects

Baseline Characteristics	
Age	53±12
Male	49%
Hypertension	42%
Diabetes	3%
Hispanic	58%
African Descent	33%
White	9%
Hunt Hess	2.4±0.9
Aneurysm coiled	78%

Preconditioning in SAH

- On average 7.7±2.4 conditioning sessions
- Mean VAS 3.6±3.4
- 3 DVTs in 5 min group
  - One in the leg of a patient with arm preconditioning
  - One leg DVT with contralateral leg being preconditioned
  - One leg DVT (asymptomatic) with ipsilateral leg preconditioning

Conclusions

- Safe and feasible
- Could do it repetitively in conscious patients
- 10 min ischemia times were tolerated

Phase II

- More safety data
- Included control group with placebo [sham] preconditioning.

UCLA Experience
Nestor Gonzalez

- Looked at preconditioned muscle to see if ischemia is being induced
- Assessed acute effects of preconditioning on cerebral blood flow [MOCAIP] with TCD and found vasodilatory effect
- Investigated cerebral microdialysis data and found reduction in lactate and pyruvate and preservation of glycerol
- Most recently genetic expression

PreLIMBS 2

- **Specific Aim 1:**
  - Investigate the effect of 4 different preconditioning strategies on cerebral vasoreactivity in subjects with subarachnoid hemorrhage
  - 4x 5min daily
  - 4x 5 min every other day
  - 3x 10min daily
  - 3x 10min every other day
- **Specific Aim 2:**
  - Assess clinical outcomes DCI, 90 day mRS and cognitive assessment (MOCA)

PreLIMBS 2

- **Specific Aim 3:**
  - Serum biomarkers baseline, day 1, 6, 10
  - Genetic expression
  - Cerebral metabolism- microdialysis in a subset

Inclusion Criteria

- **Subarachnoid hemorrhage due to cerebral aneurysm secured by either coiling or surgical clipping. Subarachnoid hemorrhage is defined as the sudden onset of a headache accompanied by neuroimaging (brain CT, MRI) or lumbar puncture evidence of subarachnoid blood and demonstration of cerebral aneurysm, felt to have caused the bleeding, by CT angiogram, MR angiogram or conventional catheter angiography.**

- **Ability to start treatment within 4 days from symptom onset (rationale: the high risk period for DCI typically starts at day 4. Starting the intervention before day 4 would optimize preconditioning for ischemia prior to its onset).**

- **Hunt Hess scale of 2,3 or 4. (rationale: this will allow enrichment of study cohort for subjects at higher risk of developing DCI)**

- **Minimum age of enrollment: 18 years.**

Exclusion Criteria

- **Inability to start limb preconditioning within 4 days from subarachnoid hemorrhage.**
- **Unsecured cerebral aneurysm.**
- **Subarachnoid hemorrhage not related to cerebral aneurysm such as: trauma, coagulopathy, arteriovenous malformation, any other non-aneurysmal bleeding (rationale: the inclusion of only aneurysmal subarachnoid hemorrhages ensures a homogenous study population).**
- **Impaired historical functional status defined as mRS>2.**
- **Hunt Hess scale > 4 (rationale: patients with scores > 4 have severe disability and very poor prognosis) or < 2 (rationale: this will allow enrichment of the cohort for subjects at higher risk of DCI).**
- **Patients with DCI at the time of enrollment (rationale: the intention is to treat subject before the onset of ischemia).**
- **Lower extremity soft tissue, orthopedic or vascular injury which in the judgment of the investigator would preclude leg conditioning (e.g. superficial wounds, venous, arterial ulcers, gangrene).**
- **History of peripheral vascular disease.**
- **Ankle:brachial index (ABI)<0.9 (rationale: to exclude patients with asymptomatic peripheral vascular disease).**
- **Hemiplegic leg (absence of antigravity effort) with contralateral leg having been used for angiographic access (rationale: leg paralysis promotes venous stasis and angiographic vascular access may be prone to leakage or pseudoaneurysm formation from back pressure induced during blood pressure cuff inflation).**
- **History of lower extremity deep vein thrombosis.**
- **Hypotension (systolic blood pressure ≤90 mm Hg) refractory to fluid therapy.**
- **Neurogenic pulmonary edema or cardiac failure requiring inotropic support.**
- **Pregnancy, breastfeeding or positive pregnancy test. (Women of childbearing potential must have a negative pregnancy test prior enrollment).**
- **Severe or unstable concomitant condition, disease or chronic condition, which, in the opinion of the investigator, could affect assessment of the safety or efficacy of study intervention.**
- **Participation in a concurrent clinical therapeutic trial.**
- **Refractory and uncontrolled intracranial pressure.**
- **Patient unlikely, in the investigator's opinion, to complete the study and return for follow-up visits (e.g. active drug or alcohol dependence, out of country residency).**
- **Inability to obtain informed consent from subject or health care proxy.**

Study Overview

- Acute SAH admission
- Treatment of aneurysm
- Screening, consent, randomization
- Baseline evaluations
  - Vasomotor reactivity
  - Blood draw serum and genetic markers
- Preconditioning daily or alternate days
- Blood draws day 1, 6 and 10
- Repeat vasoreactivity day 10-12
- 90 day: DCI, mRS and MOCA

Preconditioning Protocol

- Apply blood pressure cuff over thigh
- Inflate to 30mmHG over systolic BP
- Leave inflated for 5-10 min
- Confirm ischemia by loss of Doppler signal
- Reperfuse for 5 min
- 3 or 4 cycles
- Confirm reperfusion by reconstitution of Doppler signal

Vasoreactivity

- Transcranial Doppler- CO_2 based at baseline and Day 10-12
- Monitor bilateral MCA flow
- Inhalation of 5% CO_2 for 2 minutes
- Measure increase in flow velocities
- Measure end tidal CO_2 during procedure
- Percent increase in flow velocities per end tidal CO_2 unit partial pressure change

Vasomotor Reactivity

- 7.7± 2.2 % / CO_2 mmHg based on 20 subjects with SAH and 57 TCDs

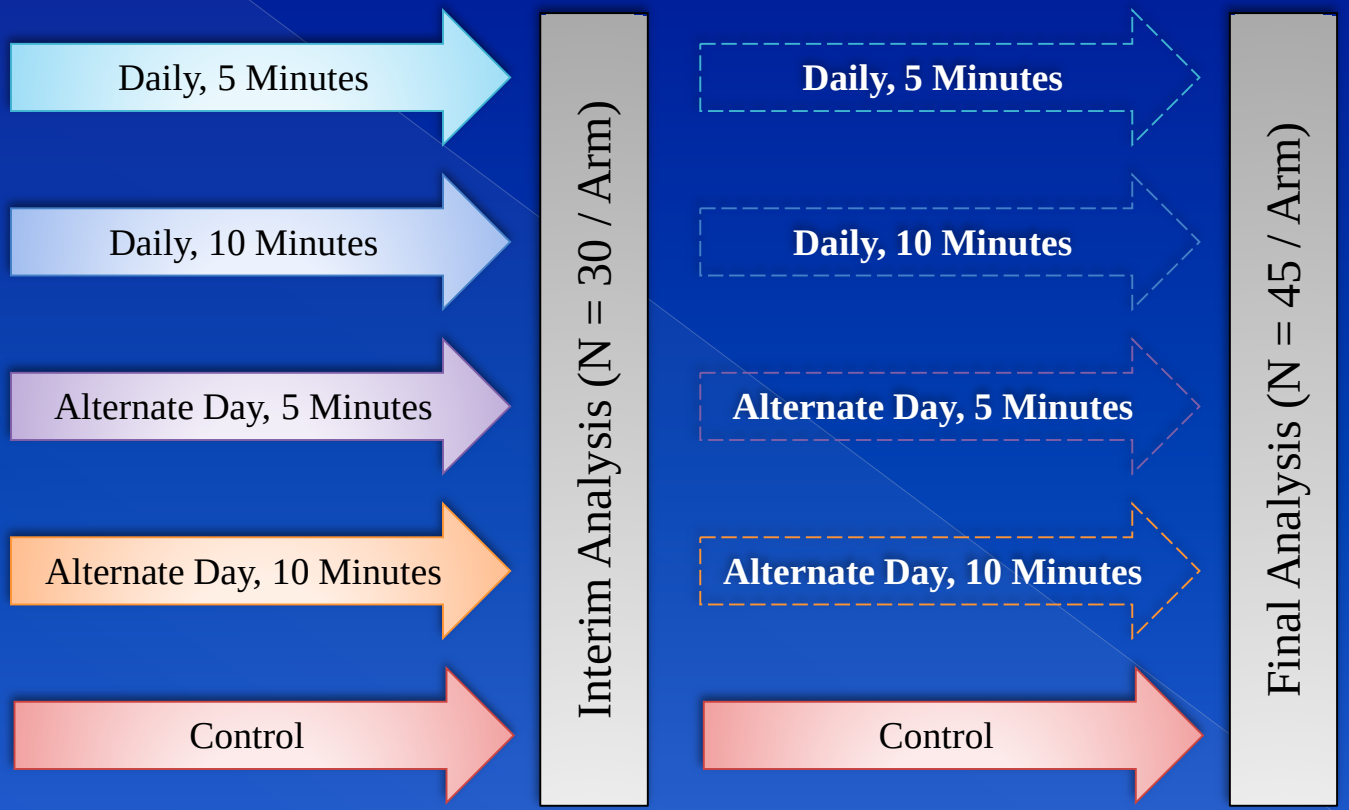
  (Frontera 2006)

- Why TCD VMR?
  - Biological rationale
  - Impaired VMR correlates with DCI
  - Several preconditioning studies have assessed effects of brachial vasoreactivity

    (Loukogeorgakis 2005, Kimura 2007)
  - Improvements in coronary blood flow

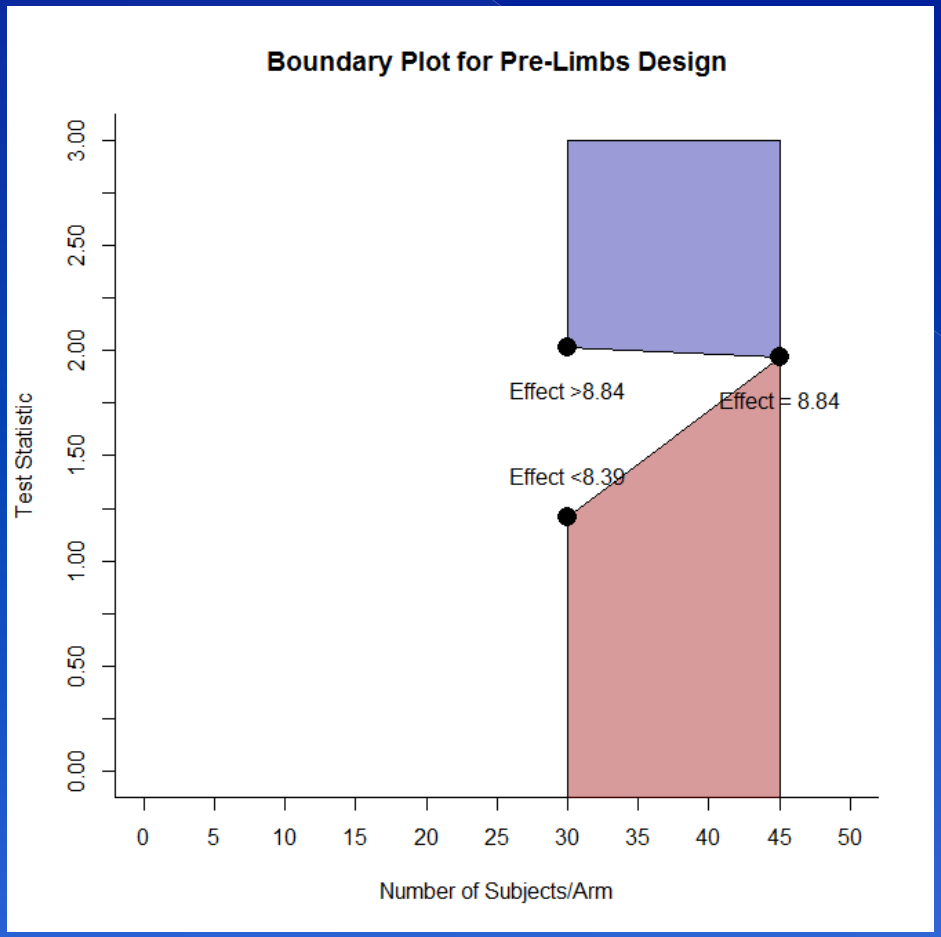
    (Zhou 2007)

Multi-Arm Multi-Stage Trial Design (Max Sample N=225)



- Enrollment is terminated to any arm that crosses the futility boundary
- Trial is terminated once an arm crosses efficacy boundary
- Control group continues until trial is stopped
- Futility boundaries are currently specified as binding

Decision Boundaries



- Interim Analysis
  - N=30 subjects per arm
  - If mean treatment effect for arm is < 8.39, arm is terminated for futility
  - If mean treatment effect for any arm is > 8.84, trial is stopped for efficacy
- Final Analysis
  - If treatment effect is >8.84, arm is successful
  - If treatment effect is <8.84, arm is futile
  - What decision do we make if more than one arm is efficacious at end of trial?
- Statistical properties are NOT guaranteed if decisions are not followed (i.e. failing to stop a futile decision)

Power Analysis

Scenario	Simulated Effect Size					Probability of Stopping after Stage I		Probability of Rejecting the Null Hypotheses (H_0)			
	μ_c	μ_1	μ_2	μ_3	μ_4	Futility	Efficacy	$\mu_1 - \mu_c$	$\mu_2 - \mu_c$	$\mu_3 - \mu_c$	$\mu_4 - \mu_c$
1	7.70	7.70	7.70	7.70	7.70	71.7	7.8	2.2	2.9	2.6	2.9
2	7.70	8.49	8.49	8.49	8.49	14.9	56.7	28.8	26.1	28.4	27.7
3	7.70	8.49	9.33	8.49	9.33	1.3	90.8	26.8	79.2	28.4	79.9
4	7.70	8.11	8.52	8.92	9.33	2.7	86.2	10.4	28.1	56.4	80.2
5	7.70	9.33	9.33	9.33	9.33	0.5	96.7	80.2	78.4	78.9	81.7
6	7.70	7.70	7.70	7.70	9.33	5.0	78.5	2.0	2.9	2.7	80.1
7	7.70	8.49	8.49	8.49	9.33	3.0	84.0	27.4	26.1	27.2	81.7