



# **Multicentre Preclinical Animal Research Team**

Multi-PART

**Work Package 3 – Experimental Design**

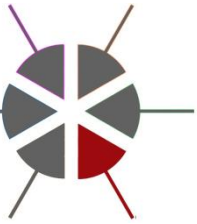
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# WP 3 – Beta-Test Design

## Background

- The primary aim is to assess whether multi-centre preclinical trials of a test compound for stroke are feasible.
- Secondary aims are
  - to determine whether glyceryl trinitrate (GTN) administered at a clinically relevant dose and time reduces infarct volume and/or improves neurological score assessed at 48 h after middle cerebral artery occlusion (MCAO) in the mouse;
  - to assess within-laboratory and between-laboratory variation of effect sizes in view of refining future experimental designs.



# WP 3 – Beta-Test Design

## Experimental questions

- Does glyceryl trinitrate (GTN, a nitrous oxide donor)
  - affect infarct volume (TTC staining)?
  - improve neurological score?
  - affect grip strength?



# WP 3 – Beta-Test Design

## Animal stroke models

- Transient **intraluminal filament** MCAO in mice
- Permanent distal (**diathermy**) MCAO in mice



# WP 3 – Beta-Test Design

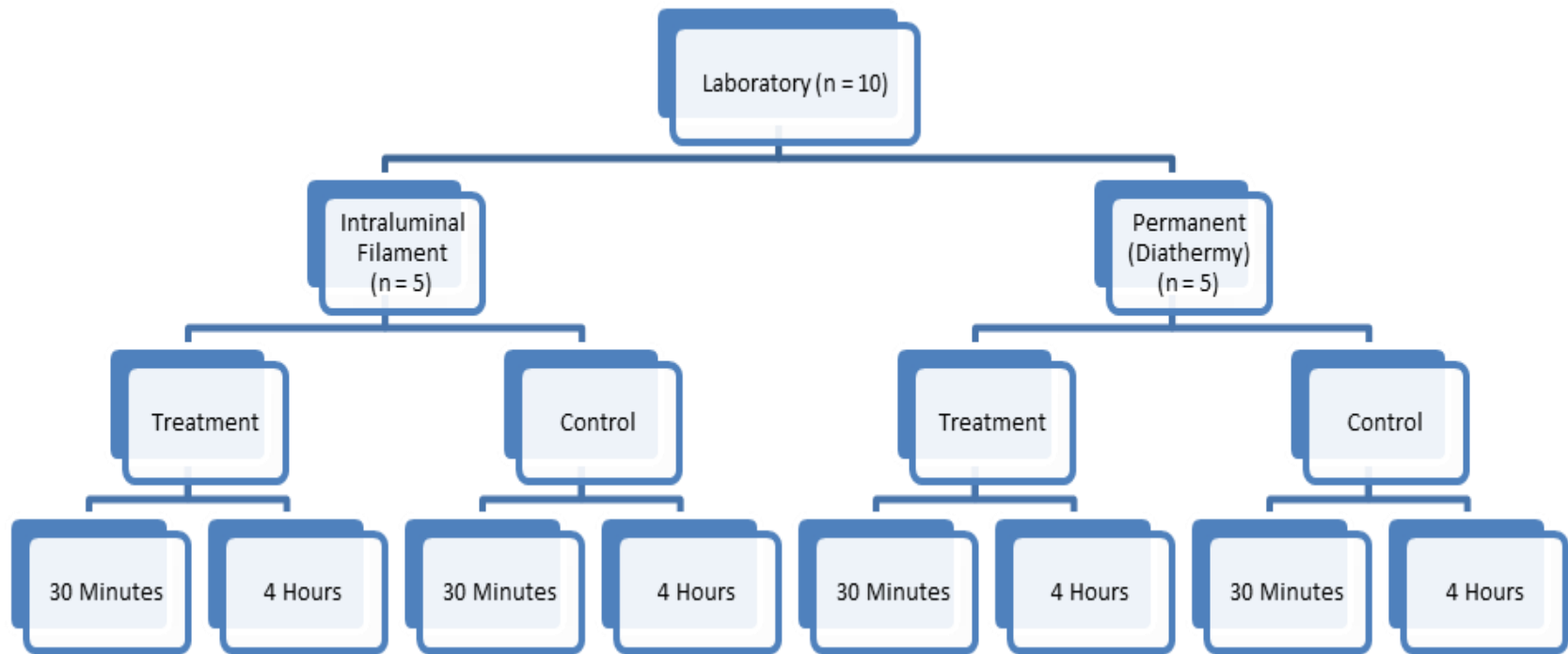
## Selection of labs

Centre	No.	Strain	Male	Intraluminal filament	Diathermy
Nottingham	32	C57BL/6	1	1	
Manchester	32	C57BL/6	1	1	
VHIR	48	C57BL/6 or Balb/C	1		1
CAEN	48	C57BL/6 or Swiss	1	1	1
Edinburgh	48	C57BL/6J	1	1	1
Melbourne	30	C57BL/6	1	1	
Berlin	32	C57BL/6	1	1	
Utrecht	-	-	-	-	-
Glasgow	32	C57BL/6	1	1	1
CSIC	32	Balb/C	1		1
Cho lab	48	C57BL/6	1	1	
Cipolla lab		rats?			
<b><u>TOTAL</u></b>	<b><u>382</u></b>		<b><u>10</u></b>	<b><u>8</u></b>	<b><u>5</u></b>



# WP 3 – Beta-Test Design

## Hierarchical structure (Primary model)





# WP 3 – Beta-Test Design

## Primary model - Factors

- **Outcome Variables (n =3)**
  - Neurological score
  - Grip strength for distal model
  - Infarct volume (TTC staining)
- **Predictors (n =2)**
  - Effect of Treatment (2 levels: Treatment, Control)
  - Time Points (2 levels: 30 minutes, 4 hours)
- **Control/Heterogenization Factors**
  - Laboratory nested within model of stroke (n =5)

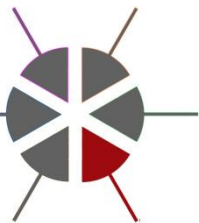


# WP 3 – Beta-Test Design

## Effect size

- Reference values for effect sizes Cohen's  $f$ :  $\frac{\sigma_{between}}{\sigma_{within}}$ 
  - Very small effect = 0.1
  - Small effect = 0.2
  - Medium effect = 0.35
  - Large effect = 0.50
  - Very large effect = 0.7





# WP 3 – Beta-Test Design

Sample size calculation ( $n_{\max} = 320, 32/\text{lab}$ )

Effect Size (Cohen's f)		$\alpha$	Power (1- $\beta$ )	n (Total)	n (Per Lab)
Very Small	0.10	0.05	0.80	1094	109
			0.85	1234	123
			0.90	1421	142
			0.95	1721	172
Small	0.20	0.05	0.80	277	28
			0.85	311	31
			0.90	358	36
			0.95	433	43
Medium	0.35	0.05	0.80	93	9
			0.85	104	10
			0.90	120	12
			0.95	144	14
Large	0.50	0.05	0.80	48	5
			0.85	53	5
			0.90	61	6
			0.95	73	7
Very Large	0.70	0.05	0.80	27	3
			0.85	29	3
			0.90	33	3
			0.95	39	4



# WP 3 – Beta-Test Design

Sample size calculation ( $n_{\max} = 320, 32/\text{lab}$ )

Effect Size (Cohen's f)	$\alpha$	Power (1- $\beta$ )	n (Total)	n (Per Lab)	
Very Small	0.10	0.01	0.80	1551	155
			0.85	1714	171
			0.90	1930	193
			0.95	2273	227
Small	0.20	0.01	0.80	392	39
			0.85	433	43
			0.90	487	49
			0.95	573	57
Medium	0.35	0.01	0.80	132	13
			0.85	145	15
			0.90	163	16
			0.95	191	19
Large	0.50	0.01	0.80	68	7
			0.85	74	7
			0.90	83	8
			0.95	96	10
Very Large	0.70	0.01	0.80	37	4
			0.85	41	4
			0.90	45	5
			0.95	52	5



# WP 3 – Beta-Test Design

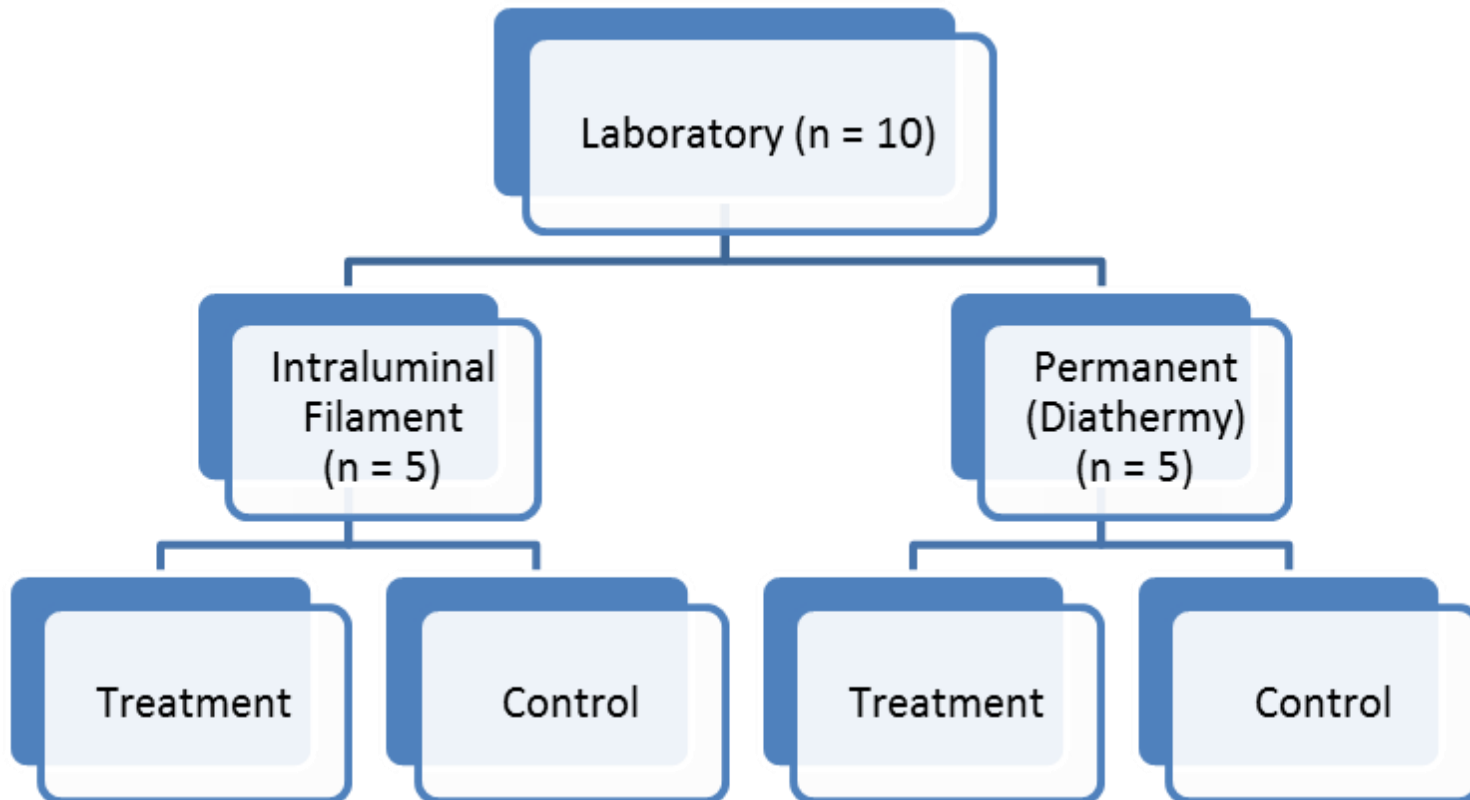
Sample size calculation ( $n_{\max} = 320, 32/\text{lab}$ )

Effect Size (Cohen's f)		$\alpha$	Power (1- $\beta$ )	n (Total)	n (Per Lab)
Very Small	0.10	0.001	0.80	2163	216
			0.85	2352	235
			0.90	2602	260
			0.95	2994	299
Small	0.20	0.001	0.80	547	55
			0.85	594	59
			0.90	657	66
			0.95	755	76
Medium	0.35	0.001	0.80	184	18
			0.85	200	20
			0.90	220	22
			0.95	252	25
Large	0.50	0.001	0.80	94	9
			0.85	102	10
			0.90	112	11
			0.95	128	13
Very Large	0.70	0.001	0.80	52	5
			0.85	56	6
			0.90	61	6
			0.95	89	9



# WP 3 – Beta-Test Design

## Hierarchical structure (Alternate model)





# WP 3 – Beta-Test Design

## Alternate model - Factors

- **Outcome Variables (n =3)**
  - Neurological score
  - Grip strength for distal model
  - Infarct volume (TTC staining)
- **Predictors (n =2)**
  - Treatment
  - Control
- **Control/Heterogenization Factors**
  - Laboratory nested within model of stroke (n =5)



# WP 3 – Beta-Test Design

## Effect size

- Reference values for effect sizes Cohen's  $d$ :  $\frac{X_1 - X_2}{SD}$ 
  - Very small effect = 0.25
  - Small effect = 0.5
  - Medium effect = 0.75
  - Large effect = 1.0
  - Very large effect = 1.5



# WP 3 – Beta-Test Design

Sample size calculation ( $n_{\max} = 320, 32/\text{lab}$ )

Effect Size (Cohen's d)		$\alpha$	Power (1- $\beta$ )	n (Total)	n (Per Lab)
Very Small	0.25	0.05	0.80	504	50
			0.85	577	58
			0.90	674	67
			0.95	834	83
Small	0.50	0.05	0.80	128	13
			0.85	146	15
			0.90	170	17
			0.95	210	21
Medium	0.75	0.05	0.80	58	6
			0.85	66	7
			0.90	77	8
			0.95	94	9
Large	1.00	0.05	0.80	33	3
			0.85	38	4
			0.90	44	4
			0.95	54	5
Very Large	1.50	0.05	0.80	16	2
			0.85	18	2
			0.90	21	2
			0.95	25	3

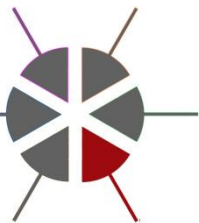


# WP 3 – Beta-Test Design

Sample size calculation ( $n_{\max} = 320, 32/\text{lab}$ )

Effect Size (Cohen's d)		$\alpha$	Power (1- $\beta$ )	n (Total)	n (Per Lab)
Very Small	0.25	0.01	0.80	751	75
			0.85	838	84
			0.90	956	96
			0.95	1143	114
Small	0.50	0.01	0.80	190	19
			0.85	212	21
			0.90	241	24
			0.95	288	29
Medium	0.75	0.01	0.80	86	9
			0.85	96	10
			0.90	109	11
			0.95	130	13
Large	1.00	0.01	0.80	50	5
			0.85	56	6
			0.90	63	6
			0.95	75	8
Very Large	1.50	0.01	0.80	24	2
			0.85	27	3
			0.90	30	3
			0.95	35	4

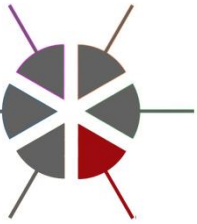




# WP 3 – Beta-Test Design

Sample size calculation ( $n_{\max} = 320, 32/\text{lab}$ )

Effect Size (Cohen's d)		$\alpha$	Power (1- $\beta$ )	n (Total)	n (Per Lab)
Very Small	0.25	0.001	0.80	1098	110
			0.85	1204	120
			0.90	1343	134
			0.95	1584	158
Small	0.50	0.001	0.80	279	28
			0.85	305	31
			0.90	340	34
			0.95	395	40
Medium	0.75	0.001	0.80	127	13
			0.85	139	14
			0.90	154	15
			0.95	179	18
Large	1.00	0.001	0.80	74	7
			0.85	80	8
			0.90	89	9
			0.95	103	10
Very Large	1.50	0.001	0.80	36	4
			0.85	39	4
			0.90	43	4
			0.95	49	5



# WP 3 – Beta-Test Design

## Randomisation

- **Factors to consider** (within labs)
  - treatment group
  - time of treatment
  - cage



# WP 3 – Beta-Test Design

## Levels of blinding

- **Allocation concealment**
- **Blinding experimenters**  
(care takers, surgeons, experimenters)
- **Blinding data collectors**  
(technicians, experimenters)
- **Blinding outcome assessors**  
(experimenters, external assessors)
- **Blinding data analysts**  
(experimenters, external analysts)



# WP 3 – Experimental Design

## Deliverables

- **Month 18:** Preliminary solutions for sample size calculation and systematic variation for further discussion and refinement
- **Month 24:** Final report describing the most appropriate design of future preclinical trials (randomisation, blinding, sample size calculations, systematic variation)