

MOST Ancillary Study 10-02 (AS10-02)
“Nerve Growth Factor Serum Levels and Pain and Radiographic Severity of Knee Osteoarthritis” (Barton Wise)

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1. Dataset description and Analyst Notes

Dataset: AS1002_bioassay.sas7bdat

Observations: 337 records (337 participants, 1 assay; 2 visits)

Documentation:

- VariableGuide_AS1002_bioassay.pdf
- Distributions_AS1002_bioassay.pdf

The Nerve Growth Factor Analysis Dataset (AS1002_bioassay.SAS7BDAT) includes assay data from the two samples (one sample from the baseline visit and one sample from the 30-month visit), of fasting blood serum from 337 participants. Analyses were performed at the UC Davis laboratory on baseline and 30-month serum paired samples.

Note: the laboratory performing the assays was blinded to subject ID but provided with paired link to ensure that both samples collected at baseline and 30-month time points from the same participant were included in the same batch.

Commercially available NGF ELISA (NGF-Emax-ImmunoAssay) kits were used to measure the NGF levels on baseline and 30 month serum samples at the UC Davis Center for Healthy Aging.

Analyst Notes:

- Selection indicator variables are included in the analytical dataset (variables #5 Selection1 and #6 Selection2). See selection criteria for each case/control sub-study below.
If participant met selection criteria for more than one sub-study as a case or as a control, the paired blood samples from baseline and 30-month serum were tested only once. There is one record per participant in the analytical dataset.
- The NGF assay was performed from the same blood sample in two trials. Variables V0NGF1 and V0NGF2 represent results from the baseline serum for trial 1 and trial 2; variables V2NGF1 and V2NGF2 represent results from the 30m serum for trial 1 and trial 2 accordingly.
- When assay results were not obtained due to below low detection level, laboratory used value=0 for results. According to the protocol, if V0NGF1=0 result from the first trial was below detection level, the second trial was not performed and result for the second trial marked as dot or missing (V0NGF2=.).

	All	BL NGF pg/mL first trial		BL NGF pg/mL second trial			30M NGF pg/mL first trial		30M NGF pg/mL second trial		
		zero	value>0	missing	zero	value>0	zero	value>0	missing	zero	value>0
Female	203	87	116	87	7	109	97	106	95	6	102
Male	134	45	89	45	4	85	53	81	53	4	77
All	337	132	205	132	11	194	150	187	148	10	179

- If there was insufficient volume or some other reason assay could not be performed, and all assay values were missing, the record is not included in the analytical dataset.

2. Selection plan

Table 1. Final selection and overlapping between two sub-studies.

Baseline participants n=3026					
Selection 1		Selection 2 bilat case	Selection 2 bilat control	Selection 2 unilat case	Selection 2 unilat control
FKP and KL 2,3,4 and bilateral	N=63	4	26	9	0
No FKP and KL 2,3,4 bilateral	N=68	6	46	10	0
Subtotal ppts	N=131	10	72	19	0
Selection 2	Not included in Selection 1	Already included in Selection 1		Selection 2 (final)	
KL2,3 bilateral and bilateral progression	N=8	10		18	
KL 2,3 bilateral and no progression	N=22	72		94	
KL 2,3 unilateral and unilateral progression	N=64	19		83	
KL 2,3 unilateral and no progression	N=115	0		115	
Subtotal ppts	N=209	N=101		N=310	
Total ppts	N=340*				

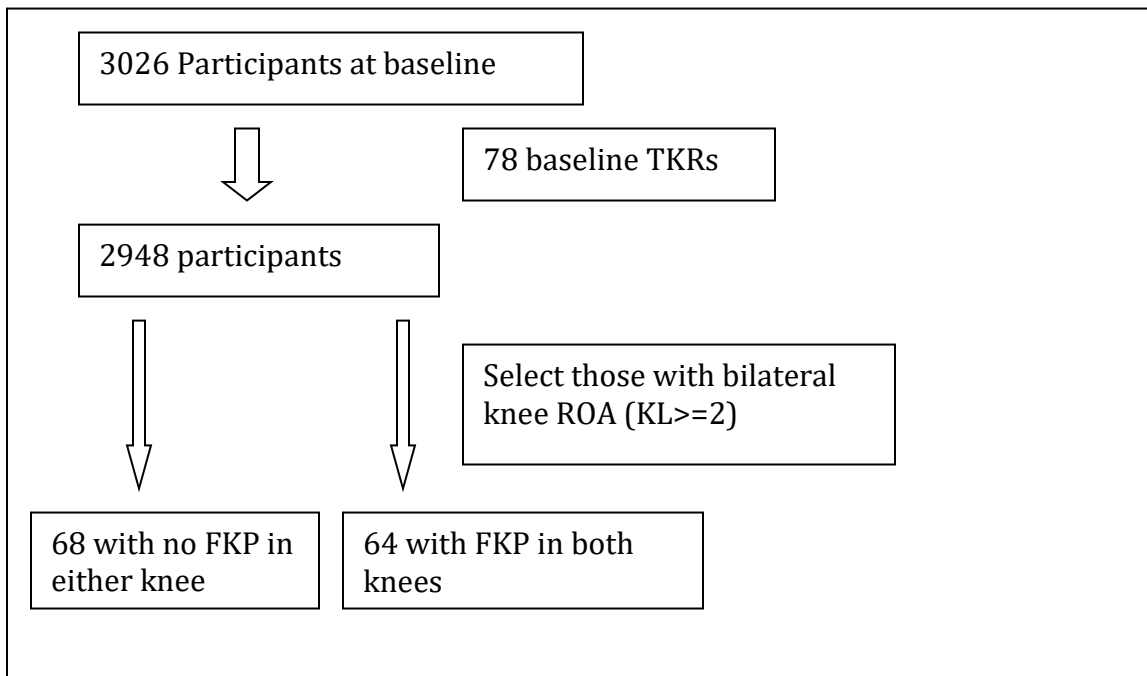
* Total 340 participants/ 680 serum cryovials (baseline and 30-month pairs)

3. Excerpt from Ancillary Study Proposal (1/20/11 B. Wise):

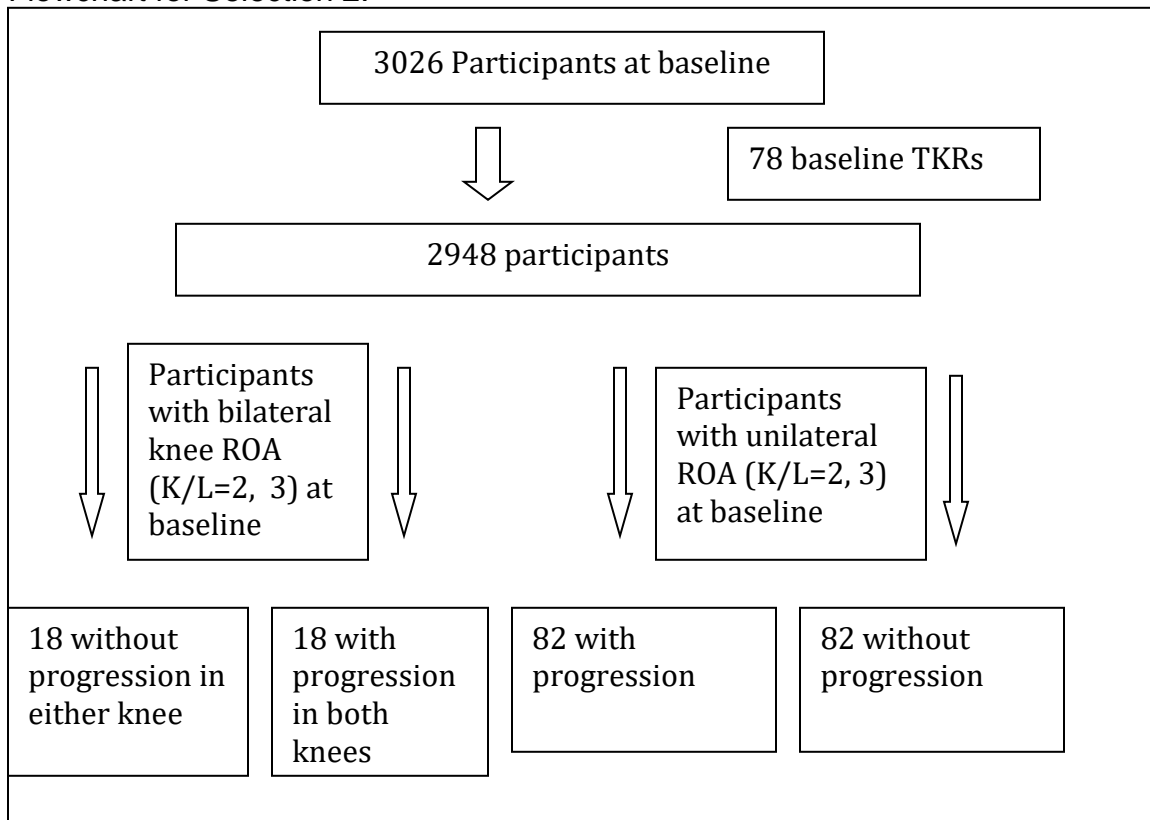
Our sample will total approximately 332, and will be selected according to specific hypotheses we are going to test:

1. 64 participants with frequent knee pain by FKP question (cases); and 68 participants without frequent knee pain by FKP question (controls). Both cases and controls have bilateral radiographic knee OA.
2. 82 participants with either unilateral or bilateral radiographic knee ROA (i.e., K/L of 2 or 3) at baseline who had one knee ROA progressed over 30 months; 82 participants with either unilateral or bilateral knee ROA (i.e., K/L of 2 or 3) at baseline who had neither knee ROA progressed over 30 months; 18 participants with bilateral ROA at baseline and who had both knees progress over 30 months; 18 participants with bilateral knee ROA at baseline who had neither knee progress over 30 months. Thus, there are 100 participants who had experienced OA progression in at least one knee (cases) and 100 participants who had no ROA progression (controls).

Flowchart for Selection 1:



Flowchart for Selection 2:



4. Appendix A. Reliability analysis results done in the laboratory.

In addition to testing the 2 trials, laboratory selected a random set of vials (one random set per visit) and re-ran the NGF assay using the same kit and protocol. When the first trial result was below detection level (value equal zero), the second trial was not performed and therefore the reliability analysis for the second trial has a lower number of observations.

The proposed NGF value for analysis – average from 2 trials – was a basis to create a calculated categorical variable:

- 0 = if the first trial value was zero;
- 1 = if average of two trials was from 0.1 to 100;
- 2 = if average of two trials was above 100.

Table 1. Weighted Kappa and 95% confidence interval on NGF assay analyzed as 3 levels (3 levels category: 0, <=100, >100), original run and rerun by visit (V0:baseline, V2:30-month)

Random sample/trials	N of levels in analysis	N of participants	Weighted kappa (95% CI)
V0 NGF average of two trials	3 levels	38	0.5476(0.2855,0.8098)
V0 NGF 1st trial	3 levels	38	0.5156(0.2567,0.7744)
V0 NGF 2nd trial	3 levels	35	0.4982(0.2271,0.7693)
V2 NGF average of two trials	3 levels	18	0.4706(-0.1532,1.0000)
V2 NGF 1st trial	3 levels	18	0.4706(-0.1532,1.0000)
V2 NGF 2nd trial	3 levels	17	0.7848(0.3790,1.0000)