MOST Ancillary Study 14-03 (AS14-03)
"The predictive values of novel plasma metabolic markers for early knee osteoarthritis changes" (G. Zhai)

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1. Dataset description and Analyst Notes

Dataset: AS1403_bioassay.sas7bdat
Observations: 1246 records (1246 participants, 6 selection groups, 157 assays; 1 visit)
Documentation:

- VariableGuide_ AS1403_bioassay.pdf
- Distributions_ AS1403_bioassay.pdf

AS1403_bioassay dataset contains 1246 records (one record per participant) with assays results performed on baseline plasma samples at the Genetics Research Centre in St. John's, Newfoundland, Canada.

Note: the study laboratory was blinded to subject ID and any clinical characteristic.

The Multicenter Osteoarthritis Study (MOST) collected fasting blood from all study participants at the baseline study visit. An ancillary study, The predictive values of novel plasma metabolic markers for early knee osteoarthritis changes (AS14-03), under the direction of Dr. G. Fhai, was funded to investigate the relation of serum metabolic analytes content and incidence of radiographic knee OA at 30m/60m clinic visit; incidence of Sx knee OA at 30m/60m clinic visit; TF ROA progression and Early OA changes based on MRI. There are 6 selection groups in this study.

Analyst Notes:

- If results were missing for all time points due to insufficient volume or some other reason assay could not be performed, the record was not included in the analytical dataset.
- Variables #5 to #10 are indicator for the selected groups described below in details (see Selection details below).
- QA analysis by batch was performed by the laboratory and results were used in publication (not provided to Coordinating Center).

2. Reference.

Zhai G, Sun X, Randel E, Liu M, Wang N, Tolstykh I, Rahman P, Torner J, Lewis CE, Nevitt MC, Guermazi A, Roemer F, Felson DT. Phenylalanine is a novel marker for radiographic knee osteoarthritis progression: the MOST study. J Rheumatol. 2020 May 1. pii: jrheum.200054. doi: 10.3899/jrheum.200054. Epub ahead of print. PMID: 32358162 https://www.ncbi.nlm.nih.gov/pubmed/32358162

3. Proposal from investigator.

Metabolic profiling on plasma: In addition to performing metabolic profiling using Biocrates P180 kits as the initial proposal, we would like to propose to further measure eicosanoids (n=37) on all the samples. This is because that we recently identified that lysophosphatidylcholines (LysoPCs) to phosphatidylcholines (PCs) ratio can predict end-stage knee OA in a cross-sectional and longitudinal study[11]. The LysoPCs to PCs ratio is related to release of polyunsaturated fatty acids including arachidonic acid, linoleic acid, and linolenic acid from the sn-2 position of the PC phospholipid glyceryl backbone. As precursors to the eicosanoids, a family of bioactive and inflammatory mediators, examination of this group of molecules may hold great promise to refining the apparent prognostic relationship between the LysoPCs to PCs ratio and OA outcomes.

Fasting plasma samples for the MOST study participants will be shipped to our lab from the USA. We will perform metabolic profiling on plasma using Biocrates AbsoluteIDQ® p180 kit. For this part, we request 50 µl plasma samples. We have excellent experience using this commercially available targeted metabolomics assay kit [12, 13]. The profiling will be done using a Waters Xevo® TQ Mass Spectrometry with an ESI source coupled with a Waters ACQUITY UPLC® System (Milford, Massachusetts, USA). The kit measures a total of 186 metabolites including acylcarnitines (n=40), amino acids (n=22), biogenic amines (n=18), hexoses (sum of hexoses) (n=1), and phopho-and sphingolipids (n=105).

Further, we will perform a targeted metabolic profiling with the method as previously described[14] but with modifications for analysis using the Waters Xevo® TQ Mass Spectrometry with an ESI source coupled with a Waters ACQUITY UPLC® System (Milford, Massachusetts, USA) instrumentation available in our laboratory to examine bioactive lipids (not covered by the Biocrates AbsoluteIDQ® p180 kit) produced from arachidonic acid, linoleic acid, and linolenic acid released into the extracellular environment from hydrolysis of membrane lipids. Briefly, up to 300 µL of plasma will be thawed on ice, spiked with internal standard solution and antioxidant solutions, and extracted using Waters Oasis HLB cartridges (60 mg of sorbent, 30 µm particle size) and solvent system previously described[14]. The final eluate will be dried under nitrogen stream, reconstituted in methanol and 10 µL injected onto a Waters BEH C18 column (2.1 mm × 150 mm, 130 Å, 1.7µm particle size) at a flow rate of 300 µL/min and a solvent system consisting of 0.1% acetic acid in water and acetonitrile:isopropanol (90:10) and gradient system previously used[14]. Our in-house Waters Xevo TQ mass spectrometer will be tuned for detection and quantification of relevant eicosanoids and endocannabanoids in the eluate. Pure standards of each eicosanoid and endocanabinnoid will be purchased for preparation of calibration standards for quantification and MS/MS tuning. All analyses will be done using selective ion monitoring transitions specific for the metabolite of interest based on optimized tuning parameters.

OA outcome measures:

Early OA changes will be defined based on the criteria proposed by *H. Madry, et al*[9]. People will be classified as having early knee OA changes if they have 1). Knee pain (most days of a recent month); 2). KL score <2; and 3). MRI WORMS score for cartilage morphology grade 3-6, meniscus grade 2, and for bone marrow lesions (BML) grade 2 and 3. We will examine both existent early changes, e.g. subjects with early OA changes at baseline, and incident early OA changes, e.g. healthy subjects at baseline but with early OA changes at follow-up.

X-ray OA incidence and incident symptomatic OA will be defined as KL<1 at baseline but ≥2 at follow-up on joint X-ray for x-ray incident OA plus knee pain for most of the days for incident symptomatic OA.

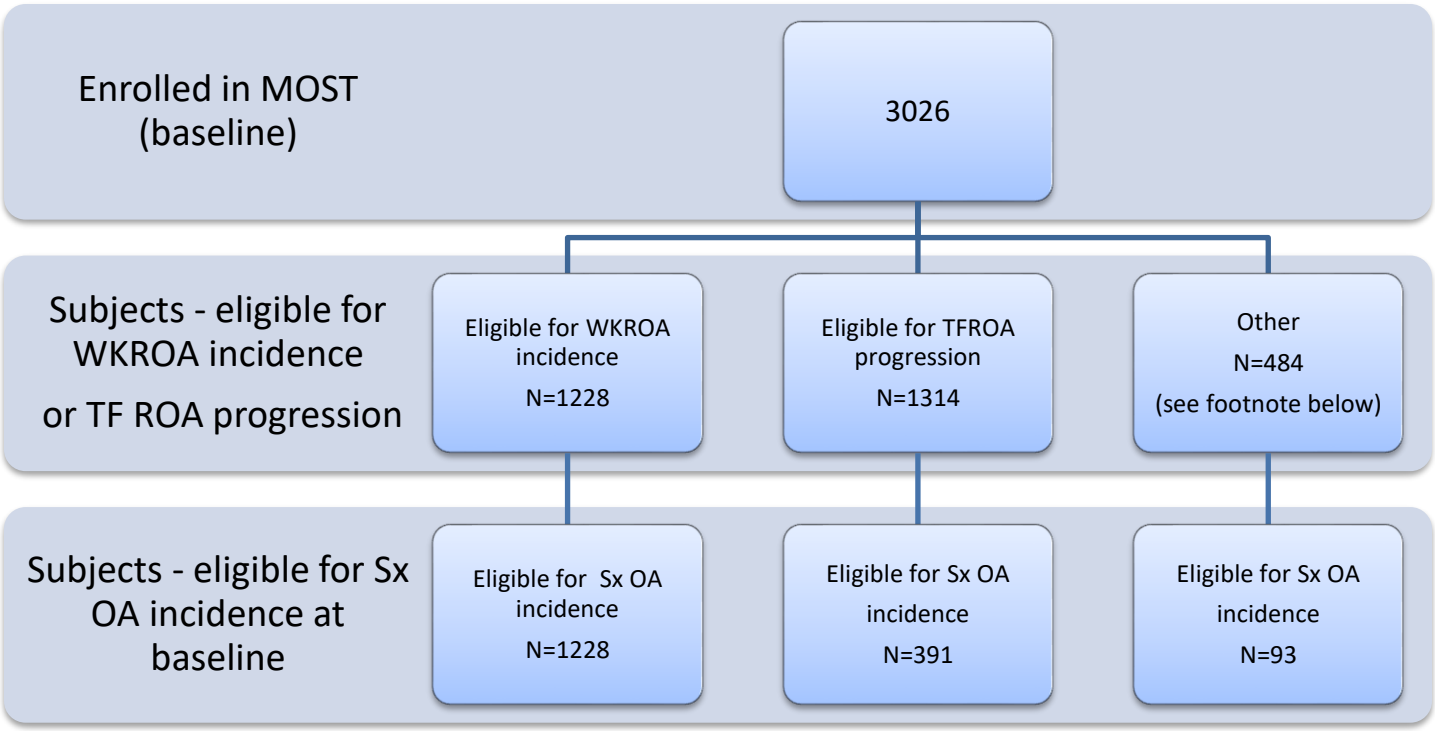
OA progression will be defined as KL ≥2 at baseline and at least a half-grade worsening in joint space width in any compartment at follow-up. The progression definition was validated in the MOST and produce a cumulative incidence of progression about 50% at 30 months follow-up[10].

4. Eligibility at Baseline for study selection.

Participants enrolled in MOST: N=3026 subjects / 6052 knees

Summary of selection:
Total N = **1248 (614 UAB + 634 UI)** vials selected for assay - 6 outcomes groups; cases and controls frequency matched by clinic site:
N=550 (286 UAB + 264 UI) selected as a control for one or more of the 6 outcomes;
N= 94 (35 UAB + 59 UI) selected as a case for one more of the 6 outcomes and as a control for one or more of the 6 outcomes;
N=604 (293 UAB + 311 UI) selected as a case for one or more of the 6 outcomes.

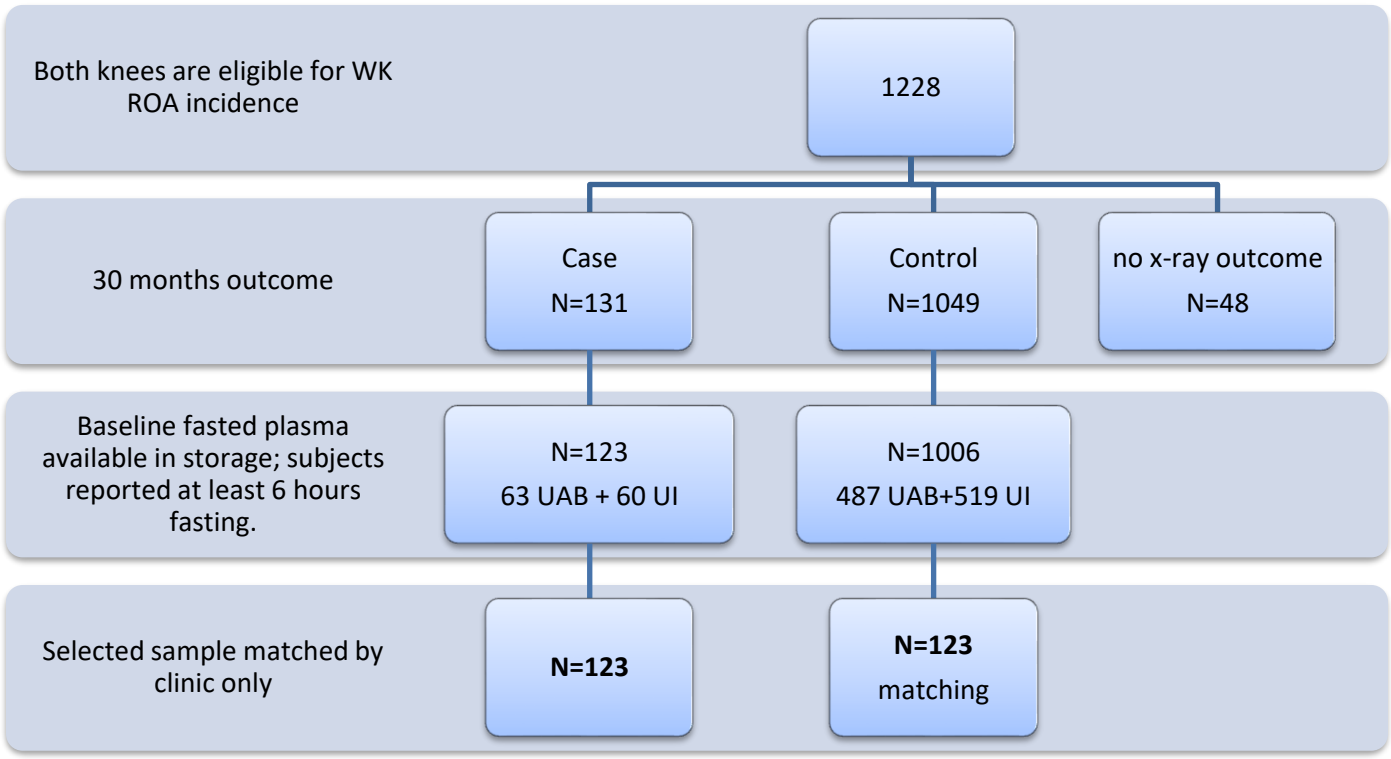
Figure 1 – FLOW chart for MOST study selection



Other N=484 – baseline status:
103 subjects have unilateral KR or other x-ray exclusion (RA, amputation, missing patella, necrosis, etc);
83 subjects – unilateral TF ROA with end stage (JSN=3) - 10 subjects eligible for Sx OA incidence;
107 subjects – bilateral TF ROA; both knees end stage – 5 subjects eligible for Sx OA incidence;
91 subjects – no TF ROA (bilateral), but PF ROA determined (not eligible for WK ROA incidence) – 39 subjects eligible for Sx OA incidence;
100 subjects – no TF ROA (bilateral, PF ROA was not read; not eligible for WK ROA incidence, no follow up images) – 39 subjects eligible for Sx OA incidence.

5. Whole Knee Radiographic OA (WK ROA) incidence outcome selection.

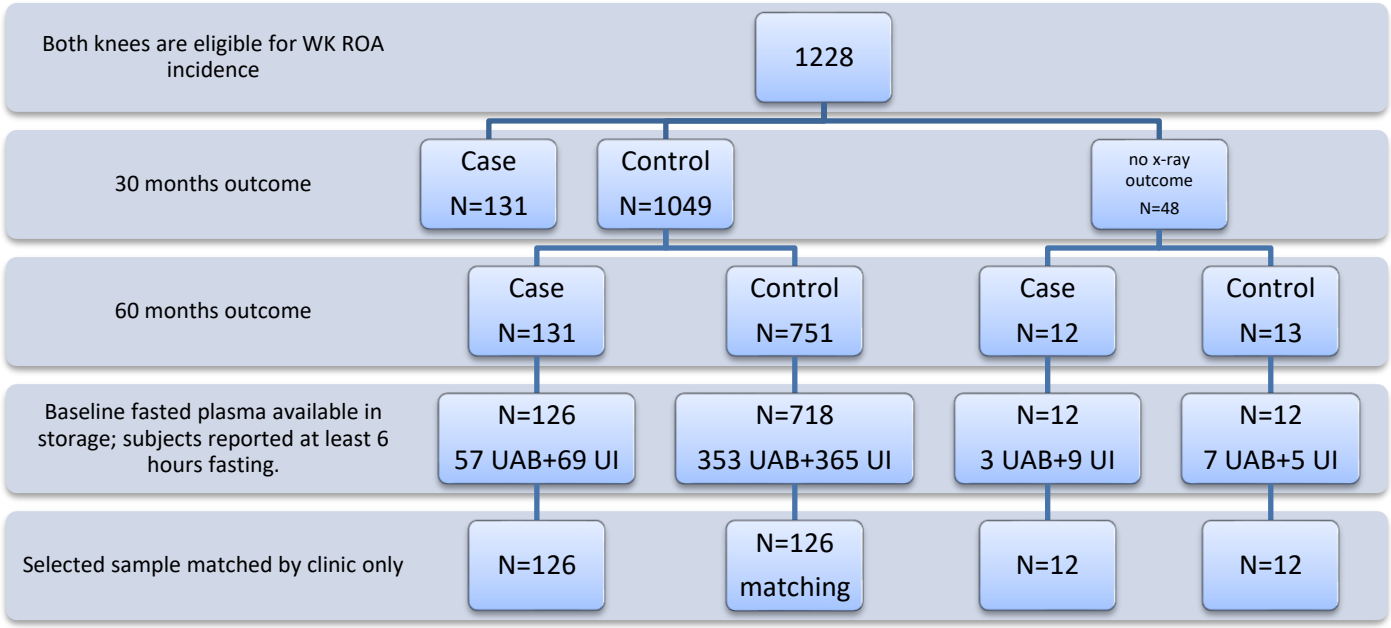
Figure 2.A. WK ROA incidence selection - 30 months



Eligible for the WK ROA incidence at 30 months:
Subject status: both knees without TF ROA or PF ROA at baseline.
Variables and code: V0XWKROAk=0
WK ROA incidence case N=123 selected (63 UAB + 60 UI):
Subjects who developed WK ROA or KR at 30m.
Variables and code: V0XWKROAk=0 and (V2XWKROAk>0 or V2R_TKR=1 or V2L_TKR=1)
Note: to be included as a case, subjects have to complete the 30m Visit with x-ray obtained and read (PA view, left and right lateral view) and TF ROA or PF ROA condition determined in at least one knee. Those eligible and developed KR could be with or without 30m clinic visit (could be self-reported by phone).

WK ROA incidence control N=123 selected matching by clinic site (63 UAB + 60 UI):
Subjects who completed 30m visit with x-ray and did not developed WK ROA or KR at 30m.
Variables and code: V0XWKROAk=0 and V2XWKROAk=0
Note: to be included as a control, subjects have to complete the 30m Visit with x-ray obtained and read (PA view, left and right lateral view) and neither TF ROA nor PF ROA condition determined in both knees.

Figure 2.B. WK ROA incidence selection – 60 months.



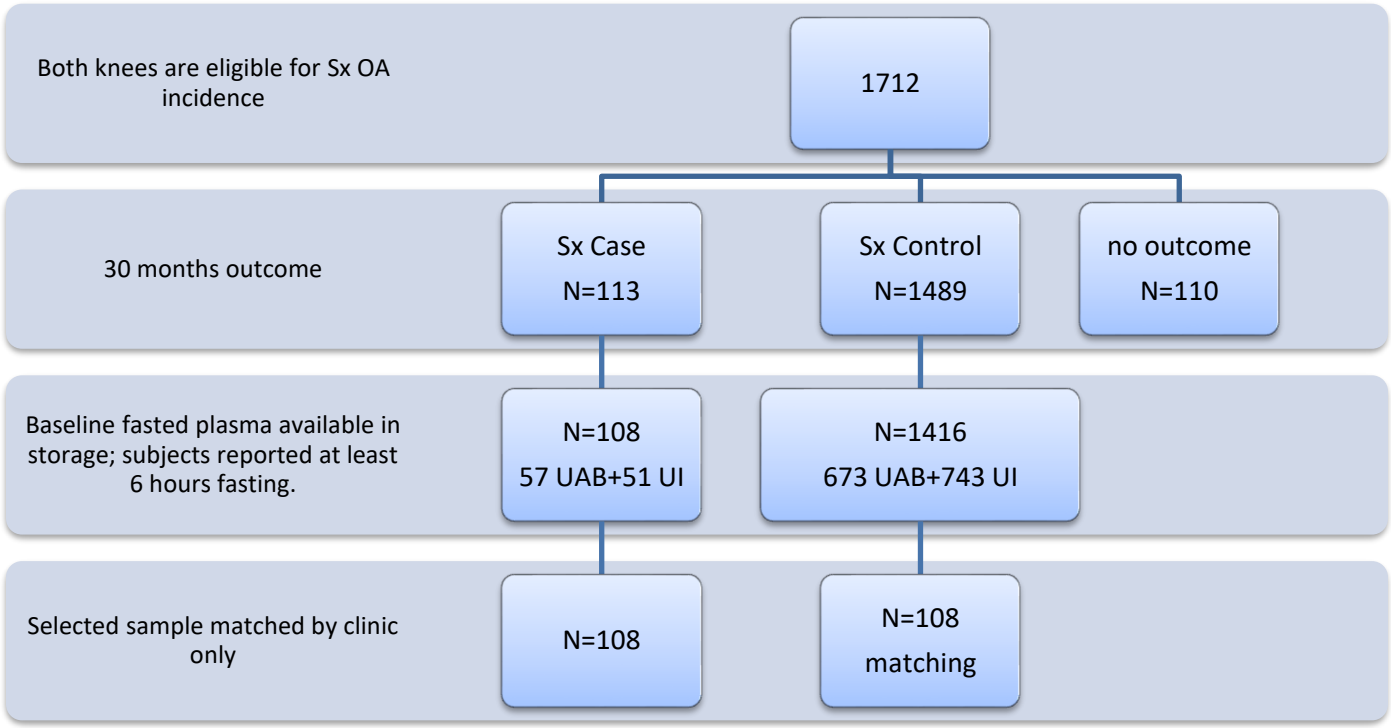
Eligible for the WK ROA incidence at 60m:
Subject status: both knees without TF ROA or PF ROA at 30m or at baseline if 30m visit was not completed.
Variables and code: V2XWKROAk=0 or (V0XWKROAk=0 and V2_CV=0)

WK ROA incidence case N=138 (126+12) selected (60 UAB + 78 UI):
Subjects who developed WK ROA or KR at 60m.
Variables and code: (V2XWKROAk=0 or (V0XWKROAk=0 and V2_CV=0)) and (V3XWKROAk>0 or V3R_TKR=1 or V3L_TKR=1)
Note: to be included as a case, subjects have to complete the 60m Visit with x-ray obtained and read (PA view, left and right lateral view) and TF ROA or PF ROA condition determined in at least one knee. Those eligible and developed KR could be with or without 60m clinic visit (could be self-reported by phone).

WK ROA incidence control N=138 (126+12) selected (60 UAB + 78 UI):
Subjects who did not developed WK ROA at 60m.
Variables and code: (V2XWKROAk=0 or (V0XWKROAk=0 and V2_CV=0)) and V3XWKROAk=0
Note: to be included as a control, subjects have to complete the 60m Visit with x-ray obtained and read (PA view, left and right lateral view) and neither TF ROA nor PF ROA condition determined in both knees.
Note 2: all available cases and all available controls from the group of 48 (eligible without 30m outcome) were selected mandatory. The remaining group of controls was selected among 713 available to frequency match the cases and controls 1:1 by clinic.

6. Symptomatic OA (Sx OA) incidence outcome selection

Figure 3.A. Sx OA incidence selection – 30 months.



Eligible for the Sx OA incidence:
Subjects with both knees without (TF ROA or PF ROA) or without frequent knee pain at baseline (V0XSXOAk=0). Subjects with baseline KR or other x-ray exclusion (RA, amputation, missing patella, necrosis) are not included. Additionally - knees with whole knee radiographic OA but with pain at ONLY one time - either phone call or clinic are NOT eligible for incident SxOA. For subject to be eligible for incidence, both knees need to be eligible.
Variables and code:

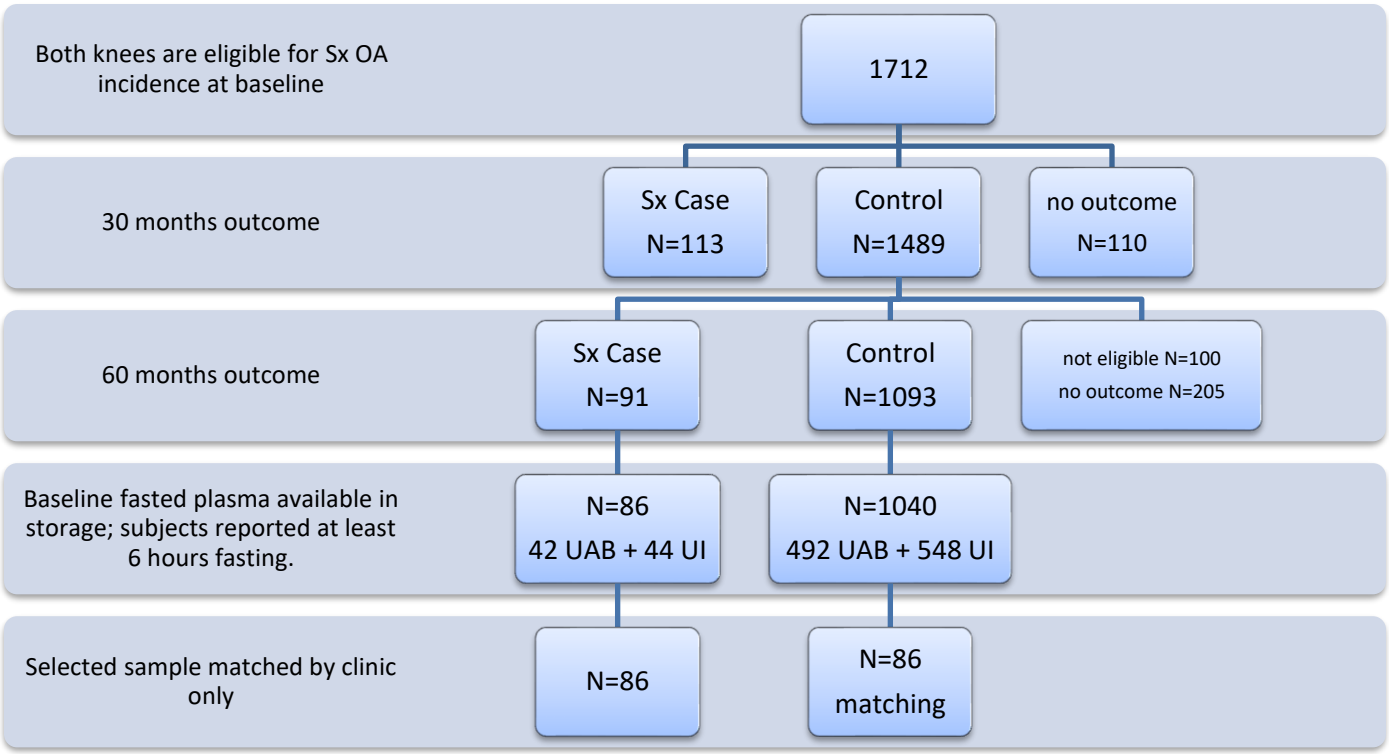
```
if v0xRTFROA=0 and V0xRPFROA=0 then right_eligSx=1; else
if 0<=v0xRKL<=4 and v0r_fkp in ("NN","NM","MN") then right_eligSx=1; else
if (v0xRTFROA>0 or V0xRPFROA>0) and index(v0r_fkp,"Y")>0 then right_eligSx=0;
if v0xlTFROA=0 and V0xlPFROA=0 then left_eligSx=1; else
if 0<=v0xLKL<=4 and v0l_fkp in ("NN","NM","MN")  then left_eligSx=1; else
if (v0xlTFROA>0 or V0xlPFROA>0) and index(v0l_fkp,"Y")>0 then left_eligSx=0;
if right_eligsx=1 and left_eligsx=1 then elig_sx=1;
```

Sx OA incidence case at 30 months N=108 selected (57 UAB + 51 UI):
Subjects who met both conditions: presence of WK ROA and frequent knee pain at 30m on at least one knee or eligible subjects with KR reported at 30m.
Variables and code: elig_sx=1 and (V2XSXOAk>0 or V2R_TKR=1 or V2L_TKR=1)
Note: to be included as a case, subjects have to complete the 30m visit with knee pain questions answered as Yes during phone interview and Yes during clinic interview.
If TF ROA/PF ROA was not present at baseline, subjects required to have x-ray obtained and read (PA view, left and right lateral view) and TF ROA or PF ROA condition determined in at least one knee.
If TF ROA/PF ROA was present at baseline, this information was projected to 30m status determination.

Sx OA incidence control N=108 selected (57 UAB + 51 UI):
Subjects who did not developed Sx OA at 30m.
Variables and code: V0XSXOAk=0 and elig_sx=1 and V2XSXOAk=0
Note: to be included as a control, subjects have to complete the 30m Visit with x-ray obtained and read (PA view, left and right lateral view) and neither TF ROA nor PF ROA condition determined in both knees or have to have knee pain questions answered as No at least one time (by phone or during the clinic

interview) during 30m contact. The TF ROA or PF ROA status determined at baseline was projected to 30m with or without x-ray confirmation.

Figure 3.B. Sx OA incidence selection – 60 months.



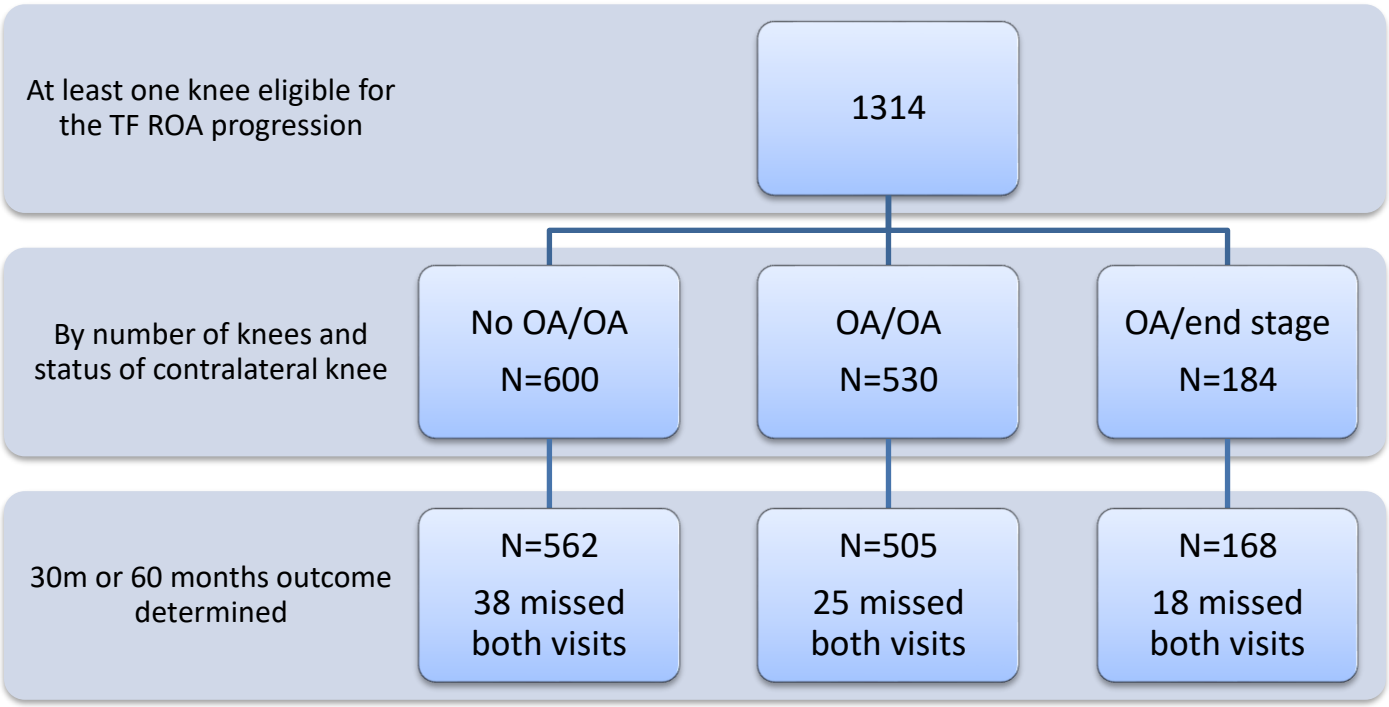
Eligible for the Sx OA incidence at 60 months:
Subjects with both knees without (TF ROA or PF ROA) or without frequent knee pain at 30m (V2XSXOAk=0). Subjects with KR or other x-ray exclusion (RA, amputation, missing patella, necrosis) are not included. Additionally - knees with whole knee radiographic OA but with pain at ONLY one time - either phone call or clinic are NOT eligible for incident SxOA. For subject to be eligible for incidence, both knees need to be eligible.
There are 194 subjects who were not eligible at baseline (OA knee reported knee pain), but became eligible at 30 months (OA knee without pain). Those subjects were not selected and determined as not eligible based on the baseline ineligibility.

Sx OA incidence case N=86 selected (42 UAB + 44 UI):
Subjects who met both conditions: presence of WK ROA and frequent knee pain at 60m on at least one knee or eligible subjects with KR reported at 60m.
Note: to be included as a case, subjects have to complete the 60m visit with knee pain questions answered as “Yes” during phone interview and “Yes” during clinic interview.
If TF ROA/PF ROA was not present at 30m, subjects required to have x-ray obtained and read (PA view, left and right lateral view) and TF ROA or PF ROA condition determined in at least one knee.
If TF ROA/PF ROA was present at baseline or 30m, this information was projected to 60m status determination.

Sx OA incidence control N=86 selected (42 UAB + 44 UI):
Subjects who did not developed Sx OA at 60m.
Note: to be included as a control, subjects have to complete the 60m Visit with x-ray obtained and read (PA view, left and right lateral view) and neither TF ROA nor PF ROA condition determined in both knees or have to have knee pain questions answered as No at least one time (by phone or during the clinic interview) during 60m contact. The TF ROA or PF ROA status determined at baseline or 30m was projected to 60m with or without x-ray confirmation.

7. Tibio-Femoral Radiographic OA (TF ROA) progression outcome selection

Figure 4. TF ROA progression selection.



Eligible for the TF ROA progression:
Knee is eligible for TF ROA progression if KL grade>=2.
Knees with KL grade=4 or one of the TF JSN score=3 (either medial or lateral, either on PA view or lateral view) are considered “end stage OA” and not eligible for progression. The PF JSN score is ignored.
Person is eligible for progression if at least one knee is eligible for progression.
Variables and code:

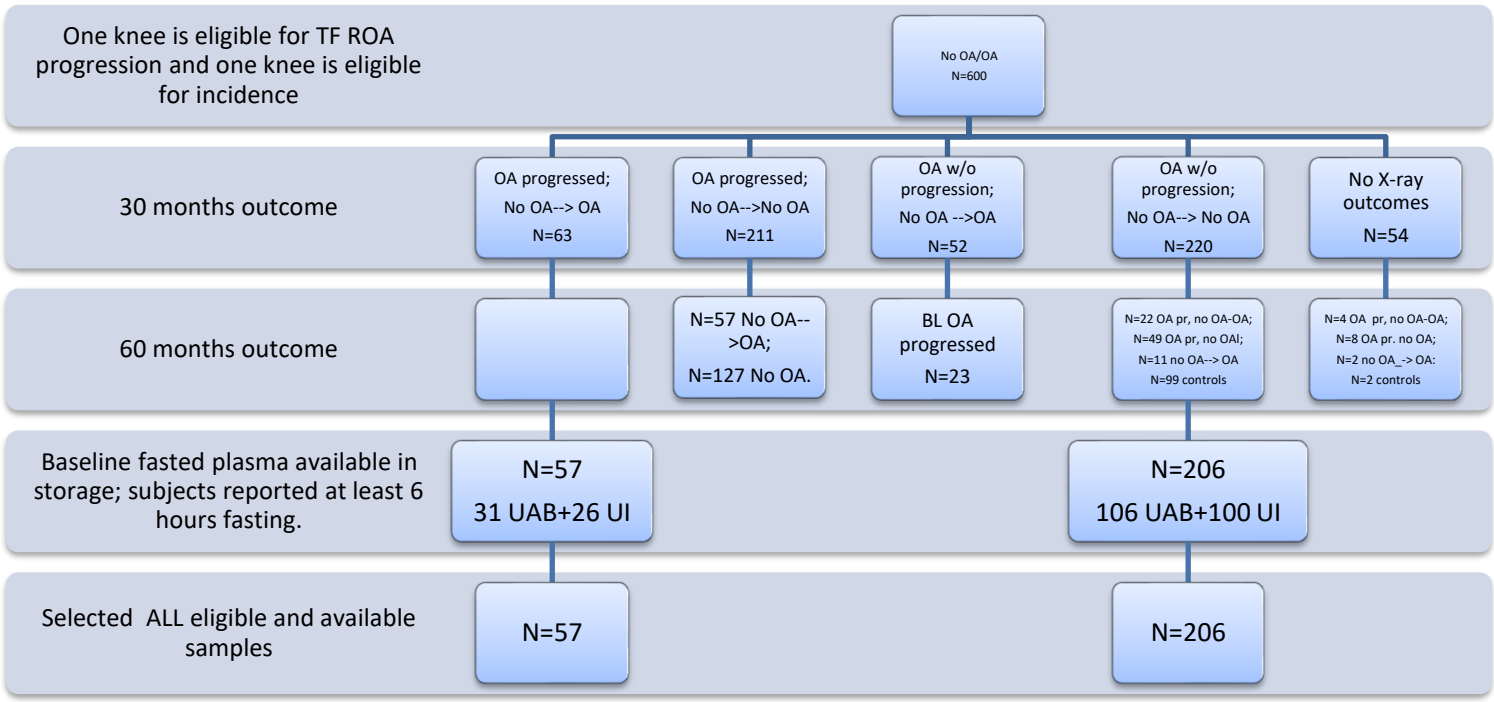
```
if V0XLTFROA=1 then l_jsn3=(v0xlkl=4 or V0XLJSL=3 or V0XLJSM=3 or V0LXLJSL=3 or V0LXLJSM=3);
if V0XrTFROA=1 then r_jsn3=(v0xrkl=4 or V0XrJSL=3 or V0XrJSM=3 or V0LXrJSL=3 or V0LXrJSM=3);
if V0XLTFROA=1 then elig left=(l jsn3=0);
if V0XrTFROA=1 then elig_right=(r_jsn3=0);
elig prog=sum(elig left,elig right);
if elig_left=1 and v02xltfjs_p>=0 then left_prog2=v02xltfjs_p;
if elig_right=1 and v02xrtfjs_p>=0 then right_prog2=v02xrtfjs_p;
if elig_right=1 and right_prog2=. and v0r_tkr=0 and v2r_tkr=1 then right_prog2=2;
if elig_left=1 and left_prog2=. and v0l_tkr=0 and v2l_tkr=1 then left_prog2=2;
if elig_prog=1 and max(r_jsn3,l_jsn3)=1 then elig_prog=3;
```

There are 3 groups of participants eligible for TF ROA progression at baseline:
Group 1 – marked as “no OA/OA”:
OA knee is eligible for progression and contralateral knee is eligible for TF ROA incidence.
Group 2 – marked as “OA/OA”:
Both knees are OA knees eligible for progression.
Group 3 – marked as “OA/end stage”:
One OA knee is eligible for progression and contralateral knee is “end stage OA” knee.

The OA knee (KL grade >=2 at baseline) progression is an increase in at least one TF JSN score, this will include partial grade increase (e.g. Grade 1 going to 1.5). Also, if the knee has KL >=2 and that knee gets a TKR, it is defined as progressed.

Each eligibility group is described in details on the pages 8, 9 and 10.

Figure 4.A. TF ROA progression selection from Unilateral OA subjects with OA/no OA knees.



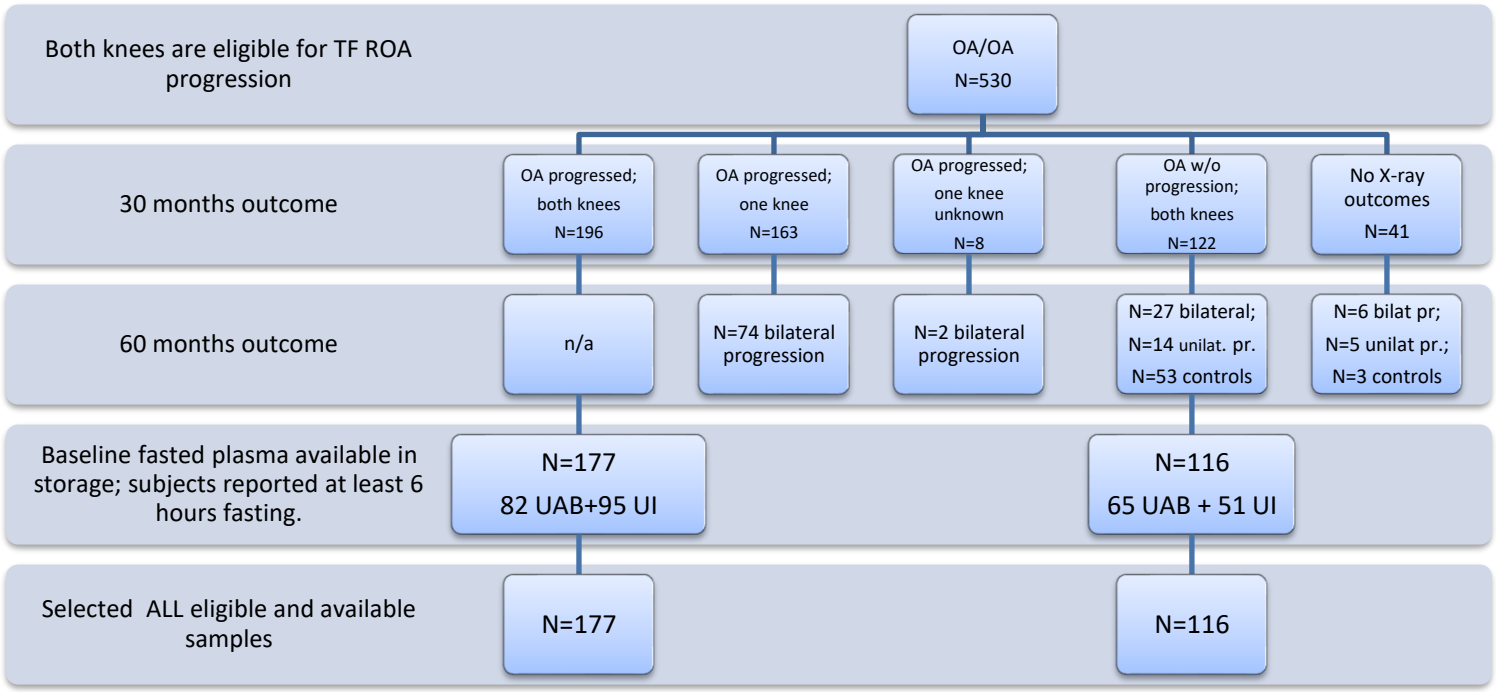
Group 1 = subjects with No OA/OA (not end stage) knees N=600
Progression – means OA knee with increase JSN at least 0.5 (any of the 4 TF JSN scores) or KR
No OA → OA – means cross the KL=2+ at follow up visit or KR
Variables and code:

```
if elig_prog=1 and elig_right=1 then do;
if right_prog2>0 and (v2xltfroa=1 or v2l_tkr=1) then cat_prog=1; else
if right_prog2>0 and (v2xltfroa=0) then cat_prog=2; else
if right_prog2=0 and (v2xltfroa=1 or v2l_tkr=1) then cat_prog=3; else
if right_prog2=0 and (v2xltfroa=0) then cat_prog=4; else
if right_prog2>0 then cat_prog=8; else
cat_prog=9;
end;
if elig prog=1 and elig left=1 then do;
if left_prog2>0 and (v2xrtfroa=1 or v2r_tkr=1) then cat_prog=1; else
if left_prog2>0 and (v2xrtfroa=0) then cat_prog=2; else
if left_prog2=0 and (v2xrtfroa=1 or v2r_tkr=1) then cat_prog=3; else
if left_prog2=0 and (v2xrtfroa=0) then cat_prog=4; else
if left prog2>0 then cat_prog=8; else
cat_prog=9;
end;
```

Eligible cases and controls are defined at 30m:
Case: Unilateral progression and contralateral knee incidence OA: N=63
Variables and code: V2_TFPROG=11
Selected: N=57 (31 UAB + 26 UI)

Controls: OA knee without progression at 30m and contralateral knee remain without TF ROA (KL<2) N=220
Variables and code: V2_TFPROG=10
Selected: N=206 (106 UAB + 100 UI)
Note – bilateral progression cases and controls for the 30 m progression are selected without matching.

Figure 4.B. TF ROA progression selection from Bilateral OA subjects.



Group 2 = subjects with OA/OA (not end stage) knees. N=530
Progression – means OA knee with increase JSN at least 0.5 (any of the 4 TF JSN scores) or KR
Variables and code:

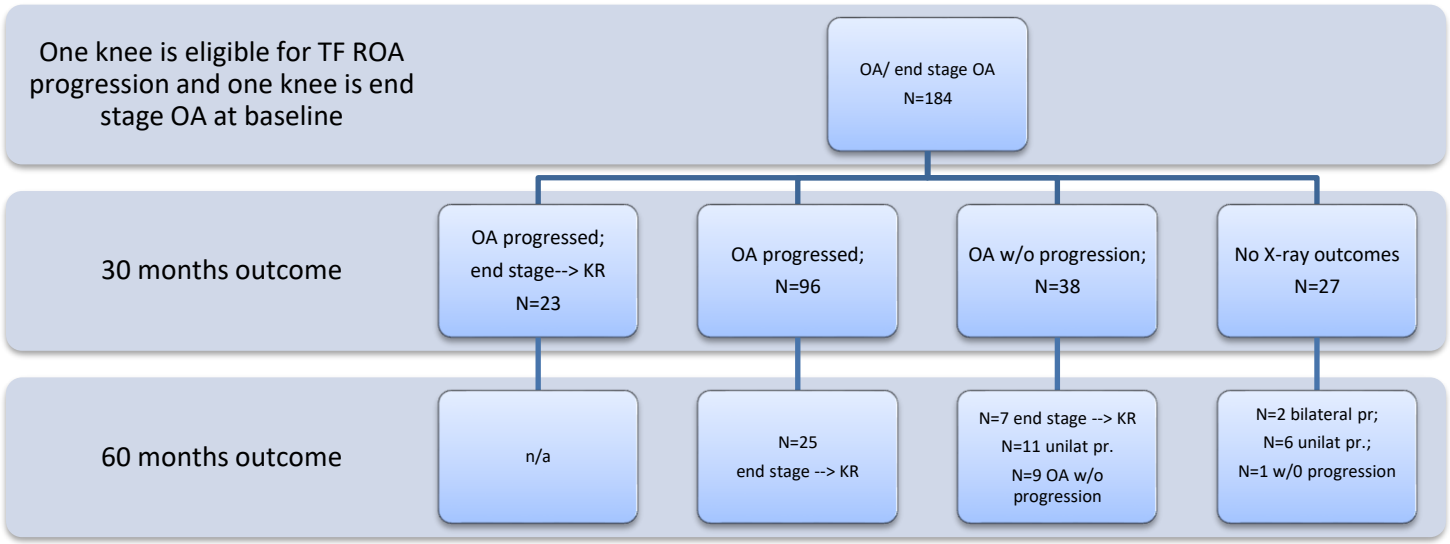
```
if elig_prog=2 then do;
if right prog2>0 and left prog2>0 then cat prog=1; else
if (right_prog2>0 and left_prog2=0) or (right_prog2=0 and left_prog2>0) then cat_prog=2; else
if (right_prog2>0 and left_prog2=.) or (right_prog2=. and left_prog2>0) then cat_prog=3; else
if right_prog2=0 and left_prog2=0 then cat_prog=4; else
if (right_prog2=. and left_prog2=0) or (right_prog2=0 and left_prog2=.) then cat_prog=8; else
cat_prog=9;
end;
```

Eligible cases and controls are defined at 30m:
Case: Bilateral progression: N=196
Variables and code: V2_TFPROG=21
Selected: N=177 (82 UAB + 95 UI)

Controls: both OA knees without progression at 30m N=122
Variables and code: V2_TFPROG=20
Selected: N=116 (65 UAB + 51 UI)

Note – bilateral progression cases and controls for the 30m progression are selected without matching.

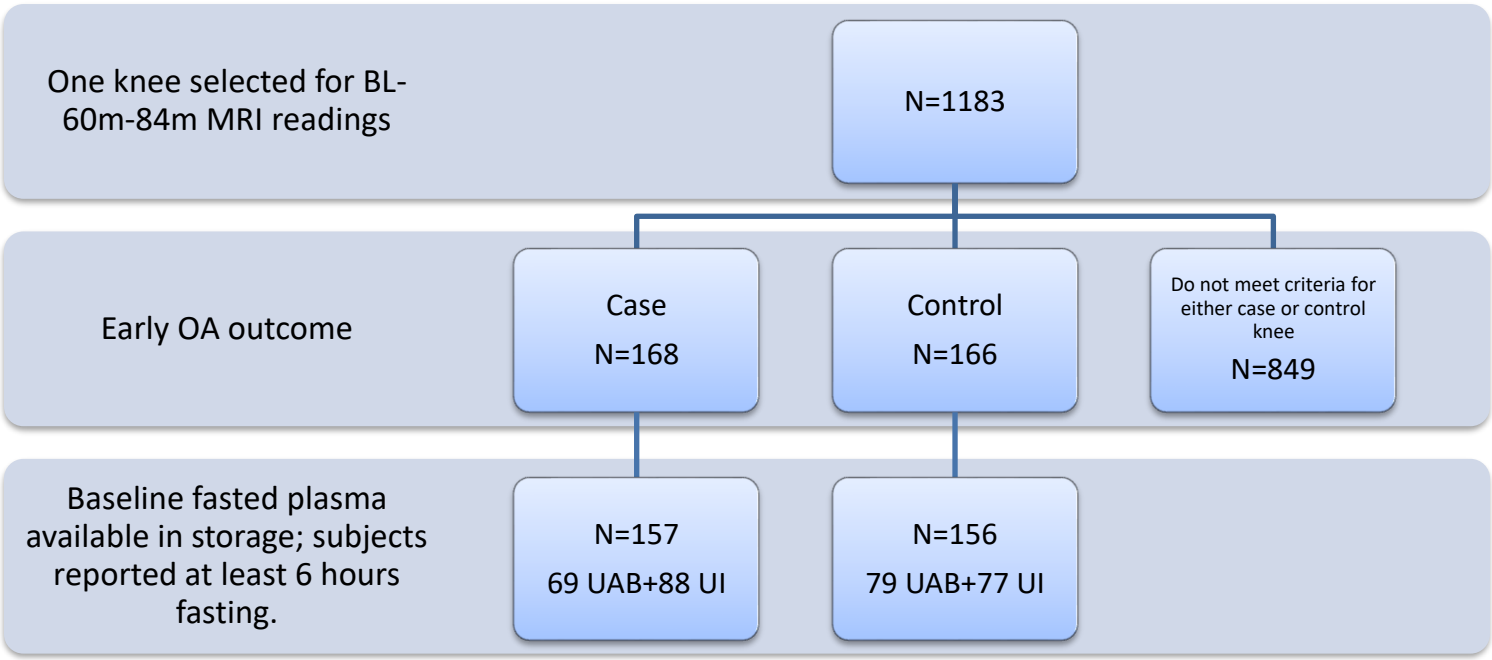
Figure 4.C. TF ROA progression selection from Bilateral OA subjects with one knee eligible for progression (contralateral knee is end stage at baseline).



Group 3 = subjects with OA/ end stage OA knees. N=184
Progression – means OA knee with increase JSN at least 0.5 (any of the 4 TF JSN scores) or KR
End stage OA progression – means KR.
There are no eligible cases or controls.

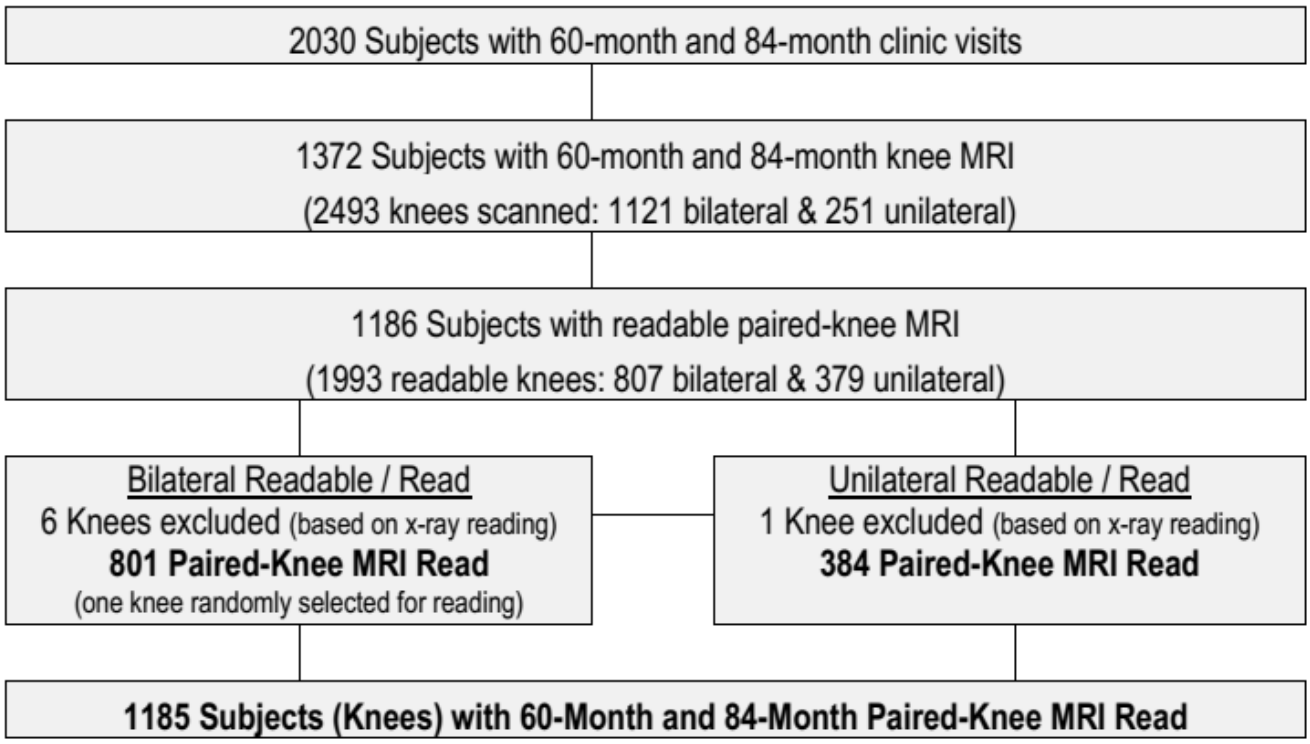
8. Early OA (BL-60m-84m MRI readings) outcome selection

Figure 5. Baseline-60m-84m MRI readings – Early OA knee definition.



The MRI selection for BL-60m-84m reading summary:

60-Month and 84-Month Paired MRI Reading Summary



Note – an additional 2 knees were determined as meeting the 84m x-ray exclusion criteria after the reading was completed. Therefore the final number of knees with MRI readings done in this group is N=1183.

Eligible cases and controls are defined at baseline.
Early OA case and control definition: contralateral knee OA status was ignored.

Case knee: KL grade 0 or 1 plus Frequent Knee Pain (most days in the past 30 days) at clinic visit interview=Y plus at least one MRI score met the criteria:
- cartilage morphology (CM) grade 3-6
- meniscus grade 2
- bone marrow lesions (BML) grade 2 or 3.

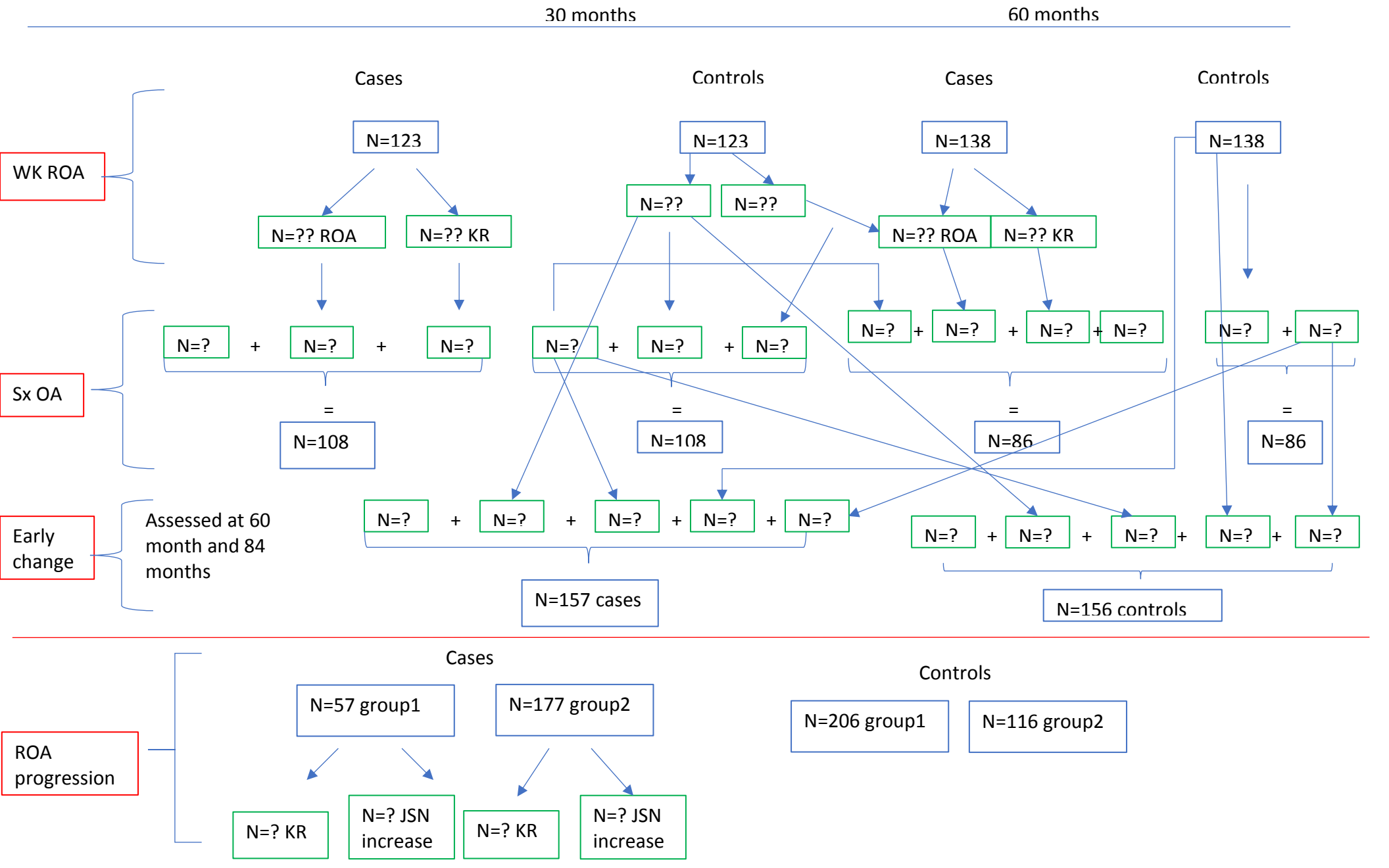
Variables and code: **V0_earlyOA** =1
Selected: N=157 (69 UAB + 88 UI)

Control knee: KL grade 0 or 1 plus Frequent Knee Pain (most days in the past 30 days) at clinic visit interview=N plus none of the MRI scores met criteria of the case (see above).

Variables and code: **V0_earlyOA** =0
Selected: N=156 (79 UAB + 77 UI)

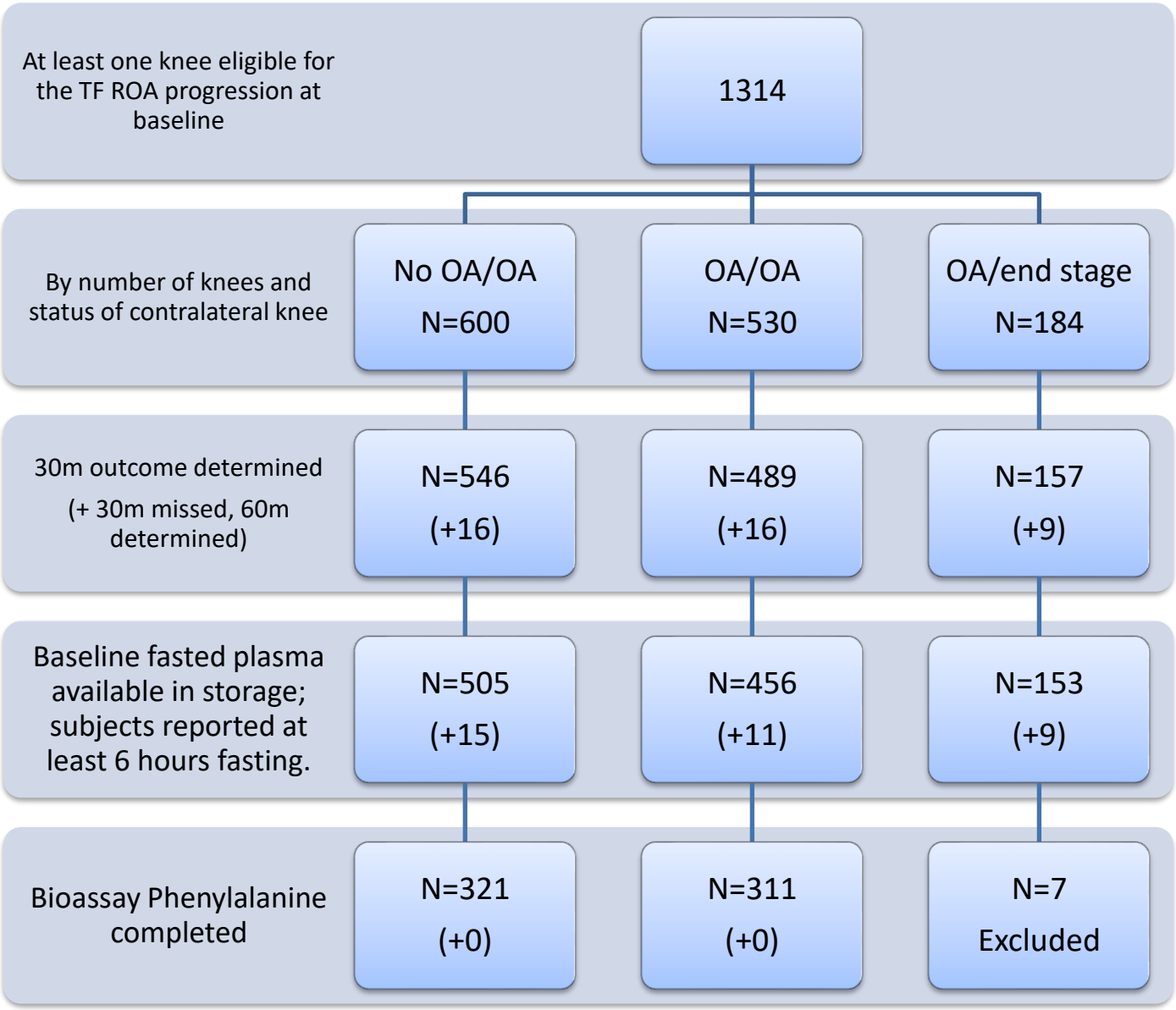
Note 1 – the Frequent Knee Pain at telephone interview was ignored (different from Sx OA definition).
Note 2 – early OA cases and controls from MRI are selected without matching (everything available was selected).

9. Appendix 1. Potential overlap between different selection groups



10. Appendix 2. Additional details about 30m progression group.

Figure 6. TF ROA progression selection.



Eligible for TF ROA progression:
A knee is eligible for TF ROA progression if KL grade>=2.
Knees with KL grade=4 or one of the TF JSN score=3 (either medial or lateral, either on PA view or lateral view) are considered “end stage OA” and not eligible for progression. The PF JSN score is ignored.
A person is eligible for progression if at least one knee is eligible for progression. There are 3 groups of participants eligible for TF ROA progression at baseline: “No OA/OA”, “OA/OA” and “OA/end stage”.

30m outcome:
OA knee (KL grade >=2 at baseline) progression is an increase in at least one TF JSN score, this will include partial grade increases (e.g. Grade 1 going to 1.5). Also, if the knee has KL >=2 and that knee gets a TKR, it is defined as progressed.

Note: the group “OA/end stage” (One OA knee is eligible for progression and contralateral knee is “end stage OA” knee) was excluded from progression analysis.

Group 1 – marked as “no OA/OA”:
OA knee is eligible for progression and contralateral knee is eligible for TF ROA incidence.
Progression outcome:
Bilateral progression = (JSN progression or KR) in OA knee and (incidence OA or KR) in “no OA” knee
Unilateral progression = (JSN progression or KR) in OA knee and “no OA” knee remains “no OA”
No progression = no JSN progression in OA knee and “no OA” knee remains “no OA”

Note: if the OA knee did not progress and the “no OA” knee developed incident OA, the person was excluded from analysis.

Bioassay Phenylalanine completed N=321-13=308 ppts could be included in the analysis and plasma available for additional bioassay N=149 ppts

Table 1-1. Cross tab with selection status for Sx or MRI (early OA) case/control

Variable Cat_prog	30m outcome	N	Selection for the assay				**Plasma available**	
			V2_TFPROG	V2_SXOA	V3_SXOA	V0_EARLYOA	30m outcome	60m outcome
1	Bilateral progression	57	57 cases	5 cases	1 case	7 cases 1 control	0	4
2	Unilateral progression on OA knee	45		16 cases 1 control	10 cases 1 control	5 cases 15 controls	149	7
3	No progression on OA knee and incidence on “no OA” knee. **Exclude from analysis.**	13		7 cases	2 cases	5 cases	35	2
4	No progression on both knees	206	206 controls	6 cases; 9 controls	7 cases; 6 controls	12 cases 7 controls	0	2
Total		321					184	15

SUMMARY:

#1 there are 45 participants in the group 1 “no OA/OA” with unilateral progression from BL to 30m and bioassay Phenylalanine completed and could be included in analysis. Note – those are selected as cases/controls for different outcome and not a random sample of all eligible (see details in the Table 1-3 below).

#2 there are 149 in the group 1 “no OA/OA” with unilateral progression from BL to 30m and with baseline fasted plasma available.

Table 1-2. Summary and rate for 30m progression outcome by eligibility group and bioassay completeness.

Eligible for progression at baseline and known 30m outcome as group 1:”no OA/OA”		30m outcome: N (%)		
		Bilateral progression	Unilateral progression	No progression
Total 30m outcome developed	N=494	63 (12.8%)	211 (42.7%)	220 (44.5%)
Total plasma available (potential group for analysis)	N=457	57(12.5%)	194 (42.4%)	206 (45.1%)
Total phenylalanine completed (sample for analysis)	N=308	57(18.5%)	45 (14.6%)	206 (66.9%)

Table 1-3. Detailed cross tab for the N=321 (bioassay completed)

Variable Cat_prog	30m outcome	subject_selected	Frequency	Selection status details
1	Bilateral progression N=57	xx_1x_1_1	1	Case for progression, SxOA at 30m, early OA
		xx_1x_1_x	4	Case for progression, SxOA at 30m
		xx_x1_1_x	1	Case for progression, SxOA at 60m
		xx_xx_1_0	1	Case for progression, control for early OA
		xx_xx_1_1	6	Case for progression, early OA
		xx_xx_1_x	44	Case for progression
2	Unilateral progression on OA knee N=45	xx_00_x_x	1	Control for Sx OA at 30m and 60m
		xx_1x_x_0	2	Case for SxOA at 30m, control for early OA
		xx_1x_x_x	14	Case for SxOA at 30m
		xx_x1_x_x	10	Case for SxOA at 60m
		xx_xx_x_0	13	Control for early OA
		xx_xx_x_1	5	Case for early OA
3	No progression on OA knee and incidence on “no OA” knee. **Exclude from analysis.** N=13	xx_1x_x_1	1	Case for SxOA at 30m, early OA
		xx_1x_x_x	6	Case for SxOA at 30m
		xx_x1_x_x	2	Case for SxOA at 60m
		xx_xx_x_1	4	Case for early OA
4	No progression on both knees N=206	xx_00_0_x	5	Control for progression and SxOA at 30m and 60m
		xx_0x_0_1	1	Control for progression; control for SxOA at 30m, early OA case*
		xx_0x_0_x	3	Control for progression and SxOA at 30m
		xx_1x_0_0	1	Control for progression and early OA, case for SxOA at 30m*
		xx_1x_0_x	5	Control for progression and case for SxOA at 30m*
		xx_x0_0_x	1	Control for progression and SxOA at 60m
		xx_x1_0_x	7	Control for progression and case for SxOA at 60m
		xx_xx_0_0	6	Control for progression and early OA
		xx_xx_0_1	11	Control for progression and early OA case*
		xx_xx_0_x	166	Control for progression
Total			321	

*for sensitivity analysis these 17 controls could be dropped (as 30m cases for another endpoint selection)

Group 2 – marked as “OA/OA”:
Both knees are OA knees eligible for progression.
Progression outcome:
Bilateral progression = (JSN progression or KR) in both OA knees
Unilateral progression = (JSN progression or KR) in only one OA knee
No progression = no JSN progression in both OA knees

Bioassay Phenylalanine completed N=311 and plasma available for additional bioassay N=137

Table 2-1. Cross tab with selection status for Sx or MRI (early OA) case/control

Variable Cat_prog	30m outcome	N	Selection for the assay				**Plasma available**	
			V2_TFPROG	V2_SXOA	V3_SXOA	V0_EARLYOA	30m outcome	60m outcome
1	Bilateral progression	177	177 cases	6 cases 3 controls	2 cases 2 controls	0	0	4
2	Unilateral progression	18		12 cases 2 controls	4 cases 1 control	0	137	6
4	No progression on both knees	116	116 controls	6 cases; 4 controls	2 cases; 1 control	0	0	1
	Total	311					137	11

SUMMARY:

#1 there are 18 participants in the group 2 “OA/OA” with unilateral progression from BL to 30m and bioassay Phenylalanine completed and could be included in analysis. Note – those are selected as cases/controls for different outcome and not a random sample of all eligible (see details in the Table 2-3 below).

#2 there are 137 in the group 2 “OA/OA” with unilateral progression from BL to 30m and with baseline fasted plasma available.

Table 2-2. Summary and rate for 30m progression outcome by eligibility group and bioassay completeness.

Eligible for progression at baseline and known 30m outcome as group 2:”OA/OA”		30m outcome: N (%)		
		Bilateral progression	Unilateral progression	No progression
Total 30m outcome developed	N=481	196 (40.7%)	163 (33.9%)	122 (25.4%)
Total plasma available (potential group for analysis)	N=448	177(39.5%)	155 (34.6%)	116 (25.9%)
Total phenylalanine completed (sample for analysis)	N=311	177(56.9%)	18 (5.8%)	116 (37.3%)

Table 2-3. Detailed cross tab

Variable Cat_prog	30m outcome	subject_selected	Frequency	Selection status details
1	Bilateral progression N=177	xx_00_1_x	2	Case for progression, control for SxOA at 30m and 60m
		xx_0x_1_x	1	Case for progression, control for SxOA at 30m
		xx_1x_1_x	6	Case for progression, SxOA at 30m
		xx_x1_1_x	2	Case for progression, SxOA at 60m
		xx_xx_1_x	166	Case for progression
2	Unilateral progression N=18	xx_00_x_x	1	Control for Sx OA at 30m and 60m
		xx_0x_x_x	1	Control for Sx OA at 30m
		xx_1x_x_x	12	Case for SxOA at 30m
		xx_x1_x_x	4	Case for SxOA at 60m
4	No progression on both knees N=116	xx_00_0_x	1	Control for progression and SxOA at 30m and 60m
		xx_01_0_x	1	Control for progression; control for SxOA at 30m, case for SxOA at 60m
		xx_0x_0_x	2	Control for progression and SxOA at 30m
		xx_1x_0_x	6	Control for progression and case for SxOA at 30m*
		xx_x1_0_x	1	Control for progression and case for SxOA at 60m
		xx_xx_0_x	105	Control for progression

*for sensitivity analysis these 6 controls could be dropped (as 30m cases for another endpoint selection)

11. Appendix 3. Metabolic profile method (information from laboratory)

Metabolomic profiling method:

Frozen (-80°C) fasting human plasma collected at baseline was used. Metabolic profiling was performed with 10 ul of undiluted plasma using Biocrates *AbsoluteIDQ® p180 kit* (Biocrates Life Sciences AG, Innsbruck, Austria, catalog # 20073), which assesses 186 metabolites including acylcarnitines (n=40), amino acids (n=21), biogenic amines (n=19), hexoses (sum of hexoses) (n=1), and phospho-and sphingolipids (n=105) (see **Table 1**). The profiling was done using an API4000 Qtrap® tandem mass spectrometry instrument (Applied Biosystems/MDS Analytical Technologies, Foster City, CA) equipped with Agilent 1100 HPLC system at The Metabolomics Innovation Centre (https://www.metabolomicscentre.ca). The complete analytical process (e.g., the targeted metabolite concentration) was performed using the MetIQ software package, which is an integral part of the *AbsoluteIDQ®* kit. A total of 157 metabolites were successfully analyzed. Concentrations of all metabolites are reported in μM.

Table 1. List of metabolites assessed using the Biocrates AbsoluteIDQ p180kit

Metabolite class	Number	Metabolite name or abbreviation
Amino acids	21	Alanine, arginine, asparagine, aspartate, citrulline, glutamine, glutamate, glycine, histidine, isoleucine, leucine, lysine, methionine, ornithine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine
Carnitine	1	C0
Acylcarnitine	25	C2, C3, C3:1, C4, C4:1, C5, C5:1, C6(or C4:1-DC), C6:1, C8, C9, C10, C10:1, C10:2, C12, C12:1, C14, C14:1, C14:2, C16, C16:1, C16:2, C18, C18:1, C18:2
Hydroxy- and dicarboxyacylcarnitines	14	C3-OH, C4-OH(or C3-DC), C5:1-DC, C5-DC(or C6-OH), C5-M-DC, C5-OH(or C3-DC-M), C7-DC, C12-DC, C14:1-OH, C14:2-OH, C16:1-OH, C16:2-OH, C16-OH, C18:1-OH
Biogenic amines	19	acetylornithine, asymmetric dimethylarginine, symmetric dimethylarginine, total dimethylarginine, alpha-Aminoadipic acid, carnosine, creatinine, histamine, kynurenine, methioninesulfoxide, nitrotyrosine, hydroxyproline, phenylethylamine, putrescine, sarcosine, serotonin, spermidine, spermine, taurine
Lyso-phosphatidylcholines	14	lysoPC a C14:0/C16:0/C16:1/C17:0/C18:0/C18:1/C18:2/ C20:3/C20:4/C24:0/C26:0/C26:1/C28:0/C28:1
Diacyl-phosphatidylcholines	38	PC aa C24:0/C26:0/C28:1/C30:0/C30:2/C32:0/C32:1/C32:2/ C32:3/C34:1/C34:2/C34:3/C34:4/C36:0/C36:1/C36:2/C36:3/C36:4/C36:5/C36:6/C38:0/C38:1/C38:3/C38:4/C38:5/C38:6/C40:1/C40:2/C40:3/C40:4/C40:5/C40:6/C42:0/C42:1/C42:2/C42:4/C42:5/C42:6
Acyl-alkyl-phosphatidylcholines	38	PC ae C30:0/C30:1/C30:2/C32:1/C32:2/C34:0/C34:1/C34:2/C34:3/ C36:0/C36:1/C36:2/C36:3/C36:4/C36:5/C38:0/C38:1/C38:2/C38:3/C38:4/C38:5/C38:6/C40:1/C40:2/C40:3/C40:4/C40:5/C40:6/C42:0/C42:1/C42:2/C42:3/C42:4/C42:5/C44:3/C44:4/C44:5/C44:6
Sphingomyelines	10	SM C16:0, SM C16:1, SM C18:0, SM C18:1, SM C20:2, SM C22:3, SM C24:0, SM 24:1, SM C26:0, SM C26:1
Hydroxysphingomyelines	5	SM (OH) C14:1, SM (OH) C16:1, SM (OH) C22:1, SM (OH) C22:2, SM (OH) C24:1
Hexose	1	H1
Total	186	