PSTC Workshop 20 FEB 2023

Japanese Healthy Volunteer Study

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PSTC
CRITICAL PATH INSTITUTE

Contents

1. Project background
2. Study background
    1.1 Study objective
    1.2 Study design
    1.3 Analysis considerations
3. Study results
    2.1 Demographics
    2.2 Biomarker: baseline
    2.3 Biomarker: inter- and intra-subject variabilities
    2.4 Biomarker: specificity
    2.5 Biomarker: effect of covariates
4. Summary

1. Project background

Kidney Safety Urine Biomarker Project

The Critical Path Institute's (C-Path) Predicative Safety Testing Consortium (PSTC) is seeking regulatory endorsement of eight urinary biomarkers for use in drug development for the detection of exposure to a nephrotoxicant in clinical studies.

CLAIM:

Qualified renal safety biomarkers are proposed to be used together with conventional kidney biomarker monitoring (e.g., sCr, BUN) in early clinical drug development research (under an IND or CTA, etc.) to support conclusions as to whether a drug is likely or unlikely to have caused a mild injury response in the renal tubule at the tested dose and duration.

STUDY POPULATION:

Use in healthy volunteers and patients with normal renal function, taking into account age, gender and ethnicity.

Biomarker Qualification Definition

- Qualification is a formal regulatory review and endorsement/acceptable process of biomarkers for their use as Drug Development Tools (DDT) by **US FDA**, **EMA**, and **PMDA**
- "Qualification is a conclusion that within the stated context of use, the DDT can be relied upon to have a specific interpretation and application in drug development and regulatory review. "

Drug Development Tool (DDT) Qualification Programs | FDA

Japanese Qualification Strategy: Enabling Studies

General study strategy

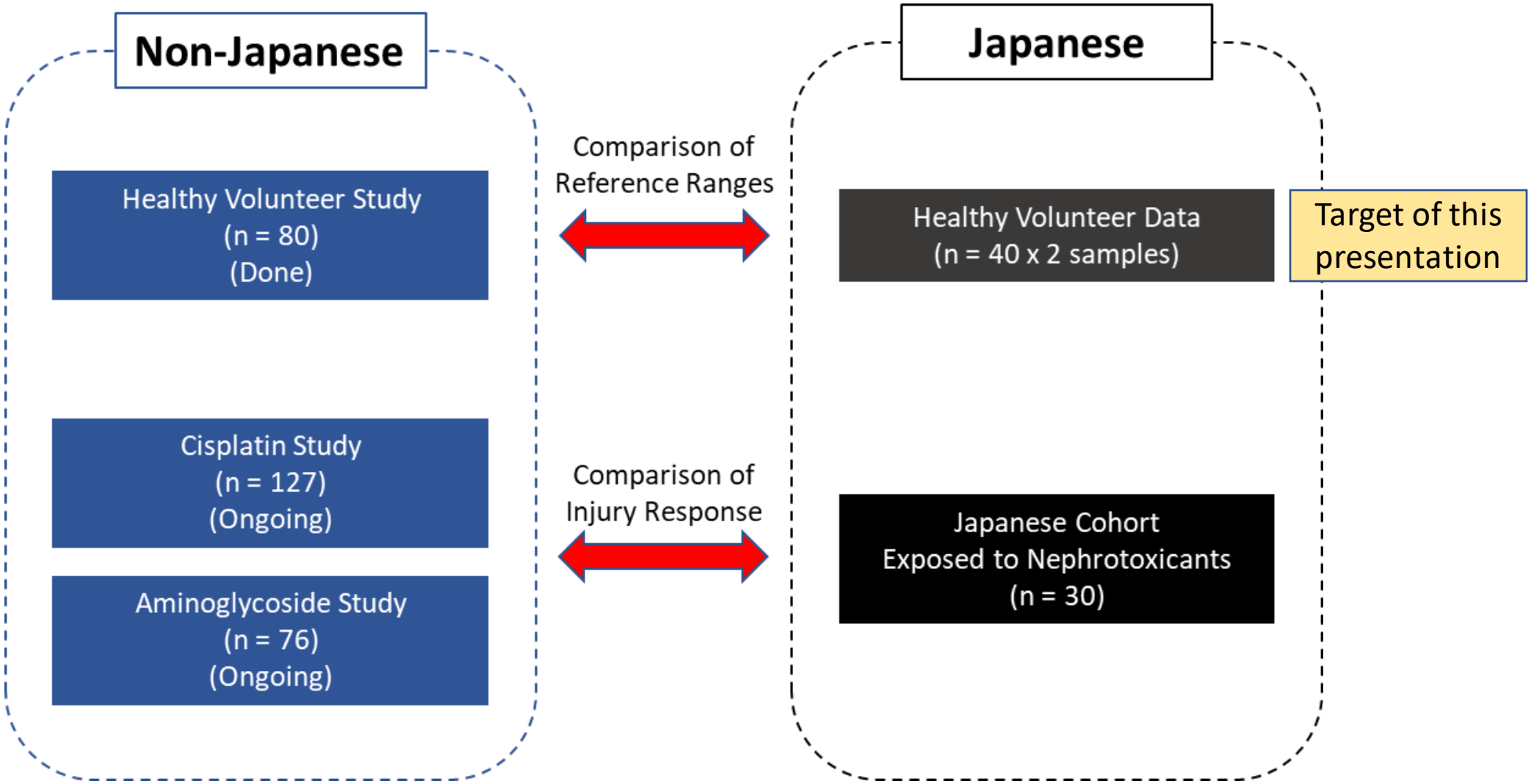
Japanese healthy volunteer samples

- Demographics similar to Phase 1 heathy volunteer population in US
- Urine and serum samples (quantity sufficient for defining the reference ranges of multiple biomarkers)

Samples from Japanese subjects with organ injury

- Demographics similar to Phase 1 heathy volunteer population in US
- Urine and serum samples (quantity sufficient for sensitivity and specificity studies across multiple biomarkers)

Japanese Qualification Strategy



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Samples from Japanese subjects with organ injury

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2. Study background

2.1 Study objective

- Primary Objectives:
  - To characterize the mean values and inter- and intra-subject variability of renal biomarkers (**BM**) in healthy Japanese subjects.
  - To compare the mean values and inter- and intra-subject variability of renal biomarkers in healthy Japanese subjects to historical data obtained in healthy US subjects.
- Secondary Objectives:
  - To assess whether the mean values or variability are influenced by age or sex in the Japanese population.
  - To create a well-annotated sample set of urine, serum, and plasma for evaluation in future biomarker qualifications submitted by the PSTC.

2.2 Study design

- This is a non-intervention study. Subjects will not receive any treatments.
- A total of 40 subjects will complete the study (10 subjects in each category).

	Age 20-39 years	Age 40 to 70 year
Female	10	10
Male	10	10

- Subjects will be screened to document health status and study eligibility
  - Healthy Japanese subjects meeting the entry criteria (Details in Backup slides)
- Those meeting the entry criteria will be asked to return for Visit 1 (Baseline visit) and Visit 2 (5 to 7 days from Visit 1) for collection of urine and blood samples.

Screening Visit | **Visit 1 (Baseline)** | **Visit 2 (5 to 7 days from Visit 1)**

Measured biomarkers

- Eight (8) BMs were measured in the study
- Six of the 8 BMs were selected as components of the Composite Measure [1)]

	Abbreviation	Biomarker
Composite Measure (6 components)	ALB	Albumin
	CLU	Clusterin
	CysC	Cystatin-C
	KIM1	Kidney injury molecule-1
	NAG	N-acetyl-beta-D-glucosaminidase
	NGAL	Neutrophil gelatinase-associated lipocalin
	OPN	Osteopontin
	TPRT	Total protein
Normalizing factor	uCr	Urine creatinine

1) User's Guide, Kidney Safety Composite Measure Biomarker for Use in Clinical Development, Ver. 1.1, May 15, 2019.

Composite Measure

From the User's Guide Document (2019) [1)]

The CM at a given timepoint, t, for an individual subject, i, is calculated as:

$$CM_{it} = exp\left[(1/6) \times \log\left(FC_{CLU,it}\right) + (1/6) \times \log\left(FC_{OPN,it}\right) + (1/6) \times \log\left(FC_{NAG,it}\right) + (1/6) \times \log\left(FC_{KIM-1,it}\right) + (1/6) \times \log(FC_{CysC,it}) + (1/6) \times \log(FC_{NGAL,it})\right]$$

where $FC_{CLU,it}$, $FC_{OPN,it}$, $FC_{NAG,it}$, $FC_{KIM-1,it}$, $FC_{CysC,it}$, and $FC_{NGAL,it}$ are the CLU, OPN, NAG, KIM-1, CysC and NGAL fold changes from baseline for individual subject i, at timepoint t, as defined above. Note that log refers to the natural log transformation of the fold changes from baseline. In the event values are below the limit of quantitation, the value should be assigned as < LLOQ = LLOQ-0.1*LLOQ (i.e. 90% of the LLOQ value).

1) User's Guide, Kidney Safety Composite Measure Biomarker for Use in Clinical Development, Ver. 1.1, May 15, 2019.

3. Study results (DRAFT)

Draft results as of 27 DEC 2022

3.1 Demographics and vital signs (baseline)

			JAPAN			USA		
			Age category (years)		Total	Age category (years)		Total
			20-39	40-70		20-39	40-70	
Total number of subjects			**20**	**20**	**40**	**41**	**40**	**81**
Age		Mean	28.3	48.2	38.2	29.4	50.8	40.0
Sex: Female		N (%)	10 (50.0)	10 (50.0)	20 (50.0)	21 (51.2)	20 (50.0)	41 (50.6)
Race	Asian	N (%)	20 (100)	20 (100)	40 (100)	0 (0)	0 (0)	0 (0)
	Black or AA[1)]	N (%)	0 (0)	0 (0)	0 (0)	9 (22.0)	4 (10.0)	13 (16.0)
	White	N (%)	0 (0)	0 (0)	0 (0)	32 (78.0)	36 (90.0)	68 (84.0)
Weight (kg)		Mean	60.3	59.5	59.9	79.2	81.7	80.4
Height (cm)		Mean	164.8	162.8	163.8	171.2	170.5	170.9
BMI (kg/m^2)		Mean	22.0	22.4	22.2	26.8	28.0	27.4
BMI category[2)]	Underweight	N (%)	2 (10.0)	1 (5.0)	3 (7.5)	0 (0)	0 (0)	0 (0)
	Healthy weight	N (%)	14 (70.0)	17 (85.0)	31 (77.5)	18 (43.9)	8 (20.0)	26 (32.1)
	Overweight	N (%)	4 (20.0)	1 (5.0)	5 (12.5)	10 (24.4)	18 (45.0)	28 (34.6)
	Obese	N (%)	0 (0)	1 (5.0)	1 (2.5)	13 (31.7)	14 (35.0)	27 (33.3)

1) AA: African American

2) BMI category: Underweight (<18.5), Healthy weight (18.5-<25.0), Overweight (25.0-<30.0), Obese (>=30)

Demographics and vital signs (continued)

- Japan study
  - Ten (10) subjects in each of 4 sex/age category was enrolled by study design
  - All 40 subjects completed the study
  - 50% were females, mean age was 38.2 years, and mean BMI was 22.2 kg/m^2
- Comparison with US study
  - BMI of US study subjects was higher compared to the Japan study
    - Mean BMI: 27.4 kg/m^2 (USA) *vs* 22.2 kg/m^2 (JPN)
    - Proportion of subjects in the overweight or obese category: 67.9% (USA) *vs* 15.0% (JPN)

Biomarker analysis considerations

- Normalization
  - Each BM was **normalized** by urine creatinine (uCr) value measured at the same visit
  - Example
    - uCr normalized ALB = unnormalized ALB / uCr

Biomarker analysis considerations (continued)

- Handling of BM values below the limit of quantification (**BLQ**)
  - Each BM has lower limit of quantification (**LLoQ**), as shown in the table below
    - LLoQ of Japan and US studies differ for KIM1 and NGAL
  - When the measured value is BLQ, the following value will be substituted (imputed) in the data analysis [1)]
    - **Imputed value = 0.9 × LLoQ**

LLoQ used in each study	ALB (mg/L)	CLU (ng/mL)	CysC (ng/mL)	KIM1 (ng/mL)	NAG (U/L)	NGAL (ng/mL)	OPN (ng/mL)	TPRT (mg/dL)
Japan study	1.0	10	1.4	0.156	0.70	1.0	40	3.5
US study	1.0	10	1.4	0.012	0.31	0.4	40	3.5

1) User's Guide, Kidney Safety Composite Measure Biomarker for Use in Clinical Development, Ver. 1.1, May 15, 2019.

3.2 BM: Baseline

- Unnormalized BM
  - Geometric mean (GM) and coefficient of variation (CV) were calculated for each BM
  - For GM, greater than 2-fold difference was seen for NGAL (larger for USA). The differences were smaller for other BMs.
  - CV was generally larger for USA compared to JPN for all BMs, except for OPN

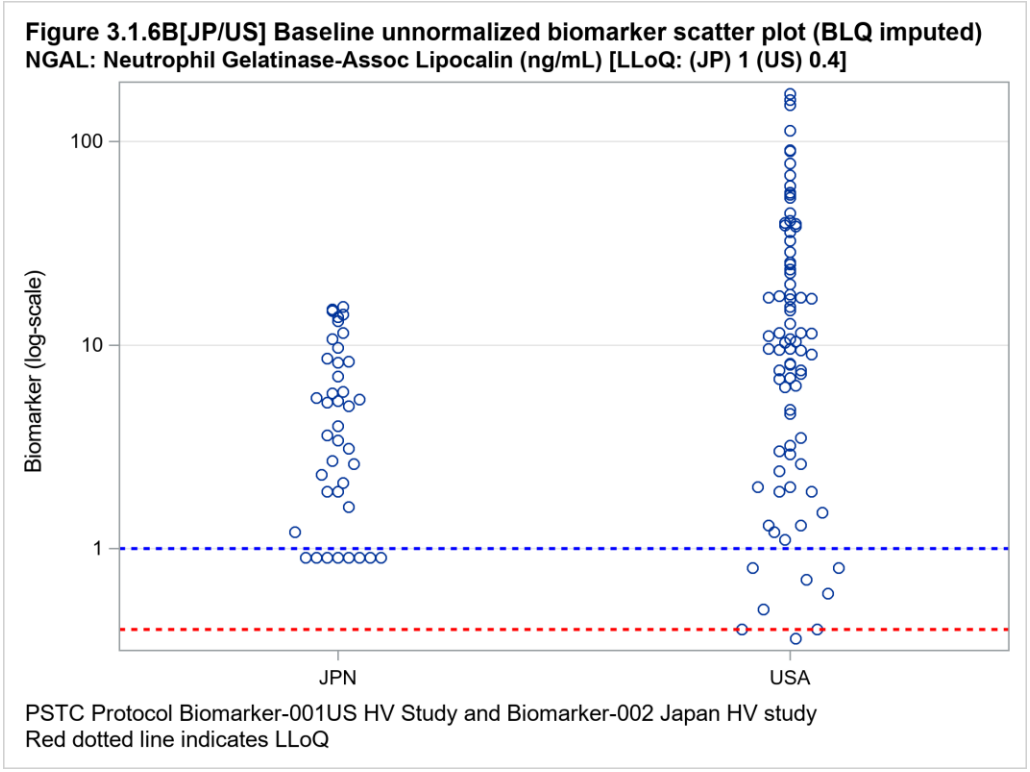
Unnormalized BM (BLQ imputed)	ALB		CLU		CysC		KIM1		NAG		NGAL		OPN		TPRT	
	JPN	USA	JPN	USA	JPN	USA	JPN	USA	JPN	USA	JPN	USA	JPN	USA	JPN	USA
N	40	81	40	80	40	81	40	81	40	80	40	81	40	81	40	81
GM [1)]	2.71	3.44	133.52	93.16	18.48	20.21	0.32	0.30	1.41	1.34	3.75	9.36	706.24	837.23	5.16	5.08
CV(%) [2)]	86.2	105.2	139.6	166.0	157.7	266.3	92.4	242.4	75.7	133.6	127	316.0	219.4	185.5	37.9	59.2

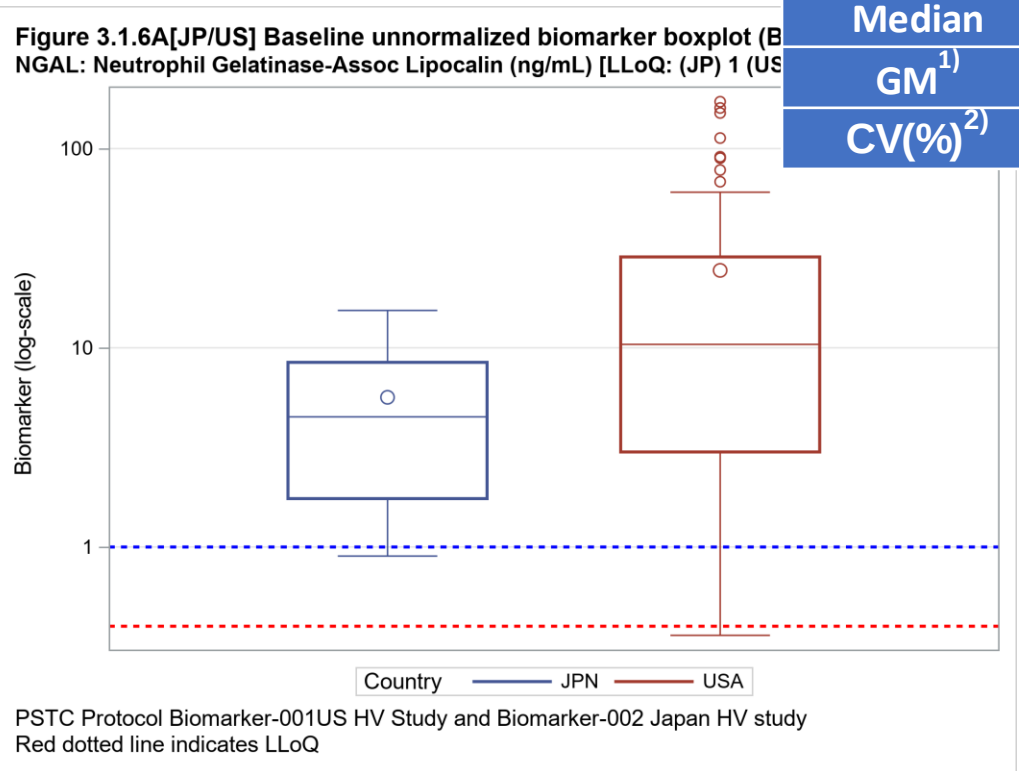
1) GM: geometric mean, 2) CV: geometric coefficient of variation

NGAL (unnormalized)

- Scatter plot (left) and the corresponding boxplot (right) are shown for JPN and USA
  - LLoQ differ between JPN (LLoQ=1) and USA (LLoQ=0.4)
  - GM for USA (9.36) was >2 time higher than JPN (3.75)
  - CV for USA (316.0) was >2 times higher than JPN (127.0)

BLQ imputed	JPN	USA
N	40	81
Mean	5.64	24.48
Median	4.5	10.4
GM[1)]	3.75	9.36
CV(%)[2)]	127.0	316.0





1) GM: geometric mean, 2) CV: geometric coefficient of variation

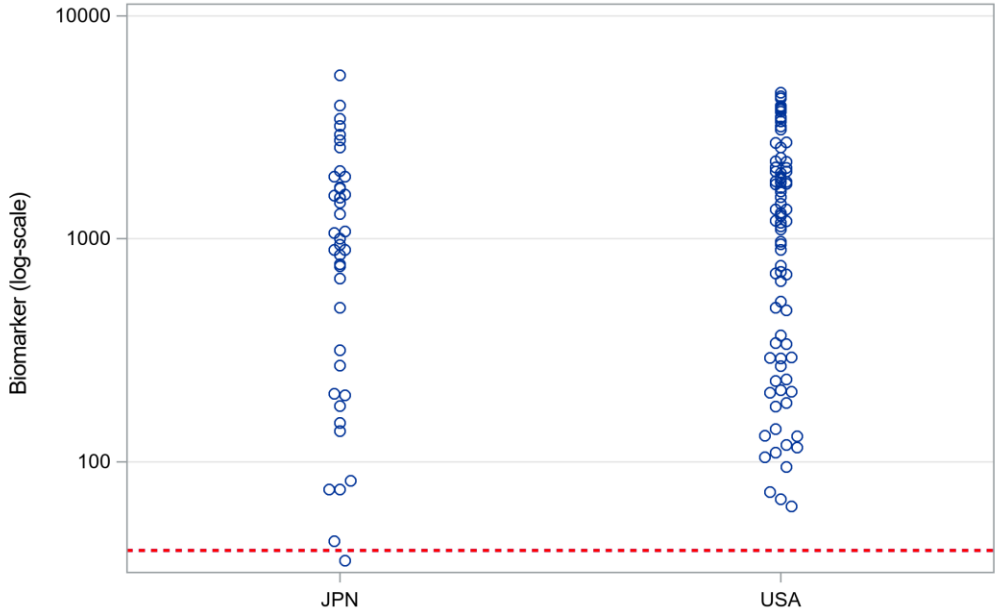
Box: Q_1, Q_2 (median), Q_3
Circle: mean
Whisker: min/max within 1.5xIQR from Q_1/Q_3

OPN (unnormalized)

- Scatter plot (left) and the corresponding boxplot (right) are shown for JPN and USA
  - LLoQ was 0.4 for both JPN and USA
  - GMs and CVs were comparable between USA and JPN

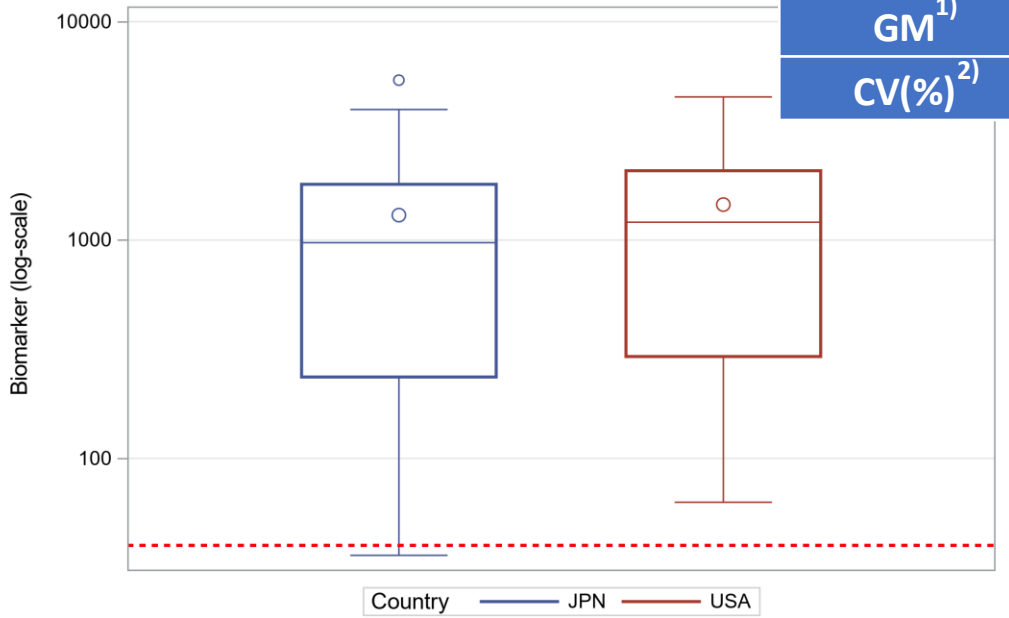
BLQ imputed	JPN	USA
N	40	81
Mean	1301	1454
Median	973	1207
GM[1)]	706	837
CV(%)[2)]	219.4	185.5

Figure 3.1.7B[JP/US] Baseline unnormalized biomarker scatter plot (BLQ imputed)
OPN: Osteopontin (ng/mL) [LLoQ: (JP) 40 (US) 40]



PSTC Protocol Biomarker-001US HV Study and Biomarker-002 Japan HV study
Red dotted line indicates LLoQ

Figure 3.1.7A[JP/US] Baseline unnormalized biomarker boxplot (B
OPN: Osteopontin (ng/mL) [LLoQ: (JP) 40 (US) 40]



PSTC Protocol Biomarker-001US HV Study and Biomarker-002 Japan HV study
Red dotted line indicates LLoQ

1) GM: geometric mean, 2) CV: geometric coefficient of variation

Box: Q_1, Q_2 (median), Q_3
Circle: mean
Whisker: min/max within 1.5xIQR from Q_1/Q_3

BM: Baseline (continued)

- uCr normalized BM value
  - GM for JPN was greater than 2-times that of USA for CLU (smaller difference for other BMs)
  - CV was generally larger for USA compared to JPN for all BMs, except for OPN
  - GM of the normalizing factor (uCr) was larger in the USA study (90.82) compared to the JPN study (53.87)

Normalized BM (BLQ imputed)	ALB		CLU		CysC		KIM1		NAG		NGAL		OPN		TPRT	
	JPN	USA	JPN	USA	JPN	USA	JPN	USA	JPN	USA	JPN	USA	JPN	USA	JPN	USA
N	40	81	40	79	40	81	40	81	40	79	40	81	40	81	40	81
GM[1)]	5.01	3.79	247.75	110.51	34.28	22.25	0.60	0.33	2.62	1.65	6.95	10.31	1310.88	921.90	95.83	55.89
CV(%)[2)]	53.2	132.8	54.5	61.7	35.9	54.3	54.0	85.0	45.9	47.8	120.1	138.4	94.6	56.1	69.3	96.5

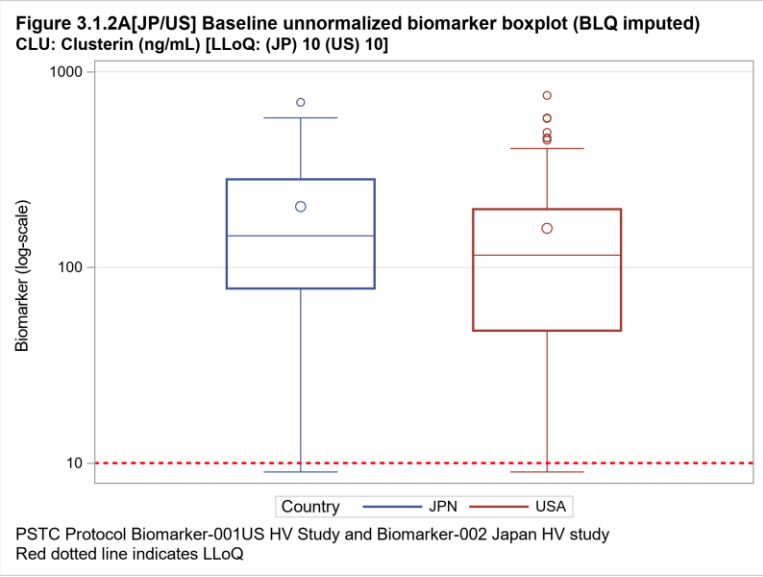
uCr	JPN	USA
N	40	81
GM[1)]	53.87	90.82
CV(%)[2)]	104.7	157.3

1) GM: geometric mean, 2) CV: geometric coefficient of variation

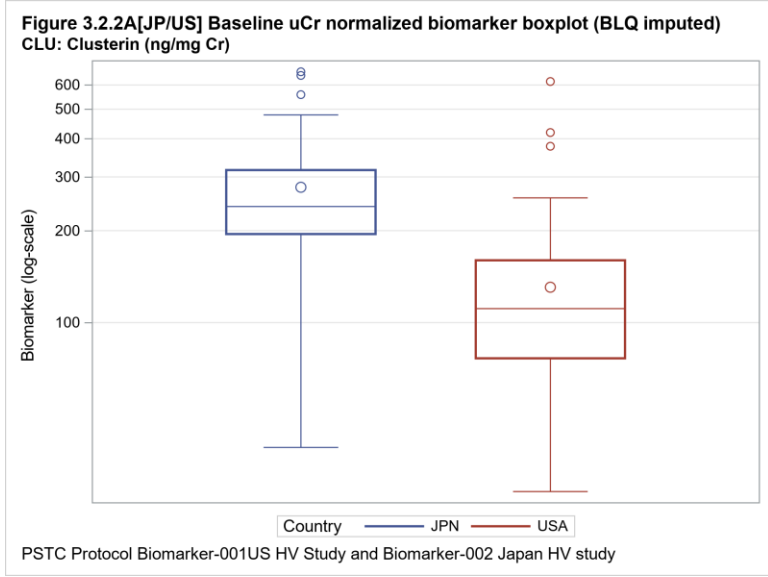
CLU and uCr

(See backup for other BMs)

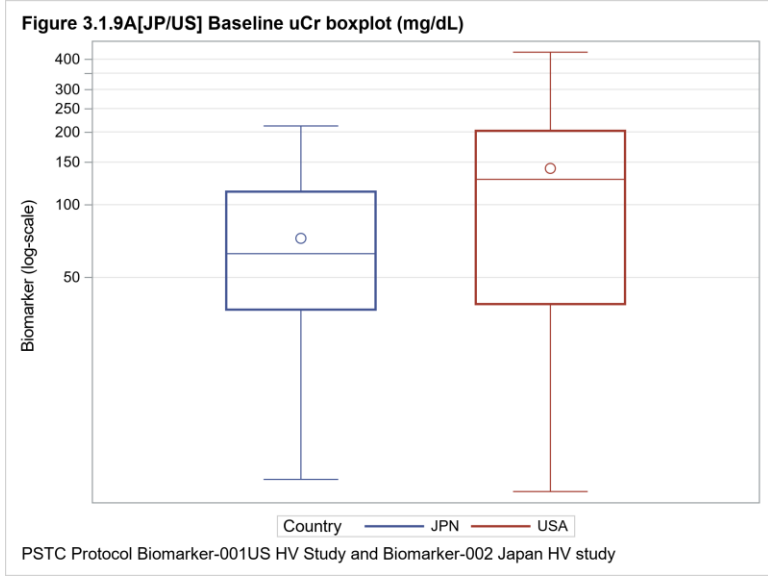
CLU (unnormalized)



CLU (uCr normalized)



Normalizing factor (uCr)



Unnormalized	CLU	
	JPN	USA
N	40	80
Mean	204.63	158.49
Median	145.00	115.50
GM [1)]	133.52	93.16
CV(%) [2)]	139.6	166.0

Normalized	CLU	
	JPN	USA
N	40	80
Mean	277.54	130.60
Median	240.00	111.11
GM [1)]	247.75	110.51
CV(%) [2)]	54.5	61.7

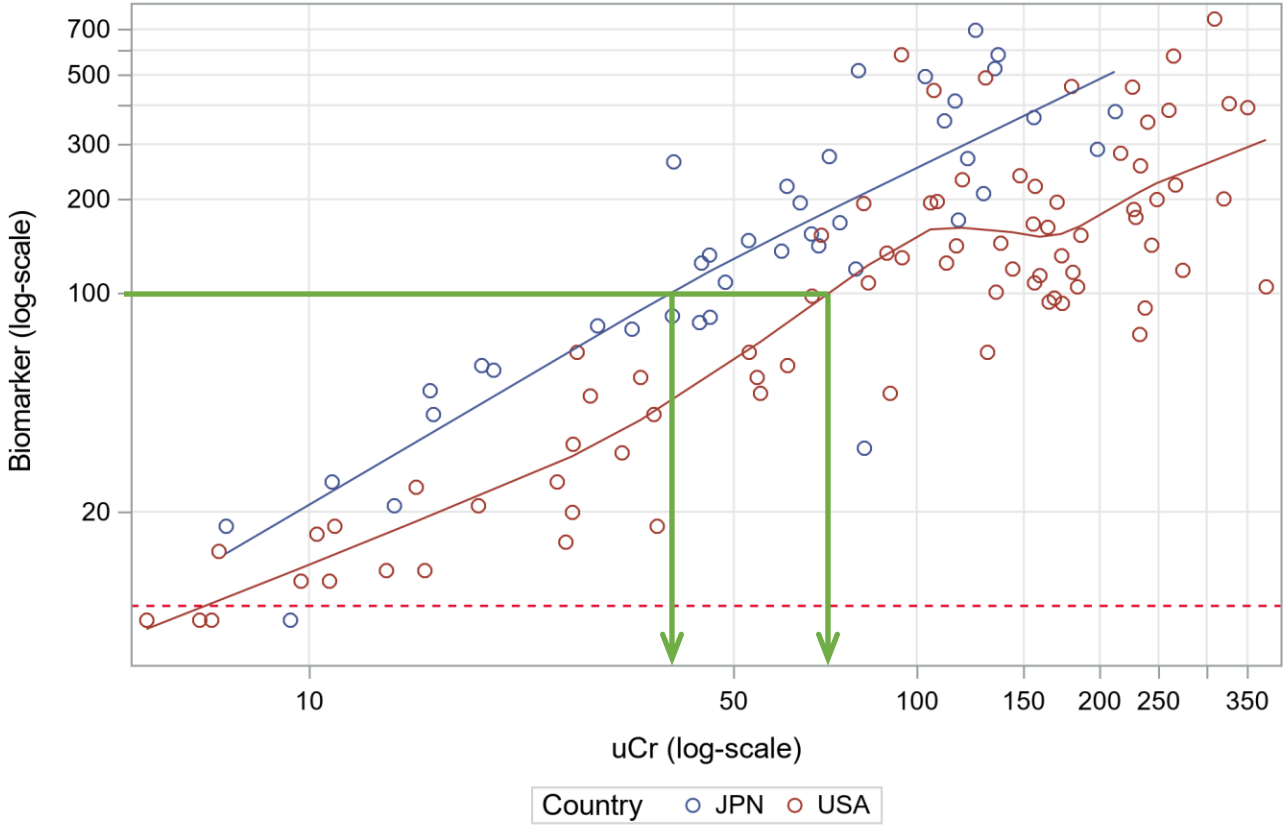
5	uCr	
	JPN	USA
N	40	80
Mean	72.57	141.28
Median	62.70	127.30
GM [1)]	53.87	90.82
CV(%) [2)]	104.7	157.3

Exploratory analyses of CLU *vs* uCr

Relationship between unnormalized CLU and the uCr was explored.

- The trend lines are approximately parallel between the Japan study (blue) and US study (red).
- The trend line is higher for the Japan study
- uCr values corresponding to the same CLU value (e.g., =100) is lower for JPN study compared to the US study.

Figure 9.1.2 Baseline biomarker (unnormalized) vs. uCr
CLU: Clusterin (ng/mL) [LLoQ: 10 (JP), 10 (US)]



PSTC Protocol Biomarker-001US HV Study and Biomarker-002 Japan HV study

2.3 BM: Inter- and intra-subject variabilities

- For each BM, measurements from 2 visits were used to calculate the inter- and intra-subject coefficient of variations (CV).
- Study visits for the Japan study
  - Visit 1 (Baseline), Visit 2 (5 to 7 days from Visit 1)
- Study visits for the US study
  - US study consisted of 4 visits
    - Visit 1 (Day 1), Visit 2 (Day 2), Visit 3 (Days 5 to 7), Visit 4 (2 weeks after Visit 3)
  - For the comparative analyses ,Visit 1 and Visit 4 were used, since all BMs were measured in Visit 4, while only 2 BMs were available in Visit 3. (see backup for details)

2.3 BM: Inter- and intra-subject variabilities

Model without covariates		Number of obs.	Inter-subject CV(%)	USA/JPN ratio of inter-subject CV	Intra-subject CV(%)	USA/JPN ratio of intra-subject CV
Biomarker	Country					
ALB	JPN	71	20.24		64.12	
	USA	160	75.87	3.748	63.92	0.997
CLU	JPN	79	32.97		100.64	
	USA	155	71.88	2.180	93.10	0.925
CysC	JPN	78	55.47		94.84	
	USA	152	86.55	1.560	101.62	1.071
KIM1	JPN	59	37.81		60.32	
	USA	157	93.79	2.481	100.19	1.661
NAG	JPN	60	36.13		48.60	
	USA	136	40.90	1.132	74.17	1.526
NGAL	JPN	70	51.69		57.57	
	USA	161	169.99	3.289	113.21	1.966
OPN	JPN	79	61.80		116.22	
	USA	161	81.75	1.323	101.88	0.877
TPRT	JPN	67	16.42		25.98	
	USA	100	32.09	1.955	40.99	1.578

BM: Inter- and intra-subject variabilities (continued)

- For the Japan study, inter-subject CV ranged between (16%, 62%) and intra-subject CV ranged between (26%, 116%).
- The ratio of inter-subject CVs between Japan and US ranged between (1.13, 3.75), indicating larger values for the US study.
- The same ratio for intra-subject CV ranged between (0.88, 1.97).
  - It should be noted, however, that for the US study, time between the visits were 2 weeks longer compared to the Japan study. The possible impact of this difference may warrant further assessment.

2.4 BM: Specificity

- The aim of the kidney safety BMs is to identify potential kidney function problems with high sensitivity and specificity.
  - **Sensitivity** is the probability of positive BM test results (indicating potential kidney problems) among those subjects with true kidney problems.
  - **Specificity** is the probability of negative BM test results among those subjects without kidney problems.
- Since the current study subjects are normal healthy adults, only the specificity can be evaluated.

BM: Specificity (continued)

The specificity of each BM was evaluated using the statistically significant thresholds (T_{ss})

- For ALB and TPRT, Visit 2 value greater than 1.0×ULN was considered as "positive" for potential kidney problems
- For all other BMs, the **Fold Change** ratio (Visit 2/Visit 1) greater than the indicated T_{ss} value was considered "positive" for potential kidney problems.

(see Backup for more details)

BM	ULN	Statistically Significant Thresholds (T_{ss})[1)]
ALB	30 µg/mg uCr	> 1.0×ULN
CLU	301 ng/mg uCr	FC > 2.6
CysC	0.052 µg/mg uCr (52 ng/mg uCr)	FC > 2.2
KIM1	1.19 ng/mg uCr	FC > 3.1
NAG	3.54 mU/mg uCr	FC > 3.9
NGAL	87.6 ng/mg uCr	FC > 3.5
OPN	2.10 µg/mg uCr (2100 ng/mg uCr)	FC > 3.1
TPRT	0.20 mg/mg uCr (200 µg/mg uCr)	> 1.0×ULN

1) FC: fold change

BM: Specificity (continued)

Specificity (BLQ imputed)					
	No. of subjects		Specificity[1)]		
	Total	Negative / Positive	Estimate	95%CI	
				Lower	Upper
ALB	40	40 / 0	1.000	0.912	1.000
CLU	40	38 / 2	0.950	0.831	0.994
CysC	40	39 / 1	0.975	0.868	0.999
KIM1	40	39 / 1	0.975	0.868	0.999
NAG	40	40 / 0	1.000	0.912	1.000
NGAL	40	36 / 4	0.900	0.763	0.972
OPN	40	34 / 6	0.850	0.702	0.943
TPRT	40	37 / 3	0.925	0.796	0.984

1) Proportion of BM negative subjects

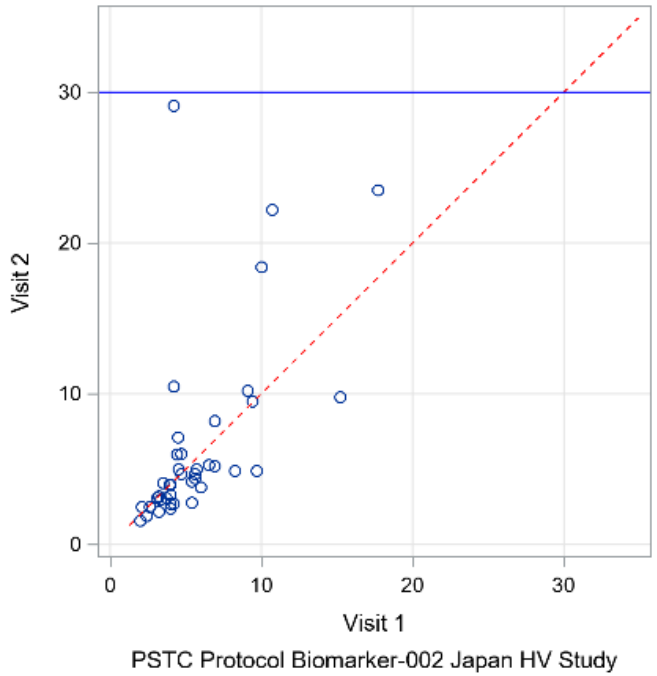
BM: Specificity (continued)

- The estimate of specificity ranged between 0.850 and 1.000.
- Also, the lower limit of the 95%CI of specificity was above 0.7 for all BMs
  - This satisfied the prespecified criteria in the protocol
- Results of analysis that excluded subjects with BLQ were similar (see backup).

Graphical presentation of specificity

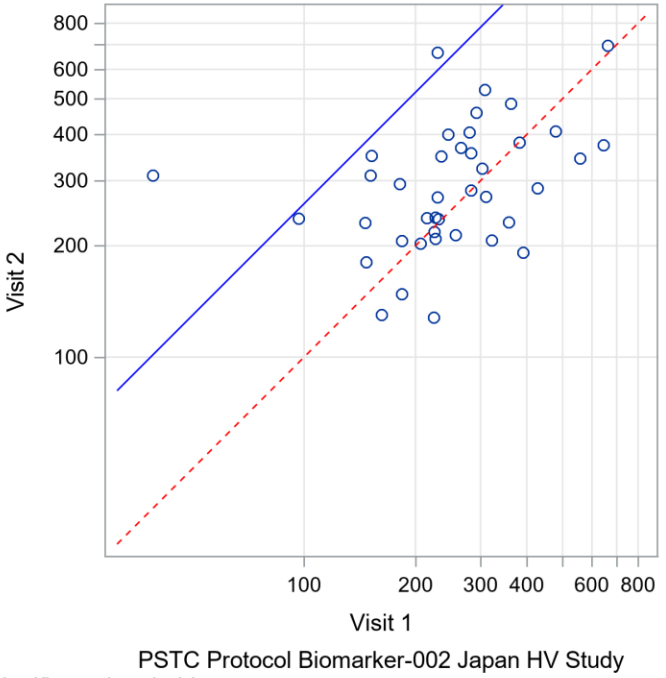
- Visit 1 vs. Visit 2 values of uCr normalized BM (BLQ imputed) for evaluation of specificity
- ALB with T_{ss}: 1xULN (left panel); CLU with T_{ss}: FC>2.6 (right panel)
- Blue line indicates T_{ss} criteria for each BM, red dotted line indicates 45-degree line.

Figure 7.1.1B uCr normalized biomarker values and Tss (BLQ imputed, log-scale)
ALB: Albumin (µg/mg Cr) [Tss: Visit2>30]



Tss: statistically significant threshold

Figure 7.1.2B uCr normalized biomarker values and Tss (BLQ imputed, log-scale)
CLU: Clusterin (ng/mg Cr) [Tss: FC>2.6]



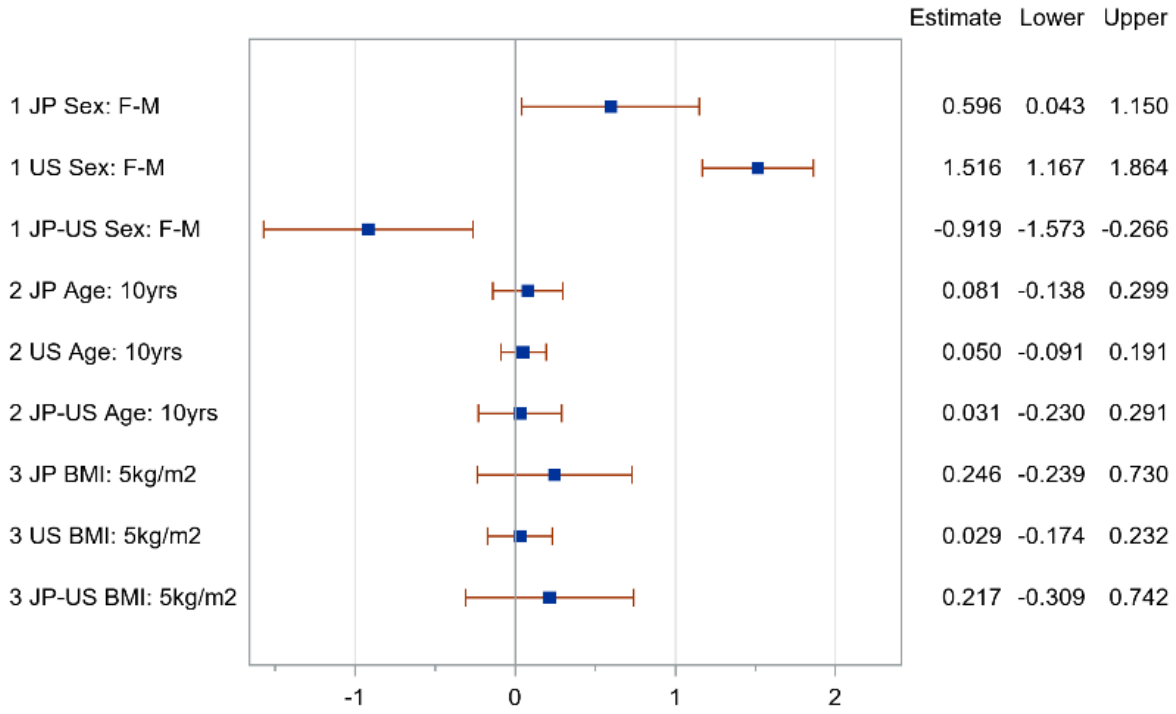
Tss: statistically significant threshold

(see Backup for other BMs)

2.5 BM: Effect of covariates

- For the unnormalized BMs, there were no inconsistency in trend between the Japan and US studies for all covariates. Also, there was no covariate with positive (or negative) effect in both studies.
- For the uCr normalized BMs, there was a consistent trend toward larger BM values for females compared to males in both studies for KIM1, NAG, NGAL, and TPRT. Also, the degree of female/male difference was more extreme for the US study for NGAL.

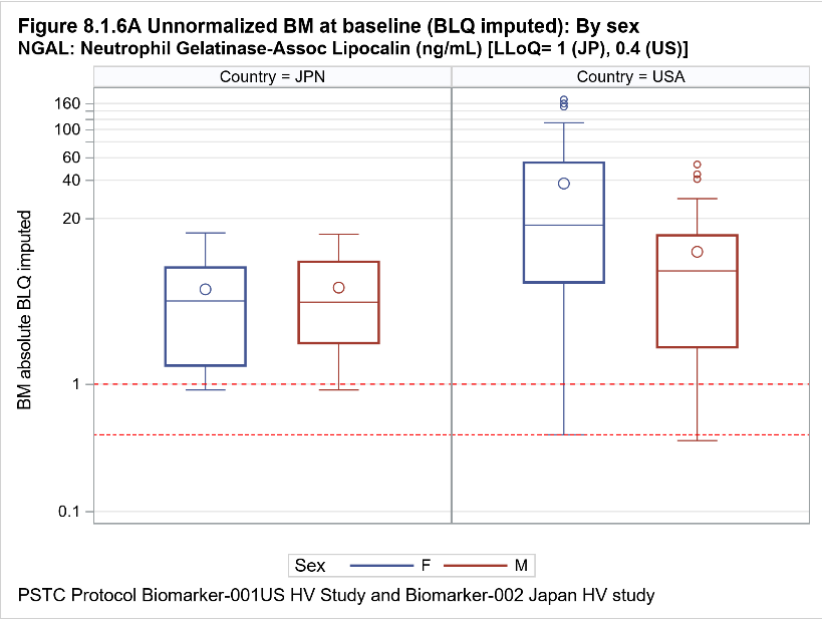
Display 8.2.6 Effect of covariates on BM based on linear model (Estimate, 95%CI)
Baseline value: log-transformed, uCr normalized, BLQ imputed
Biomarker: NGAL



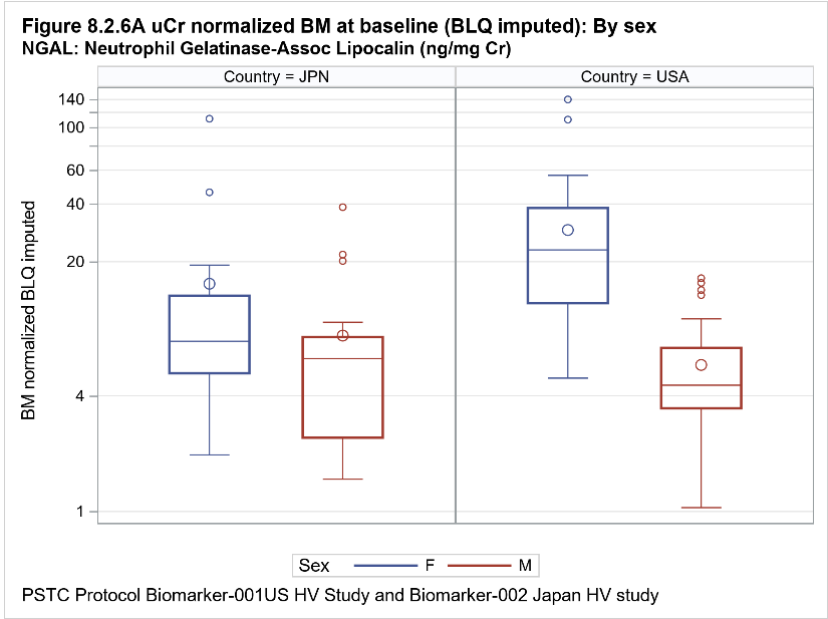
PSTC Protocol Biomarker-001US HV Study and Biomarker-002 Japan HV study

BM: Effect of covariates (continued)

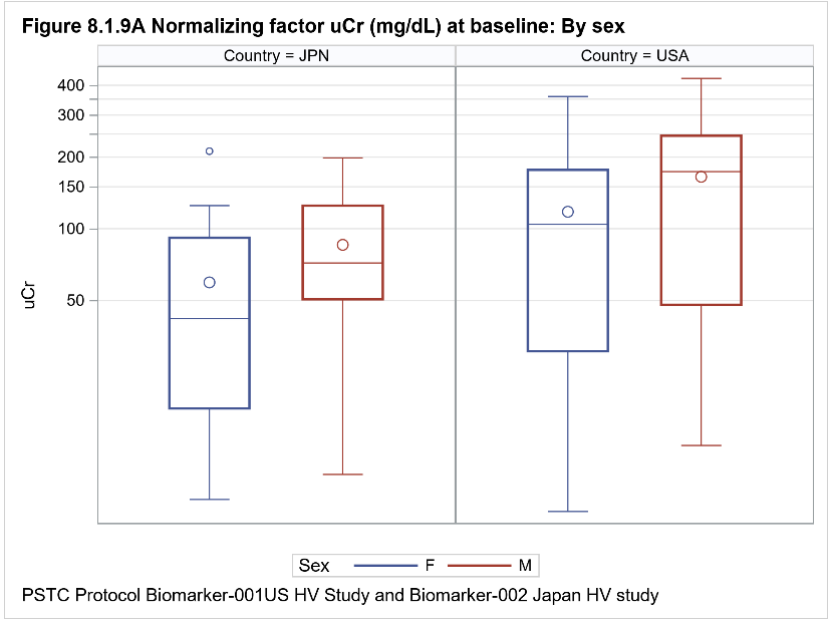
NGAL (unnormalized)



NGAL (uCr normalized)



Normalizing factor (uCr)



- Female/male difference is amplified for uCr normalized values compared to unnormalized values
- uCr values tend to be lower for females compared to males in both studies.

Box: Q_1, Q_2 (median), Q_3
Circle: mean
Whisker: min/max within 1.5xIQR from Q_1/Q_3

3. Summary

- Distribution of age and sex was similar for the 2 studies. Body weight and BMI were higher in the US study.
- There were some differences in mean level and variability between Japan and US studies for some BMs.
- The specificity of BMs ranged between 0.850 and 1.000.
- There were some differences in mean level of uCr normalized BM values between males and females.
- For the next step
  - Assess the impact of the observed differences on the next stage of the project

Thank You

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