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Kidney safety biomarkers in humans & approach to interpret emerging exploratory biomarkers

EUFEMED Conference

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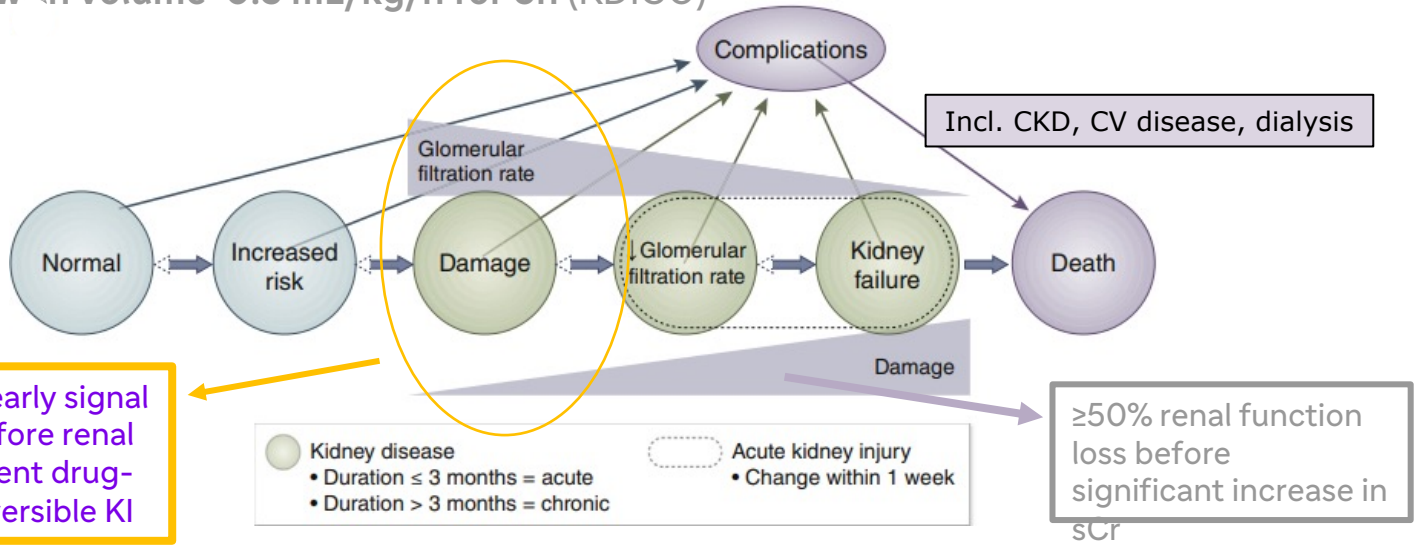
Drug-Induced Kidney Injury (DIKI) & safety biomarkers

- **DIKI contributes up to 25% of all cases of acute kidney injury (AKI)**
  - **AKI defined with acute rise of serum creatinine (sCr) by ≥0.3 mg/dl** (>26.5 µmol/l) **within 48h OR increase in sCr ≥1.5 x BSL known or presumed within the prior 7 days OR decrease in urine output with volume<0.5 mL/kg/h for 6h** (KDIGO)



Need BMs to detect early signal of kidney damage before renal function loss, to prevent drug-induced AKI and irreversible KI

≥50% renal function loss before significant increase in sCr

Source: Kidney International (2020) 97, 1117–1129 - Reprinted from The Lancet, Volume 382, Eckardt KU, Coresh J, Devuyst O, et al. Evolving importance of kidney disease: from subspecialty to global health burden, Pages 158–169, Copyright 2013, with permission from Elsevier

Drug-Induced Kidney Injury (DIKI) & safety biomarkers

- **Conventional renal BMs with clear limitations** (in animals & humans)
  - **Serum creatinine (sCr)** (Creat. clearance as indirect evaluation of GFR) & **blood urea nitrogen (BUN)**
  - **Lack of specificity** (change w/o KI)**/sensitivity** (KI w/o change) **& w/o information on KI site**
    - *Little & delayed change of BUN & sCr until ≥ 50% of renal function loss (in both human and animal)*
    - *Factors of variation (renal/extra-renal: tubular secretion/reabsorption, gender, muscle, age, diet, etc)*
  - **Functional BMs (not lesional) may increase due to an impaired reabsorption when tubular damage**
  - **Common BMs in urine** (spot Urinary Protein-to-Creat Ratio UPCR, Albumin-to-Creat Ratio UACR)
    - *Urinalysis, Total protein (incl. albumin, Ig, microglobulins), Albumin or microalbumin: glomerular damage and/or decreased re-uptake of proteins by proximal tubules when proximal tubular damage*
- **Other GFR marker with performance > sCr: serum cystatin C** (% change from BSL)
- **Urinary early** (within ≥ 24-48h of injury) **& sensitive BMs of kidney damage**

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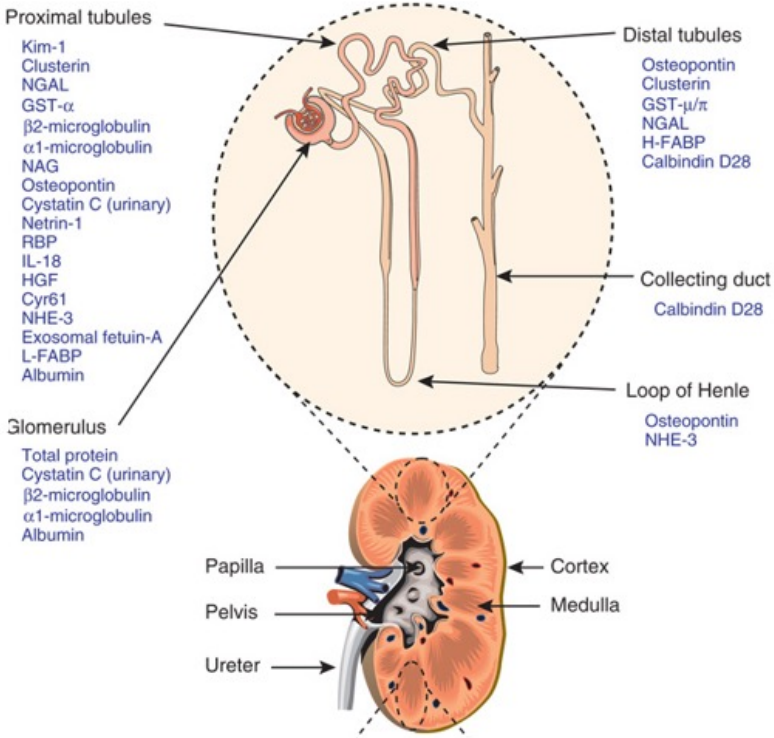
Translational BM strategy for clinical monitoring

Sensitive (early diagnosis) **& specific** (minimal false +) **BMs of DIKI**

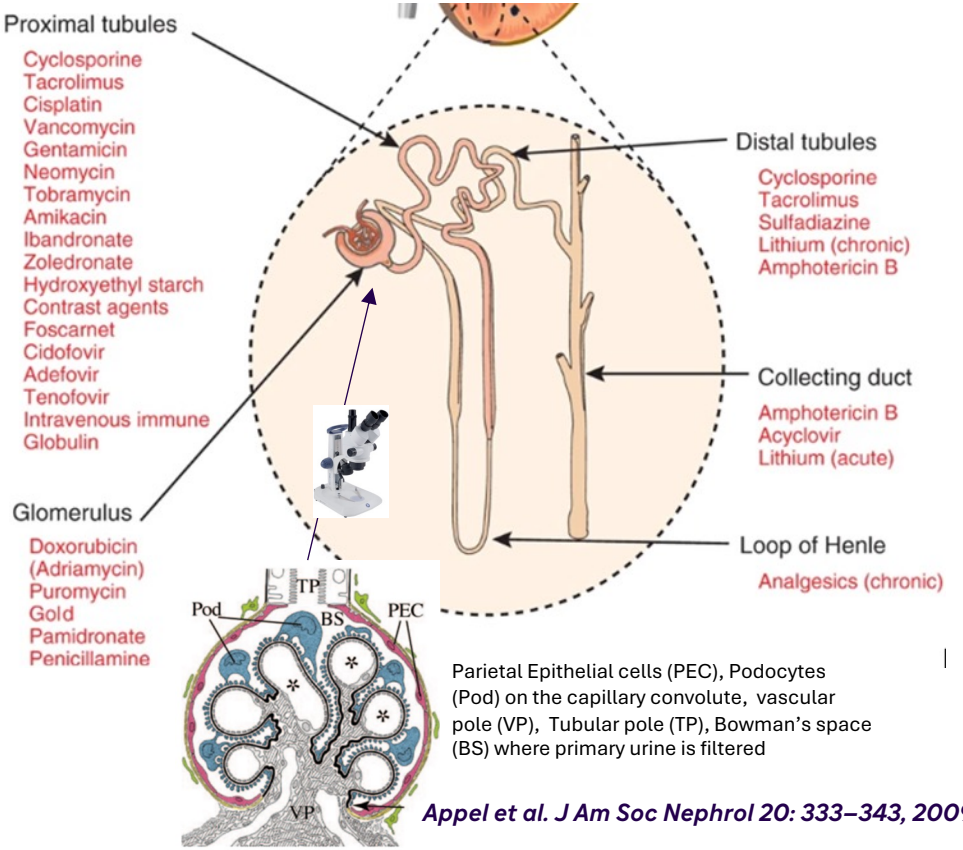
- **To ensure optimal healthy volunteer/patient renal safety**
  - **Across multiple animal species & in humans, with similar reliability**
  - **Timing, severity, reversibility, injury site, underlying KI mechanisms** (tubular reab., necrosis, inflammation, regeneration)
  - **Easily accessible** (spot urine) **& BMs normalized to Ur creat**
- **To permit early detection of DIKI at an early stage of drug dev.**
  - **HA encouragement for exploratory use of novel Ur BMs in preclinical & early clinical studies**
  - **Appropriate assay performance to further assess sensitivity/specificity of each BM**
  - **Study design & time course of BM testing based on time to threshold/time to maximal change, drug PK, time course and pattern of preclinical injury**

Biomarkers to detect DIKI



BMs to detect injury to specific nephron segments affected by various nephrotoxicants

Bonventre JV et al. Nat Biotechnol 2010;28(5):436-40



Parietal Epithelial cells (PEC), Podocytes (Pod) on the capillary convolute, vascular pole (VP), Tubular pole (TP), Bowman's space (BS) where primary urine is filtered

Appel et al. J Am Soc Nephrol 20: 333–343, 2009

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Exploratory use of Ur BM panel for early detection of DIKI

Urinary BMs	Preclinical* (species-dep.) C-Path PSTC	Clinical/IMI safe T (2016) (w/o NAG, incl. cis-treated)	Clinical/ FDA** (2018) (w/o GSTα, incl. cis-treated)	Clinical/Transbioline (EU) **** (2019-2025)
Glomerular	Total protein (b), cystatin C, β2-microg., albumin (μ)	cystatin C	cystatin C	Podocyte BMs: NHPS1, NPHS2, PODXL / Vascular BMs: MMP3, VCAM1
Prox. Tubular	KIM-1 (a, slightly for b), clusterin (b ++), NGAL (a), NAG, OPN (a), cystatin C (a), albumin(μ) (a, b)	KIM-1, clusterin, NGAL, **GST-α**, OPN, (***): cystatin C	KIM-1, clusterin, NGAL, **NAG**, OPN, (***): cystatin C	KIM-1, clusterin, NGAL, OPN, (***): cystatin C, AMBP, RBP4
Dist. Tubular	OPN (a) (dist. Incl. loop of Henle > prox.), clusterin (b++), NGAL	OPN, clusterin, NGAL	OPN, clusterin, NGAL	Presumably : OPN, clusterin, NGAL
Loop of Henle	OPN	OPN	OPN	
Collecting duct	Clusterin	Clusterin	Clusterin	

(*) Qualified in rats (2008 C-Path PSTC Nephrotoxicity WG /reg agencies FDA, EMA, PMDA); (**) FDA qualified Safety BM panel (2018); (***) freely filtered by glomerulus, then reabsorbed, except if tubular damage; (****) Analytical validation of new candidate BM podocalyxin with a 5-plex assay (nephrin, podocin, podocalyxin, NGAL, KIM-1) of mixed glomerular/tubular injury BM panel assay; (**a**) + mice (Adeyemi et al. 2021); (**b**) + dog (Adeyemi et al. 2022)

NGAL: Neutrophil Gelatinase-Associated Lipocalin, KIM-1: Kidney Injury Molecule-1, NAG: N-acetyl-β-D-glucosaminidase, OPN: osteopontin, GST α : Glutathion S-Transférase α, NHPS1 : nephrin, NPHS2 : podocin, PODXL: podocalyxin, MMP3: matrix metalloproteinase 3, VCAM1: vascular cell adhesion molecule 1, AMBP: α1-microglobulin/bikunin precursor, RBP4: retinol binding protein 4

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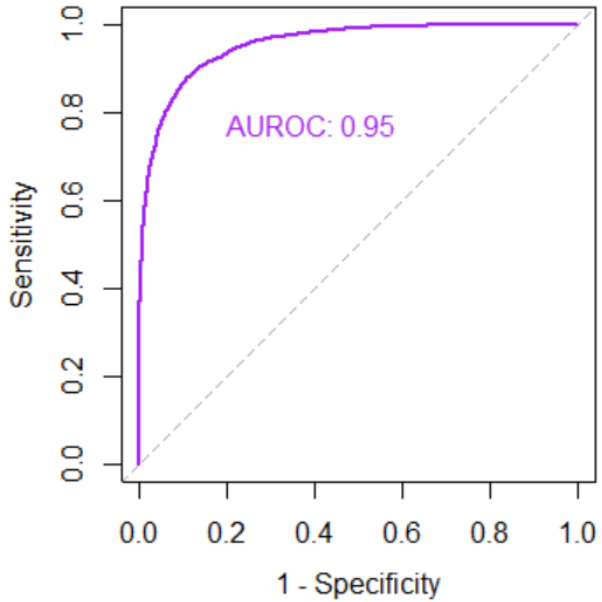
Qualification of selected novel BMs of kidney injury

Qualification for the proposed context of use (COU) to detect DIKI, using AKI criteria & Ur BM corrected for Ur creatinine with % change from BSL

- **BM accuracy/performance based on supportive fit-for-purpose clinical studies**
  - ROC (Receiver Operating Charateristics) as plot of the true positive rate (sensitivity) vs. the false positive rate (1 - specificity) & AUROC (Area Under the ROC Curve) to assess discriminatory performance of each BM



Selected Novel Biomarkers – Performance

(IMI SAFE-T consortium/DIKI group – supported by EMA & FDA)

Table 11-1: Biomarker accuracy based on maximum percent change from baseline of urinary creatinine corrected values comparing treated patients versus non-treated control patients (Cisplatin study)

Biomarker	Number of subjects cisplatin Treated	Non treated	Biomarker performance AUROC and 95% CIs	
BUN	102	17	0.95 [0.89;0.99]	Good performance
u. osteopontin	88	17	0.95 [0.90;0.98]	
u. albumin	92	17	0.95 [0.90;0.98]	
u. KIM-1	88	17	0.95 [0.87;0.99]	
u. total protein	95	17	0.93 [0.84;0.99]	
s. cystatin C	103	16	0.92 [0.85;0.97]	
s. creatinine	103	17	0.84 [0.74;0.92]	Relatively good performance
estimated GFR	103	17	0.84 [0.73;0.92]	
u. α-GST	88	17	0.84 [0.71;0.93]	
u. cystatin C	88	17	0.79 [0.64;0.90]	Relatively poor performance
u. clusterin	88	17	0.78 [0.60;0.90]	
u. NGAL	88	17	0.71 [0.52;0.85]	

Creatinine-corrected values were used for urinary biomarkers.

Single BM vs. Panel of BMs → May lead to loss of specificity vs. Improved sensitivity

Selected Novel Biomarkers – Time course of BM change

(IMI SAFE-T consortium/DIKI group – supported by EMA & FDA)

Table 11-3: Biomarker time to maximum change from baseline in AKI patients determined by adjudication committee (Cisplatin study) *Approx. 70% (70/103) AKI adjudicated with Multiple BM*

		Time to maximum change from baseline (days)			
Biomarker	**N**	**25th percentile**	**Median**	**75th percentile**	**90th percentile**
u. α-GST	70	1.0	1.0	4.0	7.0
s. cystatin C	70	2.0	2.0	4.0	7.0
u. KIM-1	70	2.0	2.0	7.0	7.0
u. osteopontin	70	1.0	3.0	7.0	7.0
u. clusterin	70	1.0	4.0	7.0	7.0
u. cystatin C	70	1.0	4.0	7.0	7.0
u. total protein	69	2.0	4.0	7.0	7.0
u. NGAL	70	1.0	4.0	7.0	14.0
BUN	70	2.0	5.5	7.0	7.0
u. albumin	68	4.0	7.0	7.0	7.0
s. creatinine	70	4.0	7.0	7.0	14.0

Creatinine-corrected values were used for urinary biomarkers.

From the Table 11-3 above, it can be seen that most novel urinary biomarkers reach peak change from baseline a median of 2 to 4 days after nephrotoxin exposure. Serum creatinine reaches maximum change from baseline a median of 7 days after exposure.

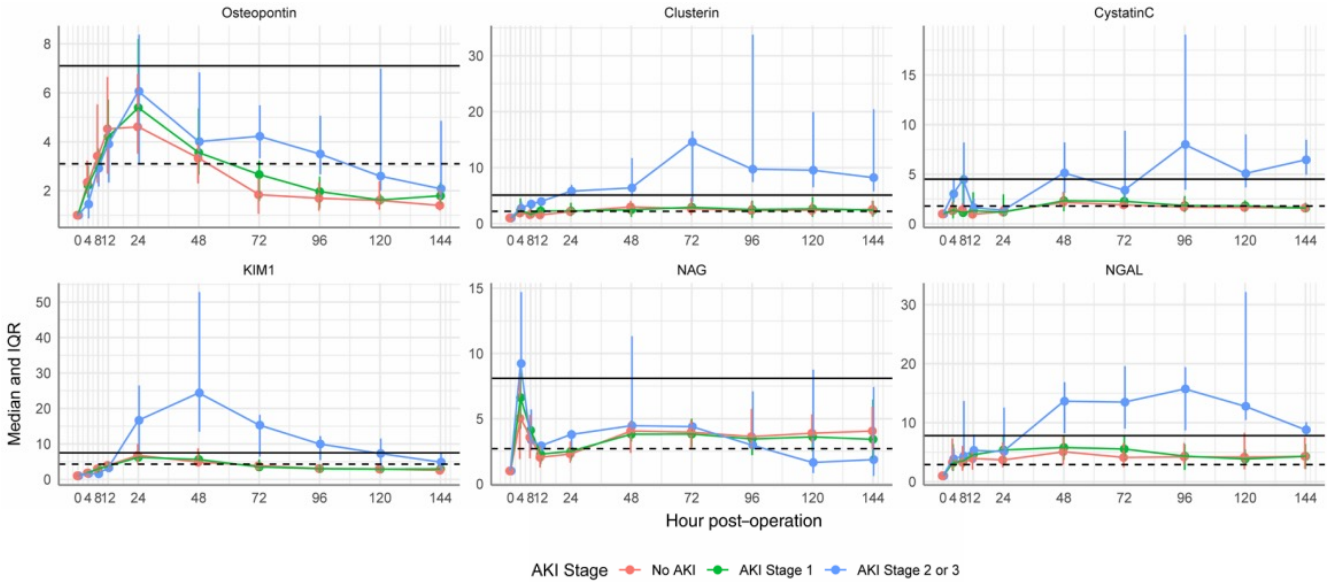
Overall take-home message

- Exploratory data (limitations)
- Use % change from baseline for uCr corrected values
- BSL value from sensitive assays
- At least 1-week follow-up

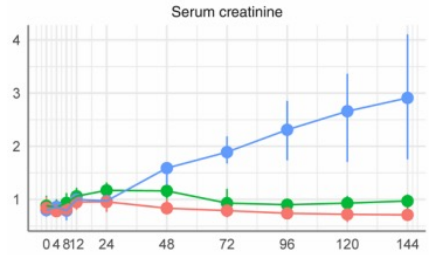
FDA qualified safety BM panel: recent review in oncology pts

Longitudinal change in Ur BMs in mesothelioma surgery pts (resection & intrathoracic CIS)

UCr-normalized fold-changes from BSL: **early detection of DIKI**



Fold-changes from BSL



Vertical bars: 25th and 75th percentile values
-- line: BM's "**statistically significant threshold**", differentiate mesothelioma cis-treated with AKI (n=24) vs. HV (n=81)
— line: BM's "**medically significant threshold**", differentiate mesothelioma pts with (n=24) & without AKI (n=26)

Waikar SS et al. Urinary Kidney Injury Biomarker Profiles in Healthy Individuals and After Nephrotoxic and Ischemic Injury. Clin Pharmacol Ther. 2025 doi:10.1002/cpt.3531

FNIH/PSTC: Composite Measure (User's Guide 2019)
Interpretation of novel BM panel in addition to traditional measures

- **Help early detection of renal tubular injury in Ph1 studies in normal healthy vol rs (NHV) when preclinical signal identified**
- **Panel of 6 urinary creatinine-normalized BMs** (CLU, CysC,
- **Formula to calculate the composite measure (CM) at each p**
  - **Calculate CM for each subject as geometric mean (GM) of the 6 BM**
  - **Calculate group GM CM in a dose cohort** (separately for active and p
- **Compare against CM threshold selected based on study design, cohort size, and probability of threshold exceeding normal variability**
- **Qualified by FDA in Sep 2018** (can be used for INDs, CTAs, NDAs
  - **Non-drug biomarker study in healthy volunteers** (n=81)
  - **Retrospective study in cisplatin-treated mesothelioma patients** (n=

Probability level **commensurate with the unmet medical need** in the indication under investigation

Not (yet) qualified for **individual** safety monitoring of normal healthy volunteers or for Patient **studies**

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Application to FIH in Healthy Volunteers

- **Assessing a drug study group alone or vs a comparator/placebo group:**

Table 1: Thresholds for the observed GM CM across various sample sizes (n/group) that would be expected with less than 5% probability from a drug study group consistent with an NHV population

	Thresholds based on NHV population: observed measures greater than or equal to thresholds are expected to be observed with less than 5% probability in an NHV population	
Sample Size (n/group)	Threshold when assessing a drug study group alone GM CM	Threshold when assessing a drug study group relative to a comparator/placebo group (ratio of GM CMs)
6	1.28	1.33
8	1.25	1.27
10	1.23	1.24
12	1.22	1.22
14	1.21	1.20
16	1.20	1.19
18	1.19	1.18
20	1.18	1.16

GM = geometric mean
CM = composite measure of the fold change from baseline of urine CLU, CysC, KIM-1, NAG, NGAL and OPN, normalized to urine creatinine

Table 2: Observed GM CM thresholds when assessing a drug study group *alone* with an NHV population (samples sizes of n = 6 to 20 per group)

Sample	Thresholds based on NHV population: observed measures greater than or equal to thresholds are expected to be observed with less than P% probability in an NHV population				
Size (n/group)	P = 50%	P = 20%	P = 10%	P = 5%	P = 1%
6	1.07	1.18	1.23	1.28	1.38
8	1.07	1.16	1.21	1.25	1.33
10	1.07	1.15	1.19	1.23	1.30
12	1.07	1.14	1.18	1.22	1.28
14	1.07	1.14	1.18	1.21	1.26
16	1.07	1.13	1.17	1.20	1.25
18	1.07	1.13	1.16	1.19	1.24
20	1.07	1.13	1.16	1.18	1.23

GM = geometric mean
CM = composite measure of the fold change from baseline of urine KIM-1, CLU, OPN, CysC, NAG, and NGAL normalized to uCr

Less conservative, when few treatment options

- **Table based on single timepoint without adjustment for multiplicity** (multiple post-BSL timepoints) – greater risk of false positive
- Threshold not to be compared with individual CM value, only at dose cohort level

From FNIH/PSTC Kidney Safety CM Biomarker User's Guide, May 2019

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20	1.18	1.16

GM = geometric mean
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Table 3: Observed ratio of GM CM thresholds when assessing a drug study group *relative to a comparator/placebo* with an NHV population (samples sizes of n = 6 to 20 per group)

Sample	Thresholds based on NHV population: observed measures greater than or equal to thresholds are expected to be observed with less than P% probability in an NHV population				
Size (n/group)	P = 50%	P = 20%	P = 10%	P = 5%	P = 1%
6	1.00	1.15	1.24	1.33	1.49
8	1.00	1.12	1.20	1.27	1.42
10	1.00	1.11	1.18	1.24	1.37
12	1.00	1.10	1.16	1.22	1.34
14	1.00	1.10	1.15	1.20	1.30
16	1.00	1.09	1.14	1.19	1.28
18	1.00	1.08	1.13	1.18	1.26
20	1.00	1.08	1.12	1.16	1.25

GM = geometric mean
CM = composite measure of the fold change from baseline of urine KIM-1, CLU, OPN, CysC, NAG, and NGAL normalized to uCr

Less conservative, when few treatment options

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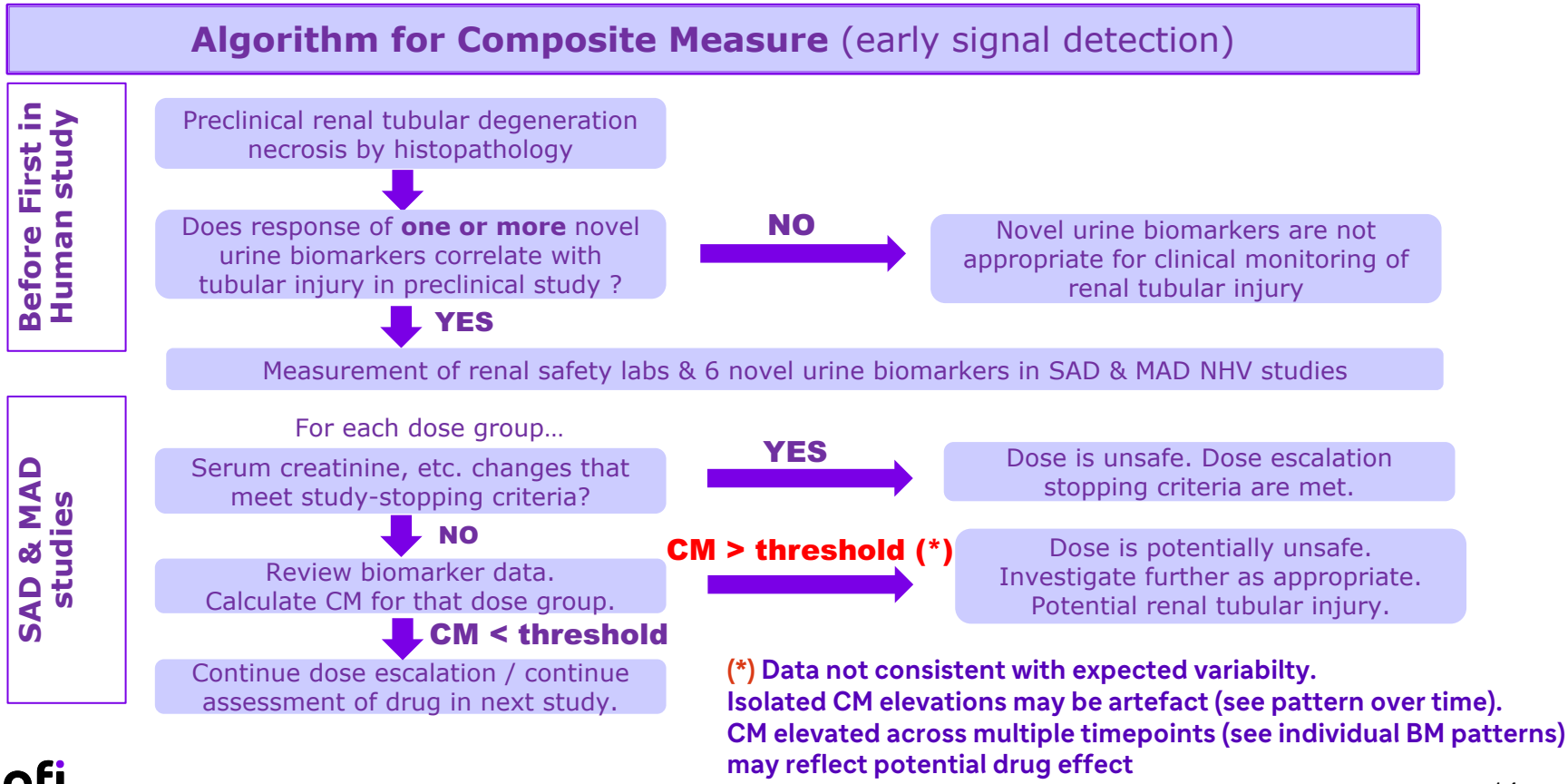
- Threshold not to be compared with individual CM value, only at dose cohort level

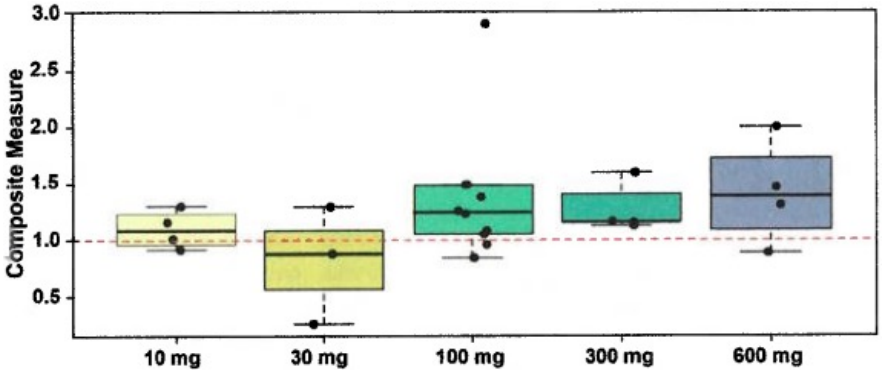
From FNIH/PSTC Kidney Safety CM Biomarker User's Guide, May 2019

Composite Measure: Application to FIH in Healthy Volunteers



Case studies

- **Pfizer (1) presenting the exploratory use of the composite measure with the BM panel:**
  - **In Phase 1 HVs** (n=12; incomplete block crossover; SAD):
    - *Adverse kidney findings observed in toxicology study, with no significant change to BUN and sCr*
    - *Consistent with preclinical data, traditional serum BMs were unaffected by PFE-1 relative to placebo*
    - *Composite measure in HVs per PFE-1 dosing regimen normalized to placebo:*



	10 mg	30 mg	100 mg	300 mg	600 mg
	n=4	n=3	n=10	n=4	n=4
GM CM (vs Pbo):	1.09	0.66	1.29	1.25	1.36

(1) Ravindra, K. C., Fader, K. A., Potter, D., Radi, Z. A., Friedman, G. S., Brenneman, K. A., ... & Vaidya, V. S. (2024). Qualified kidney injury biomarkers demonstrate value during early clinical drug development. Toxicological Sciences, 201(2), 206-215.

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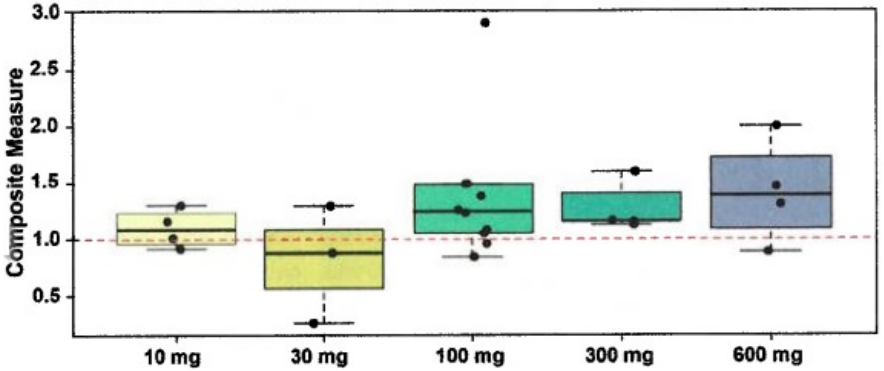

	10 mg	30 mg	100 mg	300 mg	600 mg
	n=4	n=3	n=10	n=4	n=4
GM CM (vs Pbo):	1.09	0.66	1.29	1.25	1.36

n ≤ 4 too small to make direct comparison to thresholds from CM User's guide

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Case studies

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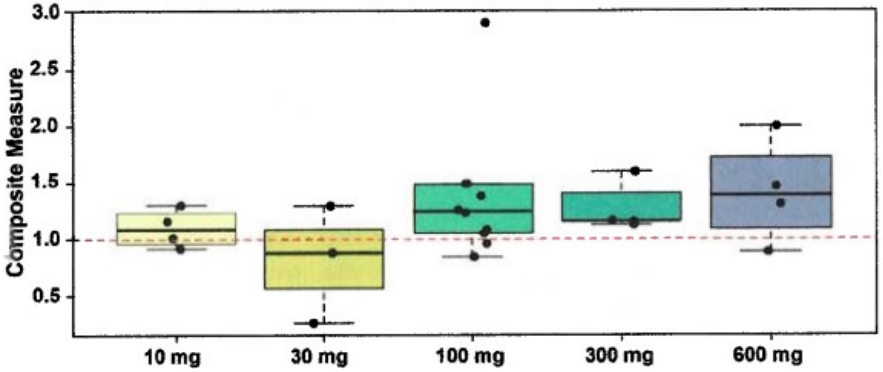
    - *Consistent with preclinical data, traditional serum BMs were unaffected by PFE-1 relative to placebo*
    - *Composite measure in HVs per PFE-1 dosing regimen normalized to placebo:*



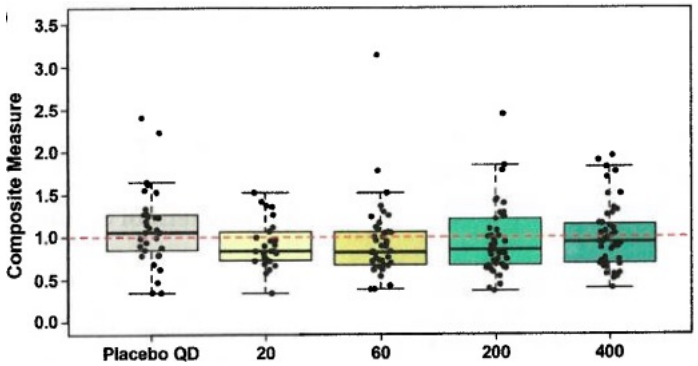
	10 mg	30 mg	100 mg	300 mg	600 mg
	n=4	n=3	n=10	n=4	n=4
GM CM (vs Pbo):	1.09	0.66	1.29	1.25	1.36

The GM CM for 100 mg (1.29) exceeds the 5% threshold for n=10 (1.24), suggesting potential kidney tubular injury

(1) Ravindra, K. C., Fader, K. A., Potter, D., Radi, Z. A., Friedman, G. S., Brenneman, K. A., ... & Vaidya, V. S. (2024). Qualified kidney injury biomarkers demonstrate value during early clinical drug development. Toxicological Sciences, 201(2), 206-215.

Case studies

- **Pfizer (1) presenting the exploratory use of the composite measure with the BM panel:**
  - **In Phase 2 rheumatoid arthritis (RA) patients** (n=266):
    - *Adverse kidney findings observed in toxicology study, with few significant change to BUN and sCr*
    - *Consistent with preclinical data, traditional serum BMs were unaffected by PFE-2 relative to placebo*
    - *Composite measure in patients treated with PFE-2:*



	Placebo QD	20	60	200	400
	n=33	n=30	n=45	n=45	n=45
GM CM:	1.02	0.91	0.86	0.89	0.94
GM CM (vs Pbo):	1	0.89	0.84	0.87	0.92

The GM CM of each dose cohort did not exceed the 5% threshold (established for HV), suggesting no evidence of kidney tubular injury in RA pop., but **limitations**

NB: Levels of 5 out of 6 ur BMs (except CystC) are elevated in RA pts vs. HV at baseline

(1) Ravindra, K. C., Fader, K. A., Potter, D., Radi, Z. A., Friedman, G. S., Brenneman, K. A., ... & Vaidya, V. S. (2024). Qualified kidney injury biomarkers demonstrate value during early clinical drug development. Toxicological Sciences, 201(2), 206-215.

Conclusion

To optimize drug development & therapeutic drug monitoring

- Race to the BM qualification for early detection of DIKI
- US/EU Consortia, supported by HAs, promoting exploratory use of safety kidney BM panel for testing, validation, qualification
- To date, **exploratory use → Need for more clinical data**
  - To better interpret the clinical significance of the CM for early DIKI detection in healthy population
  - To assess beyond the healthy population in diverse patient populations for both drug development & therapeutic drug monitoring
- Applicants should discuss the approach with HAs, when rationale for Ur BM-based decision-making

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Thank *you* ☺

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