SAFE-T: A consortium for the regulatory qualification of human kidney biomarkers to monitor drug-induced kidney injury

J. Keenan, F. Dieterle, S. Sultana, M. Pinches, R. Schindler, and the other members of the SAFE-T Work-package 2

ABSTRACT

Drug-induced kidney injury (DIKI) is not an uncommon adverse event in drug development. The greatest issue is the late identification of Acute Kidney Injury due to the current standards (i.e. serum creatinine (sCr) and blood urea nitrogen (BUN)) which are late indicators of injury and may not be significantly changed until 2/3 of the kidneys function has already been lost.

Current Standards
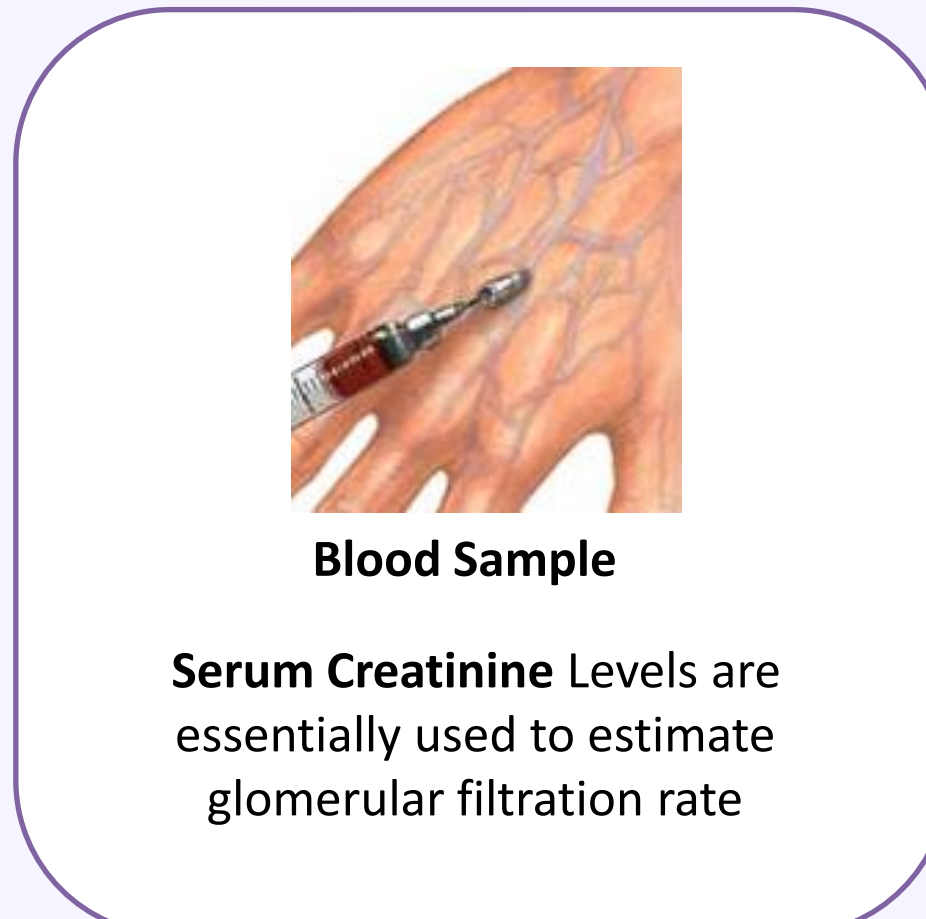
Blood Sample

Serum Creatinine Levels are essentially used to estimate glomerular filtration rate

Markers being investigated
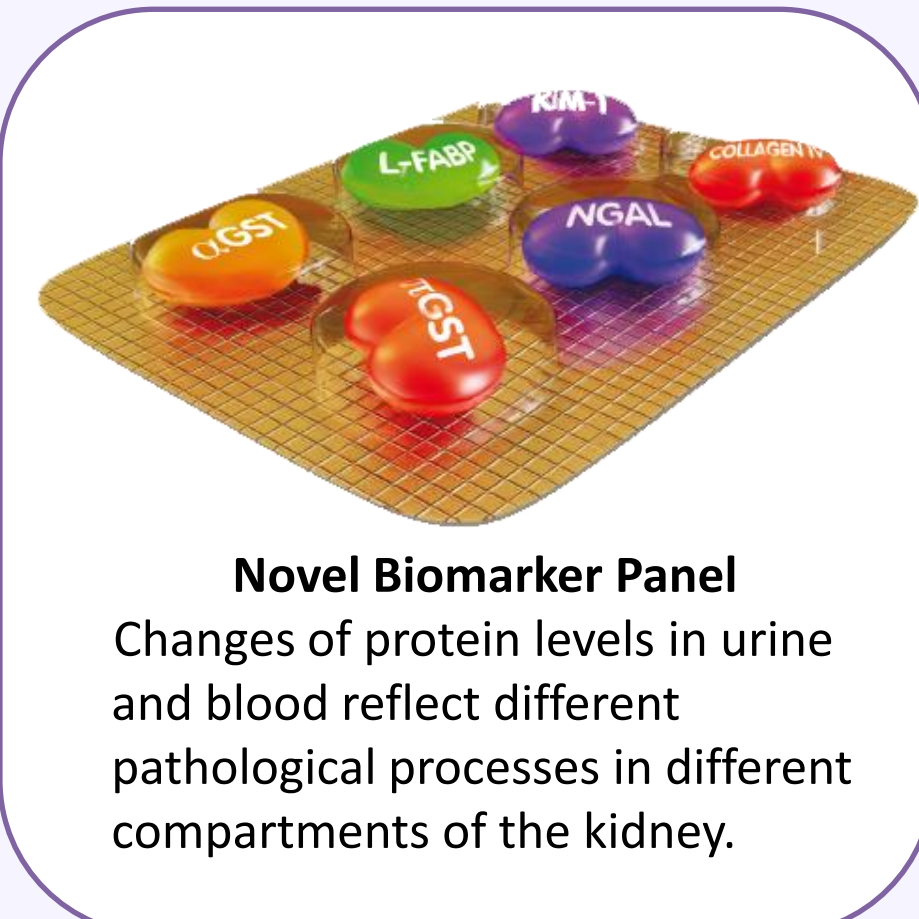
Novel Biomarker Panel

Changes of protein levels in urine and blood reflect different pathological processes in different compartments of the kidney.

Over the last three years there has been great progress with preclinical qualification processes for kidney biomarkers . The PSTC and ILSI HESI have both had rat kidney biomarkers qualified by both the FDA and EMA. These landmark qualifications mean that drug companies may now use certain novel preclinical markers for **real** decision making within their qualification context.

Comprehensive rat Data set submitted in 2005

Comprehensive rat Data set submitted in 2008

Agencies evaluated and then awarded Qualification in 2009

Agencies evaluated and then awarded Qualification in 2010

1. Kim-1
2. Albumin
3. Total Protein
4. β2 Microglobulin
5. Cystatin C
6. Clusterin
7. Osteopontin

1. RPA-1
2. Clusterin

The principal objective of this new project is to collect and generate sufficient **clinical** data from a number or candidate kidney biomarkers, that will provide convincing evidence for the health authorities to endorse these biomarkers for the detection and monitoring of drug induced kidney injuries in specific clinical situations. To address this, a European-based partnership called the SAFE-T Consortium was formed from 20 participants from the pharmaceutical industry, small-medium enterprises, academic institutions and clinical units.

SAFE-T PARTICIPANTS 2011
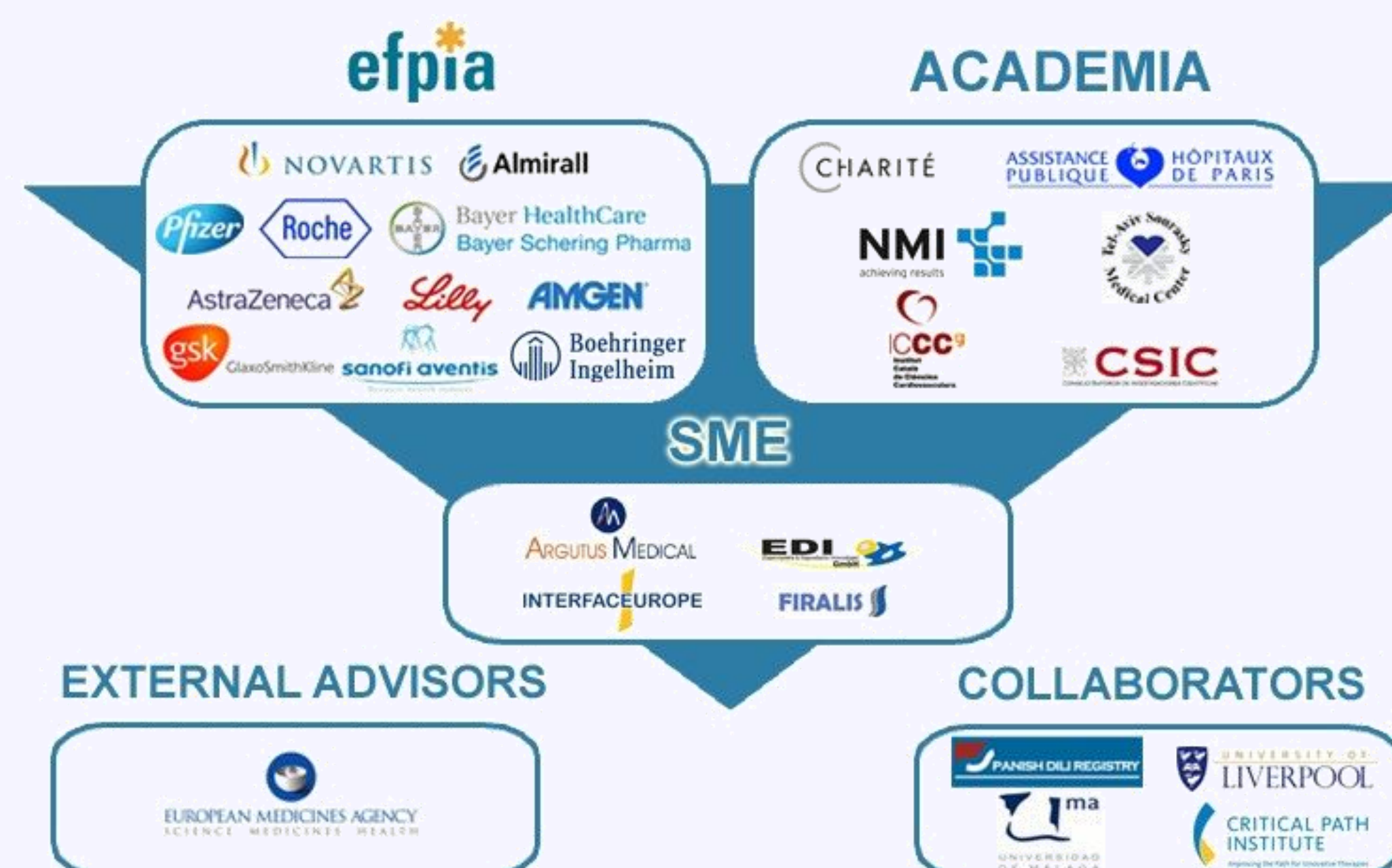
PROBLEM STATEMENT

Being more effective than serum creatinine and BUN for AKI diagnosis is easy. Evidence based proof to submit to the regulators is more of a challenge! How does one compare new biomarkers with a *flawed bronze standard* (sCr)?

➡ **There is an urgent need for the qualification of biomarkers that detect and monitor drug induced kidney injury**

SAFE-T APPROACH:
THE KIDNEY BIOMARKER QUALIFICATION WORKFLOW
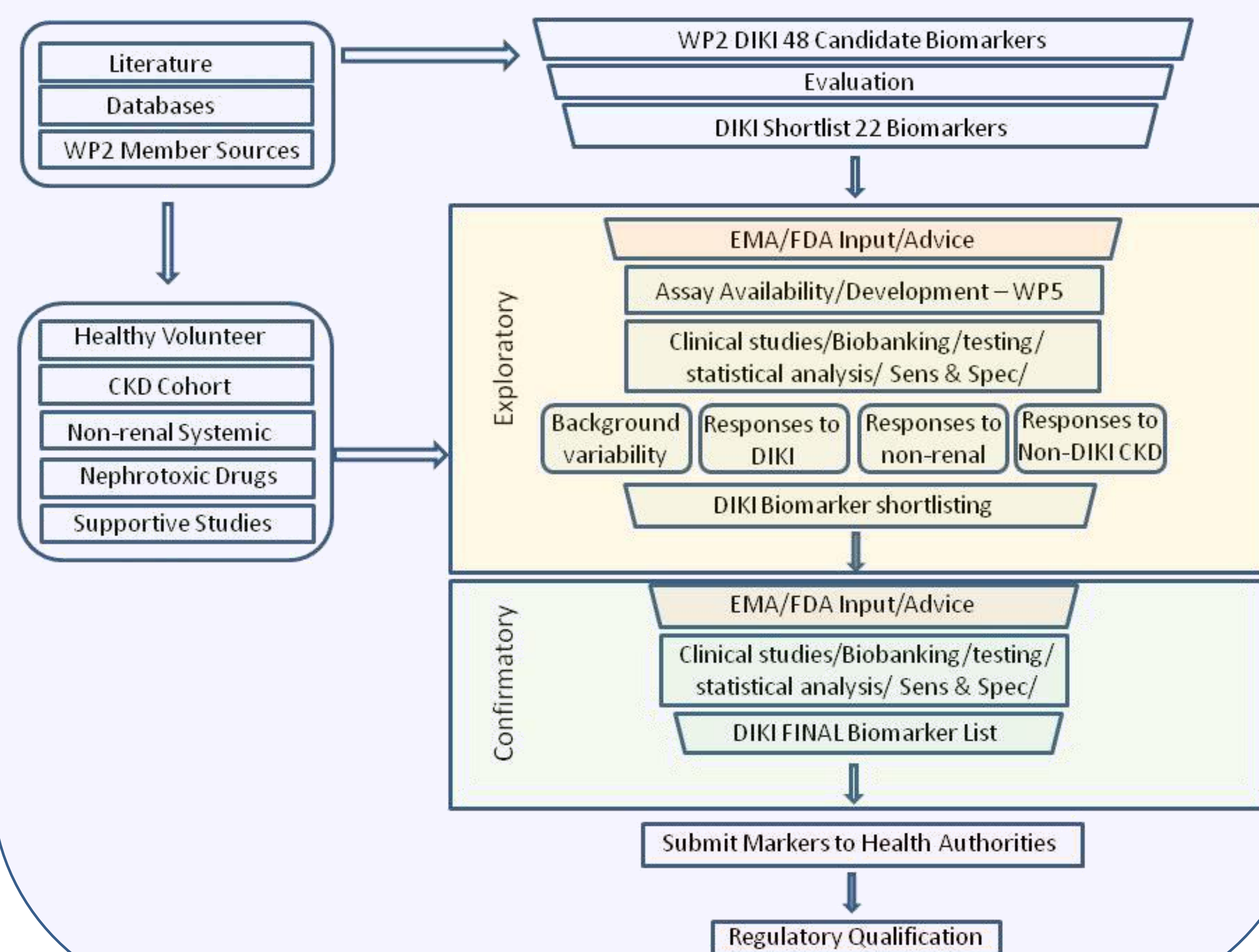
SAFE-T OBJECTIVES

• **Primary objectives**

1. To develop and qualify biomarkers that detect DIKI earlier in man than the currently used standards (sCR & BUN) and with greater sensitivity
2. To gain scientific acceptance and regulatory endorsement for the use of these biomarkers in defined clinical and potentially translational contexts

• **Secondary objectives**

To characterize selected renal biomarkers with respect to their utility as follows;

a. Assessment of sensitivity & specificity of biomarkers for injury in specific compartments of the kidney (e.g. glomeruli, tubules, collecting ducts) and to different pathologies (e.g. fibrosis, necrosis, degeneration)

b. Characterization of the effect on biomarkers of acute and chronic non-renal organ diseases, injuries and systemic conditions e.g. diabetes, congestive heart failure, liver diseases

c. Determination of inter- and intra-individual variability of the candidate biomarkers in healthy individuals and patients with chronic kidney diseases, associated normal ranges and relevant covariates

d. Evaluation of the animal-to-human translational potential of kidney biomarkers, which have been demonstrated to monitor kidney injury in preclinical species, to enable the safe conduct of clinical studies with compounds which had shown nephrotoxic potential in one or several pre - clinical species.

e. Evaluation of the potential of biomarkers to track not only the onset of kidney injury but also to monitor the resolution of kidney injury

f. Exploration of the clinical utility when these biomarkers are used to trigger intervention (e.g. cessation of treatment or initiation of renal rescue therapy) in a narrow clinical context.

RESULTS

CANDIDATE KIDNEY BIOMARKERS UNDER ASSESSMENT

Tissue Injury Response	Tissue Injury Leakage	Functional
○ KIM-1	○ Alpha GST	○ Microalbumin
○ NGAL (urine & serum)	○ Pi GST	○ α1 Microglobulin
○ Clusterin	○ L-FABP	○ Cystatin C (urine & serum)
○ TFF3	○ NAG	○ RBP 4
○ Osteopontin	○ Collagen IV	
○ Timp-1	○ Podocin	
○ CTGF	○ Nephrin	
○ IL-18	○ Aquaporin 2	
○ MCP-1	○ Calbindin D28	

ASSAY VALIDATION PROCESS

SAFE-T are using commercially available assays for the candidate biomarkers selected where feasible. Through a series of low and high bar validation processes the markers will be assessed for their suitability for entry into the clinical sample assessment. For some of the markers that are available on multiple technologies (ELISA, Luminex, Mesoscale, etc..) the consortium has undertaken dual technology assessment in an attempt to cover any potential weaknesses lent to a particular technology. The priority will be lent to the technology that has lead to most publications in the literature to date.

Biomarker	Technology 1	Technology 2
KIM-1	Microtitre Elisa (Bioassayworks)	Multiplex Bead KTP1 (EDI)
NGAL (urine)	Microtitre Elisa (Bioporto)	Multiplex Bead KTP2 (EDI)
NGAL (serum)	Microtitre Elisa (Bioporto)	Multiplex Bead STP1 (EDI)
Clusterin	Microtitre Elisa (Biovendor)	Multiplex Bead KTP1 (EDI)
TFF3	Multiplex Bead KTP3 (EDI)	
Osteopontin	Multiplex Bead KTP2 (EDI)	
Timp-1	Microtitre Elisa (RnD Systems)	Multiplex Bead KTP2 (EDI)
CTGF	Multiplex Bead Based KTP1 (EDI)	
IL-18	Mesoscale Discovery (Firalis)	
MCP-1	Mesoscale Discovery (Firalis)	
Alpha GST	Microtitre Elisa (Argutus)	Multiplex Bead KTP1 (EDI)
Pi GST	Microtitre Elisa (Argutus)	
L-FABP	Microtitre Elisa (CMIC)	
Collagen IV	Microtitre Elisa (Argutus)	
Podocin	LC/MS (Sanofi)	
Nephrin	LC/MS (Sanofi)	
Aquaporin 2	LC/MS (Sanofi)	Microtitre ELISA (Dev)
Calbindin D28	Multiplex Bead Based KTP1 (EDI)	
α1 Microglobulin	Microtitre ELISA (Immunodiagnostik GmbH)	
Cystatin C (urine)	Microtitre Elisa (Biovendor)	Multiplex Bead KTP1 (EDI)
Cystatin C (serum)	Microtitre Elisa (Biovendor)	Multiplex Bead STP (EDI)
RBP 4	Mesoscale Discovery	

Low Bar Validation

Low bar refers to a basic technical validation of the assay whereby the functionality is tested to see if the manufacturer's specifications can be met. This stage will be further developed with the testing of clinical samples.

For the Low bar Validation, under current assessment are factors including;

➢ Precision - Inter and Intra assay variation of standard curve and samples (Low, Medium and High)

➢ Sensitivity - Lower limit of detection determination (Lowest standard and negative urine matrix testing)

➢ Recovery - Spiking urine matrix with known concentration of analyte and observing the analyte recovery

➢ Linearity - dilution series of a high sample

➢ Robustness - Freeze-thaw stability of samples

High Bar Validation

High bar refers to a full technical validation of the assay. More detailed experiments will be carried out such as, any interference factors which may exist, reference range generation (age, gender etc) and lot to lot variability. This will then lead to the establishment of a final QC SOP and then the further study of the assay according to GLP standards.

Assays CURRENTLY VALIDATED (23rd March 2011)

The below table represents the list of currently validated candidate biomarkers that have been assessed and passed the LOW BAR validation as set by the consortium

Biomarker	Technology	Status
KIM-1	Microtitre Elisa (Bioassayworks)	VALIDATED
NGAL	Microtitre Elisa (Bioporto)	VALIDATED
Clusterin	Microtitre Elisa (Biovendor)	VALIDATED
Alpha GST	Microtitre Elisa (Argutus)	VALIDATED
Pi GST	Microtitre Elisa (Argutus)	VALIDATED
L-FABP	Microtitre Elisa (CMIC)	VALIDATED
Collagen IV	Microtitre Elisa (Argutus)	VALIDATED

Following the validation of all the candidate biomarkers the consortium will collect 1000's of samples from a number of clinical cohorts to assess the biomarkers performance in these different situations.

### SUCCESSFUL BIOMARKER SELECTION PROCESS

• **Exploratory Phase**

➢ The biomarkers will be assessed in healthy volunteer cohorts. Markers with high variability in these samples will be dropped

➢ Next the candidates will be assessed for their response to drug induced kidney injury. Those markers with bad discrimination in these cohorts will be dropped

➢ A number of non-renal disease cohorts will be collected. These samples should not elicit a significant response in the candidates.

➢ Finally a cohort of CKD samples will be collected. Candidate patterns in CKD will be very important to understand.
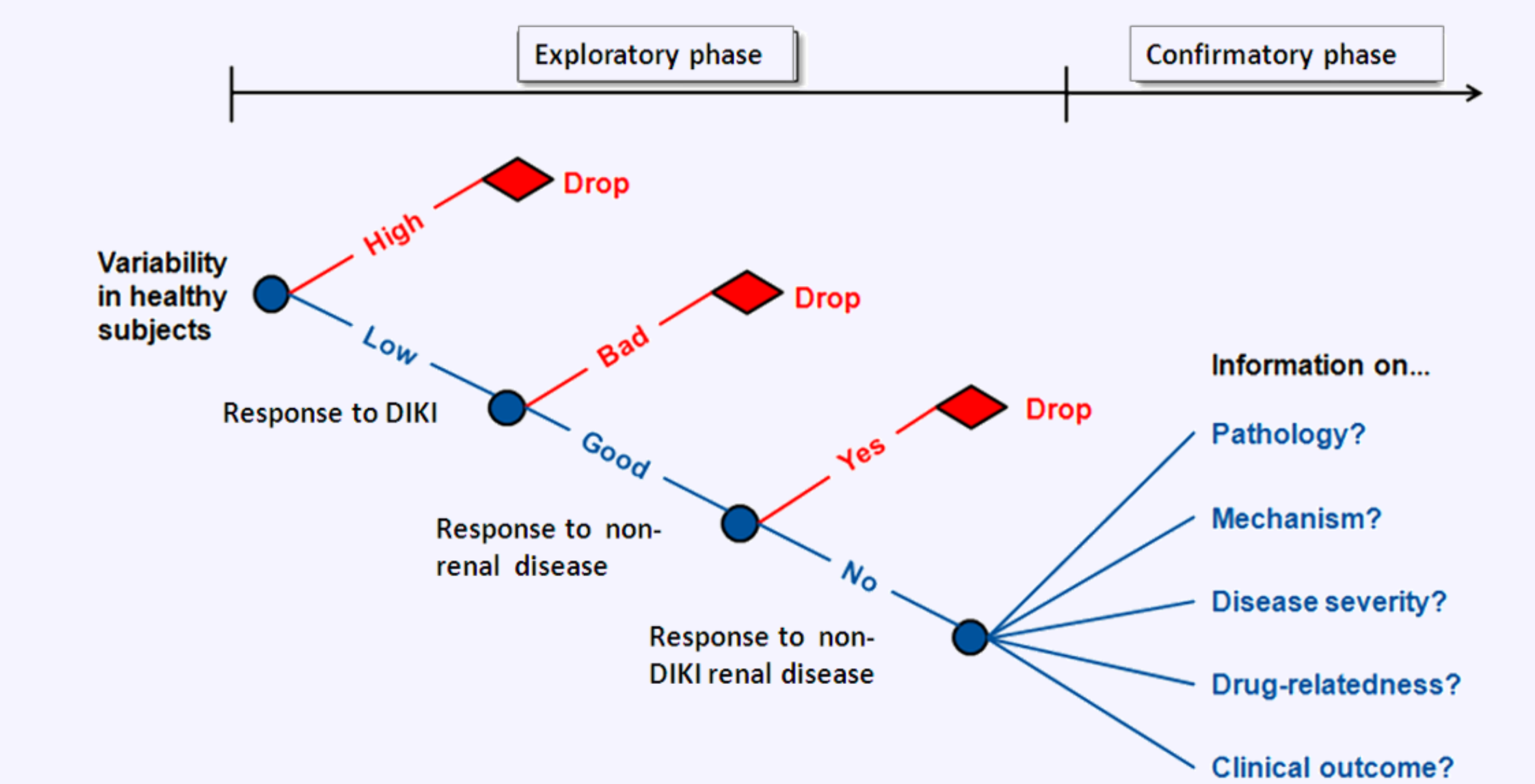
• **Confirmatory Phase**

➢ The confirmatory phase anticipated will look closely at the above issues in large scale clinical trials

NEPHROTOXIC DRUG SELECTION
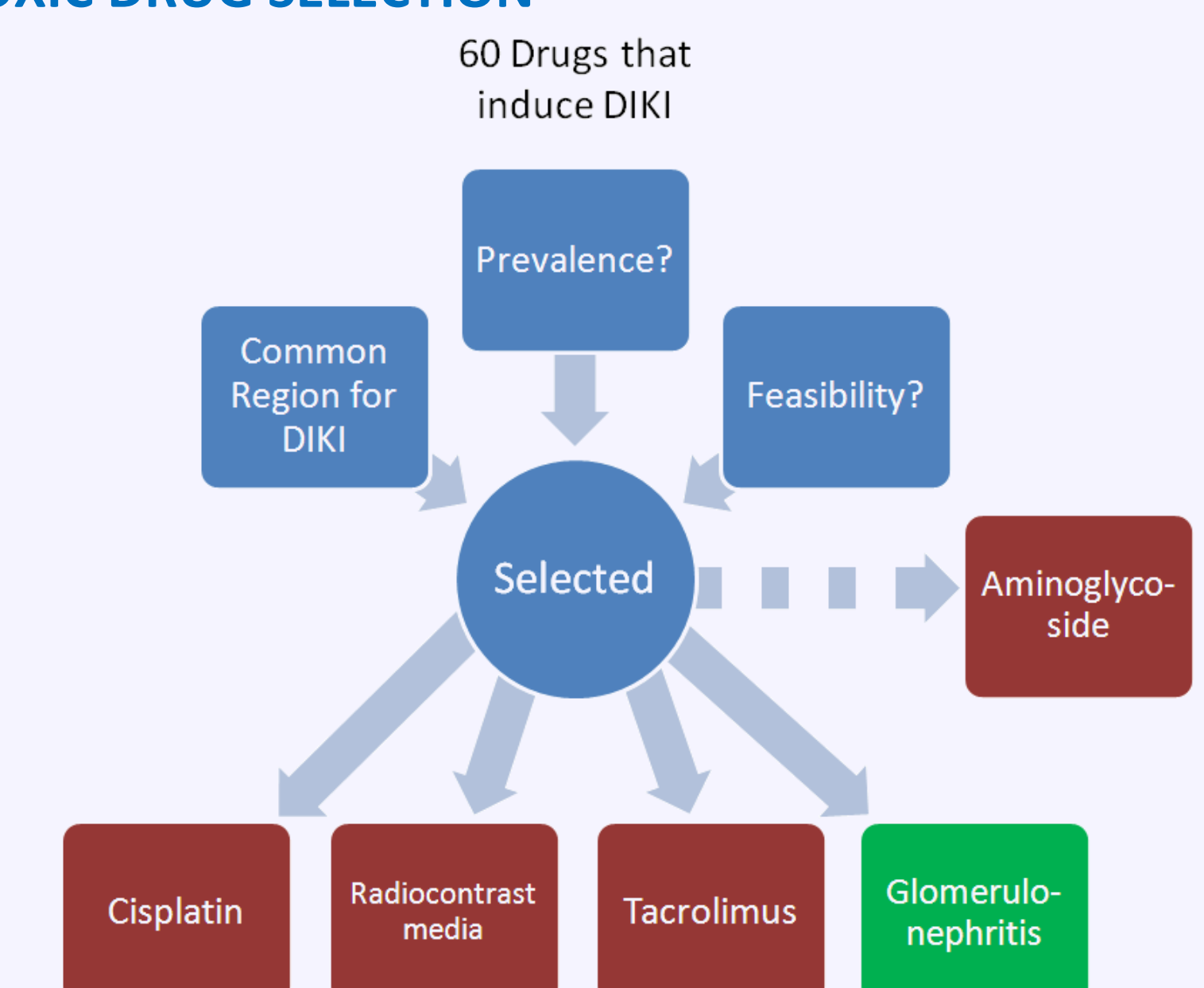
ASSESSING PERFORMANCE OF NOVEL BIOMARKERS vs ???

SAFE-T have adopted the AKIN criteria for the definition of acute kidney injury and its three stages. This classification is dependent on serum creatinine which has been described as a weak standard for AKI.

➡ **How do you assess a novel biomarker against Scr without histology?**

SAFE-T has proposed an approach to novel biomarker assessment (which has been discussed with the regulatory authorities). The approach involves the establishment of adjudication committees to determine the relevance of increases of biomarkers in the absence of increases of serum creatinine and whether these are true positive or false positive signals (i.e. post-hoc judgment of the patient assignments [control vs nephrotoxicant] on the basis of blinded review of
1) Standard clinical parameters
2) Biomarker profiles
3) Biomarker profiles and clinical parameters)?

Further the adjudication committees will determine if changes of standard clinical parameters are due to:
1) Prerenal azotema
2) Non-progressive renal disease
3) Acute kidney injury