Biomarkers are commonly used in clinical trials to identify organ injury before it becomes clinically evident. While they add value in early discovery work and in human trials to identify major safety issues, their potential has not yet been fully reached in preclinical development in animals. This paper explores the current state of biomarker use in preclinical development and examines possible steps for future use.

**Current State**

**One Piece of the Puzzle**

It’s important to note that biomarkers must be used *in conjunction* with other traditional clinical and pathology endpoints. When used with other data, they can be used to identify premonitory signals significantly earlier than when utilizing traditional tests alone.

But there may be instances in which biomarker results can confound data interpretation. Due to the sensitivity of many biomarker assays, a biomarker may show evidence of a certain toxicity in the absence of correlative findings among other, more traditional study endpoints. In these cases the biomarker may be identifying a submicroscopic effect that cannot be detected through traditional means. Since biomarker use in preclinical studies is not a regulatory requirement, some scientists are hesitant to use biomarkers because they are concerned about explaining these results. Therefore, it’s important to discuss the benefits and potential drawbacks of using biomarkers during the planning stages of a toxicity study. The more that is understood about the mechanisms involved in the biomarker’s signaling pathways, the easier it is to explain its results. A pathologist should be consulted pre-study to determine how and when to use the biomarker most effectively. Understanding the timing of the pathologic processes involved is critical to yielding meaningful results. For example, it may be necessary to draw blood samples within hours or days of dosing to capture the positive signal instead of waiting until the end of the study when the signals may no longer persist.

**Quality over Quantity**

Ideal biomarkers must function both analytically and from a pathological perspective, so while analytical teams are continually discovering new biomarker targets, there must be collaboration with pathologists to ensure these markers will be useful in practice.

**What are the characteristics of a good biomarker?**

A good biomarker must be present in body tissue and/or fluids, easy to detect in affordable assays, and “its appearance must be associated as specifically as possible with damage of a particular tissue, preferably in a quantifiable manner.”

**What are the characteristics of a validated biomarker?**

While it’s fairly straightforward what makes a good biomarker, characteristics of a validated biomarker are still up for debate. The Predictive Safety Test Consortium (PSTC) was created by scientists in academia and industry with the goal of qualifying new biomarkers “for the detection and monitoring of drug-induced toxicity in preclinical and clinical studies.”

- In 2008, Kidney injury molecule-1 (Kim-1, in rodents), along with six other markers, was approved by the FDA as an acceptable biomarker “for the detection of acute drug-induced nephrotoxicity in rats and can be included along
with traditional clinical chemistry markers and histopathology in toxicology studies.\textsuperscript{3,4}

- In 2010, the FDA published guidance on the Qualification Process for Drug Development Tools, which stated that “qualification is a conclusion that within the stated COU [context of use], the [drug development tool] can be relied on to have a specific interpretation and application in drug development and regulatory review.”\textsuperscript{5} Qualification through the FDA is voluntary, and the COU can be expanded incrementally over time with appropriate data.

- In the last several years, troponins have been established as a reliable biomarker for cardiovascular injury.\textsuperscript{6} In 2012, they were concluded by the FDA to be qualified biomarkers for use in rats and dogs, in certain preclinical circumstances.\textsuperscript{7}

While organizations like the PSTC continue to make strides in developing tests for biomarkers for preclinical use, minimum threshold criteria for an acceptable validation of a preclinical biomarker have not been published (the way they have been for clinical biomarkers), so there is still a spectrum of what is considered a validated or qualified biomarker.

Many preclinical labs involved in biomarker work adopt similar validation practices, which usually include inter/intra-assay precision and accuracy, dynamic range, upper and lower limits of quantitation, dilution linearity, spike-and-recovery, parallelism, and stability, but agencies have yet to create standardized guidance for all labs to follow.

Lack of Oversight
The manufacture of biomarker kits used in preclinical studies is not currently regulated, so one cannot assume that every kit on the market is functional. Individual labs must do the due diligence for each kit to ensure analytical performance, as well as performance from a biological perspective (e.g., using serum samples from animals with a known pathology to verify kit functionality). This can be a large investment upfront, but establishing a reliable kit supplier and using preclinical biomarkers to detect injury early can save considerable time and money once a drug program is underway.

Individual methods must also be taken into account: two different kits may use completely different methods for the same biomarker, so it’s advisable to maintain consistency of methods and manufacturer throughout a program to ensure an apples-to-apples comparison between results from different studies. This may not always be possible between species as species-specific reagents are not available for all biomarkers.

Future State
In order for preclinical studies to truly reap the full range of benefits that biomarkers can offer, we hope to see a few changes over the next several years.

Standardization is Crucial
The industry needs more guidance from regulatory agencies on the minimum requirements for validation, so that each lab can universally adhere to one set of standards. This will help to remove the subjective nature of what a “good” biomarker is and will result in a consistent set of standards used across the industry.

Establishing a Trustworthy Set
Biomarker development needs collaboration between pathologists and analytical groups. Both types of specialists are critical to developing biomarkers that are useful in preclinical studies. Once more standards for validation and data submission are established by regulatory agencies, teams can work together to streamline the discovery of preclinical biomarkers and submission for review. As more biomarker data is collected and made accessible to the community, more time can be saved in choosing the appropriate marker for a study, and in understanding sensitivity and interpreting results.
Reliable Test Kits
Commercial kits must become more reliable. With the proper reagent manufacture standards in place, confidence in biomarker kits used on preclinical studies could become more like the confidence we have in over-the-counter glucose meters (which don’t require the consumer to verify their functionality). This will require oversight on reagent manufacture and kit quality. The challenge here is that the responsibility doesn’t fall on one body. Ideally:

- Regulatory agencies will provide more standards and supervision over reagent manufacture and release
- Companies will take responsibility for proving that their kits function in the ways they claim
- Scientists in industry and academia will develop new automated technologies for preclinical biomarker kits (the current technologies are labor intensive and time-consuming)

Closing Thoughts
With collaboration across the industry and development of a consistent process for validation, the use of preclinical biomarkers can become more standardized. Researchers will be able to identify signs of injury and recovery more quickly, so that decisions for new drug candidates can be made more efficiently. Use of reliable biomarkers will safely expedite preclinical studies and allow resources to be allocated more effectively.

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