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Target Engagement in Lead Generation

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ABSTRACT

The pharmaceutical industry is currently facing multiple challenges, in particular the low number of new drug approvals in spite of the high level of R&D investment. In order to improve target selection and assess properly the clinical hypothesis, it is important to start building an integrated drug discovery approach during Lead Generation. This should include special emphasis on evaluating target engagement in the target tissue and linking preclinical to clinical readouts. In this review, we would like to illustrate several strategies and technologies for assessing target engagement and the value of its application to medicinal chemistry efforts.

Great attention has been dedicated in the past few years to analysis of the current challenges in the pharmaceutical industry. In spite of the sequencing of the human genome and the exponential rate of learning in the area of human health, no significant improvements in clinical success rate have been realized. Multiple authors have analyzed the underlying causes for this inefficiency trend. Many concluded that a lack of efficacy in Phase 2 clinical studies was the major reason for failure.[1–3] The inability of preclinical disease models to predict clinical outcomes and the frequent irreproducibility of literature findings further increases the difficulty of modern drug discovery.[4,5] To both internal and external observers, it is clear the pharmaceutical industry is in a state of paradigm shift. The industry is moving away from older strategies and business models for selecting targets and molecules for clinical investigation. Recent strategies focus more on developing a deeper understanding of mechanisms of action, pathway biology, and the relation of a biological target to human disease. To increase the probability of technical success, it is crucial to start investing during preclinical research in target validation, target selection, and development of integrated drug discovery strategies.[2,6]

Identifying potential clinical readouts or biomarkers that can be used pre-clinically should help connect discovery research (from hit identification to candidate selection) to the ultimate test of the clinical hypothesis in man. At minimum, being able to demonstrate sufficient clinical target engagement at the site of action would unequivocally establish the validity of a given target for a specific disease indication.[7]

This concept is supported by recent analyses conducted by major pharmaceutical companies. A retrospective analysis by Pfizer of 44 drug programs in Phase 2 identified 'lack of efficacy' as the most common cause of attrition in their discovery programs.[8] To improve drug discovery effectiveness, the authors suggested a model of 'three pillars' for evaluating potential investment in non-validated drug targets: (1) sufficient exposure of ligands at the site of action; (2) proof of target engagement; (3) expression of functional pharmacological activity. The authors' conclusion was that projects being able to demonstrate all 'three pillars of survival' should have the highest probability of translating in human clinical studies.

AstraZeneca has also recently published an exhaustive review of their small molecule pipeline from 2005 to 2010.[9] They identified five critical technical determinants of project success (coined the 'five R's'): the right target, the right patient, the right tissue, the right safety and the right commercial potential. In particular, the 'right tissue' is defined as the appropriate exposure of the candidate drug in the target organ leading to sufficient pharmacological activity. To assess the 'right tissue', it is necessary to evaluate pharmacokinetic properties and target engagement to develop an understanding of the PK/PD correlations relative to the target organ. Interestingly, it was pointed out by the authors that less than 10% of the projects reviewed had demonstrated a strong correlation between target occupancy and pharmacological activity.

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This review is composed of two halves. The first half presents background and discussion on how target engagement can be used in Lead Generation drug discovery along with a brief overview of various established methods for its measurement. In the second half, we explore specific examples from recent literature where target engagement is being interrogated within Lead Generation and highlight emerging technologies that can assess target engagement.

Target engagement in Lead Generation: Traditionally, drug discovery teams build a testing scheme progressing compounds from in vitro testing (to measure binding, affinity, and selectivity) to assessing ADMET properties and efficacy in preclinical animal models.[10–12] A target engagement assay (Fig. 1) linking compound performance in vitro to compound performance in vivo is critical to ensure the appropriate compound concentration reached the intended target. Ideally, an integrated discovery approach will link target engagement with relevant clinical endpoints. It is important to emphasize that there are different types of biomarkers: (1) diagnostic markers, to assess the presence or absence of disease; (2) disease activity markers, to assess severity of the disease; (3) drug effect biomarkers, markers of target engagement and PD effects; (4) drug kinetic biomarkers, to assess genetic variants on drug metabolizing enzymes and drug transporters. For the purpose of this review, we will refer to drug effect biomarkers.

An excellent example of the use of drug effect biomarkers is the development of sitagliptin, a DPP4 inhibitor for the treatment of diabetes. Preclinical studies demonstrated that 80% inhibition of the enzyme generated maximal lowering of blood glucose. Similar degrees of DPP4 inhibition in the first human studies were associated with reduced glucose levels. Those correlations significantly facilitated Phase 2 clinical studies, and even shortened clinical development time.[13] A second example where target engagement has been clearly linked to efficacy is anti-psychotic drugs targeting the dopamine D2 receptor. It is now well established that achieving ~60% receptor occupancy correlates to positive benefits in patients.[14,15]

Being able to assess the degree of target engagement, pharmacodynamics and duration of effect (time on target) relative to preclinical measures of efficacy (e.g., behavioral measures, biomarkers, etc.) are crucial for compound selection and further hypothesis generation (Fig. 2). Once the correlation is built between in vitro activity, target engagement and in vivo efficacy, a target engagement assay should supply a mechanism for rapid decision making. Such an approach has the potential to require less use of iterative preclinical animal models, which supports the responsible use of animals for research.[16,17]

There is a wide variety of methods to measure target engagement biomarkers. The use of a particular method is influenced by the ease of access to the relevant tissue and the nature of the downstream pharmacological effect.[18] For example, within the field of chemical biology, Cravatt and co-workers have highlighted the use of chemical probes to engage their intended targets in vivo to validate protein function.[19] Optimized functional chemical probes can measure occupancy inside the cell and facilitate unbiased selectivity determination in a more physiologically relevant environment.

Imaging techniques like positron emission tomography (PET) have received great attention since they can enable non-invasive target engagement assays compatible with human clinical studies.[20,21] Recent developments in liquid chromatography coupled to tandem mass spectrometry (LC–MS/MS) methods, have enabled the rapid assessment of chemical space for a suitable tracer in a preclinical setting.[22] It takes advantage of the same biology that PET measures by comparing levels of the tracer in a total binding region to that of a null (target deficient) region, distinguishing specific binding from background. Several examples from different companies applying this methodology to their medicinal chemistry efforts will be discussed later in this review.

Key breakthrough advances in imaging technology have allowed for an increase in imaging resolution resulting in a significant number of applications to early drug discovery. In vivo bioluminescent imaging (BLI) is a sensitive tool based on detection of light emission from cells or tissues. Reporter gene technology enables the analysis of specific cellular and biological processes in a living animal through imaging methods. Combining animal engineering with molecular imaging techniques, it is possible to conduct dynamic studies of specific molecular processes in living animals. BLI-based models founded on the same reporter assays used in high-throughput screening (HTS), could offer a bridge between in vitro biological assays and preclinical animal model efficacy studies. In comparison with animal models, mechanistic BLI reporter models require less resources. They also have a higher throughput and generate quantitative data for compound ranking in three formats: primary cells, tissues and whole animals.[23] This approach could dramatically impact cycle times during Lead Generation efforts. An interesting recent example is the generation of a bioluminescence transgenic mouse model for detecting ligand activation of GPCRs by Polites and co-workers at Sanofi.[24]

Another example of molecular imaging is biodistribution studies confirming that a compound reaches the target tissue. This also allows assessment of accumulation in non-target sites. Investigations in whole-animal imaging using micro-PET have garnered increased interest. This is due to the technique's ability to assess biodistribution not only for CNS targets but also for oncology targets where up-regulation of pumps excluding drugs from tumors are a significant issue.[25–27]

Target engagement studies are most valuable when there is a robust hypothesis regarding the extent of target engagement needed for a pharmacological effect. In those cases, data relating

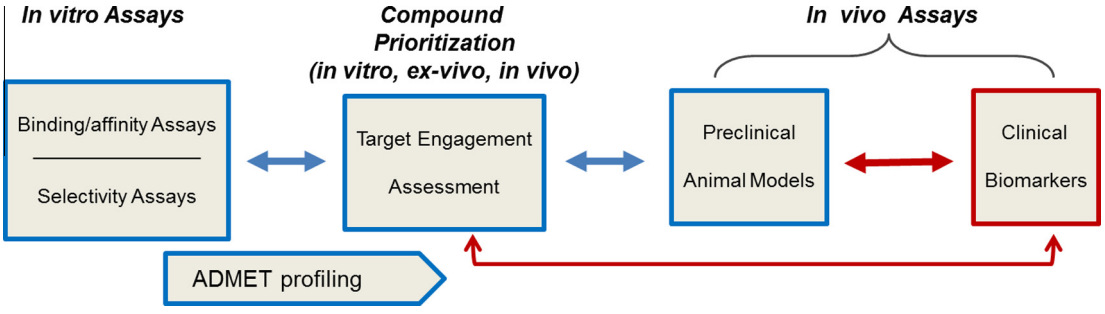

Figure 1. Flowscheme representation: building correlations across the drug discovery paradigm. Blue boxes represent preclinical assays and red boxes clinical readouts. There should be a connection between preclinical and clinical readouts. Clinical results should also inform future discovery projects. Arrows represent critical data correlations along the drug discovery paradigm.

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