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Target (In)Validation: A Critical, Sometimes Unheralded, Role of Modern Medicinal Chemistry

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ABSTRACT: Small molecule drug discovery commonly ventures into previously unknown and unexplored target space. For such programs, an important role of medicinal chemistry is to generate molecules that enable the most reliable conclusions from a preclinical target validation/invalidation study. Multiple facets of chemistry that provide the most rigorous results for such an experiment are highlighted.

KEYWORDS: *Target validation, target invalidation, proof-of-concept, biomarkers, inactive controls*

The last 15 years of small molecule drug discovery have been characterized by dichotomous flux. Medicinal chemistry departments have actively hired new scientists and have undergone drastic headcount reductions. New initiatives have proclaimed more efficient ways to conduct drug discovery, while others have fallen into obscurity. Philosophies, technologies, "hot" therapeutic areas, and even collaborations have come and gone, as the field scrambles to increase the output of clinical candidates in an era of poignant, almost caustic oversight. We have witnessed a steady stream of published "dogmas" of small molecule drug discovery that been rapidly followed by manuscripts detailing exceptions. There are several questions underlying the many waves of change that have manifested among the community of medicinal chemists: To what degree should chemists adhere to the rule-of-five approach?[1] How much effort should be invested in traditional high-throughput screening (HTS) follow-up relative to fragment-based ones?[2] Which is the more valid screening method, a phenotypic- or target-based one?[3] To what extent should chemists actively investigate irreversible target inhibition relative to its perceived risk?[4] What contributes more to success: the traditional acquired instinct of an experienced drug-hunter or the modern computing powers of advanced in-silico design?[5]

The persistent search for the "better" has enabled the community to engage in a healthy dialogue around these topics. Consequently, the vast majority of medicinal chemists are arguably better equipped to succeed today than ever before. Yet these popular topics have tended to overlook one fundamental success-enabling aspect in all work that is conducted: the integrity of the foundational biology underlying any small-molecule drug discovery program. Too often, medicinal chemistry teams blindly accept the biological underpinnings of a new program as sound and are unaware that their ensuing efforts at compound optimization may confirm or debunk the link between the biological target and the disease. In many instances, this event occurs very early in the discovery process, at the level of preclinical interrogation. As such, several medicinal chemistry departments have dedicated teams focused on target validation. The principle of using small-molecules to validate or invalidate biological targets is very basic indeed, and the exercise is not, nor should it be, exclusive to just dedicated chemistry teams. In the broadest sense, there are only two types of small molecule drug discovery programs: (1) preclinically validated ones and (2) all the others. The features of how chemistry is utilized to produce a validated program, or invalidate a program, are highlighted below.

INTERROGATE MEASURABLE BIOMARKERS

Regardless of the stage of a program, the ability to measure and quantify modulation of a biological target is essential for progression both into the clinic and through it.[6] Medicinal chemists are very adept at recognizing the precarious situation of trying to prosecute a program in the absence of a biomarker. Not coincidentally, resources tend to gravitate away from such projects over time, especially when advancement proves difficult. When a measurable biomarker exists, however, teams often trust that efficacy in their primary assay will seamlessly translate to effective modulation of the biomarker, either in a cellular context or in vivo. In some cases, error or lack of reliability/robustness of the biomarker measurement is not realized until very late in the program, when a medicinal chemistry team has triangulated its focus onto one optimized chemical series. By this stage of the project, considerable investment has been made into compound optimization. This is not the best time for troubleshooting. The infrastructure behind any drug discovery program should be stress-tested at a very early stage. Fear of obtaining suboptimal results from a suboptimal compound should not be a reason to defer such system testing. It is not uncommon for medicinal chemists to challenge their primary assay and note when occasional aberrations in output are observed. This feedback to biologists is quite helpful in establishing the durability of the primary assay. Accordingly, this philosophy should extend to downstream assays as well. As in most aspects of drug discovery, strict linearity in data acquisition will delay obtaining answers to important questions underlying a program, and it will usually result in failure.

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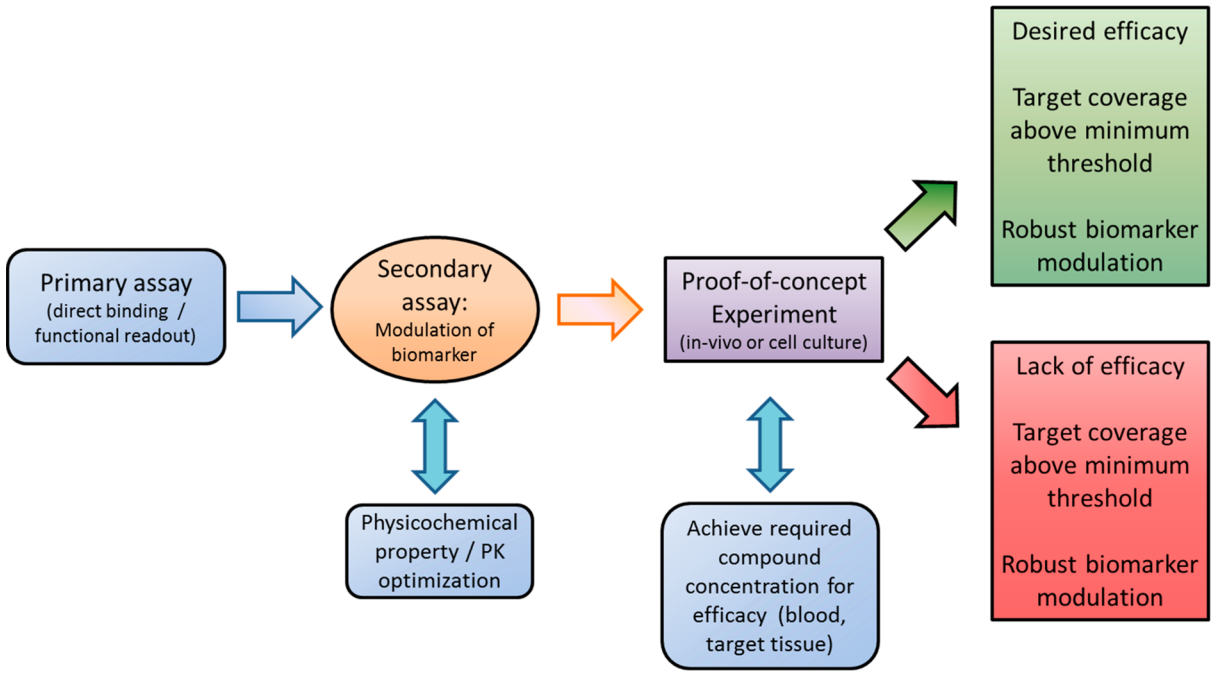

Figure 1. General pathway toward biological target (in)validation with optimized small molecules.

CHARACTERISTICS OF A ROBUST TARGET VALIDATION EXPERIMENT

A widely accepted standard of preclinical target validation consists of the in vivo proof-of-concept (POC) study. Testing the target-to-disease link in an animal model of the human disease allows investigators to simultaneously gauge efficacy, assess the pharmacokinetic (PK) properties of the molecule, and measure the biomarker modulation after dosing (Figure 1). The medicinal chemistry effort that is required to discover a suitable "tool" molecule for this can be quite involved. Optimization of potency, PK, and selectivity are necessary prerequisites that can take months or even years to realize. In some cases, the profile of a tool molecule needed for an in vivo POC study is not that far removed from the characteristics of a preclinical development candidate. There is more flexibility in certain requirements however. For example, intraperitoneal injection can substitute for oral dosing in the event sufficient exposures of compound cannot be achieved with the later route. The sometimes extensive medicinal chemistry effort behind a target validation study is justified by the existence of a robust and reliable in vivo animal model of disease that is supported by the ability to measure (1) compound concentration and (2) movement in a translatable biomarker. The outcome of this type of study provides confidence in the validity of the biological target to the disease of interest. An optimal scenario is that of achieving desired efficacy coupled with adequate compound exposures, confirmed by robust biomarker changes. The target may be considered preclinically validated at this stage. A clear "no-go" decision is usually made in the scenario where no efficacy is observed despite clear target engagement above levels expected to achieve efficacy (>IC_{50} or IC_{90}), confirmed by robust biomarker modulation. This highlights the basic paradigm of using small molecules for target validation. It is an overly simplified analysis, however, that is subject to misinterpretation. As will be explained below, chemistry can be effectively utilized to add another layer of confidence in arriving at go/no-go decisions based on the initial POC experiment.

PRESENTING THE CASE WITH MULTIPLE CHEMICAL SERIES

Rarely do initial hypotheses at the start of a drug discovery program remain unchanged as the project progresses. The act of synthesizing compounds and obtaining data on their ability to modulate the target frequently leads to refined hypotheses, additional insights, and in rare cases, drastically different conclusions. Arriving at compounds with favorable results, whether they derive from rational design or empiricism, understandably leads to excitement among a project team. The excitement quickly evolves into a sentiment of confidence and validation for the project. For example, the discovery of a highly potent compound that completely suppresses (or fully activates) a downstream biomarker would rationally entice a team to test whether this compound would provide the expected phenotype in a cellular context or in vivo. At this point, human nature can lead to inaccurate conclusions. Observation of a robust phenotype (tumor regression, weight loss, increased memory, reduced sensation of pain) is embraced as confirmation of the project hypotheses, and critique may understandably be restrained by the desire to move the program forward and succeed.

The certainty of target validation/invalidation is far from absolute. It is akin to a lawyer's task of establishing judgment beyond a reasonable doubt to a jury. A highly effective medicinal chemistry team is commonly lauded for intense focus. However, this focus should not preclude a critical assessment, from the broader perspective of drug discovery, of the compounds being used to make project go/no-go decisions. The proteome is vast, and "druggable" targets are estimated to be in the thousands.[6,7] Based on one, or a few compounds, how certain can anyone be that there is a *causative* correlation between target engagement (in vitro and in vivo) and an observed phenotype? This confidence is further weakened if the

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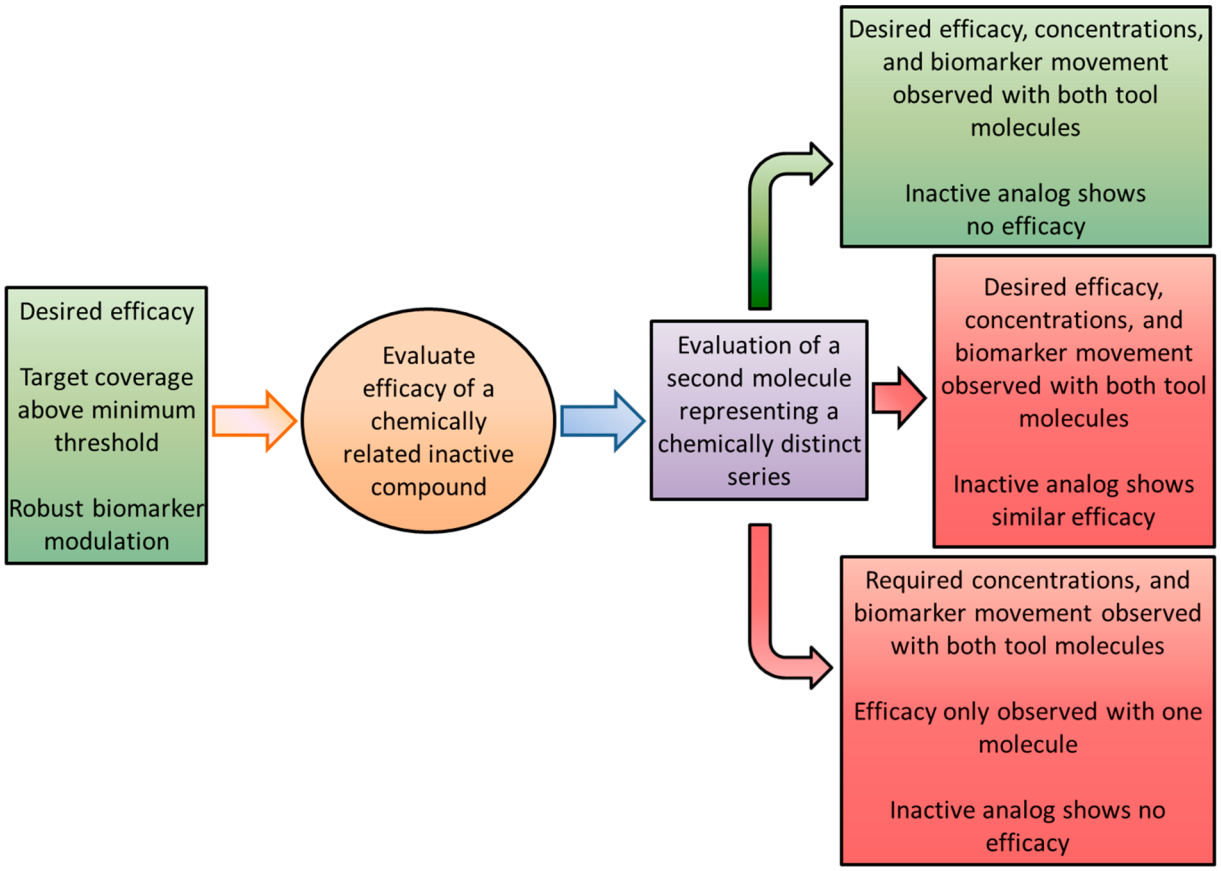

Figure 2. Additional level of target (in)validation enabled by small molecule tools.

"tool molecule" is a representative of a chemical series that has been investigated for numerous drug targets in the literature. It is widely accepted that such implied promiscuity can be reasonably debunked by in vitro selectivity-panel readouts, whether it be relative to similar proteins or as a part of a broader survey. A compound that appears selective against dozens, if not hundreds, of other proteins should be suitable as a tool compound from which target validation/invalidation conclusions can be drawn, and from which further development may ensue en-route to a clinical candidate. The causative link between target engagement and observed phenotype is predicated on the assumption that counter-screening panels effectively derisk the possibility of off-target or nonspecific effects resulting in the observed phenotype. It is debatable how sound this assumption is, and it is highly dependent on the general knowledge of the chemical structure/series represented by the molecule[8] and familiarity with the protein class for which the target is a member. This is precisely where chemistry can help support such a position or refute it (Figure 2). In scenarios where other appealing chemical series[9] exist and can be optimized, they should not go ignored. Development of an additional distinct tool molecule can provide an important readout. Use of different molecules that can modulate the same target may, with higher certainty, establish the link between the target and the phenotype. Two structurally dissimilar compounds of equal potency and equal functional efficacy (as measured by a biomarker) should both show disease-modifying efficacy. Examples where they do not will cast doubt on the integrity of the initial target hypotheses and may provide the basis for target invalidation very early in the discovery effort.

SOLIDIFYING THE CASE WITH NEGATIVE CONTROLS

The use of multiple chemical series to establish a firm foundation for target validation or invalidation is a luxury that is not commonly available. The experiences of scientists in the current age of drug discovery serve as constant reminders that the "low-hanging fruit" has become a rare occurrence. Not only are new targets being explored but also new target classes for which structural binding features are little understood. Furthermore, low hit rates are often observed with current compound screening collections. As such, exploratory programs often commence with only one chemical series to optimize for an eventual target assessment. How then, can medicinal chemistry enhance the odds of arriving at a sound decision for validation or invalidation of a target? The answer is in utilizing something of which almost no chemistry program is short of having: inactive compounds. Similar to the case made previously, the euphoria of arriving at a compound with optimized target engagement and a robust phenotypic response should be tempered with critical evaluation of plausible scenarios. Is the functional efficacy observed caused by the target engagement? Utilizing a structurally related but inactive control in the same experiment may add granularity. If similar efficacy is observed, the project team should be very dubious in linking the biological target to the phenotype being observed.

SUMMARY

The concepts highlighted in this perspective are quite intuitive, requiring little extra effort or resources to implement. They reflect a philosophical approach to target-based small-molecule

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drug discovery that utilizes chemistry to challenge the biological theories underlying the drug-hunting effort. Assessment of a biological target based on genetic evidence or knockdown models (i.e., siRNA or shRNA) is fraught with caveats. For example, the distinction between protein ablation and modulation with a small molecule is now a well-documented one.[10] Therefore, methods such as RNAi and newer gene editing techniques (i.e., CRISPR/Cas9)[11] should mainly be used as part of a broader data package to decide whether commencement of a medicinal chemistry discovery effort is warranted. It is only when (1) multiple optimized small molecules are discovered, ideally representing structurally divergent series that robustly alter levels of the biomarker, along with (2) closely related inactive controls, that a proof-of-concept experiment linking a phenotype to the target can carry the most weight. It is prudent to note that this does not mean success will be realized in the clinic, as weak translatability from animal models remains a factor behind attrition and is beyond the scope of this Viewpoint.[12] The modern medicinal chemist can, however, contribute to the robustness of *preclinical* validation and should routinely do so. Medicinal chemistry departments have invented numerous subclassifications of drug-discovery efforts, such as "lead optimization", "hit-to-lead", "hit-to-tool", and "fast-follower". Classifying these in a more simplified manner as either (1) *advancing* preclinically validated targets or (2) *establishing* preclinical validation should provide a more pragmatic context by which this discipline can influence success. Robust invalidation of programs is of great value to drug discovery, allowing efforts and resources to be repositioned rather than following false paths (a costly mistake). The philosophy of failing early/failing cheaply depends on the efficiency of medicinal chemists to discover drug-like tools. As such, creativity and innovation supporting early target validation work is every bit as critical as the late stage optimization phase. High quality medicinal chemistry is essential to both.

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Notes

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