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TARGET IDENTIFICATION AND VALIDATION IN THE DRUG DISCOVERY PROCESS

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Abstract

Target identification and validation are decisive early-stage activities in modern drug discovery because they determine whether a molecular intervention is biologically plausible, technically tractable, and sufficiently safe to justify downstream investment. This article provides a structured literature synthesis of contemporary approaches used to nominate and validate therapeutic targets. Fifteen peer-reviewed international sources were analysed across six evidence domains: target/druggability landscape, human genetics, functional genomics and CRISPR, chemical biology and target engagement, phenotypic deconvolution, and integrative informatics. The analysis shows that no single technology is sufficient for robust validation. Human genetic evidence strengthens causal inference and may clarify the direction of therapeutic modulation; CRISPR and related functional-genomic tools test gene dependency and pathway function in disease-relevant systems; chemical probes and proteomic methods confirm pharmacological tractability and intracellular target engagement; phenotypic strategies reveal context-dependent mechanisms; and integrated resources such as Open Targets enable evidence aggregation and prioritisation. A practical validation framework is proposed in which disease causality, directionality, tractability, target engagement, safety, translational biomarkers, and patient context are evaluated iteratively. The central conclusion is that orthogonal evidence triangulation, rather than reliance on a single assay or model, provides the most defensible basis for target nomination and reduces avoidable biological risk before lead discovery.

Keywords: target identification; target validation; drug discovery; human genetics; CRISPR; chemical probes; proteomics; target engagement; phenotypic screening; Open Targets.

1. INTRODUCTION

Drug discovery is often presented as a sequence that begins with a biological hypothesis and ends with a clinically useful therapeutic, yet the most consequential decisions are made well before a candidate molecule reaches formal preclinical

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development. Among these decisions, the choice of the therapeutic target is fundamental. A target can be a protein, nucleic acid, complex, pathway node, or another biological entity whose modulation is expected to alter a disease-relevant process. Target identification asks which entity should be perturbed; target validation asks whether perturbing that entity, in a defined direction and biological context, is sufficiently likely to produce a meaningful therapeutic effect without unacceptable toxicity. Early drug discovery therefore depends on a chain of reasoning that links disease biology to a modifiable molecular mechanism (1,2).The expansion of genomic, transcriptomic, proteomic and chemical data has dramatically increased the number of plausible targets, but it has not eliminated the central problem of prioritisation. The human proteome contains many proteins that may be biologically interesting yet difficult to modulate with conventional small molecules or biologics. The concept of the 'druggable genome' was introduced to distinguish proteins whose structural or functional properties make them more accessible to pharmacological intervention (2). Subsequent analyses showed that approved medicines remain concentrated in a comparatively limited set of target families, even though new modalities are progressively broadening the space of addressable biology (3,7). Thus, biological relevance and druggability are separate questions and must be evaluated together.

A useful target must satisfy several conditions at the same time. First, there should be credible evidence that the target has a causal or functionally important relationship to disease. Second, the desired direction of modulation should be defined: inhibition, activation, degradation, stabilisation, agonism, antagonism or another modality may have very different consequences. Third, the target should be tractable by a realistic therapeutic modality. Fourth, its modulation should be measurable through target-engagement or pharmacodynamic biomarkers. Fifth, the safety margin should be compatible with the tissue distribution, biological function and degree of pathway redundancy. Finally, the relevant disease population must be identifiable so that target biology is tested in the patients most likely to benefit. These conditions convert a biological observation into a therapeutic hypothesis (1,4).Historically, target identification frequently began with physiology, biochemistry or pharmacology. Modern programmes increasingly begin with large-scale human data. Genome-wide association studies, rare-variant sequencing, Mendelian disease genetics, expression quantitative trait loci and other forms of human genetic evidence can provide natural experiments that connect variation in a gene or regulatory element to disease risk or protection. Because these experiments occur in humans, they can strengthen causal inference beyond what is available from many preclinical models. Plenge and colleagues argued that human genetics can contribute not only to target identification but also to estimating the consequences of long-term target perturbation, while later empirical analyses reported an enrichment of successful drug mechanisms among targets with human genetic support (4,5).

The value of genetics is not absolute. Disease-associated loci may contain several genes, regulatory variants may act over long genomic distances, and a genetic effect present throughout life may not be equivalent to pharmacological modulation initiated in adulthood. Directionality can also be difficult to infer if the functional consequence of a variant is uncertain. These limitations are important because a target can be genetically associated with disease yet remain a poor pharmacological hypothesis if the causal gene is

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misassigned or if the pharmacological direction of effect is wrong. For this reason, genetics is most powerful when integrated with functional genomics, molecular phenotyping, protein biology and experimental perturbation (4,6).Functional genomics provides a second major line of evidence. Earlier approaches included antisense oligonucleotides, RNA interference, gene overexpression and model-organism genetics. The introduction of CRISPR-Cas systems substantially expanded this toolkit by enabling efficient knockout, repression, activation and precise editing across disease-relevant cell types. Genome-scale CRISPR screens can identify genes required for a phenotype, while focused perturbation can test a specific therapeutic hypothesis in isogenic systems. CRISPR also enables construction of disease models carrying patient-relevant variants and can reveal context-specific dependencies, synthetic lethal interactions and mechanisms of drug resistance (12). Nonetheless, the biological meaning of a CRISPR phenotype depends on the model, guide performance, gene essentiality and the difference between complete genetic loss and partial pharmacological inhibition.

Chemical biology adds a distinct form of validation because pharmacological perturbation can behave differently from genetic perturbation. A gene knockout removes or strongly reduces a gene product, whereas a drug may inhibit one activity domain, alter conformation, induce degradation, stabilise a complex or modulate only part of the protein's function. Carefully characterised chemical probes can therefore test whether a target is pharmacologically actionable and whether acute modulation produces the expected phenotype (9). Such experiments are especially convincing when structurally distinct probes generate concordant effects, inactive analogues are negative, resistance mutations alter compound sensitivity, and direct target engagement is demonstrated in cells.Target engagement has become a central requirement in rigorous validation. An apparent phenotypic effect cannot be confidently attributed to the intended target unless exposure at the site of action and molecular engagement are demonstrated. Chemical proteomics, affinity-based methods, activity-based protein profiling, cellular thermal shift approaches and thermal proteome profiling can identify direct or indirect interactions and characterise off-target binding. The thermal profiling work of Savitski and colleagues illustrated how ligand-induced changes in protein stability can be scaled across thousands of proteins to study drug action in living cells (10). These methods provide an essential bridge between biochemical potency and mechanism in a cellular context.

Phenotypic drug discovery introduces a complementary route to target discovery. Instead of beginning with a predefined molecular target, phenotypic screening starts from a measurable disease-relevant cellular or organismal phenotype and identifies interventions that reverse or modify it. This can capture complex mechanisms that are missed by reductionist assays, but it creates a target-deconvolution challenge: once an active compound is found, its relevant molecular target or network of targets must be identified (8,11). The most reliable deconvolution strategies combine biochemical capture, genetic sensitisation or resistance, omics signatures and orthogonal pharmacology rather than relying on a single readout.The increasing scale of biomedical data has also made target identification an informatics problem. Knowledge platforms integrate genetics, expression, pathways, animal models, existing drugs, adverse-event information and tractability data to rank target-disease associations. Open Targets is an important example of this approach. Its

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platform was designed to bring heterogeneous evidence into a traceable framework in which users can inspect the provenance of target-disease associations and consider tractability and safety alongside biological evidence (14,15). Such resources do not replace experimental validation; instead, they support systematic hypothesis generation and help identify evidence gaps before resources are committed.

A recurring lesson across these approaches is that target validation is not a binary event. It is an iterative accumulation of evidence. Confidence should increase when independent perturbations converge on the same mechanism in multiple relevant systems and when findings can be connected to human disease. Conversely, confidence should decrease when results are highly model-dependent, when genetic and pharmacological perturbations disagree, when target engagement is absent, or when safety liabilities are inseparable from efficacy. This perspective is important because late discovery failures are often the consequence of assumptions made too early about causality, relevance or mechanism (1,4).The objective of this article is to synthesise the principal approaches used for target identification and validation and to translate them into a practical, evidence-based framework suitable for early drug discovery. The review focuses on six domains: the druggable-target landscape, human genetics, functional genomics and CRISPR, chemical biology and target engagement, phenotypic target deconvolution, and integrative informatics. Particular attention is given to the way these domains can be combined to support a go/no-go decision before lead discovery.

2. MATERIALS AND METHODS

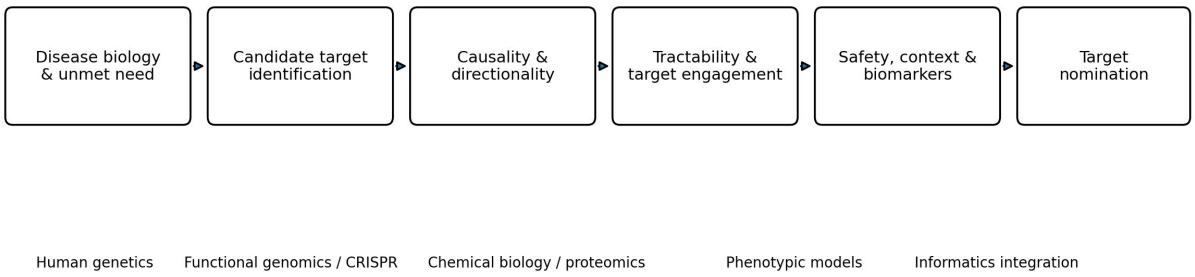
This study was designed as a structured narrative review and evidence synthesis rather than a quantitative meta-analysis. The purpose was to examine how different evidence classes contribute to target identification and validation and to derive a practical decision framework for early drug discovery. The review corpus consisted of 15 international peer-reviewed publications published between 2002 and 2023. The selected papers included foundational descriptions of the druggable genome and approved drug-target landscape, empirical analyses of human genetic support, reviews of genetic and phenotypic target validation, chemical-biology methods, proteome-wide target-engagement approaches, CRISPR-enabled functional genomics, and successive versions of the Open Targets platform (1-15).Public bibliographic records and publisher pages were used to verify article titles, authorship, journal details, publication year, pages and digital object identifiers where available. Papers were selected because they contributed directly to at least one of the following questions: (i) how candidate targets are identified; (ii) how causal relevance to disease is tested; (iii) how pharmacological tractability is assessed; (iv) how target engagement and mechanism are confirmed; (v) how safety and context are incorporated; or (vi) how heterogeneous evidence can be integrated for target prioritisation. The sample was intentionally methodologically diverse and was not intended to represent every publication in the field.

For analysis, each paper was coded into one or more overlapping evidence domains: target/druggability landscape, human genetics, functional genomics/CRISPR, chemical biology/target engagement, phenotypic screening/deconvolution, and informatics/data

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integration. Because a single paper could contribute to more than one domain, category counts were allowed to overlap. This approach was chosen to reflect the actual practice of target validation, in which evidence types are complementary rather than mutually exclusive.The extracted conceptual outcomes were organised around seven validation dimensions: disease causality, direction of effect, tractability, target engagement, biological context, safety, and translational measurability. Disease causality was defined as evidence that the target is mechanistically related to disease initiation, maintenance or a therapeutically relevant phenotype. Direction of effect referred to whether inhibition, activation or another mode of modulation is predicted to be beneficial. Tractability referred to the feasibility of modulating the target with small molecules, biologics or emerging modalities. Target engagement referred to direct evidence that an intervention interacts with the intended molecular target in a relevant system. Biological context encompassed cell type, tissue state, genetic background and disease stage. Safety referred to on-target and off-target liabilities. Translational measurability referred to biomarkers that connect target modulation to downstream biology and clinical response.

The review did not pool effect sizes because the source literature was heterogeneous in design, outcome and unit of analysis. Instead, results were synthesised qualitatively and converted into a decision matrix. A candidate target was considered to have higher validation confidence when several independent evidence types converged, particularly when human data, genetic perturbation and pharmacological perturbation pointed in the same direction. Conflicting evidence was treated as an information gap rather than averaged away. This is consistent with the principle that target validation should be iterative and falsifiable rather than a checklist that can be passed by accumulating weak evidence.Two visual summaries were generated from the review. Figure 1 presents an integrated workflow from disease biology to target nomination. Figure 2 shows the overlapping thematic coverage of the 15-source literature corpus. The latter is descriptive of the selected references only and should not be interpreted as a bibliometric estimate of the broader field. Tables were developed to compare method classes and to translate the evidence synthesis into practical go/no-go criteria.



Iterative evidence triangulation: orthogonal methods should converge before a high-cost discovery programme advances

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Figure 1. Integrated target identification and validation workflow.

3. RESULTS

3.1. Comparative landscape of target-identification and validation approaches

The literature synthesis showed that modern target validation is best understood as a sequence of risk-reduction steps rather than as a single assay. The earliest stage is broad candidate generation from disease biology, genetics, omics, literature and phenotypic observations. Candidate targets are then progressively challenged with evidence about causality, directionality and context. Only after these biological questions become sufficiently coherent does tractability become a dominant concern. The final transition to target nomination requires a connection between molecular engagement, downstream pharmacology, safety and a measurable translational strategy (1,4,6,14).The sources also demonstrate a shift from target lists toward evidence graphs. Earlier work sought to estimate how much of the genome could in principle be drugged and how many protein targets were already used by approved medicines (2,3). More recent resources integrate multiple data types and permit disease-centric or target-centric exploration with explicit evidence provenance (14,15). This change matters because a target should not be prioritised simply because it is novel or technically druggable. Its value depends on the coherence and quality of evidence connecting it to a disease mechanism.

Table 1 summarises the major method classes identified in the corpus. Each method contributes a different type of inference. Human genetics is comparatively strong for causal association in patients but may be weak for pharmacological tractability. CRISPR is powerful for testing gene dependency and pathway function but may exaggerate the effect of complete loss of function. Chemical probes can test acute pharmacology and tractability but require high selectivity and rigorous controls. Proteomic target-engagement approaches can verify physical interaction but do not by themselves prove therapeutic benefit. Phenotypic screening captures complex biology but may require extensive deconvolution. Informatics platforms improve integration and transparency but remain dependent on the quality of underlying datasets.

Table 1. Major evidence classes used in target identification and validation.

Evidence class	Primary question	Typical tools	Strength	Main limitation
Human genetics	Is the target causally linked to disease, and what direction may be beneficial?	GWAS, rare variants, Mendelian genetics, eQTL/pQTL, colocalisation	Direct relevance to human biology; may support causality and direction	Variant-to-gene mapping and lifelong genetic effects may not equal drug effects

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Evidence class	Primary question	Typical tools	Strength	Main limitation
Functional genomics	Does changing target function alter a disease-relevant phenotype?	CRISPR knockout, CRISPRi/a, RNAi, overexpression, isogenic models	Direct perturbation; scalable; context-specific dependency mapping	Complete genetic loss can differ from partial pharmacology; model dependence
Chemical biology	Can the target be modulated pharmacologically with the expected phenotype?	Selective probes, inactive controls, resistance mutations, structure-activity analysis	Tests acute pharmacology and tractability	Off-target effects and inadequate probe quality can mislead
Proteomics / engagement	Does the intervention physically engage the intended target in cells or tissue?	CETSA/thermal profiling, affinity capture, ABPP, chemoproteomics	Mechanistic confirmation in complex biological systems	Engagement alone does not prove disease efficacy
Phenotypic discovery	Which intervention reverses a disease-relevant phenotype, and what target explains it?	Cell-based screens, organoids, high-content imaging, deconvolution	Captures integrated biology and unknown mechanisms	Target identification can be difficult; assay phenotype may not translate
Informatics integration	How strong and coherent is the total evidence for a target-disease pair?	Open Targets, knowledge graphs, multi-omics integration	Transparent aggregation; prioritisation; identifies evidence gaps	Dependent on source quality, ontology mapping and scoring assumptions

3.2. Human genetics as a source of causal and directional evidence

Human genetics emerged as one of the strongest evidence domains because it can connect naturally occurring variation to disease phenotypes before a therapeutic

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programme exists. Plenge, Scolnick and Altshuler framed genetic variants as 'experiments of nature' that can help test therapeutic hypotheses in humans (4). Nelson and colleagues subsequently examined approved drug indications and reported that disease-associated genes were enriched among successful drug targets, supporting the practical value of genetic information in target selection (5). Finan and colleagues expanded this concept by linking disease-associated loci to a curated druggable genome, illustrating how genetics can identify both new discovery opportunities and repurposing hypotheses (6).The most important contribution of genetics is not simply a ranked list of genes. It can help answer whether reduced or increased target function is associated with protection or risk. Loss-of-function variants that reduce disease risk may support pharmacological inhibition, whereas gain-of-function biology may suggest a different strategy. However, directionality must be inferred cautiously. Many common variants have regulatory effects that are small, tissue-specific or difficult to assign to a single gene. Linkage disequilibrium can connect the lead association signal to several plausible causal variants, and the nearest gene is not necessarily the causal gene. Therefore, genetic evidence is strongest when fine-mapping, colocalisation, functional annotation and experimental perturbation converge on the same target (4,6).

The selected literature also indicates that genetic support changes probability rather than guaranteeing success. King and colleagues re-examined the claim that genetically supported drug mechanisms are 'twice as likely' to reach approval and showed that the magnitude of enrichment depends on definitions, datasets and analytical assumptions (13). This is an important qualification. Genetic support should increase confidence in a target-disease relationship, but it does not resolve chemistry, pharmacokinetics, tissue exposure, adverse effects or the temporal difference between inherited perturbation and therapeutic intervention. A genetically compelling target can still be undruggable or unsafe, while a target with limited genetic evidence may be valid through other mechanisms.In practice, genetic evidence is most informative when integrated early, before a target is locked into an expensive programme. The key operational questions are: Is the association replicated? Is the causal variant credible? Is the effector gene supported by functional data? Does the variant alter expression or protein function in the disease-relevant tissue? Does the direction of effect align with the proposed drug mechanism? Are there human phenotypes suggesting tolerability of partial or complete target inhibition? Answering these questions turns a statistical association into a pharmacologically useful hypothesis (4-6,13).

3.3. Functional genomics and CRISPR-based validation

Functional genomic methods provide a controlled way to test whether a candidate target is necessary, sufficient or modulatory for a disease-relevant phenotype. CRISPR-Cas systems transformed this stage by allowing efficient gene knockout, transcriptional repression, transcriptional activation and precise sequence editing. Fellmann and colleagues described how CRISPR can accelerate target identification, create more faithful disease models and support large-scale genetic screens (12). In early discovery, the technology is particularly useful for moving from correlation to perturbation: a candidate derived from genetics or omics can be directly modified and its effect on the phenotype

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measured.Genome-scale screens are valuable when the relevant biology is incompletely understood. They can reveal dependencies that are specific to a disease genotype, identify synthetic lethal interactions or uncover genes that confer resistance to a pharmacological agent. Focused CRISPR libraries can then test pathways and candidate sets with higher experimental depth. CRISPRi and CRISPRa are especially informative when complete knockout is lethal or biologically unrealistic because they can approximate graded reduction or activation. Precise editing can also reconstruct patient variants in isogenic backgrounds, helping distinguish causal effects from background genomic noise (12).

Nevertheless, a CRISPR hit is not equivalent to a validated drug target. First, complete knockout may produce a phenotype that cannot be achieved safely with a medicine or may overstate the effect of partial inhibition. Second, sgRNA efficiency, copy-number effects and off-target editing can create false-positive or false-negative results. Third, cell lines may lack the tissue architecture, immune interactions or chronic disease state required for translation. Fourth, a genetic perturbation changes protein abundance or function over a longer period than many drugs. For these reasons, orthogonal confirmation with multiple guides, rescue experiments, independent perturbation technologies, pharmacological probes and disease-relevant models is essential.The strongest CRISPR-based validation therefore uses a hierarchy of models. Initial screens can be performed in tractable cell systems, but priority targets should progress into primary cells, patient-derived cells, induced pluripotent stem cell models, organoids or in vivo systems when these add disease relevance. The purpose is not to maximise experimental complexity; it is to test whether the phenotype survives increasingly realistic biological contexts. A target that disappears when the model becomes more disease-relevant should be reconsidered before substantial medicinal-chemistry resources are committed.

3.4. Chemical probes, target engagement and proteomic confirmation

Genetic perturbation establishes biological relevance, whereas chemical perturbation tests whether that relevance can be translated into pharmacology. Bunnage and colleagues emphasised that well-characterised chemical probes are essential for rigorous target validation because poor selectivity can create misleading biological narratives (9). A high-quality probe should have sufficient potency, defined selectivity, appropriate cellular activity and a negative control or inactive analogue where possible. Confidence increases when more than one chemically distinct probe produces the same target-dependent phenotype.Target identification after a phenotypic hit often requires direct biochemical or proteomic deconvolution. Schenone and colleagues grouped target-identification strategies into direct biochemical methods, genetic interactions and computational inference, arguing that combinations are often required to resolve on-target and off-target effects (8). Direct approaches such as affinity capture can identify binding proteins, whereas resistance mutations and chemogenetic interactions can reveal mechanistic dependencies. The weakness of direct binding data is that not every binder explains the phenotype; the weakness of functional data is that it may identify pathway dependencies rather than the direct molecular target. Convergence between the two is therefore particularly powerful.

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Cellular target-engagement measurements address another critical source of uncertainty: whether the candidate drug physically interacts with its intended target at relevant concentrations in an intact biological system. Thermal proteome profiling extends the principle that ligand binding can alter protein thermal stability and can survey thousands of proteins simultaneously. Savitski and colleagues demonstrated this approach in living cells, providing a proteome-wide view of drug-target interactions and potential off-targets (10). Such information can separate failures of exposure or engagement from failures of target biology.

Target engagement should be connected to downstream pharmacology through an exposure-engagement-response relationship. If increasing compound concentration produces greater target engagement but no change in the predicted downstream biomarker, the biological hypothesis may be incomplete. Conversely, if the phenotype changes at concentrations where the intended target is not engaged, an off-target mechanism should be considered. This logic is particularly important in target validation because apparent potency in a phenotypic assay can create false confidence when the true mechanism is unknown.

Proteomic and chemical-biology methods also contribute to safety assessment. Off-target proteins detected at clinically relevant concentrations may explain undesirable phenotypes or help define a selectivity window. The ideal validation package therefore includes evidence that the intended target is engaged in the relevant cell or tissue, that engagement produces a mechanistically consistent biomarker response, and that the phenotype is not better explained by another target. This package provides a pharmacological counterpart to genetic causality.

3.5. Phenotypic discovery and target deconvolution

Phenotypic drug discovery remains important because many diseases are driven by network-level dysfunction that is difficult to reduce to a single predefined target. Moffat and colleagues highlighted both the opportunities and challenges of this strategy in industry (11). A phenotypic assay can preserve aspects of cellular context, pathway interaction and compensatory biology that are absent from a purified target assay. It may therefore identify therapeutically useful mechanisms that would not have been prioritised from target-based reasoning alone.

The major challenge is deconvolution. A compound that corrects a phenotype may act through one target, several targets or a downstream physicochemical effect unrelated to the hypothesised pathway. Target identification requires a set of independent experiments. These may include affinity methods, thermal profiling, resistant clone sequencing, CRISPR sensitisation or resistance screens, transcriptomic signatures, metabolomics, pathway rescue and comparison with annotated chemical probes (8,10,11). The objective is not merely to name a binding protein but to identify the target or target set whose modulation is necessary and sufficient to explain the phenotype.

Phenotypic models also make context dependence explicit. A target may be active only in a particular genotype, differentiation state, tissue microenvironment or disease

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stage. This is not a weakness if it can be translated into a biomarker-defined patient subgroup. Indeed, context-specific activity can become a precision-medicine advantage. The validation challenge is to distinguish a meaningful disease context from an artefact of the assay. Replication across models that preserve the relevant biology and demonstration of a mechanistic biomarker are therefore essential.

3.6. Evidence integration and data-driven prioritisation

The transition from individual experiments to target nomination requires integration. Open Targets was developed to support systematic target-disease identification and prioritisation by combining evidence from genetics, genomics, drugs, pathways, literature and other sources. The 2021 platform update described evidence from multiple data sources together with CRISPR cancer-screen information, genetics and annotations related to tractability and safety (14). The later next-generation platform expanded the architecture and user experience while preserving the central principle that target prioritisation should be evidence-based and traceable (15).

Integrated platforms are useful for three reasons. First, they reduce search fragmentation by linking evidence that would otherwise be distributed across separate databases. Second, they expose disagreement: a target may have strong genetics but little tractability, or strong expression evidence but known safety concerns. Third, they support prioritisation before experimental investment. Importantly, an association score is not a substitute for scientific judgment. Scoring models depend on evidence weighting, ontologies and data availability, so users should inspect the underlying evidence rather than treat a rank as a definitive answer (14,15).

The literature suggests that the most defensible target-selection process is therefore 'triangulated'. A target receives progressively higher confidence when human genetics, functional perturbation, pharmacological modulation and target-engagement data agree. Confidence is further increased when a biomarker connects target modulation to disease biology and when human or preclinical safety information indicates a feasible therapeutic window. This triangulation approach is conceptually more robust than requiring a fixed number of experiments because evidence quality differs between targets and disease areas.

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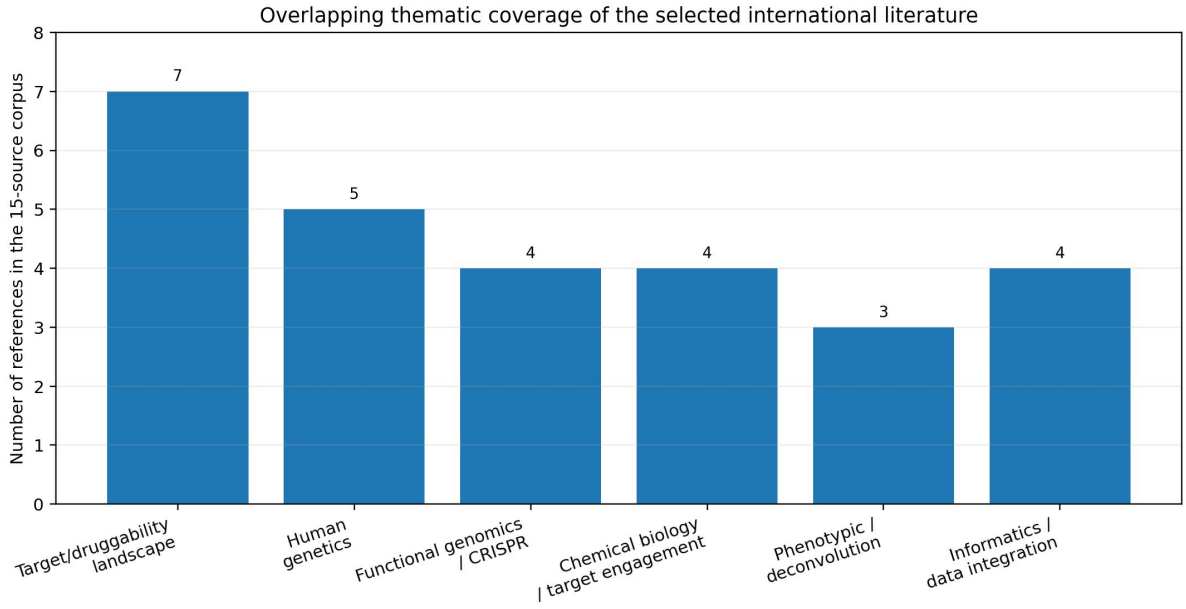


Figure 2. Overlapping thematic coding of the 15 selected references. Categories are not mutually exclusive and the figure is descriptive of this review corpus, not of publication frequency in the field.

3.7. Practical target-validation criteria for go/no-go decisions

A practical consequence of the synthesis is that target validation can be organised around explicit decision questions. These questions should be answered before a programme moves from exploratory biology into sustained lead-generation effort. The first question is causal relevance: does perturbing the target change a disease mechanism rather than merely correlate with it? The second is directionality: what form and degree of modulation are required? The third is tractability: can a therapeutic modality reach and modulate the target? The fourth is target engagement: can molecular interaction be measured in the relevant system? The fifth is context: which tissues, genotypes or disease stages determine response? The sixth is safety: what are the predictable consequences of on-target and off-target modulation? The seventh is translation: what biomarker will demonstrate that the mechanism is operating in humans?

These criteria should not be treated equally for all programmes. For a genetically validated extracellular target with an obvious antibody modality, tractability may be relatively straightforward while safety and patient selection dominate. For an intracellular scaffold protein, tractability may be the principal uncertainty. For an oncology target identified from a synthetic-lethal CRISPR screen, context specificity and resistance mechanisms may require the greatest attention. The go/no-go framework therefore works best as a risk map that identifies the weakest link in the therapeutic hypothesis.

Table 2. Proposed target-validation decision matrix.

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Dimension	Go signal	Warning signal	Recommended next experiment
Disease causality	Human genetic support or convergent perturbation across relevant models	Association only; inconsistent model effects	Orthogonal genetic perturbation, rescue, patient-derived model
Direction of effect	Clear benefit from defined increase/decrease in target activity	Mixed or U-shaped effects; uncertain functional consequence	Dose/allelic series, CRISPRi/a, agonist/antagonist comparison
Tractability	Accessible binding site or validated modality with cellular activity	No feasible modality or poor tissue access	Structural analysis, modality switch, degrader/biologic feasibility work
Target engagement	Engagement demonstrated at concentrations producing biomarker response	Phenotype without measurable engagement	CETSA/TPP, occupancy assay, chemoproteomic confirmation
Context / patient selection	Reproducible activity in disease-relevant genotype or tissue	Effect restricted to artificial model conditions	Primary cells, organoids, stratified models, biomarker mapping
Safety	Therapeutic window supported by genetics, tissue biology or selective pharmacology	Essential normal-tissue function or narrow selectivity window	Conditional models, safety panels, tissue-selective strategy
Translation	Mechanistic biomarker links exposure to engagement and response	No practical biomarker for human studies	Develop proximal PD marker or imaging/omics readout

4. DISCUSSION

The central finding of this review is that target validation is most reliable when framed as evidence triangulation rather than technology selection. Each method answers a different

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question, and the failure to distinguish these questions is a common source of false confidence. A genetic association can support causal relevance but does not prove that a target can be modulated with a drug. A potent biochemical inhibitor can prove tractability but does not establish disease relevance. A CRISPR phenotype can establish dependency but may reflect complete loss of function that is not pharmacologically achievable. Target-engagement data can prove molecular interaction but do not guarantee therapeutic efficacy. A robust programme therefore requires the evidence types to connect logically rather than merely accumulate numerically (4,8,9,12).

Human genetics deserves particular emphasis because it provides information in the species of ultimate interest. The empirical literature supports the view that genetically supported mechanisms are enriched among successful therapeutic targets, but the strength of that relationship varies by dataset and definition (5,13). The correct interpretation is probabilistic. Genetic evidence can shift prior confidence in a therapeutic hypothesis and can identify mechanism direction, potential safety phenotypes and biomarker subgroups. It cannot remove uncertainty created by imperfect variant-to-gene mapping or by the fact that inherited perturbation across decades differs from treatment in a patient with established disease.

The direction-of-effect problem is especially important. Drug discovery does not target genes in the abstract; it targets a particular biological function in a defined direction. A disease association may arise because increased activity causes risk, because decreased activity causes risk, or because the gene marks a regulatory process without being the causal effector. If a programme selects the correct protein but the wrong direction of modulation, even perfect medicinal chemistry will not rescue the therapeutic hypothesis. Functional studies should therefore reproduce an allelic or pharmacological series rather than rely only on binary knockout. CRISPRi, CRISPRa and calibrated chemical probes can be used to determine whether the desired phenotype follows a plausible dose-response relationship (4,12).

Model relevance is the second major challenge. Many early experiments are performed in cell lines because they are reproducible, scalable and compatible with screening. However, disease biology may depend on differentiated cell states, multicellular interactions, mechanical environment, chronic inflammation, endocrine signals or immune components that are absent from simplified systems. Validation should therefore progress toward models that capture the specific biological features required by the hypothesis. This does not mean that every target requires an animal model. In some cases, primary human cells, patient-derived organoids or genetically defined in vitro systems may be more informative than a species model with weak target homology or different pathway regulation.

Pharmacological validation must also account for the difference between gene removal and molecular modulation. Genetic knockout can eliminate scaffolding, enzymatic and structural functions simultaneously, whereas a small molecule may affect only one domain. Conversely, a degrader may remove the protein more comprehensively than a conventional inhibitor. Antibodies may block ligand binding but leave intracellular signalling architecture intact. The selected modality is therefore part of the target hypothesis, not an

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www.jadr.co.in
Volume 17, Issue 04 Received 28.08.2026 Accepted 30.08.2026 Online 06.09.2026

implementation detail that can be postponed. Early validation should use a perturbation that approximates the intended therapeutic mechanism whenever feasible (9).

Chemical probes are valuable precisely because they allow researchers to ask whether a biological target is susceptible to acute, dose-dependent pharmacology. Yet probe quality is critical. A compound with insufficient selectivity can generate a convincing phenotype through an unrelated protein and create a false target narrative. Orthogonal probes, inactive analogues and resistance mutations reduce this risk. Direct target-engagement measurements provide another layer of control by demonstrating that the intended target is physically occupied or altered in the same concentration range that produces the biological response (9,10).

The development of proteome-wide engagement technologies has changed the standard of evidence available for mechanism studies. Thermal profiling and chemoproteomics can reveal both intended and unintended targets in complex systems. This is particularly important during target validation because off-target activity is not merely a medicinal-chemistry problem; it can invalidate the biological conclusion itself. If a phenotype tracks with engagement of an off-target rather than the nominated protein, the programme should be redirected before further optimisation. Conversely, a clean exposure-engagement-response relationship can strongly increase confidence that the observed phenotype is mechanistically meaningful (10).

Phenotypic discovery illustrates why target identification and target validation are closely linked. In a target-based programme, the identity of the protein is known at the start, but its disease relevance is uncertain. In a phenotypic programme, a disease-relevant effect may be observed early, but the mechanism is uncertain. Both routes converge on the same requirement: a defensible connection between molecular perturbation and a clinically relevant biological outcome. For phenotypic hits, target deconvolution should therefore be followed by the same causal, tractability, safety and translational evaluation applied to targets discovered from genetics or omics (8,11).

Data integration platforms address a practical challenge created by the scale of modern biology. A target may have evidence distributed across thousands of publications and many specialised databases. Open Targets provides an example of how this information can be harmonised into target-disease associations, while still allowing users to inspect the underlying evidence and source provenance (14,15). This transparency is essential. Black-box prioritisation may generate rankings, but early drug discovery requires scientists to understand why a target ranks highly, what evidence is missing and whether the supporting data concern the same disease subtype, tissue and mechanism.

A useful operational model is to separate evidence into 'must be true' assumptions and 'nice to have' support. For each programme, the team should identify the small number of assumptions that, if false, would invalidate the therapeutic hypothesis. Examples include: the target is causal rather than correlated; inhibition rather than activation is beneficial; sufficient target engagement can be achieved in the disease tissue; and on-target modulation is tolerated in normal tissue. Experiments should be prioritised to attack these assumptions directly. This falsification-oriented strategy is often more informative than performing many confirmatory assays that all depend on the same underlying model.

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Safety should be considered during validation rather than after lead optimisation. A target may be mechanistically effective but unacceptable because the same biology is essential in normal tissues. Human genetic data can sometimes reveal whether loss-of-function states are tolerated, while expression patterns, conditional genetic models and selective probes can identify organ-specific liabilities. Safety assessment should also distinguish on-target toxicity from off-target toxicity. The former may require dose limitation, tissue-selective delivery or a different modality; the latter may be addressed by improved selectivity. Early distinction between these mechanisms can prevent unnecessary abandonment of valid targets or, conversely, prevent prolonged investment in intrinsically unsafe hypotheses.

Translational biomarkers are another underappreciated component of target validation. A biomarker is not simply a clinical-development tool; it is a way to make the target hypothesis testable. A proximal pharmacodynamic marker can show that target engagement causes the expected molecular consequence. A distal biomarker can connect that consequence to disease biology. In early human studies, the combination of exposure, target engagement, pathway modulation and clinical phenotype allows negative results to be interpreted. Without such biomarkers, a failed trial may remain ambiguous because investigators cannot distinguish insufficient exposure from incorrect biology.

The evidence framework also has implications for artificial intelligence and computational target discovery. Machine-learning systems can integrate high-dimensional molecular data, literature, networks and perturbational datasets to nominate targets or predict target-disease relationships. Their value depends on the quality of the evidence graph and the extent to which predictions can be experimentally falsified. Computational scores should therefore be treated as hypothesis prioritisation tools, not as validation in themselves. The same seven dimensions used for experimentally nominated targets—causality, directionality, tractability, engagement, context, safety and translation—remain necessary.

There are limitations to this review. The corpus was deliberately selective and designed to cover methodological breadth, not to provide an exhaustive systematic review of all target-validation literature. The thematic counts in Figure 2 describe the selected 15 references and should not be interpreted as estimates of the relative size or importance of the corresponding research fields. In addition, methods and platforms continue to evolve rapidly, particularly in single-cell omics, spatial biology, proteolysis-targeting technologies, base and prime editing, human organoid systems and AI-enabled data integration. The proposed framework should therefore be updated as new modalities change what is considered tractable or measurable.

Despite these limitations, the major principles are stable. Strong target validation depends on convergence of independent evidence, use of disease-relevant models, explicit testing of mechanism direction, demonstration of pharmacological engagement, and early consideration of safety and biomarkers. The most valuable outcome of validation is not certainty—biology rarely permits that—but a transparent statement of what is known, what remains uncertain and which experiment would most efficiently change the decision.

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5. CONCLUSION

Target identification and validation determine the biological foundation on which the rest of a drug-discovery programme is built. The literature reviewed here shows that target quality cannot be reduced to novelty, statistical association or biochemical tractability alone. A defensible target hypothesis requires a coherent relationship between human disease, molecular perturbation and pharmacological feasibility. Human genetics contributes causal and directional evidence; functional genomics and CRISPR test dependencies and disease mechanisms; chemical probes and proteomics establish pharmacological tractability and target engagement; phenotypic approaches preserve complex biological context; and integrative platforms organise heterogeneous evidence and expose gaps (4-15).

The most practical strategy is iterative triangulation. Before lead discovery, teams should ask whether the target is causally relevant, whether the required direction of modulation is known, whether the intended modality can engage the target in the relevant tissue, whether the phenotype is reproducible in disease-relevant systems, whether safety liabilities are manageable, and whether a translational biomarker can demonstrate mechanism in humans. A target that performs well across these dimensions is more likely to justify continued investment than one supported by a single compelling experiment. Equally important, a target with a clear unresolved weakness should trigger a focused experiment rather than automatic progression.

Accordingly, target validation should be treated as a dynamic decision process rather than a one-time milestone. The purpose is to reduce biological uncertainty early, when changing direction is still relatively inexpensive. As genomic, proteomic, genome-editing and computational technologies continue to expand, the number of candidate targets will grow, but the need for rigorous, orthogonal validation will become even more important. The strongest discovery programmes will be those that combine human evidence, precise perturbation, direct pharmacology and transparent data integration into a single testable therapeutic hypothesis.

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