
Supplementary information

Improving target assessment in biomedical research: the GOT-IT recommendations

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Supplementary Table 1 | **Members of the BMBF-Target Validation Initiative (for each project)**

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Supplementary Table 2 | **Template for a Target Assessment Project Plan**

The Target Assessment Project Plan (TAPP) defines the overall goals of a target assessment project and outlines the strategy needed to achieve the goals specified¹. The clear definition and articulation of a TAPP can help focus the work of the project team. As a general rule, projects tend to require input from several key areas and are best served when interactive discussions between regulatory, clinical, toxicological and pharmacological experts are included. This ensures that all perspectives are considered early in the process and that goals are clearly set in a broad sense.

Target Assessment Project Plan

Goals and expectations		Answer/Comment
Indication and usage	What is the indication/ disease/ condition of interest?	
	What is the medical need?	
	What will be the final drug product or treatment approach?	
	Is it for treatment or prevention?	
Patient Population	Does a defined patient population exist?	
Innovation	Are other drugs currently in use for treatment of the same clinical indication?	
Adverse Reactions	What adverse effects might be expected in response to the target class?	
	What adverse reactions have been observed in clinical studies?	
	What is the minimum safety profile which can be accepted?	
Project goals	What are project milestones?	
	What are project endpoints/outcomes? What is the drug discovery value chain endpoint of this project?	
Critical Path		Answer/Comment
For each chosen Assessment Block	What are key experiments for decision-making?	
	How can the Level of Confidence for decision-making be maximized?	
	What are Go/NoGo decision criteria?	
	Can tasks/goals be achieved with resources available? If not, what is the most meaningful compromise (and why)?	
	What are critical Quality Requirements for this Assessment Block?	

Causal relationships

1. Is the target expressed in relevant human cells/tissue (i.e. within the anticipated patient population)?
2. Does the target expression vary with disease severity or progression?
3. Can published results in support or against the causality be identified and their strengths and limitations be specified?
4. If the association is genetic, is there an equivalent modification at the target level (e.g. RNA or protein level) or target activity level (e.g. activating mutations in cancer) in diseased tissues?
5. Does dysregulation of target lead to the disease phenotype? (e.g. can a temporal relationship be established? Can a dose-response relationship be established?)
6. Can the influence of any confounding factors be excluded?
7. Are data sets to establish causality based on observational studies or prospectively planned experiments?
8. Is an association of the target with the disease based on clinical observations?
9. Does the use of tool compounds result in the same phenotype as, for instance, respective knockdown/knockout studies?

Relevance of the model system

10. Have healthy cells/tissues/animals been compared with diseased systems?
11. Can *in vitro* cell-based mechanistic studies be used to reveal characteristics of targets and the pathways in which they are involved?
12. For *in vivo* studies, does a functional connection exist between the animal model and humans?
13. Is extrapolation across species possible in order to select a relevant species?
14. Is the target functional in the preclinical species of choice?
15. Can human material be used to investigate the biology of the target?
16. If the target exists in multiple forms, do the expression levels of these variants vary across tissues and species?

Target manipulation procedures

17. Can the target-dependent effect be analysed in a bi-directional manner (e.g. rescuing the target knockout phenotype by re-expressing the protein of interest in target-deficient cells)?
18. Can highly selective target manipulation processes (e.g. KO technologies) be used rather than non-selectively induced changes in target?
19. Are knockout/knockdown studies complemented by small molecule or antibody studies that show the same phenotype?
20. Can the target be manipulated on a functional level rather than gene- or protein expression level?
21. Can naturally occurring target modification processes be analysed (e.g. gain-of-function or loss-of-function mutations)?
22. Does the target manipulation process lead to disease-affecting modifications?

Readout

23. Are readouts used therapeutically close to the clinical endpoint (e.g. a functional manifestation of disease, like muscle contraction)?
24. Can target-dependent processes be analysed on a system- or pathway-level, rather than gene- or protein-level?
25. Can disease-affecting modifications be analysed rather than symptomatic modifications?

Stimulus

26. Is the endogenous ligand/stimulus for the target and its concentration in the disease state known?

27. Can a biological system be used that intrinsically contains the appropriate disease-relevant stimulus?
28. Is the stimulation period justified by disease?
29. Target pathway: Does modulation of up- and downstream proteins lead to the same target-related phenotype?

Effect sizes

30. Is statistical significance treated as a criterion sufficient for decision making?
31. Can the therapeutic meaningfulness be treated as a criterion necessary for decision making?

Reported evidence and prior knowledge

1. Are any loss or gain-of-function mutations published in human and/or (preclinical) animal genetic databases?
2. Did any previous drug used to modify the target or a related pathway result in toxicity?
3. Do clinical study inclusion criteria provide any information on potential toxicities?
4. How unique is the target: are there conserved regions in homology of family and/or super-family members (e.g. are there redundancies)?
5. Could the modulation procedure interfere with other targets with similar sequence or other members of the target family?
6. Are there anticipated consequences of these off-target effects?

Target expression/tissue distribution

7. Is the target differentially expressed in samples representing the diseased state versus healthy controls?
8. Do the target distribution and expression patterns in humans and in the preclinical species differ?

Tool compound use

9. Does tool compound use lead to the same (toxic) phenotype compared to KO/KI animals?
10. How does the toxicity assessment of inactive enantiomers differ compared to active tool compounds?

Pathway analysis and target function

11. Is the target involved and plays a role in potentially harmful pathways and/or physiological processes
12. If understanding of the target function is limited, can pathway up- or downstream components (or specific cellular functions related to the target) be analysed?

Model system and translatability

13. Are *in vitro* or pharmacologically relevant animal models available for safety testing?
14. Are the models to establish a link between target and disease of interest (see Target-Disease Linkage) and target-related safety models derived from the same species?
15. Is there a need to use transgenic animals for safety assessment?
16. Do transgenic KO/KI animals or target knockdown studies show adverse side-effects?
17. Species specificity: Are there differences in anatomy, physiology, target biology and/or expression distribution in sensitive species that may explain any species difference?

Safety biomarker

18. Can a cross-species, easily accessible, accurately measurable safety biomarker be identified or developed?
19. Can off-target binding/effects (detected by the biomarker) lead to potential toxicity?

Safety window/risk–benefit ratio

20. Can a significant therapeutic window between pharmacological effects and onset of toxicity be identified?
21. Which safety window may be acceptable for the desired indication or patient population?
22. Is the mechanism of toxicity known that is driving the safety margin evaluation?
23. Are safety biomarkers known that allow monitoring of safety risks as well as intervention (e.g. cessation of treatment, antidote)?
24. Is there a likely benefit for the patient population?

Properties of the target and the microbial population

1. Is the target essential for growth or survival of the organism under a chosen condition (Essentiality)?
2. Is the target amenable to chemical modulation (druggability)?
3. Is the target accessible to the inhibiting/modifying agent (accessibility)?
4. Is the target present in critical species (spectrum analysis)?
5. Do permeability and efflux properties of the microbial population of interest affect treatment options?

Targeted gene modification and phenotype analysis

6. Do target mutations result in a phenotypic change (e.g. loss of bacterial viability)?
7. What is the phenotype of modified strains upon target downregulation or overexpression (e.g. in the absence and presence of a tool compound)?
8. For an essential target, does a critical expression level threshold exist below which a particular phenotype (e.g. growth inhibition) will manifest?
9. Can the target vulnerability (defined as the degree of inhibition of the target's function), which is required to significantly impact the cellular phenotype, be determined?

Tool compounds

10. For tool compound studies, can a variety of negative controls be included to show that the tested phenotypes are not caused by other classes of inhibitors not interfering with the target?
11. Can the risk of rapid resistance development be assessed with tool compounds?
12. Does antimicrobial activity track with enzyme-inhibitory potency?
13. If small molecule compounds are used as tool compounds, are they prone to chemical modification to optimize properties?

Pathway analysis

14. If one target enzyme is downregulated, does this affect another protein, e.g. in case both are part of the same pathway?
15. Pathway rescue: If a target is involved in a specific biosynthesis pathway, can the pathway end-product rescue a target-mutant phenotype?
16. For central metabolism pathways, how many pathways are available to the organism to synthesize a particular metabolite?
17. Can the organism scavenge the metabolite (or precursors) from the host?
18. Is the metabolite required throughout the course of infection, or only in certain disease states?

Safety

19. If there is a human homologue, can the microbial target be addressed specifically without interfering with the human counterpart?
20. If there is a human homologue, are there essential features in molecular structure, regulation or co-factor dependency that differentiate between them?
21. Can a significant therapeutic window between pharmacological effects and onset of toxicity be identified?
22. Are beneficial microbial populations (e.g. gut bacteria) affected by the antimicrobial treatment?

Target location, expression and species specificity

23. If the target is present only in specific species, does a tool compound show activity in only these species?
24. Is addressing only a single species sufficient cover for the intended therapeutic indication?

Resistance

25. Does the target of interest show mutations with high-fitness costs that prevent retention of resistance after removal of the selective pressure?
26. Does the target of interest show low potential for by-pass/compensatory mutations?
27. Does the target show low frequency of single-step and serial-passage mutations to resistance?

Method selection

28. Technical execution: Are current 'state-of-the-art' methods used or can the methods used be compared to the gold standard?

IP/patent situation

1. Can *screening assay* claims be used to protect newly developed target-related screening assays?
2. If the target can be modulated by RNAi and RNAi probes are known, can a patent be filed claiming the use of these probes against the target for the particular disease?
3. If the target can be modulated by an antibody, can a patent be filed claiming antibodies against the target?
4. Have antibodies (e.g. for humanization) been protected before use in any scientific publications?
5. Does the need to publish scientific results (including abstracts or public lectures) put any patent application at risk (and prohibit successful out-licensing)?
6. Defensive publication: If patenting is not possible, can 'prior art' be established by publishing a detailed description of the new target-related invention to prevent others from later being able to patent the invention?
7. Is the target modulation/compound screening approach unique in relation to existing IP?
8. Is it worthwhile to conduct a freedom to operate (FTO) analysis with regard to assays and reagents?
9. If patents by others exist, when were prior art references first published?

Unmet medical need

10. Do existing therapies have serious limitations with regard to efficacy or safety or both which may be overcome by the new approach?
11. Are the current treatment options disease modifying (or only symptomatic)?
12. Is the ratio between incidence of disease in a population and deaths in a population (because of a relevant disease) known?
13. Can biomarkers be developed for patient stratification (i.e. which patients would benefit from target modulation)?
14. Is the size, stratification and geographical distribution of the patient population known?
15. Are clinically relevant groups of patients identified?

Competitive landscape and differentiation

16. Is there a problem, inherent to the market, which would be solved by the new target-based product?
17. Are new compounds/biologics needed in addition to those discovered so far?
18. Is the new approach to modulate the target different to that used for existing medicines?
19. Is the target-based treatment approach novel (e.g. has a literature analysis been performed)?
20. Is there a rationale for target selection to drive differentiation over current standard of care?
21. Is the target of interest entirely new or have analogous targets been reported or patented?
22. What successes or failures have there been in the target area?
23. Is the new target-related treatment approach superior to the competitor's product or method?

Commercial needs

24. Can a first hit/lead compound be developed to increase the commercial value of a data package?
25. Are costs needed to reach specific decision points (see TA Project Plan) defined and covered?
26. Are there potential additional indications in which the target might also play a role?
27. Do clinical data exist that are helpful when considering a new indication for a known target?
28. Can the future market size be estimated?
29. Does the target only play a role in rare diseases with small patient populations?
30. Can the reimbursement environment be assessed?

Accessibility

1. Is the location of the target in its specific organ known?
2. Has the target location been taken into account when designing modulating compounds?
3. Do different forms or splice variants of the target influence cellular location or tissue distribution?
4. Should the target be blocked or activated? If target activation is required, can this be achieved?
5. Can a biological reaction be provoked as a consequence of a tool compound interacting with the target?

Modifiability

6. Is any bio-structural information available on the target?
7. Are 3D-structures available that enable the prediction of potential binding sites for small molecules (e.g. via structure-based druggability search engines)?
8. Are hit compounds/modulators already reported to modify the target?
9. Do different forms or splice variants of the target influence affinity for drug binding?
10. For target classes that have not been drugged before and/or are classified as difficult such as PPIs, can supporting structural data be obtained that demonstrate the druggability potential?

Assayability

11. Do meaningful surrogate endpoints exist that predict a clinical endpoint of interest?
12. Have methods already been reported for assaying the target?
13. If target splice variants exist in cells, are full-length proteins or catalytic domains more relevant for screening?
14. Is an orthogonal assay for HTS hit confirmation available to rule out assay artefacts?
15. Are secondary assays available with sufficient throughput to efficiently test the compounds from HTS?
16. What could be potential deselection assays and counter-screens (negative selection)?
17. Have the proposed discovery and orthogonal assays been tested with reference compounds and biological/genetic controls to confirm a link to target-mediated biological effects?
18. Is the assay pharmacologically predictive for the disease state?
19. Are assay reagents and volumes optimized with regard to costs required?
20. Is the Z factor of the assay greater than 0.4?
21. What final compound solvent concentrations can be tolerated or accepted?
22. Can a training set of compounds be screened to verify that the assay is performing acceptably?
23. Does the HTS meet standards for metrics such as robustness, efficiency and reproducibility?

Tool compounds

24. Can (in vivo) experiments be conducted to determine exposure-efficacy relationships?
25. If full target occupancy is confirmed for a tool modulator, does the tool modulator produce the expected therapeutic effect?
26. For tool antibodies: Can binding to the immunogen (e.g. via ELISA assays), cross-reactivity/specificity and potency be controlled?
27. For tool molecules: Can binding to the target in biophysical readouts, in biochemical assays and target engagement in cells be controlled?
28. Can target accessibility be analysed with available tool compounds?
29. Have structurally-related but inactive controls (e.g. inactive enantiomers) been used?
30. Have target mutagenesis studies to control for binding specificity been performed?
31. Has a selectivity profiling screen been performed for the tool compounds used?
32. Can the concentrations of compounds be measured inside cells to determine dose responses?
33. Does a selectivity window (on-target activity) for the tool compound exist?

34. Does the use of tool modulators result in the same phenotype as, for instance, respective knockdown/knockout studies?
35. If small molecule compounds are used as tool compounds, are they prone to chemical modification to optimize properties

Target-related tolerance

36. Is the target post-translationally modified?
37. What is the turnover rate of the target (i.e. the protein half-life in a cell)?

Biomarker

38. Are biomarkers available to monitor target modulation and/or target engagement?
39. If there is a cellular biomarker assay available, is the cellular readout compatible with *in vivo* target monitoring?
40. Is there a causal relationship between biomarker and disease (e.g. a biomarker as a disease component) or does the biomarker simply measure disease-related 'observations' without a key causal role (i.e. no impact on the disease outcome)?
41. Is there a correlative biomarker that can be monitored preclinically in the sensitive species that is clinically translatable?

Supplementary Box 6 | Guiding questions related to documentation and traceability

1. Are all notes taken directly after conducting the experiments?
2. Are all notes directly entered into the lab book?
3. Have lab notebooks been used in a structured way, e.g. by describing an experiment according to:
 - a) Aim/goal /question
 - b) Experimental procedure
 - c) Description of results
 - d) Discussion/interpretation
4. Are notes entered in chronological order?
5. Are study protocols complete and accessible?
6. Are all study outcomes documented and reported (both positive and negative results)?
7. Has the sample size been reported?
8. If several experiments overlap, can previous/subsequent parts of the protocol be identified by referring to respective page numbers?
9. If SOPs are used for the first time, are file name, version number and date added to the lab notebook?
10. If SOPs are permanently changed, are the new file name, version number, date documented in the lab notebook?
11. Are all deviations from standard protocols noted?
12. Are expectations, interpretations and observations distinguishable?
13. Are also minor observations noted, which can be important for subsequent interpretation of data sets?
14. Are empty pages/empty parts of pages crossed out in order to avoid later addition of information?
15. Are instrument/device settings and characteristics (e.g. manufacturer, model, or type) noted?
16. Are order and lot numbers and the manufacturer of chemicals / media etc. noted?
17. Have all entries been approved, signed and dated?
18. Are electronic records in compliance with FDA's 21 CFR Part 11?
19. Can a witnessing process be implemented?
20. Are all (raw) data sets traceable (related to storage, retrieval and reconstruction of data) and reusable?

Target–disease linkage (relevance of the model system)

Establishing a clear link between target and disease can be challenging if no adequate preclinical model systems exist. Often, the target environment in a complex human setting is modelled by animal systems reflecting and focusing only on individual aspects or factors influencing the target of interest. One example is the protein kinase C (PKC) enzyme family, which has been extensively studied as a target in various disease models and has been linked to cardiovascular disease and cancer. It proved difficult to translate the elaborate findings to new drugs, in part because the mice models were not ideally simulating the human system and the PKC isoforms are too tightly regulated and fine-balanced. This shows that a more robust understanding of the underlying biology and comparison of pathways identified in human specimens and the development of relevant in vivo models is essential for therapeutic progress in this field²⁻⁴.

Target–disease linkage (establishing causality)

The ‘amyloid hypothesis’ initially postulated that the accumulation and deposition of amyloid β ($A\beta$) peptide is causally related to development and progression of Alzheimer’s disease (AD)⁵⁻⁷. Several treatments have successfully been shown to reduce the $A\beta$ plaque load, but without any positive therapeutic impact. Causality claims have been based exclusively on transgenic mouse models with overexpression of APP triggering a variety of impairments but, notably, no neuronal loss or neurofibrillary tangles, which are also hallmarks of Alzheimer’s disease⁸.

These findings demonstrate the relevance of the GOT-IT CPQ #1 “Is the target perturbation a cause or consequence of the human disease process?” and the importance of rejecting the null hypothesis that the selected target of interest is not causally linked to the disease of interest as early as possible to save time and resources.

Early safety de-risking of a novel target example 1

In approaches targeting novel cancer/testis tumour-associated antigens (TAAs), target-related toxicity is a concern due to unrecognized expression of some cancer/testis antigens on rare vital cells (e.g. in the brain). In this context, however, studies addressing GOT-IT CPQ #15 “Is the tissue distribution of the target known?” generated new data on tissue distribution of the targets in question, such as MAGE A1, and could decrease the risk of occurrence of unsolicited side effects, demonstrating the importance of understanding target expression and distribution levels⁹.

Early safety de-risking of a novel target example 2

In 1999, an evaluation of 150 compounds from 12 pharmaceutical companies showed that of a total of 221 human toxicities identified, the proportion of target-related toxicities (versus off-target toxicity) in clinical trials was 35% in Phase I, 39% in Phase II and 43% in Phase III¹⁰. A later analysis by AstraZeneca of their 2005–2010 portfolio found that project closures due to safety were 82% preclinically, 62% in Phase I, 35% in Phase IIa and 12% in Phase IIb. In the AstraZeneca review, the ratio between target- and compound-related toxicity was 25% versus 75% for preclinical failures and 48% versus 52% for clinical failures¹¹. The results of this analysis led to early hypothesis testing for target-related safety risks at AstraZeneca¹², in line with the strategy of other pharmaceutical companies¹³.

Tool compound use

Many tool compounds are not fully characterized or have outdated specificity profiles, yet are still frequently used, leading to incorrect conclusions¹⁴.

Prominent examples are dorsomorphin, a chemical probe that was originally advertised and used as a TGF-beta receptor kinase inhibitor as well as a nanomolar inhibitor of AMPK signalling. However, it has been shown that at least 10 other kinases are more potently inhibited by

dorsomorphin compared to AMPK or TGF-beta kinase, questioning the value of the results obtained using this tool compound¹⁵.

Similarly, the chemical probe LY294002 was developed in 1994 and advertised as a selective PI3 kinase inhibitor. However, it became apparent in the following years that LY294002 also affects many other proteins at the concentrations used to inhibit PI3 kinase and that more specific and higher-quality probes are available. LY294002, though, is still widely used and published in scientific literature in the context of PI3 kinase research¹⁶.

Target engagement biomarker use and development

Corticotropin-releasing factor receptor 1 (CRF1) has been implicated in behavioural and cognitive responses to stress and preclinical studies predict the use of CRF1 receptor antagonists as possible treatments for depression and alcohol use disorders. Several studies have suggested the central role of CRF1 receptors¹⁷⁻¹⁹. However, clinical translation of these preclinical results has not yet been achieved. One reason for this may be the unavailability of a CRF1 biomarker (e.g. radiotracer for PET/SPECT imaging) to confirm adequate target engagement in humans^{20,21}.

Applying the GOT-IT framework would have identified the need for early development of target engagement biomarkers for projects focusing on CRF1 (CPQ #32 “Are biomarkers available to demonstrate target engagement in patients?”), which would have helped to optimize prioritization of drug candidates and dosing. Thus, the development of e.g. CRF1 PET radioligands will further validate CRF1 as a suitable drug target by controlling receptor occupancy.

Target assayability example 1

G protein-coupled receptors (GPCRs) are an important family of drug targets. Most widely used GPCR assays for compound screening focus on receptor–ligand binding or the functional analysis of downstream signalling events. However, the majority of GPCRs in the brain are orphan receptors, whose endogenous ligands and downstream signalling are unknown. Consequently, orphan GPCRs are underexploited for drug discovery due to the lack of appropriate technologies for compound library screening, despite the fact that the druggability of this target class is unquestionable. Thus, whereas screening assays can usually readily be established for de-orphaned GPCR targets, in contrast, orphan GPCRs will probably yield less favourable assay outcomes^{22,23}.

Applying the GOT-IT framework (CPQ #31 “Can the target be assayed in a relevant system?”) would help to identify this potential roadblock for further drug discovery and would re-direct resources to finding alternative solutions like receptor-internalization assays, which do not require prior knowledge of G protein coupling conditions and signalling pathways of the receptor²⁴⁻²⁶.

Target assayability example 2

In a project to identify novel, non-peptide bradykinin receptor antagonists, a receptor binding assay was set up using the guinea-pig ileum and muscarinic receptor binding, as a control for specificity, was assessed in rat brain. A hit compound acted at both receptors, so a low affinity at the bradykinin receptor was sought in a screen of novel structural derivatives. Further testing on fibroblasts of compounds with higher affinity revealed that they were not acting via bradykinin receptors but by inhibiting prostaglandin synthesis. The best compound NPC 12957 had a K_i for bradykinin receptors of 400 nM and had no effect on muscarinic receptors, but was non-specific in functional assays and the chemical class was abandoned. However, starting with a different chemical scaffold, binding affinity with a K_i of 200 pM could be achieved and these compounds were highly specific for bradykinin receptors²⁷. This example shows the importance of establishing a target screening assay with carefully defined criteria to identify potent, specific modulators and to answer the GOT-IT framework CPQ #31 “Can the target be assayed in a relevant system?”.

Antibacterial aspects example 1 (resistance development)

Drug efflux pumps can play a key role in drug resistance. This aspect is addressed by the GOT-IT EAQ #5 (Supplementary Box 3) “Do permeability and efflux properties of the microbial population of interest affect treatment options?”. The expression of drug pumps is often subjected to induction by small molecules like antibacterials, which can interact with regulatory systems. For example, pumps specific for the common natural product tetracycline (TET), of which 30 are known, possess regulatory controls that sense the presence of the antibiotic and TET therefore acts as an ‘inducer’, leading to an increased efflux pump expression level and consequently drug resistance development²⁸. This limits the use of these drugs to sensitive bacterial strains. Tigecycline, a relatively new tetracycline, was developed taking this issue into account and is much less active as a substrate for efflux pumps, exerting a broad spectrum of activity against resistant bacterial strains²⁹.

Antibacterial aspects example 2 (target validation in vitro versus in vivo)

The need to evaluate target essentiality not only *in vitro* but also *in vivo* is addressed, amongst others, by the GOT-IT guiding question EAQ #17 (Supplementary Box 3) “Can the organism scavenge the metabolite (or precursors) from the host?”. Answering this question identified a major roadblock when several pharma companies investigated fatty acid biosynthesis pathway as a potential target pathway. After having identified multiple leads and drug candidates, it was found that important Gram-positive pathogens are resistant to fatty acid biosynthesis inhibitors *in vitro*, when supplied with exogenous fatty acids in a concentration present in human serum or when human serum is added³⁰. Thus, these results challenge and compromise the development of antimicrobials targeting the fatty acid synthesis pathway used by Gram-positive pathogens, by considering the therapeutically relevant infection conditions.

Protocol for database search

The search was performed using the *PubMed Advance Search Builder* of the PubMed Database at the US National Center for Biotechnology Information³¹. It was delimited in time using the operator *AND* and the publication dates *2010/01/01 to 2018/04/30*. It was specified that the publication types 'Review' and 'Clinical trial' were not to be included in the search.

A total of 6 different searches were performed, one for each of the following indications: Cancer, Diabetes, Cardiology, Neurology, Immunology, Infectious Diseases. For this, the search terms were defined and connected as follows: (novel OR new) AND (target OR agent OR therapy OR therapeutic OR treatment) AND (association OR associated OR relevance OR link OR related OR relation) AND (validate OR validation OR validated OR validating) AND (XXX), where XXX denotes indication specific search terms. These were: (solid tumor OR leukemia) for Cancer; (diabetes) for Diabetes; (cardiovascular *NOT (All Fields) Diabetic or diabetes) for Cardiology; (neurology OR neurobiology) for Neurology; (innate immun* OR adaptive immun* OR autoimmun*) for Immunology; and (antibiotic OR antiviral OR bacteria OR virus) for Infectious Diseases. Finally, results were displayed according to the *BEST MATCH* option.

Inclusion and exclusion criteria

In order to be selected for analysis, publications had to meet the following inclusion criteria: 1) describe validation procedures of a gene, a protein, a pathway or a molecule previously identified as a potential therapeutic target of a human disease; 2) be written in scientific English; 3.) be published by academic research groups.

Excluded from analysis were publications with the following characteristics: 1) Review or clinical trial articles, as well as commentaries, editorials, letters, and similar; 2) Publications from industrial research groups; 3) Publications dealing exclusively with: validation of biomarkers for diagnostic and/or prognostic assays; genetic screening, microarray analysis/screening; papers related to expanding patient lifespan and patient quality of life; bioinformatic analysis (without presenting experimental research data); 4) Articles whose abstracts were not available via PubMed; and 5) Articles failing to meet the inclusion criteria.

Number of papers selected

Following the primary search based on the selected search terms, 3307 relevant abstracts were identified (see figure below). After reviewing of titles and abstracts by two independent reviewers (in case of discordance, a third independent reviewer classified the article as relevant or non-relevant), 2743 were excluded (due to specified exclusion criteria or failure to meet inclusion criteria). The 564 relevant publications were retrieved as full-text papers for detailed analysis.

Following the review of these papers, further 136 papers were excluded, leaving a total of 428 papers that were included in the final literature analyses (162 for cancer, 56 for cardiology, 63 for diabetes, 26 for immunology, 68 for neurobiology and 53 for infectious diseases). A full list of all 428 articles is provided below.

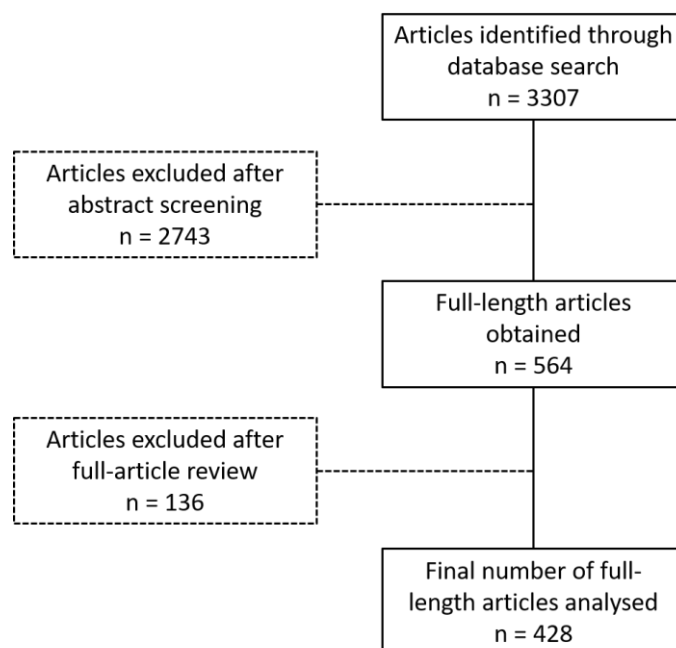


Figure | **Literature analysis.** Overview of all retrieved, excluded and selected articles for analysis of aspects related to Target Assessment in Academia.

Review of full-text publications

Full-text articles were sorted according to indication and analysed for the presence or absence of each of the pre-defined TA elements or data quality standards. The selection and definition of TA elements was based on the Validation Block categories (see main article). The percentage of papers which have discussed, used or implemented one or more of each TA element or Landis 4 criteria³², respectively, was calculated and presented in Box 1.

Potential limitations of the analysis

This analysis is based on information extracted from publications. Regarding the implementation of measures taken to avoid bias (reporting of randomisation, the blinded assessment of outcome, sample size calculations and whether samples or animals were excluded from analysis), it is possible that some poorly reported studies are in fact well-designed and well conducted, but failed to describe measures taken.

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