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Transforming Big Data into cancer-relevant insight: An initial, multi-tier approach to assess reproducibility and relevance

The Cancer Target Discovery and Development Network

Abstract

The Cancer Target Discovery and Development (CTD[2]) Network was established to accelerate the transformation of "Big Data" into novel pharmacological targets, lead compounds, and biomarkers for rapid translation into improved patient outcomes. It rapidly became clear in this collaborative network that a key central issue was to define what constitutes sufficient computational or experimental evidence to support a biologically or clinically relevant finding. This manuscript

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represents a first attempt to delineate the challenges of supporting and confirming discoveries arising from the systematic analysis of large-scale data resources in a collaborative work environment and to provide a framework that would begin a community discussion to resolve these challenges. The Network implemented a multi-Tier framework designed to substantiate the biological and biomedical relevance as well as the reproducibility of data and insights resulting from its collaborative activities. The same approach can be used by the broad scientific community to drive development of novel therapeutic and biomarker strategies for cancer.

Introduction

Large-scale molecular characterization projects are generating comprehensive data for pediatric and adult malignancies, from hundreds to thousands of patient-derived samples[1–3], transgenic mouse models[4], patient-derived xenografts, and cancer cell lines[5–7]. These data allow systematic evaluation of key biologically and clinically relevant hypotheses, such as the association between drug sensitivity and specific genetic alterations, or between specific biological features and patient outcome. As a result, biomedical discovery is being increasingly driven by the integrative analyses of large amounts of data followed by experimental evaluation both *in vitro* and *in vivo*. The challenge is to capitalize on these different data sources in a systematic way that makes the process of target discovery and translation more efficient, transparent and reproducible.

Such transition from a strictly hypothesis-driven to an increasingly hypothesis-generating paradigm presents new types of challenges. For instance, to what extent can knowledge be extracted from computational analysis of large-scale data repositories without conventional follow-up experimental validation? If experimental validation is needed, what can be considered an appropriate level of validation to justify follow-up pre-clinical and clinical studies? These questions are especially relevant in view of critical challenges to the very foundation of the biomedical research enterprise, from result reproducibility[8, 9] to biomedical impact[10].

The Cancer Target Discovery and Development (CTD^2) Network (http://ctd2.nci.nih.gov/) was established with the specific intent to accelerate the transformation of "Big Data" into novel pharmacological targets, lead compounds, and biomarkers for rapid translation into improved patient outcomes. This process includes the development of novel methods that enable the identification and validation of actionable therapeutic targets. Specifically, in the "Big Science" classification system of Sean Eddy, the CTD^2 Network aims to be a "leading wedge" – democratizing breakthrough technology for validating cancer therapeutic targets to all laboratories, an urgent medical need requiring radically improved methods[11]. With 13 Centers collaborating in the context of CTD^2 Network, the issue of what constitutes sufficient computational or experimental evidence to support a biologically relevant finding becomes central. Indeed, to a large extent, the multi-Center Network represents a microcosm of the complex interactions that drive biomedical translation forward in the broader context of academic and industrial research.

The questions discussed in this manuscript represent the specific challenges faced by this group of researchers as they began to develop successful, multi-center collaborations leading

to numerous publications and clinical translation efforts. Specifically, CTD^2 investigators quickly realized that, while each center was an expert in the methodologies related to a specific aspect of biological discovery – from Big Data analysis to large-scale chemical-biology screens to pooled functional assays – the ability to operate at the intersection of these methodologies, especially in terms of quality control and data reproducibility. For instance, the specific quality control infrastructure and mechanisms necessary to ensure reproducibility of big data analyses or *in vivo* functional assays are quite different. Thus, collaborations that leverage more than one data modality require potentially orthogonal communities to develop a cross-disciplinary understanding of their individual competencies.

This is a vast and complex undertaking and as such, this perspective cannot be interpreted as a fully finished and comprehensive framework to support interdisciplinary collaborations. Rather, it represents the first essential step in motivating the community to address several critical issues on a systematic and comprehensive basis. Indeed, we envision this effort as the first of a series of manuscripts representing both a dialog and a resource for the community, which may be especially useful to young investigators and trainees as they face the complexity and challenges of large-scale collaborative research efforts that are emerging as necessary to address an equally increasing complexity of biological discovery.

In this vein, this Perspective begins to delineate the challenges of supporting and confirming discoveries arising from the systematic analysis of large-scale data resources in a collaborative environment. We provide a framework to start addressing the pivotal question: "What level of experimental evidence is necessary to complement insights derived from Big Data analysis in order to reach its potential to impact human health positively?" While our focus in this Perspective is based entirely on a system that we have adopted within the collaborative CTD^2 Network to disseminate the hypotheses and insights resulting from this Network's research, the approach and methodology is generalizable, and is thus not limited to CTD^2 Network activities. Furthermore, we hope that the research community will use this initially sparse framework to provide increasingly in depth insight and mechanisms to address quality control and reproducibility at the boundary of the multiple and highly complementary sub-disciplines of biological investigation.

The Network implemented a system to ensure that data and insights resulting from its activities would be reproducible[12], and would thus be used by the CTD^2 or the scientific community to drive the development of novel therapeutic and biomarker strategies for cancer. We also hope to provide the scientific community a framework for the effective reporting of data generated by these and other methods in future applications of the knowledge derived from Big Data for biological insight. This Perspective should be seen as the first step in elucidating the critical challenges and deriving a framework that will begin a community wide discussion of the challenges that will lead to a more detailed description of the metrics needed for specific research technologies such as proteomics and drug screening analysis that have not already been systematically evaluated in the literature and through consensus white papers.

Data Sharing and Clarity

To enable clear description of novel therapeutic targets and pathways identified by the CTD[2] Network, the members have devised a set of classification criteria to stratify targets, pathways, associated biomarkers, and their small-molecule or biological modulators into "Evidence Tiers", based on available supporting evidence. These criteria should also enable the scientific community to understand more easily the evidence on which CTD[2] and other findings are based. A key priority of this lexicon is to minimize the misinterpretation of reported results, thereby helping the scientific community to understand the likelihood that the interpretations can be successfully progressed to human investigations[9].

Network Centers share their methods and results via publications, and all raw and analyzed data are made publicly available through the CTD[2] Data Portal (https://ctd2.nci.nih.gov/dataPortal/). This resource is regularly updated as new data are generated and additional findings are validated. All data posted in the Data Portal have undergone quality-control evaluation, but have not independently been confirmed (Figure 1).

We consider that Data Portals are necessary, but not sufficient, for ensuring clarity and reproducibility of published results. Therefore, classification of supporting evidence resulting from analysis of CTD[2] data into Evidence Tiers (see below) informs a separate web-based "Dashboard" (http://ctd2-dashboard.nci.nih.gov/) – a platform to share CTD[2] Network findings with the research community. The Dashboard is intended to house the results that connect targets, biomarkers, and modulators with evidence supporting their validation. In each Evidence Tier, we enumerate information related to three entities that are critical to the development of cancer therapeutics. These include: 1) molecular targets, 2) small-molecule or biological modulators of the targets, and 3) associated predictive or prognostic biomarkers for patient selection.

Evidence Tier Definitions

Tier 1: Preliminary positive observations

These data represent the initial results of high-throughput experiments, typically using a single experimental or computational platform; examples are given for illustration purposes only.

Small-molecule assays—Include positive results ("hits") from primary high-throughput screens (HTS) with small or large molecules such as antibodies (the term small molecule screen is used throughout to include the potential for large-molecule screens). Other means of high-throughput data generation (*e.g.*, cheminformatic analyses, correlations of genomic and cheminformatics data, patents, published literature, *etc.*) may also inform a small-molecule approach and are thus acceptable.

Genetic perturbation assays—Include results from high-throughput screening experiments (such as, whole-genome or targeted RNA interference (siRNA or shRNA) loss of function, CRISPR, or open-reading frame cDNA (ORF) gain of function screens), as well

as computational and statistical analyses that either support hit selection or filter out potential artifacts.

Prognostic or predictive biomarkers—Biomarker discovery involves the use of primary data from any number of sources (*e.g.*, cell lines isogenic for a mutant versus wild-type candidate target, sequencing, biologics screening, *etc.*). Where possible, a biomarker is linked to specific molecular targets and small-molecule modulators. Tier 1 biomarker data could enable: 1) identification of the patient population likely to benefit from a given therapeutic strategy, 2) quantification of efficacy *in vivo* for pre-clinical or clinical measurements, 3) nomination of a pharmacodynamics readout for drug activity in a patient, or 4) development of an indicator for potential undesirable off-target effects or adverse events. Tier 1 biomarkers derive directly from the analysis of the primary data and are not yet independently validated.

Molecular interactions—Discovery of molecular interactions from protein–protein interaction assays (PPI), protein–nucleic acid interaction assays, or computational analyses, among others, can be used to generate hypotheses for specific molecular targets. Examples of relevant assays include genome-wide chromatin immunoprecipitation (ChIP-seq) or cross-linking immuno-precipitation (CLIP-seq), reporter-gene activity, protein fusions (*e.g.*, luciferase or fluorescent protein) in protein-complementation assays, yeast two-hybrid assays, Förster-(fluorescence-) resonance energy transfer (FRET), protein complex identification by mass spectrometry, and reverse engineering computational algorithms.

Computational analyses—Any computational approaches and strategies including the analysis of, for example, alternatively spliced transcripts and cell surface markers and other factors that might stimulate the immune system can identify candidate cancer targets or pathways. The primary data can be from the public domain or project-specific high-throughput assays that are released into the public domain and are clearly referenced.

NOTE: In Tier 1, the data can support many concepts and not just targets, biomarkers, or small-molecule perturbagens.

Tier 2: Confirmation of primary results *in vitro*

These results meet the Tier 1 requirements AND have been confirmed by at least one of the following:

- More detailed version of the original assay, such as concentration–response *versus* single-point, high-replicate (*e.g.*, N>4) *versus* low-replicate (*e.g.*, singleton), target silencing in additional patient-relevant cell lines or models, or results from high-content microscopy experiments, *etc.*

- Orthogonal secondary assay or counter-screen

- Independent confirmatory experiment with the original assay, performed by a different Center in the Network or from the literature

- Extension or corroboration of experiments in other *in vitro* cancer models

- Experimental investigations confirming the presence on the cell surface of bioinformatically predicted cell-surface peptides and epitopes

- Generation of an antibody, chimeric antigen receptor (CAR), T-cell receptor, or other targeting molecule for cancer-specific epitope expressed on tumor surfaces

Examples of evidence for Tier 2 are given below. Sufficient detail must be captured in the Dashboard (data, methods, reagents, *etc.*; http://ctd2-dashboard.nci.nih.gov/) to enable qualified investigators to reproduce the results.

Small-molecule assays—The data include a detailed characterization of each candidate using the primary or suitable orthogonal assays. These data define the desired biological profile and may include testing in additional patient-relevant cell models. Some measure of potency and selectivity is established (*e.g.*, selective toxicity for cancer cells over an appropriate normal cell model).

Genetic perturbation assays—A more detailed characterization of gene candidates is required to meet the standard criteria of demonstrating that at least two independently designed genetic perturbation reagents produce the same effect. These experiments could use RNAi, CRISPR, TALENs, or ORF reagents. In addition, the possibility of an effect being due to miRNA seed sequences should be addressed. Validation of a reagent can occur through direct measurements (mRNA depletion, protein levels), computational approaches (measurements of reagents made in multiple cell lines or assays), or a combination thereof.

Biomarkers—The specifics of a more detailed characterization of biomarkers depend on the utility of the biomarker being developed. For biomarkers aimed at identifying the patient population likely to benefit from a given therapeutic strategy, confirmation in an independent, appropriately statistically powered population for which relevant molecular profile data can be accessed is required. Biomarkers that quantify efficacy for pre-clinical or clinical measurements require validation in independent model systems. Biomarkers providing a pharmacodynamics profile for drug activity in a patient require confirmation in a distinct, appropriately statistically powered pre-clinical model-organism cohort. Biomarkers serving as an indicator for potential undesirable off-target effects or adverse events need confirmation in an independent large sample set of cell lines or other biological samples.

Molecular interactions—Interactions require confirmation in orthogonal screening experiments in a biologically relevant context using a different readout than used in Tier 1. Computational data from the literature or public databases could also support molecular interaction-based targets in this Tier.

Computational analyses—Hypotheses inferred by computational analysis must be confirmed by experimental analysis to reach Tier 2, which could by accomplished by interventions or through data in the literature. In addition to experimental approaches highlighted above, immunotherapeutic targets or molecules, such as antibodies, chimeric antigen receptors, or T-cell receptors to novel cell-surface epitopes either in cell lines or mass spectroscopy measurements are needed and tested in cell lines.

NOTE: In Tier 2, an *in vitro*-validated target is progressing toward a substantiated hypothesis; nonetheless, the absolute connection is not yet complete.

Tier 3: Validation of results in a cancer-relevant *in vivo* model

These results meet the Tier 2 requirements AND have been validated by *in vivo* assays, including at least one of the following:

- Experiments in model organisms (*e.g.*, *Danio rerio, Mus musculus, Drosophila melanogaster, etc.*)

- A second, separate orthogonal secondary assay or counter-screen on a single gene in an *in vivo* system

- Independent validation assays *in vivo* (secondary assay or counter-screen) by the same or a different Network Center, or from the literature

- For biomarkers only, extension or corroboration of validation experiments in large independent human cancer sample cohorts with appropriate clinical data

In Tier 3, the functional hypothesis is effectively tested and, as needed, either modified or removed based on orthogonal experimental evidence. It is expected that assays are performed in carefully controlled experiments *in vivo* (*e.g.*, on a molecule-by-molecule basis in relevant models such as xenografts, genetically engineered mouse models (GEMMs), syngeneic tumor models, organoids, patient-derived xenografts, or other biological systems), at least in quadruplicate, that allow definitive conclusions. Cell-surface epitopes can be targeted either by a small molecule, an antibody, an antibody-derivative protein, or a T-cell receptor. The following types of evidence are offered at this Tier.

Small-molecule assays—The data presented include orthogonal assays that further support the profile of selected compounds as being consistent with the therapeutic hypothesis. The difference from Tier 2 is two-fold: 1) at Tier 3 the experiments are carried out *in vivo*, and 2) proof of mode-of-action is necessary (*e.g.*, mitotic arrest using an image-based assay or identify the gene or molecular alteration that leads to the cancer dependency of the small-molecule activity).

Genetic perturbation assays—Relationships that are observed by multiple groups, or a more detailed characterization of gene (cancer dependency) candidates, is required, using *in vivo* model systems for validation. Lower throughput experiments that further support the specificity of the loss of gene function (or gain), and the importance of that loss (or gain) to the proposed hypothesis are needed.

Biomarkers—Biomarkers require a more thorough demonstration of their reliability than at Tier 2 including, for example, statistically significant evidence from an appropriate clinically annotated patient cohort independent from those used for Tier 2. For all types of biomarkers (see definitions in Tier 1), the assay used is either performed independently by another Center, or with a different technology platform to measure and detect the biomarker(s). As an alternative to a different technology platform, the same platform could

be validated in terms of robustness and reproducibility meeting the requirement of a CLIA-like (Clinical Laboratory Improvement Amendments) assay.

Molecular interactions—Demonstration of direct endogenous molecular interactions, under physiologically relevant conditions, is required. Experimental evidence demonstrating that a molecular interaction-associated target is mechanistically, or at least functionally, relevant to cancer is required. This relevance is demonstrated by measuring the effect on tumor initiation, progression, or maintenance resulting from disrupting or stabilizing the interaction using mutagenesis, peptides, or small molecules. Efficacy of an antagonist peptide or small molecule in a panel of clinically relevant cell lines or *in vivo* models is required. Aberrant or neomorph-related interactions are identified as distinct from those occurring in a cell's physiologic regime.

NOTE: The evidence in Tier 3 is a robust definition of connection of small molecule (chemical or biological) to target, genotype to phenotype, and direct molecular interaction *in vivo*, such as mouse tumor models, patient-derived tumor avatars (*e.g.*, organoids, conditionally reprogrammed cells, xenografts), or patient cohorts.

Substantiated Hypotheses toward Human Investigation

CTD[2] aims to undertake and report research that can be validated by qualified investigators inside and outside the Network and that can lead to clinical applications, including the generation of therapeutic agents whose activity is predicted by specific molecular alterations in a patient's tumor. We anticipate that substantiating hypotheses from the CTD[2] Network will be based on a combination of evidence types from different Tiers, providing strong rationale for a candidate target with an agent that modulates the cancer phenotype, together with an associated biomarker for patient selection. Importantly, we expect such results to be replicated independently of the Center that generated the initial data.

Substantiated hypotheses should include all the relevant information necessary for their translation. Additional perspectives on what qualifies as a substantiated hypothesis are provided below:

Cancer target

A candidate therapeutic target should be accompanied by biomarkers for the stratification of patients most likely to derive benefit from its use, small-molecule or peptide modulators that are most likely to modify target activity, and proof of mechanism of action. Substantiated hypotheses include compelling evidence that supports translation to clinical trials.

Small-molecule assays

Small molecules should be accompanied by data that indicate appropriate properties for testing *in vivo* (*e.g.*, suitable metabolic half-life, minimal toxicity, appropriate *in vivo* exposure, *etc.*) and that substantiate statistically significant differential efficacy. This substantiation may require synthesis or purchase of analogs that address compounds with shortcomings in one of these areas. A detailed optimization strategy (chemistry, pharmacokinetics, pharmacodynamics, *etc.*) is important. Alternative acceptable surrogates

include, but are not limited to, other targeted therapeutics such as monoclonal antibodies or soluble receptors.

Genetic perturbation assays

Selective effects of targets perturbed by multiple genetic reagents (shRNA/siRNA/CRISPR) are explored with in-depth biological experimentation that includes *in vivo* interventions. This selectivity may be demonstrated in a panel of clinically relevant patient-derived xenographs, or in transgenic or xenograft mouse models using shRNA or genetic ablation of the target. Confirmation of suppression *in vivo* is necessary and multiple endpoints (tumor burden, survival, *etc.*) should be shown with appropriate statistical significance.

Biomarkers

Biomarkers as listed in Tier 3 require additional development and implementation of an analytical test system with well-established performance characteristics and cut-offs. Any associated algorithms are "locked" in terms of coefficients and other parameters. The analytic test system is used to evaluate the performance of the type-specific potential biomarkers on an independent validation patient-sample cohort. Additionally, a credible scientific framework that explains the physiologic or clinical significance of the test results is required.

Molecular interaction

Mutational analysis of cancer-specific variants could provide supporting data for molecular interactions. Aberrant or neomorph-related interactions should be identified as distinct from those occurring in a cell's physiologic regime. Biomarkers that reflect the status of the molecular interaction target should be provided for translational studies.

Examples of transition through the Evidence Tiers to clinical trials (Figure 2)

To provide examples of how this framework facilitates the discussion and comparison of different types of targets, we describe three targets that were identified by CTD[2] members and for which further validation experiments provided substantiated hypotheses, now in testing in clinical trials.

Vignette 1: WEE1 inhibitor MK1775 (AZD1775) in head and neck squamous cell carcinoma (HNSCC)

The cell cycle checkpoint kinase WEE1 is an illustrative example of advancing a target through Tiers of evidence from discovery to preclinical validation, leading all the way to a clinical trial.

The Fred Hutchinson Cancer Research Center's CTD[2] performed an unbiased siRNA kinome screen, using both mouse and human squamous cell carcinoma cells, that provided Tier 1 level of evidence for WEE1 as a cancer target (Moser et al. 2014). Specifically, siRNAs to WEE1 were among the most effective at inhibiting growth of p53-mutant head and neck squamous cell carcinoma (HNSCC) cells. Retesting with different siRNAs to

WEE1 in additional cell lines and using a small-molecule inhibitor of the WEE1 kinase, MK1775 (now AZD1775), provided Tier 2 evidence for WEE1 as a cancer target. Disruption of G2/M regulation by inhibition of WEE1, particularly in the context of p53 mutation and DNA damage, leads to apoptotic cell death.

Tier 3 evidence was obtained by inhibition of growth of p53 mutant HNSCC xenografts in mice treated twice weekly with AZD1775, as well as confirmation of target engagement by inhibition of WEE1 kinase activity in tumor extracts. These studies led to an investigator-initiated clinical trial of AZD1775, in combination with neo-adjuvant weekly docetaxel and cisplatin, prior to surgery in HNSCC (NCT02508246, E Mendez, PI). Of note, the gene encoding WEE1 is not mutated in tumors and may represent yet another example of a therapy targeting cancer-specific vulnerabilities[13].

Vignette 2: JAK2 inhibitor ruxolinitib in trastuzumab-relapsed ERBB2-amplified breast cancer

Breast cancers that present aberrant activity of the ERBB2 receptor tyrosine kinase, are treated with trastuzumab, a monoclonal antibody that acts as a specific ERBB2 inhibitor. However, a substantial number of patients who initially respond to trastuzumab (up to 70%) will eventually relapse with tumors that are drug-resistant and have poor prognosis.

The Columbia University's CTD[2] combined results from pooled RNAi screens in MCF10A cells, followed by ectopic ERBB2 expression, with network-based analysis of master regulator (MR) proteins in ERBB2-amplified breast cancer patients from The Cancer Genome Atlas (TCGA). This analysis revealed STAT3 as a critical master regulator of ERBB2-amplified tumors in ER-/ErbB2[Amp] patients, as well as a critical dependency of the transformed MCF10A cells (Tier 1). Such an approach highlighted how independent evidence from large scale computational and experimental assays can provide complementary clues that lead to identification of biological mechanisms with high potential for successful experimental validation, both *in vitro* and *in vivo*. Specifically, unless they are performed in a very large number of phenotypically relevant cellular contexts, pooled RNAi screens are often not sufficiently selective to pinpoint generalizable tumor dependency mechanisms. One concern, for instance, is that these screens may highlight idiosyncratic dependencies induced by the non-physiologic nature of the cell line context used in these assays. In contrast, network-based analysis of big data from human samples to identify master regulators of tumor cell state have shown remarkable ability to pinpoint functional drivers, with validation rates in the 70% to 80% range. Yet, the latter must still be experimentally validated to separate truly biological dependencies from potential computational artifacts[4, 14, 15]. The use of combined pooled RNAi screens with computational, network-based approaches addresses both issues providing clear complementarity and thus allowing efficient and systematic functional driver elucidation, leading to extremely high success and validation rates in follow-up in vivo and clinical studies. For instance, in this case, hundreds of potential candidates from pooled RNAi screens and tens of candidates from master regulator analysis resulted in a core of three drivers that were validated in follow-up assays, including one (STAT3) providing critical insight for the development of combination therapy in relapsed HER2+ breast cancer.

Indeed, further investigation in cell lines showed that aberrant STAT3 activity was regulated by an autocrine loop involving expression and secretion of IL6 and induced expression and secretion of the S100A and S100B hetero-dimerizing isoforms linked to aggressive breast cancer malignancy. Specifically, IL6-mediated activation of the IL6 receptor, upstream of the JAK/STAT cascade, closed the resulting signaling loop, resulting in STAT3 autoregulation. Depending on kinetics of the signaling loop, the latter could become ERBB2 independent, thus inducing trastuzumab resistance. Conversely, when combined with trastuzumab, abrogation of JAK2 activity, either genetically or via the small-molecule inhibitor ruxolitinib, abrogated STAT3 activity, reduced S100A/B expression, and induced profound synergistic loss of viability in trastuzumab resistant cell lines (Tier 2 evidence) and xenografts (Tier 3) which was reversed by ectopic S100A/B expression. These multi-Tier findings led to development of a clinical trial to test the trastuzumab-ruxolitinib combination in ERBB2-amplified breast cancer patients who had relapsed following trastuzumab therapy and no longer responded to the drug (NCT02066532)[16].

Vignette 3: TBK1 inhibitor momelotinib in lung and pancreatic cancer

Oncogenic mutations in *KRAS* occur in nearly all pancreatic cancers as well as a significant number of lung and colon cancers. Although KRAS is a well-validated oncogene involved in both tumor initiation and maintenance, targeting KRAS pharmacologically has proven challenging. The Dana-Farber Cancer Institute CTD[2] Center performed an arrayed shRNA screen to identify genes that were required in cancer cell lines that are dependent on the expression of mutant KRAS, and identified the serine-threonine kinase TBK1 as a co-dependency in such cells[17], making it a Tier 1 target. Subsequent studies by this Center and others[18, 19] identified TBK1-dependent induction of autocrine signals as the reason for this dependency and demonstrated that small-molecule inhibition of TBK1 reproduced the genetic findings[20] (Tier 2). Indeed these studies help explain why studies involving pooled shRNA screens fail to identify TBK1 since in such massively parallel screens, signaling loops involving secreted molecules are not interrupted. In addition, genetic and pharmacologic perturbation of TBK1-induced tumor regression in genetically engineered mouse models of lung cancer driven by K-ras[20] (Tier 3). Three clinical trials testing this TBK1 inhibitor, momelotinib, have been started in patients with lung and pancreatic cancer (NCT02258607, NCT02101021, NCT02244489).

Future Outlook

We introduce a multi-Tier framework designed to provide an approach to substantiate the biological and biomedical relevance, as well as the reproducibility, of novel biomedical insights arising from analysis of Big Data. Such an approach allows the systematic identification of relevant insights derived from large-scale data analyses, through a series of increasingly strict filters, rather than through a single monolithic filter, whose failure may compromise the entire validation process. The approach is not meant to be prescriptive but rather to represent the minimal data elements ensuring biological and clinical relevance. Indeed, we expect that there will be insufficient evidence to credential many targets or small molecules initially classified as Tier 1–3 as substantiated hypotheses. Nevertheless, this

framework permits one to classify potential targets, biomarkers or small molecules based on the available information.

We define Evidence Tiers to clarify the levels of validation for pharmacologically accessible therapeutic targets, associated biomarkers, and biochemical modulators. In each Tier, we enumerate information related to three entities: 1) molecular targets, 2) associated predictive or prognostic biomarkers for patient selection, and 3) small-molecule or biological modulators of the targets, that are critical to the development of cancer therapeutics. Effective representation of specific hypotheses will often involve multi-modal evidence from different Tiers (*e.g.*, a target with associated biomarkers for stratification and pharmacodynamics and a set of small-molecule modulators). We expect that systematic availability of these evidence Tiers, with additional substantiation by other CTD[2] investigators and extra-Network investigators, will motivate the use of Network-generated insight and knowledge for clinical investigation.

Experiments supporting the substantiation of any hypothesis are essential before any target is prioritized for development, either by Network Centers, other investigators or by biotechnology or pharmaceutical companies. The Evidence Tiers defined in this document help delineate and communicate the complex process of data analysis, starting from large genomic or functional data sets, and ending with the generation of pre-clinical leads for characterizing targets, small molecules, and biomarkers. We expect that new information generated by the CTD[2] Network and others will inform an improved definition of the concepts we present here. We hope that the principles of Tiers of evidence as applied in the CTD[2] Dashboard will be useful in other contexts, and thereby provide confidence in the quality, clarity and reproducibility of research performed in the public sector.

References

1. Pugh TJ, et al. The genetic landscape of high-risk neuroblastoma. Nat Genet. 2013; 45:279–284. [PubMed: 23334666]

2. Brennan CW, et al. The somatic genomic landscape of glioblastoma. Cell. 2013; 155:462–477. [PubMed: 24120142]

3. TCGA-Consortium. Comprehensive molecular portraits of human breast tumours. Nature. 2012; 490:61–70. [PubMed: 23000897]

4. Aytes A, et al. Cross-species regulatory network analysis identifies a synergistic interaction between FOXM1 and CENPF that drives prostate cancer malignancy. Cancer Cell. 2014; 25:638–651. [PubMed: 24823640]

5. Barretina J, et al. The Cancer Cell Line Encyclopedia enables predictive modelling of anticancer drug sensitivity. Nature. 2012; 483:603–607. [PubMed: 22460905]

6. Basu A, et al. An interactive resource to identify cancer genetic and lineage dependencies targeted by small molecules. Cell. 2013; 154:1151–1161. [PubMed: 23993102]

7. Garnett MJ, et al. Systematic identification of genomic markers of drug sensitivity in cancer cells. Nature. 2012; 483:570–575. [PubMed: 22460902]

8. Haibe-Kains B, et al. Inconsistency in large pharmacogenomic studies. Nature. 2013; 504:389–393. [PubMed: 24284626]

9. Begley CG, Ellis LM. Drug development: Raise standards for preclinical cancer research. Nature. 2012; 483:531–533. [PubMed: 22460880]

10. Ioannidis JP. Why most published research findings are false. PLoS medicine. 2005; 2:e124. [PubMed: 16060722]

11. Eddy SR. The ENCODE project: missteps overshadowing a success. Curr Biol. 2013; 23:R259–261. [PubMed: 23578867]

12. Kenett RS, Shmueli G. Clarifying the terminology that describes scientific reproducibility. Nat Methods. 2015; 12:699. [PubMed: 26226358]

13. Moser R, et al. Functional kinomics identifies candidate therapeutic targets in head and neck cancer. Clin Cancer Res. 2014; 20:4274–4288. [PubMed: 25125259]

14. Carro MS, et al. The transcriptional network for mesenchymal transformation of brain tumours. Nature. 2010; 463:318–325. [PubMed: 20032975]

15. Chudnovsky Y, et al. ZFHX4 interacts with the NuRD core member CHD4 and regulates the glioblastoma tumor-initiating cell state. Cell Rep. 2014; 6:313–324. [PubMed: 24440720]

16. Rodriguez-Barrueco R, et al. Inhibition of the autocrine IL-6-JAK2-STAT3-calprotectin axis as targeted therapy for HR-/HER2+ breast cancers. Genes Dev. 2015; 29:1631–1648. [PubMed: 26227964]

17. Barbie DA, et al. Systematic RNA interference reveals that oncogenic KRAS-driven cancers require TBK1. Nature. 2009; 462:108–112. [PubMed: 19847166]

18. Ou YH, et al. TBK1 directly engages Akt/PKB survival signaling to support oncogenic transformation. Molecular cell. 2011; 41:458–470. [PubMed: 21329883]

19. Xie X, et al. IkappaB kinase epsilon and TANK-binding kinase 1 activate AKT by direct phosphorylation. Proc Natl Acad Sci U S A. 2011; 108:6474–6479. [PubMed: 21464307]

20. Zhu Z, et al. Inhibition of KRAS-driven tumorigenicity by interruption of an autocrine cytokine circuit. Cancer discovery. 2014; 4:452–465. [PubMed: 24444711]
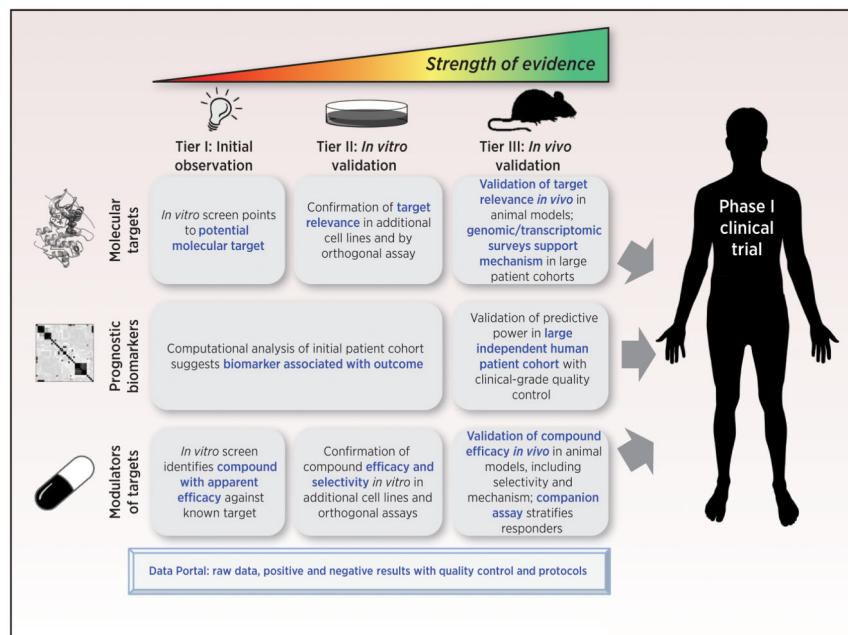
Figure 1.
As Tiers successively increase, the strength of evidence associated with data under each Tier also increases. The data in the Portal are accompanied by a statement summarizing the purpose for which they were collected and detailed experimental protocols so that others can readily reproduce them. Here are a few examples of how molecular targets, prognostic biomarkers, and modulators of targets may progress through Tier rankings toward phase 0/I clinical trials.

	Tier I: Initial observation	Tier II: *In vitro* validation	Tier III: *In vivo* validation	Clinical translation
Wee1 inhibitors for head/neck squamous cell carcinoma (HNSCC)[13]	siRNA kinome screen in HNSCC cell lines suggests Wee1 as a target	Functional genomics validates Wee1 in additional HNSCC cell lines; small molecule inhibitor identified	Small molecule inhibitor of Wee1 shows efficacy in human tumor xenografts	Phase I trial begins enrolling August 2015 (NCT02508246)
JAK2 inhibitors for trastuzumab-relapsed ErbB2-amplified breast cancer[14]	shRNA genome-wide screen and transcriptomic analysis points to STAT3 as master regulator of trastuzumab resistance	Mechanistic investigation reveals IL-6 / JAK2 / STAT3 autocrine loop drives resistance to trastuzumab, can be blocked by JAK2 inhibition *in vitro*	JAK2 inhibition synergizes with trastuzumab to delay tumor progression in mouse xenografts; efficacy correlates with loss of S100A/B expression	Phase I trial begins enrolling June 2014 (NCT02066532)
TBK1 inhibitors for lung and pancreatic cancer[15-18]	Targeted shRNA screen identifies *TBK1*, a putative essential kinase in *KRAS*-mutant tumors	Combined inhibition of TBK1, JAK and MEK pathways kills *KRAS*-mutant lung cancer cells *in vitro*	TBK1 / JAK / MEK inhibition synergizes with microtubule inhibitors to cause regression of *KRAS*-driven transgenic mouse lung tumors	Phase I trials of TBK1 inhibitor in lung and pancreatic cancer (NCT02244489; NCT02258607; NCT02206763; NCT02101021)

Figure 2.
The three vignettes that exemplify how Tier I observations were validated *in vitro* and *in vivo* as Tier II and Tier III respectively before the results were translated into the clinical setting are summarized.