INTRODUCTION

Identifying and establishing a drug target is a time-consuming, error-prone, and expensive process (1). The median time between research initiation and target establishment is 25 years, with significant lag typically caused by the difficulty of identifying quality target candidates (2). This often results in suboptimal targets being pursued and contributes to the high failure rates in drug development (3). Truvitech is developing idTRAX, a platform that can identify effective and tractable target candidates from human models of disease in a matter of weeks (4,5).

Our platform is currently focused on the kinome family of drug targets, though it can - in principle – be extended to other families of drug targets. The two core components of idTRAX are: 1) a specially formulated collection of compounds (chemical probe set), and 2) a machine learning-based algorithm for target deconvolution. The probe set and target deconvolution algorithm work together to identify target candidates with high speed and accuracy. The platform can also identify off-targets that are counterproductive for efficacy, which we will refer to as "anti-targets" (these are therefore different from toxicity off-targets and specifically relate to efficacy). Knowledge of both targets and anti-targets can guide the design of highly efficacious therapies, and has previously allowed us to develop lead compounds with very high efficacy (6). The approach is extremely versatile, and we have so far implemented it to identify drug targets for spinal cord injury (4), Human Cytomegalovirus infection (7), breast cancer (5), glaucoma (manuscript in preparation), and Lymphoma (unpublished).

SIGNIFICANCE

Drug discovery's immense need for target candidates. Drug target identification, qualification, and validation are key stages in the drug discovery process (1,8). The first step, target identification, is rate limiting (2). This is reflected in the increasing willingness of pharma companies to join precompetitive target identification consortia (3), as well as in industry-sponsored grants that solicit target candidates (e.g. Bayer's grants4targets platform). The paucity of quality candidates often results in suboptimal targets being pursued for validation and drug development, which in turn contributes to high failure rates across all steps of drug development (3).

Targets are typically identified via four main approaches (Table 1). As Table 1 indicates, each of these approaches faces fundamental challenges that complicate the generation of novel target hypotheses. Further refinements of these technologies alone are therefore not likely to solve the target identification problem. What is needed is a new approach for quickly identifying effective and tractable target candidates. This is precisely what Truvitech has set out to accomplish.

Target ID Approach	Potential weakness(es) of approach	idTRAX's Advantage(s)
Identification by genetic association with disease risk or progression	1- Genetic drivers of disease may not themselves be optimal pharmacological targets (9) 2- Discovered targets may take decades to drug (10), or may end up being entirely undruggable 3- Fails for polygenic diseases, or diseases that do not have a clear genetic driver (majority of diseases)	idTRAX identifies targets strictly by their ability to robustly modify the disease process, regardless of their mutational status
Identification from genomic, transcriptomic, and proteomic association studies	1- High degree of non-causative correlation makes it difficult to pinpoint targets that can effectively modify the disease process 2- Challenged by the lack of a comprehensive framework for connecting genomic and proteomic level data to biological functional data	idTRAX uses a carefully designed collection of chemical probes, alongside a target deconvolution algorithm, to specifically pinpoint targets that modify the disease process or pathway upon pharmacological engagement, without requirement for *a priori* understanding of the underlying molecular biology
Identification by *in vitro* gene manipulation tools (e.g. CRISPR, siRNA)	1- Downregulation effects can diverge from drug engagement effects (11) 2- Misses targets that work synergistically 3- Less than 15% of the genome is predicted to be druggable (12)	idTRAX only identifies targets that are readily druggable. Additionally, idTRAX can identify targets that work synergistically, creating opportunities for exploiting polypharmacology or drug combinations
Deconvolution from	1- High degree of non-causative correlation	By using hundreds of carefully chosen

phenotypic screening hits using proteomic approaches (e.g. mass spectrometry)	makes it difficult to distinguish between mechanistic targets and off-targets 2- Cannot be used for compounds with weak target binding or very short residence time	and highly annotated probes, information theory, and machine learning, idTRAX is able to automatically filter out non-mechanistic off-targets and pinpoint disease process-modifying targets

Table 1. Target identification approaches

idTRAX's unique value proposition for drug discovery. Following the revolution in molecular biology, the pharmaceutical industry became overwhelmingly focused on target-based approaches (13). Targets are identified as described above, validated in animal or clinical models, and then used in biochemical assays to screen large collections of compounds. The goal is to find molecules that can engage a target, with the hypothesis that engagement in the native biology of the organism would produce therapeutic effects. Industry experts believe that the near exclusive reliance on target-based approaches, compounded by the difficulty of identifying quality targets, has contributed to the striking productivity decline of the pharmaceutical industry (14–21). In search of alternatives, the industry has revisited phenotypic screening, where perturbagens are tested directly on live cells or tissues (13,22). Phenotypic screening overcomes several pitfalls of target-based screening, including: 1) highly active compounds can be found with no requirement for *a priori* target hypotheses, and 2) drugs can be discovered that engage multiple synergistic targets leading to high efficacy (23). A main difficulty with phenotypic-based screening, however, is the challenge of identifying the mechanistic target(s) (24). Knowledge of the target(s) aids chemical optimization of drug candidates and illuminates disease biology, which could be important for developing clinical biomarkers. Furthermore, the immensely efficient screening infrastructure of pharma cannot be fully exploited without the targets. idTRAX combines phenotypic screening's ability to explore novel mechanisms with the power of machine learning and target-based screening to quickly reveal mechanistic targets.

By screening the chemical probe set of known biochemical activities on cellular models of disease, then relating the resultant cellular phenotypes (e.g. cell viability, neurite outgrowth, or spheroid volume) to the probes' biochemical activities (e.g. kinase inhibition or binding), idTRAX identifies target candidates in a direct and rapid manner (**Fig. 1**). This strategy identifies targets that are pharmacologically responsive and readily druggable, because only targets that effectively induce a phenotype upon pharmacological engagement are pointed to by the target deconvolution algorithm. As an example, we previously used idTRAX to identify (and later validate) a drug target (S6K1) for axon regeneration (25) that had evaded years of extensive study in the field, including numerous genomic, transcriptomic, and proteomic analyses (26). We have also used the approach to identify novel kinase targets for Human Cytomegalovirus infection (7), breast cancer (5), and glaucoma (unpublished). The platform can also identify off-targets that are counterproductive for efficacy (4), which we refer to as "anti-targets". Knowledge of both targets and anti-targets can guide the design of highly efficacious therapies. It is worth noting here that, while idTRAX offers up targets that are readily druggable with small molecules, this does not preclude the possibility of drugging those targets with biologics where and if preferable.

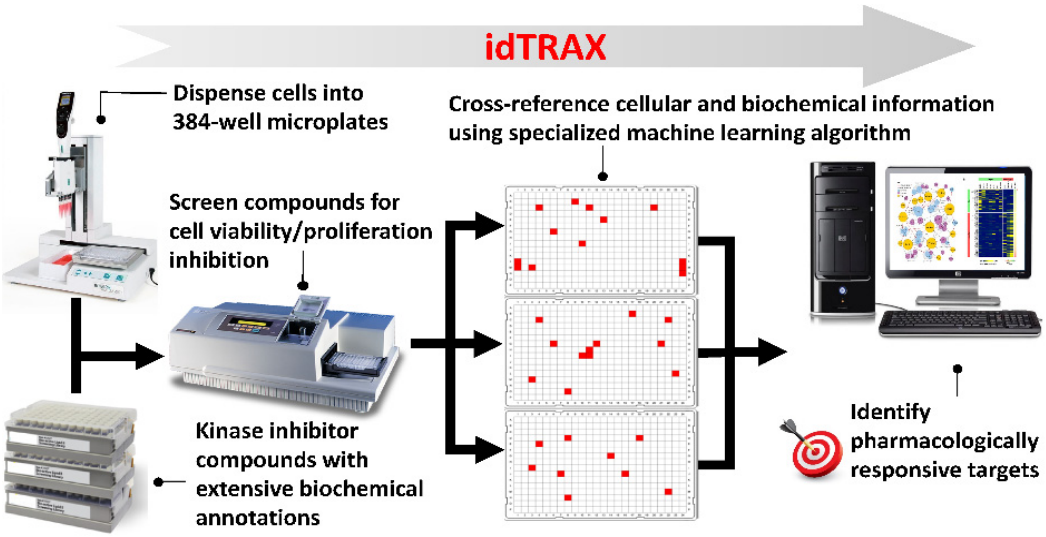

Figure 1. idTRAX flow. Cells are screened with the chemical probe set, and phenotypic data is analyzed by the target deconvolution algorithm which identifies pharmacologically responsive targets. The entire process can be completed in as little as 3 days. An example relevant to cancer is shown, though any phenotypic assay can be interrogated in this fashion.

The blue ocean of kinase drug targets. The enormous therapeutic potential of kinase inhibitors (KIs) derives from the crucial role that protein kinases play in regulating most cellular functions and disease modifying

processes (27). Initially, KIs rose to fame as oncology drugs, and for a while were perceived to be inherently toxic and only suitable in cancer. This outdated view is quickly changing as the understanding of KIs' therapeutic potential expands and their clinical safety profiles continuously improve. In 2012, Pfizer's Tofacitinib (JAK1/3 inhibitor) was approved for treating rheumatoid arthritis, marking the first approval for a KI outside of cancer. At present, hundreds of KIs are undergoing clinical development for non-oncology indications including metabolic disorders, immunology, inflammation, and even infectious disease (28–33). As the second largest drug target family after GPCRs, and with < 60 FDA approved drugs, the kinome represents a "blue ocean of opportunity" for future drug discovery and repurposing. This opportunity is further highlighted by the fact that almost half (3 out of 7) of all new molecular entities approved by the FDA in the month of writing this application (August 2019) were kinase inhibitors (Upadacitinib, Fedratinib, Entrectinib).

Additional considerations that have prompted us to develop idTRAX around the kinase family of drug targets include: 1) kinases are readily druggable targets, 2) the existence of a large number of kinase inhibitors (KI) makes it feasible to build an optimized chemical probe set for use in idTRAX, 3) reliable and commercially available kinase assays make profiling kinase activities relatively easy, 4) the polypharmacology between kinases can be used to therapeutic advantage (18,23,32,34,35), 5) our strategic partnership with Promega allows us to utilize their NanoBRET™ TE Intracellular Kinase Assays in building and implementing our technology, and finally 6) we have ten years prior experience with screening KIs in cell-based assays.

idTRAX's potential impact on drug repurposing and personalized medicine. The ability to quickly identify effective and druggable targets is also impactful for drug repurposing. Identifying binders of existing FDA approved KIs as potential targets for new disease models creates immediate opportunities for clinical validation and repurposing. If applied at the level of individual patients, the method can be used to personalize cancer treatment. We have been exploring the feasibility of *ex vivo* patient tumor screening at the University of Miami, with promising results for both liquid and solid tumors (36,37). If effective targets can be identified for a patient in a matter of a few days, then treatments (single agent or combinations) can be tailored to the patient's tumor. This will be especially valuable in the case of tumors that do not present actionable mutations, or tumors that have very high mutational burdens that preclude genomics-based predictions.

INNOVATION

The idTRAX platform is innovative in a number of ways. These include:

1) **The design of the chemical probe set.** Compound libraries intended for phenotypic screening are typically designed using compound-centric criteria (e.g. by diversifying chemical structure). That is useful for efficiently covering chemical space and identifying promising scaffolds for drug development. However, it is not useful for efficiently identifying drug targets, because diversifying chemical structure does not ensure broad target coverage or diverse biochemical activities. The purpose of idTRAX's probe set is not to serve as a source of lead compounds, but rather to work with the target deconvolution algorithm to illuminate drug target candidates. As such, we are developing a unique probe set that is entirely geared towards this purpose and specifically optimized for extracting maximum information on potential targets. In that sense, the probe set (and associated biochemical annotations) constitutes a major portion of the proprietary value of the platform.

2) **The target deconvolution algorithm**. idTRAX's target deconvolution algorithm uses information theory and machine learning, and relates data from biochemical profiling and phenotypic screening to identify candidate targets and anti-targets. The algorithm also incorporates information on the probability of co-inhibition of kinases, allowing it to better distinguish kinases that are driving the phenotype from those whose inhibition merely tracks with a mechanistic target. Additionally, while some compounds may exert effects through non-kinase targets, the use of hundreds of probes with different biochemical profiles minimizes the possibility of falsely relating these effects to kinases.

3) **Use of in-cell kinase annotations.** In our previous studies, we have used *in vitro* biochemical inhibition/binding data on KIs to perform target deconvolution. However, these data could diverge from what occurs inside intact cells (38), which could in turn affect the accuracy of target predictions. To solve this problem, we will annotate our probe set using in-cell kinase activity assays. This will be accomplished in collaboration with Promega and using their NanoBRET™ platform. Compared to cell-free assays, The NanoBRET™ platform has consistently shown better alignment with phenotypic potency measurements (38).

4) **Harnessing polypharmacology in drug development.** Once promising target candidates are identified, target validation and drug development can proceed according to well-established paths with already matured technologies. An added benefit that idTRAX offers is the ability to identify multiple targets, and also anti-targets. While drug developers might perceive pursuing an individual target as the most attractive prospect due to its

tractability, there is a steady uptick in the pursuit of two or more targets in oncology (39). Anti-targets can also be used in counter screens to deprioritize chemical series with counterproductive activities. As such, idTRAX offers the options to: 1) pursue a single target with a single agent, 2) pursue multiple targets by polypharmacology or drug combinations, and 3) incorporate anti-targets in counter screens to optimize efficacy. The decision to pursue one or more of these strategies can be made on a case-by-case basis, depending on the indication, tractability of the targets, and the relevance of anti-targets.

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