AI-Enabled Phenotypic Screens Reveal Clinically Relevant Drugs

Rare genetic disease research is an important area of unmet medical need. There are thousands of untreated rare diseases caused by tens of thousands of individual genetic defects, and each presents with unique clinical manifestations. To meet the challenge of rapidly discovering treatments for these conditions, Recursion has developed a target agnostic discovery platform that combines artificial intelligence with automated biology for massive parallelization of high throughput drug screening. Here we review a subset of results from our studies of three rare genetic conditions: Ataxia Telangiectasia (A-T), Spinal Muscular Atrophy (SMA), and Neurofibromatosis Type 2 (NF2). In this report, we highlight the unbiased re-discovery of a selection of compounds or drug classes currently in clinical development for these conditions. These results validate the discoveries generated by the Recursion platform, and underscore our ability to prioritize drug classes and differentiate best-in-class candidates at unprecedented pace and accuracy.

Table 1: Clinical-stage drugs rediscovered with Recursion platform

<table>
<thead>
<tr>
<th>Disease</th>
<th>Target Class</th>
<th>Drug (Sponsor)</th>
<th>Phase</th>
<th>Clinical trial</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ataxia telangiectasia</td>
<td>Glucocorticoid</td>
<td>Betamethasone</td>
<td>Phase 2</td>
<td>Leuzzi et. al 2015</td>
</tr>
<tr>
<td>Spinal muscular atrophy</td>
<td>Glucocorticoid</td>
<td>Dexamethasone</td>
<td>Phase 2</td>
<td>Zannoli et. al 2012</td>
</tr>
<tr>
<td>Neurofibromatosis type 2</td>
<td>HDAC</td>
<td>Valproic acid</td>
<td>Phase 2/3</td>
<td>NCT00661453; NCT0227266; NCT01671384</td>
</tr>
<tr>
<td>Neurofibromatosis type 2</td>
<td>mTOR</td>
<td>Everolimus (Novartis)</td>
<td>Phase 2</td>
<td>NCT01419639; NCT02831257</td>
</tr>
<tr>
<td>Neurofibromatosis type 2</td>
<td>VEGF c-KIT</td>
<td>Axitinib (PTCPharma)</td>
<td>Phase 2</td>
<td>NCT02129647</td>
</tr>
<tr>
<td>ERBB2/EGFR</td>
<td>Lapatinib (GSK)</td>
<td></td>
<td>Phase 2</td>
<td>NCT00973739</td>
</tr>
</tbody>
</table>

Introduction

There are approximately 6,000 rare diseases affecting an estimated 25 million people in the United States (GARD). Rare diseases disproportionately affect children, and many children with rare genetic diseases do not live to see their 5th birthday (Global Genes). Therapeutic development for these diseases has been slow, and less than 5% of rare diseases have an FDA-approved treatment (Kakkis EveryLife Foundation). Thus, there is great need for innovative new medicines for the millions of patients suffering from rare diseases.

Recursion Pharmaceuticals has developed a highly efficient, broadly applicable, and readily scalable approach to drug discovery that simultaneously leverages automated biology and artificial intelligence. Using microscopy to measure hundreds of sub-cellular structural changes caused by pathogenic perturbations, Recursion is able to rapidly discover data-rich “marker-less” high-dimensional phenotypes in vitro across many individual disease models. High-throughput drug screens on these phenotypes uncover promising drug candidates that rescue disease signatures. This unique approach allows Recursion to rapidly model and find potential treatments for hundreds of traditionally refractory diseases, making it ideally suited to tackle the urgent unmet medical need of patients with rare diseases. Recursion’s platform enables unprecedented efficiencies of scale towards dramatically increasing the pipeline of potential therapeutics and indications, and decreasing the associated costs of research and development for new therapies.
Box 1: Recursion’s Prometheus Plot

The Prometheus drug screen plot (Figure 1A) displays information on the impact of each drug in terms of both its ability to rescue morphological defects associated with knockdown of a disease-associated gene, as well as the magnitude of non-specific effects (side effects and toxicity) induced by treatment. The data are displayed relative to vehicle-treated knockdowns (Disease), shown in green and negative controls (Control), shown in yellow. Drugs are displayed in red. The disease score (depicted along the x-axis) measures the similarity between the loss-of-function disease signature under investigation and the signature of each treatment. The side effect score (depicted on the y-axis) measures the remaining effects of a treatment that are separate from the disease signature. A drug with a lower disease score (towards the left, closer to the negative controls) and a lower side effect score is more likely to successfully rescue the disease signature relative to other drugs. The size of the markers in each data plot reflect our confidence in the disease score (the larger the marker, the more confidence in the disease score).

In just under 2 years, Recursion has established hundreds of rare disease models and generated a sizeable pipeline of new drug and target candidates across several diseases. A subset of these diseases have been extensively studied by the academic and biopharmaceutical research communities, enabling us to retrospectively examine whether the Recursion platform is able to rediscover known clinically active compounds and independently validated disease targets. Here we present case studies of research performed on three rare genetic diseases: Spinal muscular atrophy (SMA), Ataxia telangiectasia (A-T), and Neurofibromatosis type 2 (NF2), to highlight the potential of our platform to quickly identify high-potential drug candidates.

Discovery of drugs in clinical development

Ataxia telangiectasia

Ataxia telangiectasia (A-T) is a rare genetic neurodegenerative disease characterized by progressive difficulty with motor control and movement coordination (ataxia) beginning in early childhood. In addition, patients with A-T develop mucosal and cutaneous lesions due to blood vessel abnormalities (telangiectasias), increased infections due to immune dysfunction, and increased risk of lymphoma. Affected individuals often succumb to early death in the second or third decade of life due to infection or cancers.

A-T affects 1 in 40,000 individuals worldwide and is caused by mutations in the DNA-repair gene ATM, a gene expressed ubiquitously in the human body. A-T causing mutations result in impaired function of the ATM protein and defects in the DNA-damage response pathway. The disease preferentially affects cells of the cerebellum, immune system, and vasculature for unknown reasons. A-T is a devastating disease and there are currently no FDA-approved treatments that delay its progression. However, based on serendipitous findings of improvement in A-T patients’ symptoms after incidental use of glucocorticoids, several human trials have been initiated to systematically evaluate their therapeutic efficacy. Betamethasone was tested in a study of 6 patients and found to improve neurological manifestations (Pignata et al. 2011). Experimenting with the route of administration of glucocorticoids, another group developed a cell-based therapeutic that

Figure 1: Glucocorticoids rescue a high-dimensional phenotype associated with ATM deficiency. A series of primary and secondary drug screens were performed using a library of ~2000 small molecules. Hits were algorithmically selected based on efficacy and screened with a higher replicate count in a tertiary screen. A) (Prometheus Plot: See Box 1) Results from a tertiary screen of the most efficacious compounds are plotted (red, shaded blue) with respect to ATM deficient controls (green) and healthy controls (yellow). Glucocorticoids are identified by the dotted red polygon. Another highly attractive molecule based on its efficacy in the screen with little to no side effect profile, REC3926, acts on a novel target for A-T. B) Impact of mometasone and novel shown in A on the twenty most prominent phenotypic disease features. The size the green bars represent changes in individual features of increasing or decreasing magnitude that best represent the ATM disease signature. The impact of each drug on individual features is overlaid in red. Drug class 2 rescues all 20 features that comprise the disease signature for ATM deficiency, while mometasone rescues a subset of features (denoted by ‘efficacy’ bracket). Note: The order of the features has been randomized between individual plots, and individual feature labels are omitted. The shade of green or red is inversely proportional to the variance of the feature measurements. C & D) Effect of glucocorticoid hits on signaling pathways associated with ATM deficiency as assayed by Western Blot. siRNA transfected AS549 cells (C) and primary fibroblasts from a patient with AT (D) were treated with H2O2 and drugs as indicated. Western blots probed for ATM and phosphorylated Chk2. All glucocorticoids except dexamethasone rescued Chk2 phosphorylation associated with ATM deficiency in our cell model. Mometasone further displayed a dose-dependent rescue of Chk2 phosphorylation in primary patient-derived cells. The lower plots represent quantification of phosphorylated Chk2 from the Western Blot, n=3. (*Healthy, * denotes p<0.05, two-sided paired t-test)

Box 2: Recursion’s AI-enabled Phenomics Platform

The power of Recursion’s platform rests on two pillars: scalability across hundreds of diseases; and rich high-dimensional data. Our broadly biologically-relevant Cell Painting protocol reveals disease phenotypes in human cells for nearly any perturbation, including hundreds of human disease genes. Robust and scalable biology provides the perfect substrate for AI-enabled phenomics. Through a combination of biological feature extraction and deep learning with convolutional neural networks, Recursion’s image analysis pipeline distills rich, high-dimensional images of cellular disease states into disease-specific phenotypic signatures. Drug candidates are then tested for their ability to restore disease signatures to a healthy state across hundreds of dimensions, and prioritized based on both efficacy and potential off-target side effects. Only the most effective and specific drugs are identified as hits.
In summary, Recursion identified significant class effects among glucocorticoids for A-T, and further highlighted the ability of the best-in-screen molecule to rescue a disease-relevant biomarker in patient-derived cells. Notably, the least efficacious compounds identified on our platform have already shown efficacy in trials of patients with A-T. These results highlight the ability of our approach to rapidly discover clinically relevant therapies, and further enable sensitive differentiation of potential best-in-class molecules.

Spinal muscular atrophy

Spinal muscular atrophy (SMA) is a devastating genetic disease characterized by progressive muscle weakness and paralysis resulting from degeneration of lower motor neurons in the spinal cord and brainstem nuclei. Onset ranges from prenatal through young adulthood. SMA is one of the most common genetic causes of mortality in children and its incidence and carrier frequency are estimated to be 1 in 10,000 and 1 in 50, respectively.

Mutations in the gene SMN1 (survival motor neuron 1) cause SMA. Humans carry a second survival motor neuron gene, SMN2, and increases in the copy number of SMN2 are known to reduce the clinical severity of SMA. Thus, an important therapeutic strategy has focused on agents that increase transcription of SMN2 or increase the functionality of the gene product through modulation of the splicing machinery. The gene products of SMN genes appear to be involved in small nuclear ribonucleoprotein (snRNP) biogenesis and function (Fischer et al. 1997, Liu et al. 1997, Pellizzoni et al. 1998) and in U2-dependent splicing events in motor neurons (Huo et al. 2014). There are currently no FDA-approved small molecules for the treatment of SMA.
SMA, and an antisense oligonucleotide based therapy has only recently been approved (FDA).

Among small molecules, HDAC inhibitors have been extensively studied in spinal muscular atrophy (reviewed in Mohseni et al. 2013). Valproic acid (VPA) was among the first HDAC inhibitors to show clinical promise for SMA. The drug increases full length SMN protein in cell-based assays and in patients, and showed a modest clinical improvement in some clinical trials (Darbar et al. 2011; Swoboda et al. 2010; Piepers et al. 2011). Studies have demonstrated that many compounds in this class increase production of SMN protein, though only VPA and phenyl butyric acid (PBA) have been evaluated in clinical studies to date (Mohseni et al. 2013). Given the therapeutic potential demonstrated by these studies, the identification of selective, potent, and CNS active drugs in this class remains an important goal.

Recursion identified a robust phenotype associated with SMN1/2 deficiency in HUVEC (Figure 2A) and performed small molecule screens for compounds that rescue this phenotype. Several promising hits were recovered from these screens, including HDAC inhibitors, a drug class which has been under intense evaluation for the treatment of SMA. HDAC inhibition is thought to function by directly increasing transcription of the SMN2 gene by sustained acetylation of the SMN2 promoter. While no compound completely rescued the SMA phenotype, among the most efficacious hits in the model is a clinical stage HDAC inhibitor (Figure 2B) and this drug rescued the 5 cellular features that are most contributory to the disease signature (Figure 2C). Follow-up studies demonstrated that this compound rescues SMN1/2 deficiency by increasing production of SMN1/2 protein, likely through action on SMN2.

In summary, Recursion identified significant class effects among multiple target classes, including HDACs, as potential treatments for the SMA. This finding further demonstrates the ability of the Recursion platform to detect distinct classes of therapeutic effects, and rapidly uncover favorable treatments that may act directly on the target.

Figure 3: Inhibitors of mTOR, VEGF, and EGFR/Her2 rescue a high-dimensional phenotype associated with NF2 deficiency. A series of primary drug screens were performed using a library of ~2000 small molecules. A small number of hits were algorithmically selected based on efficacy and screened with a higher replicate count in a confirmatory secondary screen. Compound classes with known efficacy are highlighted in red in the panels to demonstrate detection of phenotypic rescue on our platform: A) mTOR inhibitors (arrowhead identifies AZD2014, asterisk identifies an alternative potential best in class molecule). B) VEGF inhibitors (arrowhead identifies sunitinib, asterisk identifies an alternative potential best-in-class molecule). C) EGFR/Her2 inhibitors (asterisk identifies a potential best in class molecule).

**Neurofibromatosis type 2**

Neurofibromatosis type 2 is an autosomal dominant cancer syndrome characterized by a predisposition to recurrent tumors in the central nervous system. Most commonly, patients with NF2 develop bilateral schwannomas (a clinical hallmark), meningomas, and ependymomas which, while benign, can lead to hearing loss, paralysis, and early death (Martuza et al. 1988). While studies are ongoing to evaluate novel medical treatments for NF2, currently the standard of care is limited to surgical removal or radio ablation of tumors and supportive care for symptoms that arise from the disease. The disease affects an estimated 1 in 25,000 live births and exhibits near complete penetrance by 60 years of age (Asthaigiri et al. 2009).

NF2 is caused by loss of function mutations in the NF2 gene, which encodes the NF2 tumor suppressor protein. In addition to its role in neurofibromatosis, somatic inactivation of NF2 has been detected in 60% of sporadic meningiomas, a tumor that accounts for approximately 30% of intracranial neoplasms (Perry et al. 2004, Rutledge et al. 1994). An important challenge in therapeutic development for NF2 has been the characterization of complex biochemical pathways through which the protein exerts its functions. While recent results have identified multiple putative targets for medical intervention along disease relevant signaling pathways, an important challenge for the field remains understanding the most appropriate molecular target for therapeutic intervention (Evans et al. 2009).

To identify novel and effective treatments for NF2, we produced a loss-of-function model of the disease in a primary human cells and screened for molecules that rescue the disease-specific phenotype. Of 2000 small molecules screened, the Recursion platform revealed 6 target classes with rescue activity, including novel targets yet to be described in the literature.

To date, three major target classes are in clinical development for NF2: mTOR inhibitors, VEGF inhibitors, and EGFR/Her2 inhibitors (Table 1). Loss of NF2 leads to constitutive activation of mTOR complex 1 (mTORC1) signaling and thus, the mTORC1 inhibitor,
everolimus, has been evaluated for clinical efficacy in the disease. While a Phase 2 study of Everolimus failed to demonstrate efficacy (Allen et al. 2014), a Phase 2 study with a novel, highly selective mTOR inhibitor AZD2014 is currently underway (NCT02831257). Notably, in our studies everolimus showed minimal efficacy and was not advanced to secondary screens. However, AZD2014 demonstrated strong rescue albeit with an elevated side-effect profile compared to another highly selective mTOR inhibitor (Figure 3A, arrowhead vs. asterisk).

Blockade of vascular endothelial growth factor (VEGF) signaling has also been evaluated as a therapeutic approach for NF2. The VEGF receptor tyrosine kinase inhibitor sunitinib recently demonstrated activity in a Phase 2 study of recurrent, refractory meningioma, including patients with NF2 loss-of-function mutations (Omuro et al. 2015). A second VEGF inhibitor, axitinib, is currently in Phase 2 for NF2 (NCT02129647). In our drug screens, axitinib did not show sufficient efficacy to be advanced to follow-on assays. However, Sunitinib demonstrated a moderate rescue of NF2 loss-of-function phenotypes with minimal increase in side-effect profile (Figure 3B, arrowhead). As with mTOR inhibitors, we were able to identify a compound with a more striking efficacy profile that produced a complete rescue of the disease phenotype with minimal side effects (Figure 3B, asterisk).

The role of endothelial growth factor (EGFR) and Her2/ErbB2 signaling in NF2 is well documented in the literature and blockade of this signaling pathway with EGFR/Her2 inhibitors reduces proliferation of NF2-deficient glial cells (Houshmandi et al. 2009). A Phase 2 study of the EGFR/ErbB2 inhibitor lapatinib was recently carried out in patients with NF2. The study found that lapatinib was well tolerated and produced antitumor activity in a subset of patients with NF2 (Allen et al. 2012). While we did not evaluate lapatinib specifically, several EGFR/ErbB2 inhibitors rescued NF2 loss-of-function phenotypes, with one drug in this class producing a robust rescue (Figure 3C), further demonstrating that the Recursion platform can rapidly and sensitively identify clinically relevant drug classes.

Conclusion

Rare diseases represent an urgent area of great unmet medical need. Recursion is able to address this need through innovative drug discovery that leverages high-dimensional structural phenotypes across hundreds of diseases in massively parallel high-throughput drug screens. Here we presented the results of our retrospective analyses of data from studies of A-T, SMA, and NF2 to highlight the potential of our platform to rapidly uncover highly translatable drug candidates in a fraction of the time and cost of traditional drug screening. In A-T, we identified a strong disease phenotype ameliorative class effect displayed by glucocorticoids, as well as their previously unreported dichotomous grouping in terms of phenotypic side-effect profiles. Our screen specifically identified betamethasone and dexamethasone as hits, both of which have been validated independently in human trials. However, we also uncovered the ability of mometasone, which harbored a more attractive side-effect profile than betamethasone and dexamethasone, to better rescue ATM deficiency in an orthogonal disease-relevant assay. In SMA, we quickly identified HDAC inhibitors among other drug classes as potential treatments, including one specific HDAC inhibitor that has already progressed to clinical trials for the disease. In NF2, we identified the three major drug classes (mTOR, VEGF, and EGFR/Her2 inhibitors) that have known efficacy for the treatment of cancer syndromes caused by NF2 deficiency. Of note, we specifically identified the therapeutic effects of AZD2014 and sunitinib, both of which are being evaluated in advanced clinical trials for NF2 associated pathologies. Together, these data demonstrate the ability of our unique approach to rapidly uncover highly translatable drug candidates as well as differentiate them with remarkable sensitivity.

Bibliography


