ELSEVIER

The NCATS Pharmaceutical Collection: a 10-year update

Ruili Huang, Hu Zhu, Paul Shinn, Deborah Ngan, Lin Ye, Ashish Thakur, Gurmit Grewal, Tongan Zhao, Noel Southall, Mathew D. Hall, Anton Simeonov and Christopher P. Austin

Division of Pre-clinical Innovation, National Center for Advancing Translational Sciences (NCATS), National Institutes of Health (NIH), Rockville, MD 20850, USA

The National Center for Advancing Translational Sciences (NCATS) Pharmaceutical Collection (NPC), a comprehensive collection of clinically approved drugs, was made a public resource in 2011. Over the past decade, the NPC has been systematically profiled for activity across an array of pathways and disease models, generating an unparalleled amount of data. These data have not only enabled the identification of new repurposing candidates with several in clinical trials, but also uncovered new biological insights into drug targets and disease mechanisms. This retrospective provides an update on the NPC in terms of both successes and lessons learned. We also report our efforts in bringing the NPC up-to-date with drugs approved in recent years.

Introduction

There are over 7000 identified rare genetic conditions, and only 500 have approved drugs available to treat them [1]. Adopting existing active pharmaceutical ingredients (APIs) to validate the disease models can facilitate the development of drugs for patients with no current options. Drug repurposing or repositioning, defined as the application of an existing drug to treat a new disease or condition, has emerged as a promising complement to *de novo* drug development because of the significantly reduced time and cost associated with bringing treatments to patients [2]. The traditional drug development process, starting from a new chemical entity (NCE), a drug that contains no active moiety that has been approved by the US Food and Drug Administration (FDA) in any other application [3], takes 1216 years on average and it costs US$12 billion to bring a new drug to market [4]. By contrast, drug repurposing is estimated to cost on average US$300 million and take 6 years because these molecular entities have already undergone significant safety testing in humans [2]. The risk is also lower for repurposing a drug: while 10% of investigational new drug applications (INDs) gain market approval, repurposed drugs approach 30% approval rates [5]. Repositioning of existing drugs is especially attractive for rare and neglected diseases (RNDs) [1,6], for which the expected limited return on investment makes NCE development particularly challenging. Another area where repurposing becomes appealing is finding solutions for rapidly spreading infectious diseases, such as recent epidemics of Zika virus (ZIKV) [7] and Ebola virus (EBOV) [8], when a quick response is crucial; the fewer safety concerns associated with approved drugs can greatly expedite the process. Classic examples of repurposed or repositioned drugs include sildenafil for the treatment of erectile dysfunction [9], thalidomide to treat leprosy and multiple myeloma [10], and tretinoin, also known as all-*trans* retinoic acid (ATRA), for the treatment of acute promyelocytic leukemia (APL) [11].

Early success stories in drug repurposing from serendipitous discoveries have inspired systematic efforts to search for repositioning candidates taking advantage of high-throughput screening (HTS) technologies and computational modeling of big data. Toward this goal, a comprehensive collection of all currently approved and marketed drugs is fundamental. Toward this goal, scientists at NCATS started assembling the NCGC Pharmaceutical Collection, now called the NPC, a comprehensive and nonredundant collection

Corresponding author: Huang, R. (huangru@mail.nih.gov)

1359-6446/Published by Elsevier Ltd.
https://doi.org/10.1016/j.drudis.2019.09.019

of small molecule drugs amenable for HTS, as both an informatics and screening resource [12]. The first version of the NPC, a collection of 3300 drugs (72% approved and 28% investigational), including 90% of all small-molecule APIs approved by the FDA, was assembled 10 years ago. In 2011, the NPC database and browser were published and made available to the public, with all library information released as an open science initiative. The NPC screening collection has been made available to collaborators who wish to bring their projects to the National Institutes of Health Chemical Genomics Center (NCGC), now within NCATS. Providing copies of the physical library to outside collaborators has not been feasible for several reasons, including limited supply of material because of the high costs associated with procuring some difficult-to-obtain compounds, as well as federal restrictions on the shipment of controlled substances. At NCATS, assays are optimized for screening in 1536-well plate format to maximize the use of the limited material [13]. Through NCATS collaborative screening over the past 10 years, the NPC has been profiled for activity against an array of human targets and disease models, generating an unparalleled amount of data. In this retrospective, we summarize the outcomes from 10 years of screening the NPC, with lessons learned, and plans for moving forward, including the development of an updated NPC library.

NPC repurposing campaigns discovered drugs with new clinical indications

Over the past 10 years, NCATS received >300 requests for collaborations to screen the NPC, which resulted into >200 collaborative projects with main disease areas covered including RNDs, infectious diseases, and cancer. As of June 2019, the original publication on the NPC resource [12] had been cited by >200 articles (Fig. 1a) with author affiliations from 15 countries and regions and 32 US states (Fig. 1b). These articles included reports on results from screening the NPC in various assays. Several significant findings with drug leads identified from repurposing screens conducted at NCATS are summarized in Table 1 and Fig. 2. These are projects that produced drug candidates that went into clinical trials and/or high-impact publications. Patents have been filed on some of these drug leads for their use in new disease indications. The patents with NCATS scientists as co-inventors are listed in Table S1 in the supplemental information online. Here, we highlight projects that produced drugs with new indications that have entered clinical trials or have shown activities *in vivo*.

Niemann-Pick C

Niemann-Pick disease type C1 (NP-C1) is a rare, neurodegenerative, autosomal recessive disease that occurs with a frequency of one in 120 000 live births [14]. It is a genetic disease with mutations in the *NPC1* or *NPC2* genes, resulting in impaired intracellular trafficking of cholesterol and other lipids, leading to the progressive accumulation of unesterified cholesterol and other lipids in the lysosomes that affect the central nervous system (CNS) and visceral organs [15]. Currently, there is no therapy for NP-C1 approved by the FDA [16].

Phenotypic screens of the NPC drug library using NP-C1 fibroblasts identified drugs that reduce lysosomal cholesterol accumulation [17], with β-cyclodextrin [18] and δ-tocopherol as the most promising leads [19,20]. β-cyclodextrin has only been considered as an excipient and has not been approved by the FDA for use as a therapeutic agent in any drug product [21]. The mechanism of action of β-cyclodextrin against NP-C1 is not well understood, but it is thought to reduce cholesterol accumulation in NP-C1 mutant cells through induction of exocytosis [22]. δ-Tocopherol is a form of vitamin E, which is mostly known for its antioxidant effect among several proposed functions, although no specific target protein has been found or implicated [23]. Studies of the effect of δ-tocopherol on NP-C1 suggest that its pharmacological effect on NP-C1 is mediated by stimulating an increase in cytosolic calcium that enhances lysosomal exocytosis [20]. However, utilization of δ-tocopherol directly as a therapeutic agent is limited by its inability to achieve adequate plasma and brain concentrations in both animals and humans [24]. Structure optimizations to improve its pharmacokinetic properties are currently underway to better explore the potential effect of δ-tocopherol *in vivo*.

In addition to NP-C1, targeting lysosomal exocytosis might represent a useful strategy to mitigate the lysosomal burden in other lysosomal storage disorders. δ-Tocopherol has been found to reduce pathological phenotypes in fibroblasts from patients with other lysosomal storage diseases, such as Batten, Fabry, Farber, Niemann Pick type A, Sanfilippo type B, Tay-Sachs, and Wolman diseases [20]. Using this disease model, δ-tocopherol and β-cyclodextrin are under investigation, either alone or as drug combinations, in the hope of developing a new class of potential therapeutics for NP-C1 and other lysosomal storage diseases [2527].

β-Cyclodextrin, specifically 2-hydroxypropyl-beta-cyclodextrin (VTS-270), achieved clearance of the IND application in December 2012, and the first-in-human clinical trials began in February 2013 [28]. Given the strong preclinical and early clinical data, including a Phase I trial, the program was licensed to a company, Vtesse Pharma (later acquired by Sucampo Pharmaceuticals, Inc., which has since been acquired by Mallinckrodt Pharmaceuticals, Inc.), and received the Breakthrough Therapy Designation from the FDA. The Phase I trial was concluded successfully and, currently, the drug is being tested in a multicenter, multinational Phase IIb/III clinical efficacy trial (NCT02534844) sponsored by Mallinckrodt [29]. The goal of this trial is to obtain regulatory approval of VTS-270. Through this agreement, Mallinckrodt is also funding preclinical studies led by NCATS researchers to optimize the δ-tocopherol series of compounds for further testing as potential single treatments or as a combination therapy with cyclodextrin for lysosomal storage disorders. Several other drugs identified from the NPC library screen have also been exclusively licensed by Mallinckrodt specifically for their use in the treatment of this class of diseases.

Chronic lymphocytic leukemia

Chronic lymphocytic leukemia (CLL) is an adult lymphoid malignancy that is the most common leukemia in the Western world [30]. The therapeutic options for patients with CLL were limited and few therapies were under development [31]. Drug resistance often arises in relapsed disease, leaving patients with limited therapeutic options. In an effort to identify and repurpose existing drugs with anti-CLL activity, lymphocytes collected from six patients with CLL and five unaffected donors were screened against the NPC library using a cell viability assay [32]. Auranofin was identified as a potent compound that selectively killed lymphocytes from patients with CLL compared with cells from

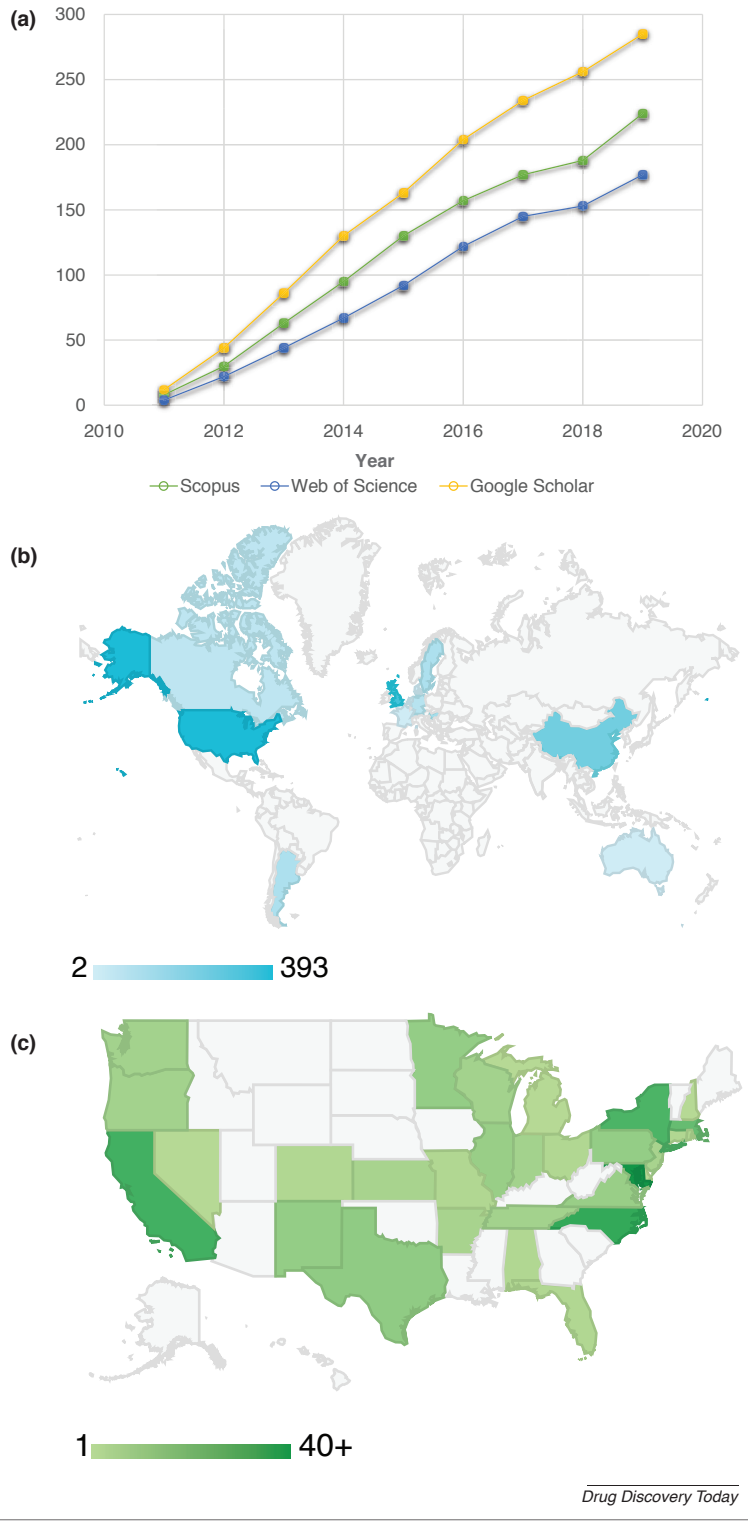

FIGURE 1

National Center for Advancing Translational Sciences (NCATS) Pharmaceutical Collection (NPC) citations. **(a)** Number of citations by year from three reporting agencies. **(b)** Region coverage by author affiliations worldwide. **(c)** Region coverage by affiliations in the USA.

normal donors, with >30-fold difference in IC_{50}. Auranofin is a gold-containing drug originally approved for the treatment of rheumatoid arthritis in 1985 [33]. Its anti-inflammatory and immunosuppressive activities were believed to be mediated by inhibiting multiple cytokines, such as interleukin 1 beta (IL-1β), IL-6, and tumor necrosis factor (TNF) α. Auranofin was also found to have potential therapeutic effects in other diseases, including cancer and infectious diseases. The mechanism of action of auranofin in these diseases is mediated by the inhibition of reduction/oxidation (redox) enzymes, which are essential for regulating intracellular reactive oxygen species [34]. Inhibition of these enzymes leads to cellular oxidative stress and subsequently

TABLE 1

Example drug leads identified from NPC screens

Disease	Target	Drug	Stage	Clinical trial	Refs
Breast cancer	NF-κB signaling	Lestaurtinib-AG14361	*In vivo*		[67]
Charcot-Marie-Tooth disease type 1A	Peripheral myelin protein 22	Fenretinide, olvanil, and bortezomib	*In vitro*		[68]
Chordoma	NF-κB signaling	Sunitinib, bortezomib	*In vivo*		[69,70]
CLL		Auranofin	Phase II complete	NCT01419691	[32]
EBOV		Multiple	Screen		[57]
Excess lipid storage	Diacylglycerol Acyltransferase 1	Multiple	*Drosophila*		[71]
Exserohilum rostratum		Multiple	Screen		[72]
Fragile X syndrome	Fragile X mental retardation protein	Multiple	Screen		[73]
Giardia lamblia	Giardia carbamate kinase	Multiple	Screen		[74]
HCV		Chlorcyclizine alone or in combination with ribavirin	Phase I complete	NCT02118012	[48,49]
Human astrocytes, protective	Oxidative stress	Multiple	*In vitro*		[75]
Lung cancer	Estrogen receptor	Fulvestrant	*In vivo*		[76]
Malaria	ABC transporter	Ketotifen	*In vivo*		[44]
		Multiple	*In vivo*		[39]
Metastatic pheochromocytoma		SAHA□epirubicin	*In vitro*		[77]
Multidrug-resistant bacteria		Colistin□auranofin□rifabutin	*In vitro*		[78]
		Rifabutin□colistin□imipenem	*In vitro*		[78]
Multiple sclerosis		Miconazole and clobetasol	*In vivo*		[79]
Mycobacterium tuberculosis	PhoP-DNA binding	Iodophthalein, dodecylbenzenesulfonic acid	*In vitro*		[80]
Myotonic dystrophy	Nuclear foci	Chromomycin A3	*In vitro*		[81]
Neuroprotection	SUMO conjugation	Multiple	*In vitro*		[82]
NP-C1	AMP-activated protein kinase (AMPK)	β-Cyclodextrin	Phase III	NCT02534844, NCT01747135	[29,83]
NP-C1 and Wolman cholesterol storage disorders	Lysosomal lipid accumulation	δ-Tocopherol	*In vivo*		[20]
Photoacoustic tomography	Macrophage	Clofazimine	*In vivo*		[84]
Primary hyperoxaluria Type 1	Glycolate oxidase	Dichromate salt, colistimethate sodium	*In vitro*		[85]
ZIKV		Niclosamide	Phase I	ACTRN12618001441202	[53]

apoptosis. The *in vitro* activity of auranofin against CLL was further validated and the drug was advanced to a Phase I/II two-step clinical trial in 2011 (NCT01419691) [35]. The collaborative project team decided to close the ongoing trial in 2018 because four promising new treatments for CLL had been recently introduced [36], significantly lessening the unmet medical need perceived when the project was initiated 10 years ago.

Malaria

Malaria remains a devastating disease with 12 million fatalities and 300500 million infections per year, largely because of the lack of an effective vaccine and because of widespread drug resistance [37]. Artemisinin combination therapies are currently the standard treatment where malaria is endemic. However, reduced sensitivity to artemisinin therapies has been observed, urging the need for new antimalarial therapies and a better understanding of drug resistance to eradicate malaria [38].

Drugs in the NPC were profiled for activity against 61 genetically diverse strains of lab-grown malaria parasites in quantitative HTS (qHTS) format [39]. The screen identified 32 drugs that were highly effective at killing at least 45 of the 61 strains. Ten of these drugs had not previously been reported to have antimalarial action, and seven were more active at lower concentrations than artemisinin. A genome-wide association study was conducted on the NPC drug screening data and single-nucleotide polymorphism (SNP) data from the 61 parasite strains. Remarkably, just three parasite genes (the same three genes that confer resistance to currently used malaria drugs) were associated with resistance to many of the screened compounds. This suggests that the malaria parasite has a limited number of ways to develop resistance following exposure to drugs. In theory, if drug combinations could be devised to target activity of all three resistance genes simultaneously, the parasite could be disarmed. Following this line of thought, pairs of drugs with complementary activities were also identified from the screen. Such complementary drug pairs, if used together, could slow the emergence of drug resistance in parasites. In this regard, several drugs killed strains of parasites resistant to chloroquine, a standard malaria drug [40]. Given that

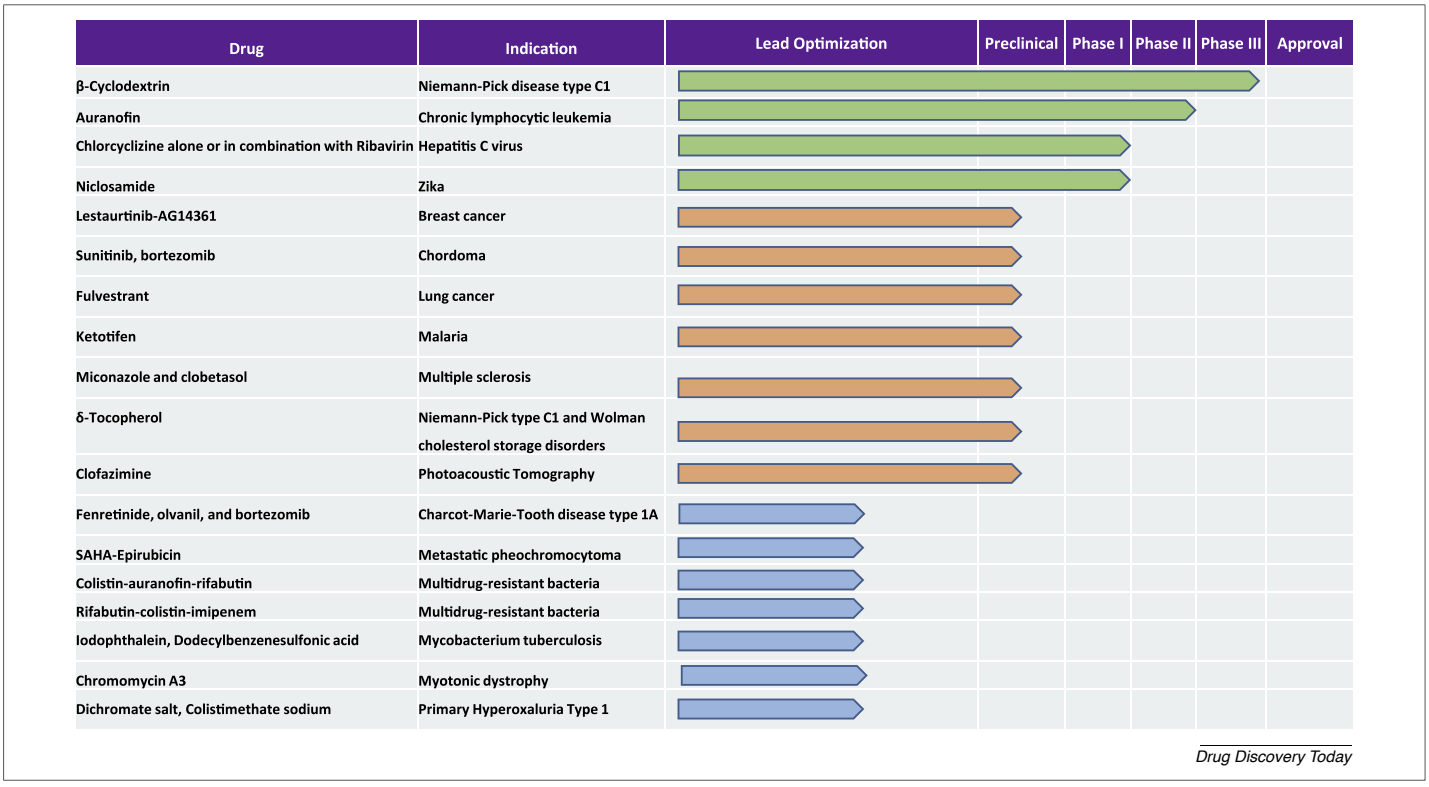

FIGURE 2

Developmental stages of example drug leads identified from National Center for Advancing Translational Sciences (NCATS) Pharmaceutical Collection (NPC) screens. The developmental status of a drug for a certain disease was determined by searching the drug and disease name in PubMed, SciFinder®, and ClinicalTrials.gov.

chloroquine-resistant parasites are widespread in many parts of the world, further studies of compounds with complementary activity could lead to new combination treatments for these drug-resistant parasites. These results provided a wealth of leads for scientists seeking to combine new or existing compounds into better multi-drug regimens against malaria. All qHTS data from the 61 malaria parasites are publicly available in PubChem (AID: 504749).

Another major limitation of current antimalarial drugs is their negligible efficacy against the transmissible mature gametocytes and/or mosquito stages [41]. To identify drugs that can interrupt parasite transmission, a malaria cell line with a disrupted ATP-binding cassette (ABC) transporter gene (*PfABCG2*), which is known to have an important role in drug transport, metabolism, and resistance, was created [42,43]. The recombinant and wild-type malaria 3D7 parasites were screened against the NPC and an antihistamine (ketotifen) was identified [44]. Ketotifen was found to be less active against the PfABCG2-disrupted parasite in culture, and was highly potent in blocking oocyst development of *Plasmodium falciparum* (human parasite) and the rodent parasite *Plasmodium yoelii* in mosquitoes, in addition to activity against asexual stages and gametocytes. Moreover, ketotifen appeared to have some activity against the relapse of *Plasmodium cynomolgi* infection in rhesus monkeys. Other tricyclic structural analogs of ketotifen showed similar activities in inhibiting transmission. These compounds could serve as new candidates for antimalarial drug development. To our knowledge, no trials have been registered for clinical testing of these compounds against malaria.

Other infectious diseases

Hepatitis C virus (HCV) chronic infection is a common disease that is spread worldwide, particularly in Asia and Africa [45]. Although treatments are available, such as standard regimens including pegylated interferon (PEG-IFN) and ribavirin, direct-acting antivirals that target viral factors are costly and have a low genetic barrier to resistance, adverse effects, and potential for drugdrug interactions [45,46]. In an effort to search for more cost-effective treatment options, cell-based qHTS of the NPC was carried out against HCV genotype 2a (JFH-1 strain) [47]. The screen identified several antihistamines, a class of drugs that are commonly used for allergy relief, which showed anti-HCV activity *in vitro* [48]. Among these compounds, chlorcyclizine, a first-generation antihistamine approved during the 1940s, showed high antiviral activity in human liver cells and mouse models, without evidence of drug resistance. It also showed selective activity for HCV against 13 other viruses, including hepatitis B. The *in vitro* antiviral effect of chlorcyclizine was found to be synergistic with other anti-HCV drugs, including ribavirin, interferon-α, telaprevir, boceprevir, sofosbuvir, daclatasvir, and cyclosporin A, without significant cytotoxicity. Antihistamines have well-established clinical safety profiles and are affordable as allergy medications, making them promising candidates for further development into new treatments for HCV infection. Chlorcyclizine alone or in combination with ribavirin entered a Phase I clinical trial in 2014 for safety, tolerability, and antiviral activity in patients with chronic hepatitis C (NCT02118012). Chlorcyclizine demonstrated some anti-HCV effects in humans, but was not potent enough to achieve the therapeutic dosing levels [49]. More potent chlorcyclizine derivatives with

better pharmacokinetic features were generated recently for further human testing and therapeutic development [50].

Recent global outbreaks of ZIKV and EBOV infections motivated efforts to repurpose existing drugs to address the urgent need for effective therapies. A large outbreak of ZIKV, a mosquito-borne flavivirus, started in Brazil in late 2014 and spread across the Western Hemisphere within 1 year [51]. With no vaccine available at the time and no drug approved to treat or prevent ZIKV infection [52], drug repurposing became an appealing alternative. *In vitro* models of ZIKV were established and the NPC, along with 3000 more known bioactive compounds, were tested in a caspase-3 ZIKV assay in human neural cells in qHTS format [53]. The screen identified >100 active compounds. The most promising leads include emricasan, an investigational drug being evaluated in a clinical trial to reduce liver injury and fibrosis, and niclosamide, a FDA-approved drug for use in humans to treat worm infections. In addition, the screen identified ten cyclin-dependent kinase (CDK) inhibitors, which are involved in the regulation of cellular processes as well as normal brain development. Emricasan was found to protect human cortical neural progenitors from cell death by inhibiting ZIKV induced caspase-3 activity, whereas niclosamide and the CDK inhibitors blocked the replication of the virus. In addition, emricasan, when combined with one of the CDK inhibitors, prevented both cell death and virus replication. The CDK inhibitors might be useful in treating nonpregnant patients who face an increased risk of GuillainBarré syndrome and other conditions sparked by ZIKV infection. All screening data have been released to the public domain via PubChem (AID: 1224857, 1224859). Testing in mouse models is currently underway to study the efficacy, neuroprotective effects, and mechanism of action of the lead compounds. In 2018, a Phase I study started in Australia to determine the safety of niclosamide as a potential therapeutic for Zika in healthy volunteers (ACTRN12618001441202).

Similar to ZIKV, an unprecedented large outbreak of EBOV started in West Africa in 2014 spreading to the other regions of the world and posted another global crisis [54]. The mortality rate of the EBOV outbreak was estimated to be 70%. There is no proven effective treatment for EBOV infection and the current supply of antibody-based therapy is limited [55,56]. A qHTS screen of the NPC against an EBOV-like particle (VLP) entry assay revealed 53 drugs that blocked EBOV VLP entry into cells [57]. The screening results, both positive and negative, have been made freely available to the public through PubChem (AID: 1117298, 1117304, 1117305, 1117310, 1117312, 1117314). Further screens of selected drug combinations revealed two sets of three-drug combinations, toremifenemefloquineposaconazole and toremifeneclarithromycinposaconazole, which blocked EBOV entry and inhibited live EBOV infection more effectively than single drugs at clinically relevant concentrations [58]. Another qHTS of the NPC using the ZIKV no-structural protein 1 (NS1) assay identified emetine, an FDA-approved drug for amoebiasis, and its structural analog, cephaeline, which potently inhibited ZIKV both *in vitro* and *in vivo* [58]. Both compounds were found to exhibit similar activities against EBOV infection via a different mechanism. Whereas their activity against ZIKV is through the inhibition of ZIKV NS5 polymerase activity, their inhibition of EBOV is through the disruption of the lysosomal function of the host, resulting in decreased viral entry. The screening-ready NPC library enabled extremely fast turnaround times on identifying drug repurposing candidates. The first EBOV assay screen was conducted in August 2014 and the manuscript reporting the screening results including the 53 drug leads was published in December of the same year [57]. Similarly, the NPC was screened against the ZIKV caspase-3 assay in March 2016 and the identified drug leads, including niclosamide, were published online in August of the same year in *Nature Medicine* [53]. The turnaround time in both cases was only 45 months.

Systematic screening data establishes drug activity signatures

The NPC has been screened in >1000 assays, targets, pathways, and cellular phenotypes in qHTS format at NCATS [13]. In addition, the NPC was tested in the Open Innovation Drug Discovery (OIDD) phenotypic assay modules publicly offered by Eli Lilly via a wide-ranging collaborative effort [59]. The results from 13 primary and 23 confirmation screens have been made publically available via the PubChem Database (https://pubchem.ncbi.nlm.nih.gov/) (AID 1117321). Phenotypic outcomes for numerous drugs were confirmed. This broad spectrum of activities observed for the NPC drugs reinforces the premise for drug repurposing [i.e., a drug is likely to hit multiple targets and/or pathways in addition to the ones for which the drug was originally developed (termed polypharmacology)]. When looking at the activities of these drugs by their developmental stage, the group of drugs that were withdrawn from the market clearly showed higher assay hit rates than the other drug categories (Fig. S1a in the supplemental information online). The major reason for drugs being withdrawn from the market is toxic adverse effects. The higher hit rates of this group of drugs might be a simple reflection of cytotoxicity because most of the assays screened were cell-based assays. The distribution of hit rates in a panel of cell viability assays from the Tox21 program confirms this point (Fig. S1b in the supplemental information online), where the group of withdrawn drugs again showed the highest hit rates. Interestingly, the group of approved drugs that are still on the market showed hit rates nearly as high as the withdrawn drugs across all assays, but markedly lower in the cell viability assays. The approved drugs are more likely to show activity toward a target, but are less cytotoxic. The preclinical compounds showed the lowest hit rates both across all assays and in the cell viability assays. Modern drug development processes are leaning more and more toward target selectivity while minimizing toxicity, and the lower promiscuity and cytotoxicity observed for the drugs currently under development reflect this tendency. When grouped by disease area, drug groups that showed the highest hit rates include hematological malignancy and oncology, which also displayed the highest hit rates in the cell viability assays (Fig. S1c,d in the supplemental information online). This is not surprising given that most cancer drugs are cytotoxic in nature. Also worth noting is the group of allergy drugs, which showed hit rates second to the cancer drugs, but were much less cytotoxic.

NPC data in the public domain provide a community resource and enable predictive modeling

The qHTS data generated from screening the NPC are being made publicly available via PubChem. Nearly 300 assay entries

(282 AIDs) have been created so far (Table S2 in the supplemental information online). As part of the Tox21 10 K compound library, the NPC was screened against over 70 Tox21 assays as 15-point titrations in triplicate [60,61]. Shown to be highly reproducible, this robust set of compound activity profiles has been shown to be instrumental in identifying mechanisms of compound action [62], and developing models for predicting *in vivo* toxicity response, such as adverse drug effects [63]. Drugs that share the same target or mode of action (MOA) were shown to cluster by similarity in their activity signature [62]. These clusters can then be used to impute the target or MOA for drugs with no such annotation available or identify new drug targets or MOAs. Adverse drug reaction (ADR) reports and animal toxicity data are available for many of these drugs, which provided the opportunity to evaluate the performance of *in vitro* drug activity data in predicting human toxicity. Using ADRs as a surrogate for human toxicity, the first meta-analysis was conducted to compare human toxicity prediction models built with *in vitro* assay data from human cell lines and those built with animal toxicity [63]. Both types of model showed similarly moderate performance in predicting human ADRs, with animal toxicity data-based models not showing a clear advantage over human cell-based data. Given that many drugs have known targets and/or MOAs, when such information was incorporated into the models, the assay data-based models showed remarkable improvements in their performance and resulted in good predictive models for human ADRs. These additional target/MOA annotations could complement the current Tox21 assays and provide a better coverage of the biological space to improve their predictive power of human *in vivo* toxicity.

Moreover, a small subset of targets/MOAs were identified that, when combined with the assay data, were shown to sufficiently expand the biological space coverage to produce predictive models. This small set of targets can serve as guide for assay development to generate *in vitro* data for better human toxicity prediction [63]. These results provide rich data sets to researchers for further data mining, generation of new hypotheses, and developing new methods for activity modeling. The other assay entries are from drug-repurposing screens encompassing an assortment of disease types, such as cancer, infectious diseases, neurological disorders, diabetes, and several rare diseases.

NPC moving forward

With the tremendous amount of screening data generated over the past 10 years, the NPC has worked well in fulfilling the goals set out when compiling this resource: (i) repurposing drugs for the treatment of RNDs; (ii) determining the activities of known drugs for improved toxicological understanding, modeling, and prediction; and (iii) determining the characteristics of small-molecule compounds that confer biological activity [12]. This is evident by the publications, citations, and drug leads produced by this resource. Ten years of screening efforts also revealed a few lessons and pointers as to how to best utilize this resource to achieve the goal (e.g., a successfully repurposed drug). The drugs in the NPC showed a wide spectrum of activities in the assays in which they were tested. This reflects the polypharmacology characteristic of older (dirty) drugs identified in broad phenotypic screens, and solidifies the premise for repurposing. However, many of the activities observed were later found to be nonspecific or a result of assay artifacts, such as cytotoxicity, as evident by the high hit rates of cytotoxic drugs. Thus, confirmation of the observed activities using secondary and orthogonal assays is crucial for hit validation. As shown by the NPC screening data, primary hits identified from the NPC are often tested in multiple follow-up assays to ensure the relevance of their activity. Moreover, having *in vivo* models available and good collaborations with disease and clinical investigators have proven essential to bring a screening hit

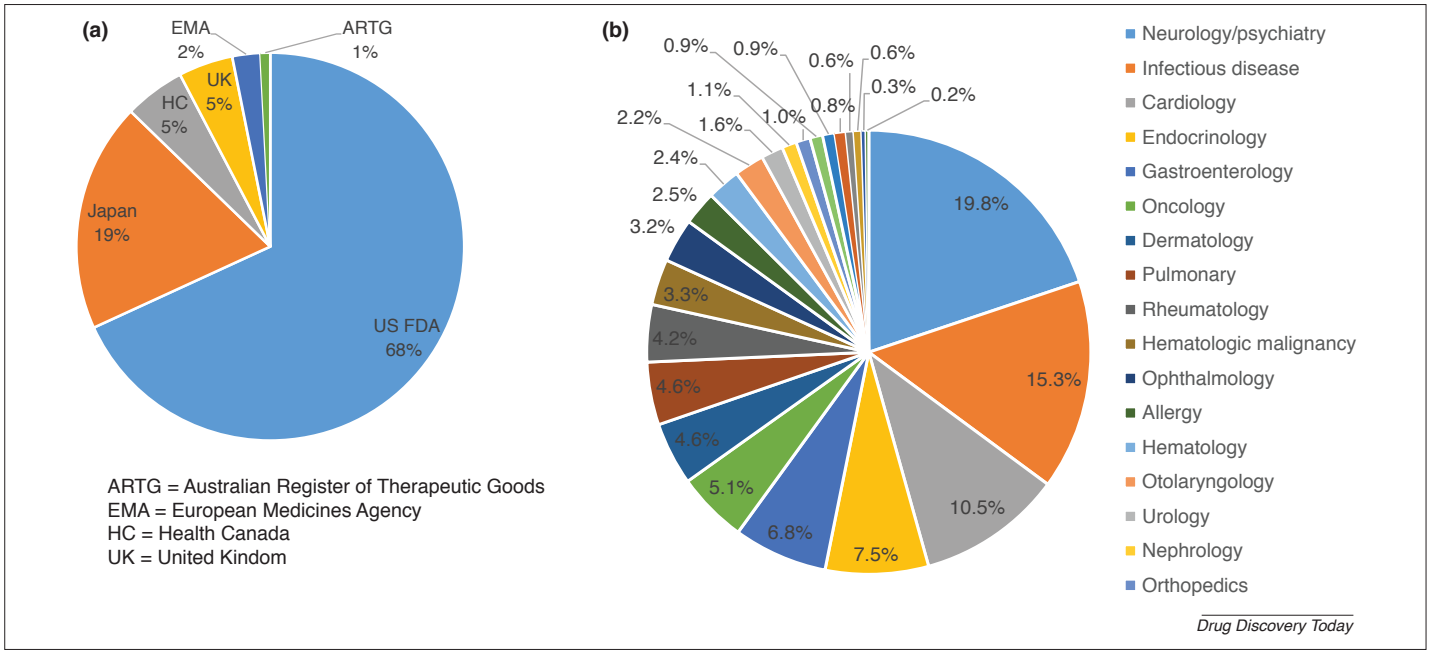

FIGURE 3

Composition of the updated National Center for Advancing Translational Sciences (NCATS) Pharmaceutical Collection (NPC) by **(a)** regulatory agency and **(b)** disease area.

to the clinic. The NPC screening efforts generated many hits, most of which were left at the *in vitro* stage because of the lack of collaborators with sophisticated disease models to advance the drug leads further. These results were simply summarized in publications with the hope that other researchers in the field would apply these published molecules to available models.

NCATS has adopted rapid dissemination of screening results as the mechanism to alert the diverse community of stakeholders of the new bioactivity of existing drugs so that they can decide how to best develop these new leads. In addition, opportunities exist within NCATS (e.g., TRND/BrIDGs or NTU) and outside it for further testing of drugs identified out of the NPC as therapeutics. The few success stories with drug candidates that went into clinical trials are all collective efforts from a large group of collaborators with different areas of expertise, including fellow researchers who can bring *in vitro* and/or *in vivo* disease models, clinicians, patient advocacy groups, and an effective project manager. For example, bringing the antihistamine chlorcyclizine into clinical trials for the treatment of HCV infection involved collaborators from three institutions across the world: NCATS and NIDDK from the USA and Hiroshima University from Japan [48]. Developing β-cyclodextrin for the NPC1 disease is another good example. The authors on the paper reporting the clinical trials in *The Lancet* are from 16 different institutions [29]. A recent review article discussed the challenges faced by the drug-repurposing community and provided recommendations as for how these challenges could be addressed to realize the full potential of drug repurposing [64].

NCATS is in the process of updating the NPC screening collection with a focus on bringing in drugs approved since the release of the first version of NPC, and adding drugs approved in additional countries such as Australia. After removing redundancy and manual curation as we did for the first version, the updated NPC drug collection comprises 2935 approved unique small-molecule molecular entities that are amenable for HTS (Table S3 in the supplemental information online). The composition of the new NPC by regulatory agency and disease area [65,66] covered is illustrated in Fig. 3. The updated NPC will continue to and better serve its purpose as a resource for the chemical genomics and drug discovery research communities, and will be updated on an annual basis with new drugs approved.

Acknowledgments

This work was supported by the Intramural Program of the National Center for Advancing Translational Sciences, NIH. R.H. coordinated the project, sourced and compiled drug lists to construct the updated NPC, helped with the NPC procurement, and wrote the manuscript; H.Z. and D.N. helped find drug sources, procured compounds for the NPC, and helped write the manuscript; P.S. helped procure compounds for the NPC and selected compounds for the NPC screening collection; L.Y. helped curate the drug list; A.T. and G.G. coordinated the custom synthesis of the new NPC compounds; T.Z. helped with the curation of the drug list; N.S. helped with the compilation and curation of the drug list; M.H. helped find drug sources; and C.P.A., A.S., and M.H. conceived and directed the project and helped write the manuscript.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.drudis.2019.09.019.

References

1 Cremers, S. and Aronson, J.K. (2017) Drugs for rare disorders. *Br. J. Clin. Pharmacol.* 83, 1607–1613
2 Nosengo, N. (2016) Can you teach old drugs new tricks? *Nature* 534, 314–316
3 FDA (2014) *New Chemical Entity Exclusivity Determinations for Certain Fixed-Combination Drug Products Guidance for Industry*. FDA
4 Scannell, J.W. *et al.* (2012) Diagnosing the decline in pharmaceutical R&D efficiency. *Nat. Rev. Drug Discov.* 11, 191–200
5 Ashburn, T.T. and Thor, K.B. (2004) Drug repositioning: identifying and developing new uses for existing drugs. *Nat. Rev. Drug Discov.* 3, 673–683
6 Feasey, N. *et al.* (2010) Neglected tropical diseases. *Br. Med. Bull.* 93, 179–200
7 Saiz, J.C. and Martin-Acebes, M.A. (2017) The race to find antivirals for Zika virus. *Antimicrob. Agents Chemother.* 61 e00411-17
8 Bixler, S.L. *et al.* (2017) Discovering drugs for the treatment of Ebola virus. *Curr. Treat. Options Infect. Dis.* 9, 299–317
9 Ghofrani, H.A. *et al.* (2006) Sildenafil: from angina to erectile dysfunction to pulmonary hypertension and beyond. *Nat. Rev. Drug Discov.* 5, 689–702
10 Jacobson, J.M. (2000) Thalidomide: a remarkable comeback. *Expert Opin. Pharmacother.* 1, 849–863
11 Chen, Y. *et al.* (2012) Acute promyelocytic leukemia: a population-based study on incidence and survival in the United States, 1975-2008. *Cancer* 118, 5811–5818
12 Huang, R. *et al.* (2011) The NCGC pharmaceutical collection: a comprehensive resource of clinically approved drugs enabling repurposing and chemical genomics. *Sci. Transl. Med.* 3 80ps16
13 Inglese, J. *et al.* (2006) Quantitative high-throughput screening: a titration-based approach that efficiently identifies biological activities in large chemical libraries. *Proc. Natl. Acad. Sci. U. S. A.* 103, 11473–11478
14 Patterson, M.C. *et al.* (2014) Niemann-Pick disease type C: a lipid trafficking disorder. In *The Online Metabolic and Molecular Bases of Inherited Disease* (Beaudet, A. L., ed.), McGraw-Hill http://ommbid.mhmedical.com/content.aspx?bookid=2709&sectionid=225545907
15 Vanier, M.T. and Millat, G. (2003) Niemann-Pick disease type C. *Clin. Genet.* 64, 269–281
16 Patterson, M.C. *et al.* (2012) Recommendations for the diagnosis and management of Niemann-Pick disease type C: an update. *Mol. Genet. Metab.* 106, 330–344
17 Karten, B. *et al.* (2003) Trafficking of cholesterol from cell bodies to distal axons in Niemann Pick C1-deficient neurons. *J. Biol. Chem.* 278, 4168–4175
18 Camargo, F. *et al.* (2001) Cyclodextrins in the treatment of a mouse model of Niemann-Pick C disease. *Life Sci.* 70, 131–142
19 Rosenbaum, A.I. *et al.* (2009) Chemical screen to reduce sterol accumulation in Niemann-Pick C disease cells identifies novel lysosomal acid lipase inhibitors. *Biochim. Biophys. Acta* 1791, 1155–1165
20 Xu, M. *et al.* (2012) delta-Tocopherol reduces lipid accumulation in Niemann-Pick type C1 and Wolman cholesterol storage disorders. *J. Biol. Chem.* 287, 39349–39360
21 Davis, M.E. and Brewster, M.E. (2004) Cyclodextrin-based pharmaceutics: past, present and future. *Nat. Rev. Drug Discov.* 3, 1023–1035
22 Chen, F.W. *et al.* (2010) Cyclodextrin induces calcium-dependent lysosomal exocytosis. *PLoS One* 5, e15054
23 Schneider, C. (2005) Chemistry and biology of vitamin E. *Mol. Nutr. Food Res.* 49, 7–30
24 Baxter, L.L. *et al.* (2012) Plasma and tissue concentrations of alpha-tocopherol and delta-tocopherol following high dose dietary supplementation in mice. *Nutrients* 4, 467–490
25 McKew, J. *et al.* US Department of Health and Human Services. Cyclodextrin for the treatment of lysosomal storage diseases. U.S. Patent No. US20150216895A1.
26 Yu, D. *et al.* (2014) Niemann-Pick disease type C: induced pluripotent stem cell-derived neuronal cells for modeling neural disease and evaluating drug efficacy. *J. Biomol. Screen.* 19, 1164–1173
27 Long, Y. *et al.* (2016) Induced pluripotent stem cells for disease modeling and evaluation of therapeutics for Niemann-Pick disease type A. *Stem Cells Transl. Med.* 5, 1644–1655

28 Ottinger, E.A. *et al.* (2014) Collaborative development of 2-hydroxypropyl-beta-cyclodextrin for the treatment of Niemann-Pick type C1 disease. *Curr. Top. Med. Chem.* 14, 330–339
29 Ory, D.S. *et al.* (2017) Intrathecal 2-hydroxypropyl-beta-cyclodextrin decreases neurological disease progression in Niemann-Pick disease, type C1: a non-randomised, open-label, phase 1-2 trial. *Lancet* 390, 1758–1768
30 Chiorazzi, N. *et al.* (2005) Chronic lymphocytic leukemia. *N. Engl. J. Med.* 352, 804–815
31 Tam, C.S. and Keating, M.J. (2010) Chemoimmunotherapy of chronic lymphocytic leukemia. *Nat. Rev. Clin. Oncol.* 7, 521–532
32 Shen, M. *et al.* (2013) Identification of therapeutic candidates for chronic lymphocytic leukemia from a library of approved drugs. *PLoS One* 8, e75252
33 Kean, W.F. *et al.* (1997) Auranofin. *Br. J. Rheumatol.* 36, 560–572
34 Gandin, V. *et al.* (2010) Cancer cell death induced by phosphine gold(I) compounds targeting thioredoxin reductase. *Biochem. Pharmacol.* 79, 90–101
35 Ghias, M. *et al.* (2013) Auranofin induces a reversible in-vivo stress response that correlates with a transient clinical effect in patients with chronic lymphocytic leukemia. *Blood* 122, 3819
36 Sharma, S. and Rai, K.R. (2019) Chronic lymphocytic leukemia (CLL) treatment: so many choices, such great options. *Cancer* 125, 1432–1440
37 Snow, R.W. *et al.* (2005) The global distribution of clinical episodes of *Plasmodium falciparum* malaria. *Nature* 434, 214–217
38 Noedl, H. *et al.* (2008) Evidence of artemisinin-resistant malaria in western Cambodia. *N. Engl. J. Med.* 359, 2619–2620
39 Yuan, J. *et al.* (2011) Chemical genomic profiling for antimalarial therapies, response signatures, and molecular targets. *Science* 333, 724–729
40 Fidock, D.A. *et al.* (2000) Mutations in the *P. falciparum* digestive vacuole transmembrane protein PfCRT and evidence for their role in chloroquine resistance. *Mol. Cell* 6, 861–871
41 White, N.J. (2008) The role of anti-malarial drugs in eliminating malaria. *Malar. J.* 7 (Suppl. 1), S8
42 Martin, R.E. *et al.* (2005) The permeome of the malaria parasite: an overview of the membrane transport proteins of *Plasmodium falciparum*. *Genome Biol.* 6, R26
43 Foote, S.J. *et al.* (1990) Several alleles of the multidrug-resistance gene are closely linked to chloroquine resistance in *Plasmodium falciparum*. *Nature* 345, 255–258
44 Eastman, R.T. *et al.* (2013) A class of tricyclic compounds blocking malaria parasite oocyst development and transmission. *Antimicrob. Agents Chemother.* 57, 425–435
45 Liang, T.J. and Ghany, M.G. (2013) Current and future therapies for hepatitis C virus infection. *N. Engl. J. Med.* 368, 1907–1917
46 Callaway, E. (2014) Hepatitis C drugs not reaching poor. *Nature* 508, 295–296
47 Hu, Z. *et al.* (2014) Novel cell-based hepatitis C virus infection assay for quantitative high-throughput screening of anti-hepatitis C virus compounds. *Antimicrob. Agents Chemother.* 58, 995–1004
48 He, S. *et al.* (2015) Repurposing of the antihistamine chlorcyclizine and related compounds for treatment of hepatitis C virus infection. *Sci. Transl. Med.* 7 282ra249
49 Koh, C. *et al.* (2019) A randomized, proof-of-concept clinical trial on repurposing chlorcyclizine for the treatment of chronic hepatitis C. *Antiviral Res.* 163, 149–155
50 Rolt, A. *et al.* (2018) Preclinical pharmacological development of chlorcyclizine derivatives for the treatment of hepatitis C virus infection. *J. Infect. Dis.* 217, 1761–1769
51 Heymann, D.L. *et al.* (2016) Zika virus and microcephaly: why is this situation a PHEIC? *Lancet* 387, 719–721
52 Abbink, P. *et al.* (2016) Protective efficacy of multiple vaccine platforms against Zika virus challenge in rhesus monkeys. *Science* 353, 1129–1132
53 Xu, M. *et al.* (2016) Identification of small-molecule inhibitors of Zika virus infection and induced neural cell death via a drug repurposing screen. *Nat. Med.* 22, 1101–1107
54 Team, W.H.O.E.R *et al.* (2014) Ebola virus disease in West Africathe first 9 months of the epidemic and forward projections. *N. Engl. J. Med.* 371, 1481–1495
55 Kanapathipillai, R. *et al.* (2014) Ebola vaccinean urgent international priority. *N. Engl. J. Med.* 371, 2249–2251
56 Qiu, X. *et al.* (2014) Reversion of advanced Ebola virus disease in nonhuman primates with ZMapp. *Nature* 514, 47–53
57 Kouznetsova, J. *et al.* (2014) Identification of 53 compounds that block Ebola virus-like particle entry via a repurposing screen of approved drugs. *Emerg. Microbes Infect.* 3, e84
58 Sun, W. *et al.* (2017) Synergistic drug combination effectively blocks Ebola virus infection. *Antiviral Res.* 137, 165–172
59 Lee, J.A. *et al.* (2015) Novel phenotypic outcomes identified for a public collection of approved drugs from a publicly accessible panel of assays. *PLoS One* 10, e0130796
60 Attene-Ramos, M.S. *et al.* (2013) The Tox21 robotic platform for the assessment of environmental chemicals from vision to reality. *Drug Discov. Today* 18, 716–723
61 Huang, R. (2016) A quantitative high-throughput screening data analysis pipeline for activity profiling. *Methods Mol. Biol.* 1473, 111–122
62 Huang, R. *et al.* (2016) Modelling the Tox21 10 K chemical profiles for in vivo toxicity prediction and mechanism characterization. *Nat. Commun.* 7, 10425
63 Huang, R. *et al.* (2018) Expanding biological space coverage enhances the prediction of drug adverse effects in human using in vitro activity profiles. *Sci. Rep.* 8, 3783
64 Pushpakom, S. *et al.* (2019) Drug repurposing: progress, challenges and recommendations. *Nat. Rev. Drug Discov.* 18, 41–58
65 Corsello, S.M. *et al.* (2017) The Drug Repurposing Hub: a next-generation drug library and information resource. *Nat. Med.* 23, 405–408
66 NCATS. Inxight: Drugs. https://drugs.ncats.io/ [Accessed 24 September 2019].
67 Vazquez-Ortiz, G. *et al.* (2014) Drug repurposing screen identifies lestaurtinib amplifies the ability of the poly (ADP-ribose) polymerase 1 inhibitor AG14361 to kill breast cancer associated gene-1 mutant and wild type breast cancer cells. *Breast Cancer Res.* 16, R67
68 Jang, S.W. *et al.* (2012) Identification of drug modulators targeting gene-dosage disease CMT1A. *ACS Chem. Bio.* 7, 1205–1213
69 Trucco, M.M. *et al.* (2013) A novel chordoma xenograft allows in vivo drug testing and reveals the importance of NF-kappaB signaling in chordoma biology. *PLoS One* 8, e79950
70 Xia, M. *et al.* (2013) Identification of repurposed small molecule drugs for chordoma therapy. *Cancer Biol. Ther.* 14, 638–647
71 Tschapalda, K. *et al.* (2016) A class of diacylglycerol acyltransferase 1 inhibitors identified by a combination of phenotypic high-throughput screening, genomics, and genetics. *EBioMedicine* 8, 49–59
72 Sun, W. *et al.* (2013) Rapid identification of antifungal compounds against *Exserohilum rostratum* using high throughput drug repurposing screens. *PLoS One* 8, e70506
73 Kumari, D. *et al.* (2015) High-throughput screening to identify compounds that increase fragile X mental retardation protein expression in neural stem cells differentiated from fragile X syndrome patient-derived induced pluripotent stem cells. *Stem Cells Transl. Med.* 4, 800–808
74 Chen, C.Z. *et al.* (2012) A homogenous luminescence assay reveals novel inhibitors for giardia lamblia carbamate kinase. *Curr. Chem. Genomics* 6, 93–102
75 Thorne, N. *et al.* (2016) High-throughput phenotypic screening of human astrocytes to identify compounds that protect against oxidative stress. *Stem Cells Transl. Med.* 5, 613–627
76 Hamilton, D.H. *et al.* (2016) Targeting estrogen receptor signaling with fulvestrant enhances immune and chemotherapy-mediated cytotoxicity of human lung cancer. *Clin. Cancer Res.* 22, 6204–6216
77 Giubellino, A. *et al.* (2014) High-throughput screening for the identification of new therapeutic options for metastatic pheochromocytoma and paraganglioma. *PLoS One* 9, e90458
78 Sun, W. *et al.* (2016) Rapid antimicrobial susceptibility test for identification of new therapeutics and drug combinations against multidrug-resistant bacteria. *Emerg. Microbes Infect.* 5, e116
79 Najm, F.J. *et al.* (2015) Drug-based modulation of endogenous stem cells promotes functional remyelination in vivo. *Nature* 522, 216–220
80 Wang, L. *et al.* (2016) A high-throughput assay for developing inhibitors of PhoP, a virulence factor of *Mycobacterium tuberculosis*. *Comb. Chem. High Throughput Screen.* 19, 855–864
81 Ketley, A. *et al.* (2014) High-content screening identifies small molecules that remove nuclear foci, affect MBNL distribution and CELF1 protein levels via a PKC-independent pathway in myotonic dystrophy cell lines. *Hum. Mol. Genet.* 23, 1551–1562
82 Bernstock, J.D. *et al.* (2016) A novel quantitative high-throughput screen identifies drugs that both activate SUMO conjugation via the inhibition of microRNAs 182 and 183 and facilitate neuroprotection in a model of oxygen and glucose deprivation. *J. Cereb. Blood Flow Metab.* 36, 426–441
83 Dai, S. *et al.* (2017) Methyl-beta-cyclodextrin restores impaired autophagy flux in Niemann-Pick C1-deficient cells through activation of AMPK. *Autophagy* 13, 1435–1451
84 Keswani, R.K. *et al.* (2016) Repositioning clofazimine as a macrophage-targeting photoacoustic contrast agent. *Sci. Rep.* 6, 23528
85 Wang, M. *et al.* (2016) High throughput cell-based assay for identification of glycolate oxidase inhibitors as a potential treatment for Primary Hyperoxaluria Type 1. *Sci. Rep.* 6, 34060