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High-content drug screening for rare diseases

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Abstract Per definition, rare diseases affect only a small number of subjects within a given population. Taken together however, they represent a considerable medical burden, which remains poorly addressed in terms of treatment. Compared to other diseases, obstacles to the development of therapies for rare diseases include less extensive physiopathology knowledge, limited number of patients to test treatments, and poor commercial interest from the industry. Recently, advances in high-throughput and high-content screening (HTS and HCS) have been fostered by the development of specific routines that use robot- and computer-assisted technologies to automatize tasks, allowing screening of a large number of compounds in a short period of time, using experimental model of diseases. These approaches are particularly relevant for drug repositioning in rare disease, which restricts the search to compounds that have already been tested in humans, thereby reducing the need for extensive preclinical tests. In the future, these same tools, combined with computational modeling and artificial neural network analyses, may also be used to predict individual clinical responses to drugs in a personalized medicine approach.

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Introduction: the challenge of rare diseases

Diseases are defined as being rare in the EU if they affects less than 1:2000 persons (Regulation (EC) N°141/2000 of the European Parliament; European Council on orphan medicinal products 16-12-1999). In the US, the Orphan Drug Act of 1983 defines a rare disease or disorder if it affects fewer than 200,000 Americans at any given time (Congressional Findings for The Orphan Drug). Taken together, these definitions imply that as many as 30 million Europeans and 25 million Americans may be affected by a rare condition. To date, more than 6000 rare diseases have been characterized, while new diseases continue to be discovered (Orphadata: Free access data from Orphanet; Orphanet: an online rare disease and orphan drug database. © INSERM 1997).

Despite remarkable advances in the diagnosis and understanding of many rare diseases, most of these disorders continue to represent an important challenge for the development of specific treatments. Assembling enough patients to test new therapies is extremely difficult. Cohorts are usually underpowered and require lowering the normal standards for clinical trials. Moreover, the clinical picture is often inhomogeneous, in particular for diseases that are highly influenced by the genetic background or by environmental factors. When diseases involve children, specific ethical limitations also apply. Finally, the rarity of these conditions reduces the interest of the industry, which often cannot justify embarking in highly expensive and time-consuming investments with limited prospect for financial return. It is currently estimated that it takes on average 14 years to proceed from the discovery of a new molecule to its commercial approval,

and that failure rates along this process exceed 95% (Paul et al 2010). According to the Tufts Center for the Study of Drug Development, for each new drug that is brought to the market, the average cost is currently estimated at 2558 million US$. Most of these costs, as well as the majority of test failures, are related to human tests (DiMasi et al 2016).

To overcome these limitations, several legislative actions have been taken over the past decades in western countries, but research and development in this field remains insufficient. One possible way to limit cost is represented by drug repositioning, where a given molecule that has already been used in human subjects, can be employed to treat a different disease.

Strategies for identifying candidate drugs

Drug discovery strategies can be based on mechanisms of action or phenotypic changes (Fig. 1) (Sams-Dodd 2006).

The mechanism-based approach, also known as target-based screening, was, until recently, the prevalent model used by the pharmaceutical industry and by academic research centers for drug discoveries. In this approach, molecular targets, such as abnormal protein functions or abnormal metabolic pathways, are identified by basic research studies. Robust biochemical assays are then developed in order to perform high-throughput screening (HTS) of chemical libraries.

Approaches based on the phenotype requires identifying a functional defect that characterizes a specific disease. Compounds are then screened in cell or animal models for their ability to correct the abnormal phenotype (Zheng et al 2013).

In phenotypic cell-based screenings, the assay is performed on a support that is suitable for high-content library screening (HCS), to identify positive hits that correct abnormal cell or tissue phenotypes.

In phenotypic animal-based screenings, tests are conducted on small animal models, such as *Caenorhabditis elegans, Drosophila melanogaster,* or Zebrafish. These models are closer to a mammalian organism, but are expensive, time consuming, and are therefore less suitable for screening large libraries of compounds. In addition, this approach provides limited information on the mechanisms of action of individual compounds and the observed effects may be related to pharmacokinetics profiles that are species-specific and may not be transposable to human subjects. The animal-based approach may be advantageously used to perform secondary screenings of a restricted number of compounds that have been identified by primary screenings performed using target- or phenotypic cell-based screenings.

From a cost-benefit standpoint, many of these strategies have an unfavorable profile when using very large collections of molecules. In general, the cost of assays is inversely proportional to the number of compounds being tested. The estimate of costs is usually in the range of $1 per compound in the primary screening, $2000–4000 per compound for subsequent validation steps and $20,000–40,000 per compound for in vivo demonstration of proof-of-concept (Bittker and Ross 2016). Overall, the process of de novo drug discovery from the initial HTS or HCS to the final marketing of a compound requires 10–17 years (Ashburn and Thor 2004). HCS on the other hand, allow multiplex measurements in vitro, such as drug uptake, efficacy, specificity and toxicity, which can be reasonably predictive of the outcome of pre-clinical and clinical tests (O'Brien 2014; Olson et al 2000). To limit cost and decrease time, academic and industry-sponsored researches increasingly tend to use more efficient chemical libraries that are smaller, but enriched with high drug-likeness compounds. In the SOSA (Selective Optimization of Side Activities) approach, a limited number of molecules that are structurally and therapeutically diverse are included in a library to be tested in cell-based phenotypic assays. These compounds have already been tested for their safety and bioavailability and most correspond to drugs that are commercially available for human use. Positive hits represent candidates for drug-repositioning approaches, or starting points for the discovery of new drugs, based on their known structure and mechanisms of action (Wermuth 2004). Compared to de novo drug discovery, drug repositioning allows shortening the timeline for marketing a given compound to 3–12 years (Ashburn and Thor 2004).

The remaining of this review will be focused on HCS strategies.

Overview of high content screening

Cell-based HCS usually use automated microscopy in order to extract from thousands of samples multidimensional information, such as cell morphology, fluorescence intensity or distribution of fluorescent markers within cells. Results usually have very high statistical power, due to the high number of signals that can be analyzed at very high speed, allowing averaging a large number of data. The major advantage however, is represented by the possibility to multiplex different assays simultaneously in integrated cell populations, cell subpopulations, or individual cells within a given population (Thomas 2010). Thus, HCS shares with HTS the speed of analysis, with flow-cytometry the capacity of analyzing single cells, and with standard microscopy the ability to analyze cell details (Zanella et al 2010; Peravali et al 2011). For these reasons, HCS is increasingly used in several steps of the drug discovery pipeline, including identification of targets, assay validation, primary screening, and secondary screening. HCS has also gained popularity in lack-of-function or gain-of-function studies, applying RNA interference or cDNA overexpression technologies. These latter approaches can be particularly interesting for genetic diseases, for which specific phenotypes can be obtained by altering gene expression in wild-type cells.

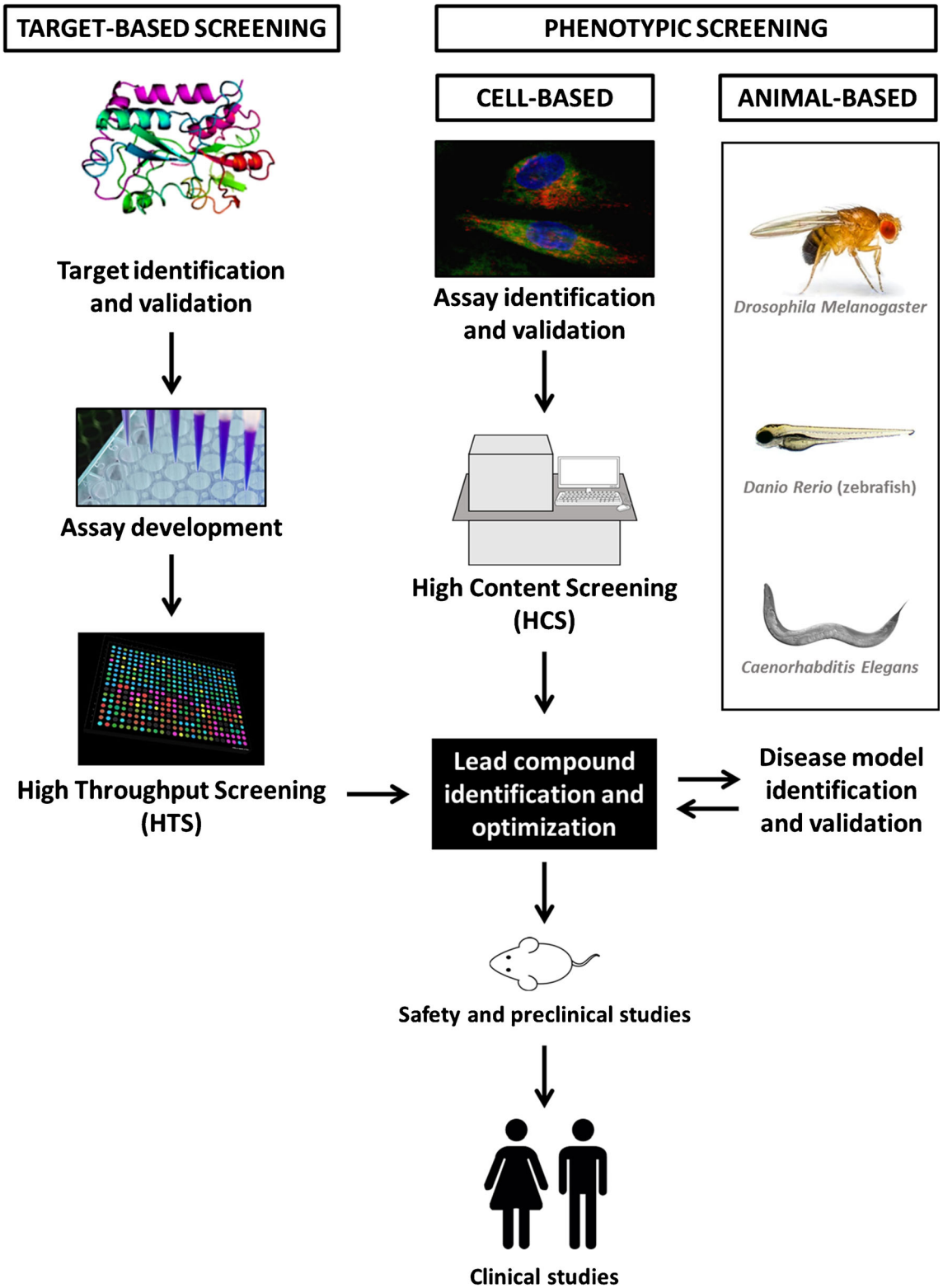

Fig. 1 Main approaches for identifying new drugs in research and development processes

Among 22 drugs that have been approved in 2016 by the U.S. Food & Drug Administration, six have been identified by HCS (https://www.fda.gov/Drugs/DevelopmentApprovalProcess/DrugInnovation/ucm534863.htm). Several limitations however, apply to HCS (Buchser et al 2004). Image acquisition and data analysis can be time consuming and requires high (sometimes

"very high") storage capacities. Limiting factors include the number of plates to be analyzed, the number of samples per plates (up to 1536), the number of fluorescent channels, light intensity (inversely correlates with the exposure time), microscope field magnification (inversely correlates with the number of fields to be acquired), and image definition. Even when all these aspects have been optimized, HCS testing thousands of compounds may last several weeks or months. The assay performance, including quality of reagents and stability of cell phenotype, should be systematically checked at planned time-points throughout the procedure to insure reproducibility.

Unquestionably, cell phenotype represents the most critical element. In the majority of cases, immortalized cell lines are preferred to primary cell cultures and to complex models, such as 3D cultures or co-cultures, because they are easier to expand and to grow on standard imaging surfaces. Their phenotypes, although frequently less differentiated, are often more stable, and these cells are easier to transfect, transduce, label or stain. The choice of the cell model is always a compromise between the need for a simple test and the requirement of high discrimination power between positive and negative hits. Since the analysis usually relies on fluorescence values, obtaining high quality fluorescence images is fundamental in most cases.

Automated microscopy and computer-assisted image analysis

Microscopy-based HCS require using automated systems that perform unbiased and fast image acquisition and storage. Several instruments have been developed and marketed in recent years (listed in Supplementary Table 1). All systems are equipped with fluorescent light sources (lamp, laser or LED), high definition cameras (cMOS or CCD) and large sets of optical objectives, to adapt to most experimental needs. X-Y transitional mechanical stages are computer-controlled. Live acquisition can be performed with instruments that are equipped with appropriate temperature, CO_2, and humidity controllers. The most critical aspect of automated image acquisition however, is the need for an autofocus system, which can use a laser beam or a specific software. Laser-based systems are fast but required very flat imaging plates, whereas software-based systems avoid this problem by using algorithms that continuously define the optimal focal plane, but are slow and may be inaccurate in the presence of large variations in cell morphology.

Images are analyzed with a dedicated software that is often provided with the instrument, or that has been developed by a third party. Each image is automatically processed within a pipeline that is adapted to each experimental setting (Supplementary Table 2). Typically, image processing starts with general adjustments (flat-field and background correction), followed by detection of primary (usually nuclei) and secondary objects (usually cell cytoplasm), to identify cells. These steps are mostly based on intensity thresholds and segmentation algorithms. Labeled organelles, proteins or cell regions can then be identified. More sophisticated analyses, such as detection of spatial distributions or identification of specific patterns, can also be performed.

Results are expressed as "positive" or "negative" hits or by comparison with control values. Alternatively, cells can be clustered into subpopulations, which can be useful, for example, to analyze co-cultures, cell differentiation, or to partition cells into different phenotypes. In these cases, the read-out corresponds to the percentage of cells showing a specific pattern. As needed, more than one parameter can be used to characterize a specific phenotype.

Highly sophisticated image analyses are usually performed with built-in protocols or with custom-made accessory modules (Supplementary Table 1). Most laboratories that are specialized in HCS have developed in house image analysis tools to increase their processing speed and versatility (Supplementary Table 2). When data sets are very large, additional software for metadata recording, normalization, visualization and plotting, as well as for quality control (QC) assessment, are often used (see Supplementary Table 3).

Additional tools that are routinely used to improve quality and speed include barcoding, automated cell seeders, semi-automated multichannel pipettes, liquid handling robots, and automated plate washers.

Quality control strategies and hit selection

Each automation step requires stringent QC assessment, which usually relies on comparisons between positive and negative controls (Zhang 2011; Bray et al 2004).

In theory, strong positive controls should be preferred because they allow better defining the dynamic range of a given assay. In practice however, positive controls should have a similar intensity to the expected intensity of positive hits. The position of controls within a multi-well plate should be random to avoid spatial biases, such as the plate edge effect. Signal-to-noise ratios (i.e., the ratio between mean positive and negative values) and signal-to-background ratios (i.e., the difference between positive and negative control values divided by the standard deviation of negative values) are sometimes used to define the dynamic range. However, the most widely used assay to measure the effect size in HCS is the Z'-factor (not to be confused with Z-score).

$$Z' \text{ factor} = 1 - \frac{3 \cdot (\sigma_{POS} + \sigma_{NEG})}{|\mu_{POS} - \mu_{NEG}|}$$

(*where μ is the mean and σ is the standard deviation of positive and negative controls*).

This parameter can be estimated using sample means and standard deviations. It allows judging if the response is large enough to be suitable for screening. In other terms, it measures the degree of separation between the distributions of positive and negative controls. A Z'-factor value between 0.5 and 0.8 indicates an optimal assay, a value lower than 0.5 indicates a poor assay, while values above 0.8 indicate assays that are too stringent and may miss valuable positive hits.

Usually, QC assessment for HCS starts with the analysis of data distribution to identify artifacts, such as intra- and inter-plate drifts. Ideally, control samples across all plates should have the same value. Often however, post-analysis normalization corrections need to be applied to compensate for spatial and illumination drifts, and for other systematic errors (Bray et al 2004; Ye et al 2012).

Hit selection depends on the strength of the assay. False negative hits can be avoided by increasing the number of replicates. False positive hits do not generally represent an important problem, unless they are very numerous, because they are discarded during secondary tests. Usually, hit selection is performed after expressing results as Z-scores or as "strictly standardized mean differences" (SSMD), for less strong phenotypes. Arbitrary thresholds are most often chosen, based on the effect size and on the number of hits above the chosen threshold. Supervised and unsupervised algorithms that dynamically identify clusters of molecules with similar effects can be used for analyzing very large libraries of molecules (Collins 2009).

Target deconvolution

A secondary step of phenotypic screening is represented by the identification of the target molecules of positive hits, a process termed "target deconvolution". This process allows understanding the mechanisms of action of molecules and of biological processes underlying specific conditions and often represent starting points to develop further drug discovery assays. Target deconvolution often requires using multiple approaches, including proteomic, genomic, and bioinformatics technologies (reviewed in Lee and Bogyo 2013, Jung and Kwon 2015).

Proteomic-based approaches include techniques such as affinity chromatography and activity-based profiling that are used to isolate molecular targets, which are then identified by MS/MS. Label-free techniques allow target identification based on changes in the thermodynamic stability of the protein-drug complex under specific conditions; unlike other proteomic approaches, this technique does not require chemical modification of molecular targets (Banfi et al 2017).

In the genomic approach, a cDNA library is expressed in an expression system (phages, in vitro translation systems, yeast, for example); proteins are then exposed to small molecules and affinity selected (Nijman 2015).

The identification of molecular drug targets is also facilitated by numerous online tools for predicting molecular interactions.

HCS for drug discovery and drug repositioning in rare diseases

While many treatments are available for common diseases, thousands of disorders affecting a small proportion of the population lack efficient therapies. Because rare diseases are poorly attractive to the pharmaceutical industry, a potential approach to shorten the timeline for drug discovery and reduce costs is to find new indications for existing drugs. This strategy is defined as "drug repositioning" or "drug repurposing", and takes advantage of the known activities of many approved drugs for human use, which are not restricted to unique biological targets, often resulting in side effects.

Until recently, drug repositioning has been mainly proposed on the bases of accidental observations, known side effects, or mechanisms of action of individual compounds. A classic example is represented by thalidomide, which was originally used as a sedative to treat insomnia, anxiety and nausea, in particular in pregnant women, and was banned after discovering that it caused phocomelia. After understanding the modes of action (Kim and Scialli 2011), this compound has been reevaluated and is currently used to treat multiple myeloma, other forms of tumor, *erythema nodosum* of leprosy, and other conditions that are characterized by granulomatous lesions. Similarly, sildenafil was developed to treat *angina pectoris*, and has thereafter been used to treat male erectile dysfunction and pulmonary arterial hypertension, based on the known vasodilatory properties (Sardana et al 2011; Ashburn and Thor 2004). Another example is represented by minoxidil, a per os antihypertensive drug that was found to increase hair growth, as a side effect. Based on this observation the molecule has been reformulated as a topical solution to teat androgenic alopecia (Rossi et al 2016).

Recently however, the development of HCS has fueled a new wave of investigations to attempt drug discover and drug repositioning in unbiased experimental settings. Herein, we briefly summarize some examples that were selected to illustrate the versatility and potentialities of these approaches in rare diseases.

A. Fibrodysplasia ossificans progressiva (FOP) is a severely disabling heritable disorder of the connective tissue that has a prevalence of approximately 1:2,000,000. It is caused by activating mutations of the activin A receptor type 1 (*ACVR1*) gene that encodes for the type I receptor for bone morphogenetic proteins (BMPs). Currently, no

treatment is available to halt the uncontrolled ossification process. Cappato et al have used a library of 1280 FDA-approved compounds to identify molecules that inhibit the *ACVR1* gene expression. They developed a cell model that stably expressed the luciferase gene under the control of the *ACVR1* promoter. The screening has identified dipyridamole, a well-known antithrombotic and vasodilator compound, as a potent inhibitor of *ACVR1* gene expression and of the downstream pathways that are up-regulated in patients with FOP. The drug has in humans a favorable safety profile and initial experimental data in mice show that it regulates chondrogenesis and osteogenesis in BMP-induced progressive heterotopic ossification (Cappato et al 2016).

B. Huntington disease is a rare (prevalence estimated at 1–9:100,000) neurodegenerative disorder of the central nervous system, characterized by uncontrolled choreatic movements. Schulte et al have performed HCS using primary neuronal cultures derived from a *Drosophila melanogaster* model of Huntington disease. Specifically, they have tested RNA interference and small molecules libraries for their ability to reduce the formation of "Hungtington aggregates" and to restore normal neuronal morphology. This lead them to identify Lkb1 kinase, an upstream kinase in the mTOR/insulin pathway, as a potential therapeutic target for HTS, and four compounds, namely 18β-Glycyrrhetinic acid, camptothecin, OH-camptothecin and carbenoxolone, that can suppress neurotoxicity in mutant flies and halt disease progression. Their HCS relied heavily on the possibility to perform live imaging, preventing the formation of artifacts that dramatically reduced the dynamic range of their assay when using immunofluorescence images of fixed cells (Schulte et al 2011).

C. Von Hippel-Lindau disease (VHL) is a familial condition predisposing to a variety of neoplasms. The estimated prevalence is 1:53,000 and the disease is caused by highly penetrant mutations in the von Hippel-Lindau tumor suppressor (*VHL*) gene. Cautain et al have used a collection of biologically active microbial natural products to identify compounds that exhibit selective cytotoxicity toward VHL-deficient cells using human renal carcinoma cell lines. The assay was set-up on a robotic workstation to multiplex several biological processes simultaneously: carboxyfluorescein succinimidyl ester was used to monitor cell cycle, thiazolyl blue tetrazolium blue was used to assess cell proliferation, and annexin V coupled with propidium iodide was used to detect early and late apoptosis. This allowed identifying a group of fungal extracts that selectively kill *VHL* deficient cells, providing informative data on the pathogenesis of the disease and allowing the identification of lipodepsipeptide MDN-0066 as a potential new treatment (Cautain et al 2015).

D. Androgen insensitivity syndrome is a rare disorder of sex development with unknown prevalence that is caused by mutations in the gene encoding for the androgen receptor (*AR*), a transcription factor involved in male sexual differentiation and reproductive viability. Using a HCS platform, Szafran et al were able to multiplex AR expression, its subcellular trafficking, reporter gene activity, cell cycle and mitotic index at the single cell level, and to test the effects of several mutations using cells derived from different patients (Szafran et al 2009). This allowed predicting clinical responses to different experimental treatments.

E. Cystic fibrosis (CF) is one of the most common genetic disorder in Caucasian children that is characterized by altered chloride transport and increased viscosity of body secretions. The incidence in Europe is approximately 1:10,000 individuals. In preparation for a HCS, Almaça et al have investigated genes encoding for proteins that are potentially involved in the regulation of transepithelial sodium reabsorption that is mediated by the ENaC epithelial sodium channel. The HCS platform was used to perform siRNA-based assays, which led them to identify ciliary neurotrophic factor receptor (*CNTFR*) and of diacylglycerol kinase iota (*DGKι*) as key regulators of *ENaC* activity and potential new targets to develop therapies for CF (Almaca et al 2013).

Summary

Most rare diseases are serious disorders with unmet medical needs. Promoting rapid development of new therapies has become a priority, in particular for diseases without available treatments. This need is increasingly recognized by national and international agencies that have developed plans to promote public-private partnerships. High failure rates of de novo drug development strategies, and low prospects for financial return, represent major obstacles for the development of drugs for rare diseases.

In recent years, the digital revolution has allowed developing new tools to perform HTS and HCS through the automatization of tasks that previously had to be handled manually. This in turn has fueled the development by academic institutions and commercial companies of small molecule libraries to be screened using these new technologies. These approaches may be very useful for the development of treatments for rare diseases based on drug repositioning strategies, which substantially reduce costs related to the development of new therapies.

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Compliance with ethical standards

Conflict of interest F. Bellomo, D. L. Medina, E. De Leo, A. Panarella, and F. Emma declare that they have no conflict of interest.

Informed consent and animal rights This article does not contain any studies with human or animal subjects performed by the any of the authors.

References

Almaca J, Faria D, Sousa M et al (2013) High-content siRNA screen reveals global ENaC regulators and potential cystic fibrosis therapy targets. Cell 154:1390–1400

Ashburn TT, Thor KB (2004) Drug repositioning: identifying and developing new uses for existing drugs. Nat Rev Drug Discov 3:673–683

Banfi C, Baetta R, Gianazza E, Tremoli E (2017) Technological advances and proteomic applications in drug discovery and target deconvolution: identification of the pleiotropic effects of statins. Drug Discov Today doi:10.1016/j.drudis.2017.03.001

Bittker JA, Ross NT (2016) High throughput screening methods: evolution and refinement. Royal Society of Chemistry, UK

Bray MA, Carpenter A, Imaging Platform, Broad Institute of MIT and Harvard (2004) Advanced assay development guidelines for image-based high content screening and analysis. In Sittampalam GS, Coussens NP, Brimacombe K, et al, eds. Assay guidance manual. Bethesda (MD): Eli Lilly & Company and the National Center for Advancing Translational Sciences.

Buchser W, Collins M, Garyantes T, et al (2004) Assay development guidelines for image-based high content screening, high content analysis and high content imaging. In Sittampalam GS, Coussens NP, Brimacombe K, et al, eds. Assay guidance manual. Eli Lilly & Co and the National Center for Advancing Translational Sciences, Bethesda

Cappato S, Tonachini L, Giacopelli F et al (2016) High-throughput screening for modulators of ACVR1 transcription: discovery of potential therapeutics for fibrodysplasia ossificans progressiva. Dis Model Mech 9:685–696

Cautain B, de Pedro N, Schulz C et al (2015) Identification of the Lipodepsipeptide MDN-0066, a novel inhibitor of VHL/HIF pathway produced by a new pseudomonas species. PLoS One 10: e0125221

Collins MA (2009) Generating 'omic knowledge': the role of informatics in high content screening. Comb Chem High Throughput Screen 12: 917–925

DiMasi JA, Grabowski HG, Hansen RW (2016) Innovation in the pharmaceutical industry: new estimates of R&D costs. J Health Econ 47: 20–33

Jung HJ, Kwon HJ (2015) Target deconvolution of bioactive small molecules: the heart of chemical biology and drug discovery. Arch Pharm Res 38:1627–1641

Kim JH, Scialli AR (2011) Thalidomide: the tragedy of birth defects and the effective treatment of disease. Toxicol Sci 122:1–6

Lee J, Bogyo M (2013) Target deconvolution techniques in modern phenotypic profiling. Curr Opin Chem Biol 17:118–126

Nijman SM (2015) Functional genomics to uncover drug mechanism of action. Nat Chem Biol 11:942–948

O'Brien PJ (2014) High-content analysis in toxicology: screening substances for human toxicity potential, elucidating subcellular mechanisms and in vivo use as translational safety biomarkers. Basic Clin Pharmacol Toxicol 115:4–17

Olson H, Betton G, Robinson D et al (2000) Concordance of the toxicity of pharmaceuticals in humans and in animals. Regul Toxicol Pharmacol 32:56–67

Paul SM, Mytelka DS, Dunwiddie CT, Persinger CC, Munos BH, Lindborg SR, Schacht AL (2010) How to improve R&D productivity: the pharmaceutical industry's grand challenge. Nat Rev Drug Discov 9:203–214

Peravali R, Gehrig J, Giselbrecht S, Lutjohann DS, Hadzhiev Y, Muller F, Liebel U (2011) Automated feature detection and imaging for high-resolution screening of zebrafish embryos. BioTechniques 50:319–324

Rossi A, Anzalone A, Fortuna MC, Caro G, Garelli V, Pranteda G, Carlesimo M (2016) Multi-therapies in androgenetic alopecia: review and clinical experiences. Dermatol Ther 29:424–432

Sams-Dodd F (2006) Drug discovery: selecting the optimal approach. Drug Discov Today 11:465–472

Sardana D, Zhu C, Zhang M, Gudivada RC, Yang L, Jegga AG (2011) Drug repositioning for orphan diseases. Brief Bioinform 12:346–356

Schulte J, Sepp KJ, Wu C, Hong P, Littleton JT (2011) High-content chemical and RNAi screens for suppressors of neurotoxicity in a Huntington's disease model. PLoS One 6:e23841

Szafran AT, Hartig S, Sun H et al (2009) Androgen receptor mutations associated with androgen insensitivity syndrome: a high content analysis approach leading to personalized medicine. PLoS One 4: e8179

Thomas N (2010) High-content screening: a decade of evolution. J Biomol Screen 15:1–9

Wermuth CG (2004) Selective optimization of side activities: another way for drug discovery. J Med Chem 47:1303–1314

Ye F, Bauer JA, Pietenpol JA, Shyr Y (2012) Analysis of high-throughput RNAi screening data in identifying genes mediating sensitivity to chemotherapeutic drugs: statistical approaches and perspectives. BMC Genomics 13(Suppl 8):S3. doi:10.1186/1471-2164-13-S8-S3

Zanella F, Lorens JB, Link W (2010) High content screening: seeing is believing. Trends Biotechnol 28:237–245

Zhang XD (2011) Illustration of SSMD, z score, SSMD*, z* score, and t statistic for hit selection in RNAi high-throughput screens. J Biomol Screen 16:775–785

Zheng W, Thorne N, McKew JC (2013) Phenotypic screens as a renewed approach for drug discovery. Drug Discov Today 18:1067–1073