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Filling the drug discovery gap: is high-content screening the missing link?

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In recent years, questions about the sustainability of the current drug discovery process have triggered a revival of interest in phenotypic drug discovery approaches. This trend has clearly been amplified by the emergence of multiple cell-based assay technologies enabling a higher degree of translatability between *in vitro* conditions and physio-pathological situations, including induced pluripotent stem cells, three-dimensional models, co-culture and organ-on-a-chip systems, complemented by advances in gene editing technologies. Progress in High-Content Screening technology has also contributed to the recent excitement for phenotypic drug discovery approaches, bringing image-capture and processing, and data-analysis, to a level of content and throughput fully compatible with large scale drug discovery efforts. Nevertheless, implementation of HCS in discovery projects must be carefully considered, to ensure optimal performance and the generation of relevant data to enable the discovery of first-in-class medicines.

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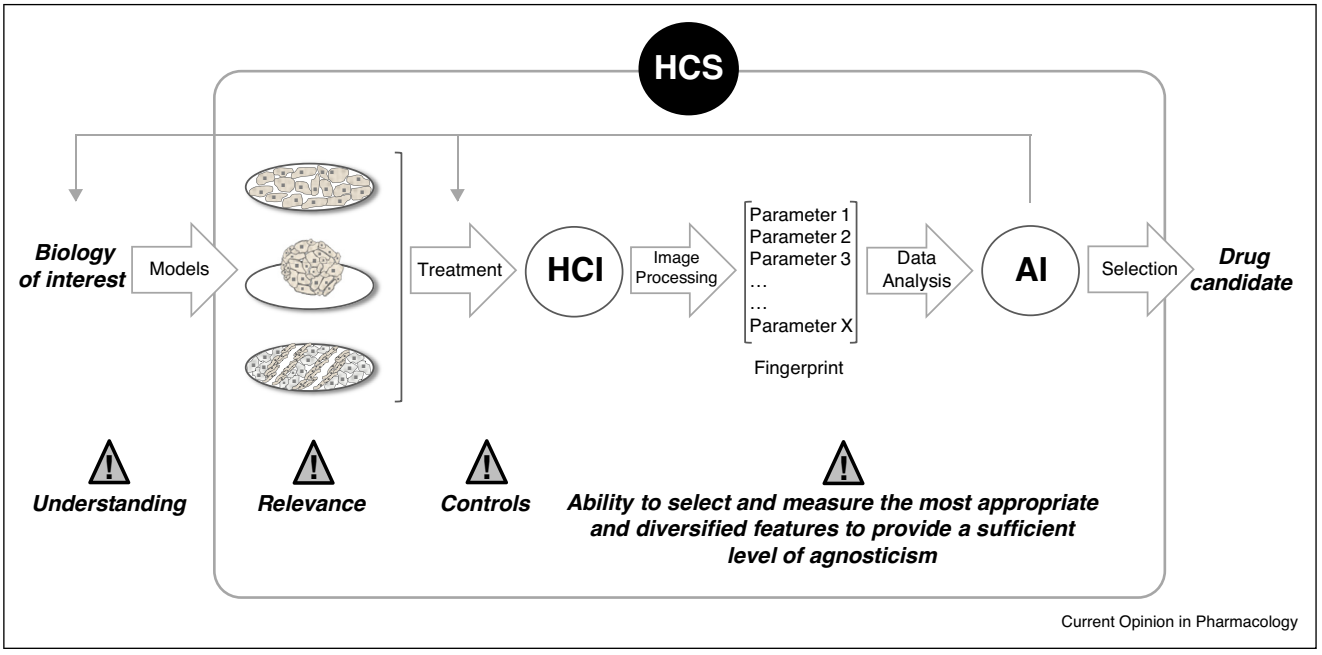
Developing new pharmaceutical drugs is well known as a high-risk, expensive, and long-term endeavor, but for almost two decades, the long-term profitability of the pharmaceutical industry and, more specifically, the sustainability of the current drug discovery model have been seriously questioned. Indeed, several analyses highlight the fact that, despite major investments in drug research and development, the pharmaceutical industry is unable to either reduce the attrition rate or to sustain sufficient innovation to replace revenue loss due to patent expiration [1–10]. Consequently, there has been a reliance on the ability of drug candidates to alter a disease phenotype of interest without any *a priori* [8,11], rather than focusing on their ability to modulate the activity of a specific target potentially involved in the disease [12]. In fact, phenotypic approaches have the potential to address the incompletely understood complexity of disease and deliver first-in-class drugs to the clinic [8,13], while target-based approaches, that have been widely used in the pharmaceutical industry in the past three decades, are perceived as being less productive [14] in terms of clinical relevance. Debates over phenotypic versus target-based approaches have concluded that successful drug discovery programs must rely on the complementarity of both strategies [15]. However, critical questions still remain on how best to deploy functional cellular assays considering the different biological and technical challenges intrinsically associated to phenotypic approaches [16••]. This short review aims to highlight how High-Content Screening (HCS) represents an attractive approach to successfully deploy such phenotypic drug discovery strategies, highlighting key benefits of high-content imaging and analysis over other multi-parametric data-generating technologies, defining the capabilities of HCS and describing current challenges to widely implement the technology to various projects (Figure 1). We will also discuss the mid-term to long-term evolutions of the technology and the expected synergy with the foreseen disruptive implementation of artificial intelligence technologies to drug discovery for first-in-class medicines.

Cell viability, cell signaling and transcription, and disease-related characteristics are the main phenotypic readouts commonly performed in lead discovery. Various assay modalities have been developed in each of these categories using a number of different technologies. For instance, cell proliferation and/or cell death have been the predominant phenotypic readout for many decades, using various types of assay, for example the Alamar Blue assay, the colorimetric MTT assay or the ATP content assay [17]. Although these types of readout are robust and informative, they clearly suffer from their lack of integration, providing only a single information readout per well. Intracellular signaling and transcription assays generally provide a simple link between a complex network of protein interactions and a specific transcription event of interest through pathway driven or active promoter reporter genes such as luciferase and beta-lactamase,

Figure 1



Key steps when implementing High-Content Screening (HCS) for target or drug discovery and critical points to consider for success.

fluorescent proteins like GFP and YFP, which produces an easily measurable luminescence or fluorescence signal [18]. Although reporter gene approaches are extensively used, they clearly suffer from a lack of integration as well as the possible risk of artifacts. However, recent advances in this field are now enabling molecular phenotyping [16••,19,20], providing the ability to run high-throughput transcriptome analysis as a primary screen. This type of approach relies on molecular phenotype gene signatures to deliver an accurate pathway-centric view of the biological system under study, hence enabling an unbiased evaluation of both the molecular state of the discovery model versus the disease state in humans, and the extent to which a given molecular treatment is beneficial or not. Many pathologies are associated to disease-specific cellular and/or functional phenotypic changes relative to healthy cells, for example morphological changes, invasion, migration, differentiation or epithelial-to-mesenchymal transition. Although, many single readout assay technologies were used to investigate these phenotypic changes, high-content screening approaches have clearly become the approach of choice to investigate these cellular and functional modifications [21–23].

This trend is exemplified by the intrinsic ability of HCS to combines automated fluorescence microscopy with quantitative image analysis, allowing the acquisition of unbiased multi-parametric data at the single cell level. Indeed, traditional cell-based High-Throughput Screening (HTS) focuses on a unique readout and measures a signal average within a well whether it is applied to target-based or phenotypic drug discovery. In contrast, HCS has the potential to combine cell viability, cell signaling and transcription, and/or phenotypic readouts in a single well across a large number of conditions [16••] in a target-based, phenotypic or combined target/phenotypic drug discovery mode. Therefore, in a single assay, HCS technology has the potential to predict and integrate key biological activities of compounds during the early stages of the drug discovery process.

Different technologies such as high-throughput flow cytometry [24], multiplex immunochemistry [25], imaging mass spectrometry [26], high-content imaging [27••] (HCI) can all provide high-content data, however, HCS provides the best overall performance in terms of throughput and the ability to perform quantitative phenotyping of integrated biological systems at the single cell level. For example, HCI/HCS is the only technology able to assess cellular morphology changes, such as neurite outgrowth [28], cardiomyocyte hypertrophy [29] or stem cell differentiation [30] whilst simultaneously monitoring disease-related and cellular differentiation-related phenotypes [31,32•]. To perform this type of investigation, various fluorescence probes [33••] can be combined, providing a powerful means of exploring complex phenotypes and quantifying cellular changes in response to treatment. Using these fluorescent tools, HCS assays can monitor the expression/redistribution of various cellular proteins including structural components, specific