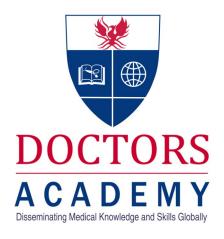
The Role of Cell-Based Imaging in Drug Discovery

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The Role of Cell-Based Imaging in Drug Discovery

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and this review will critically consider these roles. The imaging in drug discovery. Fluorescent probes label and process of drug development will be outlined followed by track 'targets' central to pathological processes; targets a discussion on how different types of cell-based imaging mainly include single genes or proteins. Targets can be assays have contributed to this.

discovered and starts with High Throughput Screening of molecular events in living cells. GFP has revolutionised (HTS), which is a scientific method that uses robotics, orphan receptor research; endogenous ligands have been data processing and control software, liquid handling identified by imaging GFP-tagged therapeutic proteins². devices, and sensitive detectors to rapidly perform GFP-tagged proteins have been used to determine the millions of pharmacological tests on compound libraries site and time course of receptor expression and to relate to identify ligands that modulate targeted pathways of receptor dynamics with therapeutic outcomes³. For disease processes. These ligands are termed'hits'. The hit example, automated imaging of fluorescent protein -to-lead phase is the follow-up of HTS and includes: hit reporters has facilitated the interrogation of the confirmation; hit expansion; and the lead optimisation Gonadotrophin Releasing Hormone Receptor (GnRHR) phase. Following on from this, pre-clinical studies occur signalling to the Raf/MEK/ERK and Ca²⁺ /calmodulin/ prior to entry into clinical trials.

Cellular imaging refers to the visual representation, treat hormone sensitive cancers of the prostate and characterisation, and quantification of cellular processes. breast⁴. Microscopy has contributed to the drug discovery pipeline by visualising the unfolding of pathological Direct and indirect immunofluorescence, which involves mechanisms and identifying targets for development. Novel innovations in microscopy have primary and secondary antibodies, has contributed to the enhanced experimental throughput by improving spatial selection, characterisation and target validation process resolution and tissue penetration and have overcome in drug discovery. To illustrate this principle direct and physical access issues. This has been achieved by: the indirect immunofluorescence has been development of super-resolution microscopes capable of characterise neurotransmitter release in multimeric resolving structures to below the diffraction limit of voltage-gated 200nm; incorporating multi-photon techniques into pharmacological implications for drug discovery in intravital and fibre-optic microscopy, which allow image disorders such as Alzheimer's disease, which are collection at greater tissue depths; and the automation of characterised by impaired neurotransmitter release from microscopy and image analysis for HTS¹.

Cell imaging has an important function in drug discovery The use of fluorescence is central to the role of cell based tagged with fluorescent proteins such as Green Fluorescent Protein (GFP), which auto-fluoresces without Drug discovery is the process by which drugs are substrates or co-factors and allows for real-time analysis calcineurin/NFAT cascades. This has contributed to the development of cetrorelix, a GnRHR antagonist used to

> drug the conjugation of fluorescently labelled proteins to (Kv1); K+ channels central Kv1 ion channels⁵.



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Fluorescence Transfer Resonant Energy the molecular proximity of two proteins⁶. Other similar are phosphorylated. high-throughput cell-imaging assays include: bimolecular fluorescence complementation; enzyme fragment Cell based imaging techniques have played a key role in complementation; and the yeast two-hybrid assay, which assessing the safety of drugs as part of the drug can detect protein-protein or protein-DNA interactions. Flow cytometry has contributed to the drug discovery the In vitro micronucleus assay, which detects pipeline. For example, flow cytometry has been used for micronuclei (damaged pieces of chromosomes), which ex vivo analysis of in vivo efficacy of chemotherapeutic serve as markers of drug-induced genotoxicity⁹. agents such as enzastaurin, a protein kinase C inhibitor, Pharmaceutical regulatory bodies require the application on intracellular phosphoprotein signalling in monocytes of tests that screen for genotoxicity prior to drug obtained from cancer patients. These results confirmed approval. the efficacy of enzastaurin by revealing reduced PKC activity following drug administration'.

imaging of miRNA's⁸. However, cell based reporter assays and cost-effective relative to current methods. are not ideal for drug discovery as they have a high false positive rate. Furthermore, luciferase reporter assays are In summary cell-based imaging assays have proven hydrolysed products of the test compound.

low-throughput methods, have facilitated either activating or inhibiting target GPCR's. They can also expectation.

(FRET) quantify second messenger responses. Further benefits microscopy is a HTS cell imaging technique based on the include generating data to: measure binding affinity by physicochemical property of an excited fluorophore saturation or competition analysis; determine doserapidly losing energy to a nearby molecule that is capable response relationships; and determine the potency and of absorbing it. Therefore, FRET is a powerful tool to efficacy of novel compounds. Other examples of low detect and locate protein interaction sites within live cells throughput cell based imaging techniques that have been and can be used to measure targeted events, such as a used in drug discovery include: conventional and confocal pharmacological intervention, which produces changes in microscopy; and western blotting to detect targets that

development process. This can be illustrated by use of

Despite the multitude of both high throughput and low throughput cell-based imaging assays currently available Cell-based reporter assays using luciferase have the future of cellular imaging in drug discovery may contributed to HTS and drug development by enabling reside with non-invasive imaging. For example, Raman the assessment of target transcriptional activity. For spectroscopy, which is a scattering technique that uses example miRNA's, which regulate gene expression, have vibrational information specific to chemical bonds and been linked to cancer and viral infections, identifying molecular symmetry, will inevitably expose novel miRNA'sas potential targets for drug discovery. HTS using approaches to non-invasively identify pharmacological luciferase reporter assays have facilitated cell-based targets whilst being equally or more accurate, predictive

unable to confirm whether the positive result is due to instrumental in drug discovery. Fine-tuning existing the test compound rather than the induction of assays coupled with the development of non-invasive alternative signalling pathways by the test compound or imaging techniques will enhance the signal to noise ratio of cell-based imaging assays down to genomic, transcriptomic and proteomic levels, with the aim of Cell imaging using radio-ligand binding assays, which are unravelling disease processes and identifying new the therapeutic targets. This will turn the hope of advancing identification of compounds capable of binding to and drug discovery into a more realistic and exciting

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