

# The Role of Cell-Based Imaging in Drug Discovery

**Dr. Jason Seewoodhary**

**July 2011**

**Volume 1**

**Issue 1**

**Doctors Academy Publications**

The World Journal of Medical Education and Research (WJMER) is the online publication of the Doctors Academy Group of Educational Establishments. Published on a quarterly basis, it's aim is to promote academia and research amongst all members of the multi-disciplinary healthcare team including doctors, dentists, scientists, and students of these specialties from all parts of the world. The principal objective of this journal is to encourage the aforementioned from developing countries in particular to publish their work. The journal intends to promote the healthy transfer of knowledge, opinions and expertise between those who have the benefit of cutting edge technology and those who need to innovate within their resource constraints. It is our hope that this will help to develop medical knowledge and to provide optimal clinical care in different settings all over the world. We envisage an incessant stream of information will flow along the channels that WJMER will create and that a surfeit of ideas will be gleaned from this process. We look forward to sharing these experiences with our readers in our subsequent editions. We are honoured to welcome you to WJMER.



**DOCTORS**  
**ACADEMY**  
Disseminating Medical Knowledge and Skills Globally



# WJMER

World Journal of Medical Education and Research

*An Official Publication of the Education and Research Division of Doctors Academy*



The Role of Cell-Based Imaging in Drug Discovery

Antigen Microarrays for Rapid Screening of Rheumatoid Arthritis and Other Autoimmune Diseases

Abstracts from the International Academic & Research Conference 2012

Osteoradionecrosis (ORN) of the jaw

**In this edition, these topics and more....**

Management of Major Trauma:  
A Malaysian Perspective

Assessment and Management of Head and  
Spinal Cord Injuries

Role of Cloud Computing in Global Healthcare  
Provision

## The Role of Cell-Based Imaging in Drug Discovery

**Dr. Jason Seewoodhary, B.Sc (Hons), MBBCh (Hons), MRCP (UK)**

Specialist Registrar in Diabetes Mellitus & Endocrinology / General Internal Medicine

University Hospital of Wales

Wales Training Programme, Wales, United Kingdom

Address for correspondence: [seewoodharyj@hotmail.com](mailto:seewoodharyj@hotmail.com)

### Keywords:

*Cell Based Imaging; Drug Discovery; High Throughput Screening; Fluorescence; Assays*

Cell imaging has an important function in drug discovery and this review will critically consider these roles. The process of drug development will be outlined followed by a discussion on how different types of cell-based imaging assays have contributed to this.

Drug discovery is the process by which drugs are discovered and starts with High Throughput Screening (HTS), which is a scientific method that uses robotics, data processing and control software, liquid handling devices, and sensitive detectors to rapidly perform millions of pharmacological tests on compound libraries to identify ligands that modulate targeted pathways of disease processes. These ligands are termed 'hits'. The hit-to-lead phase is the follow-up of HTS and includes: hit confirmation; hit expansion; and the lead optimisation phase. Following on from this, pre-clinical studies occur prior to entry into clinical trials.

Cellular imaging refers to the visual representation, characterisation, and quantification of cellular processes. Microscopy has contributed to the drug discovery pipeline by visualising the unfolding of pathological mechanisms and identifying targets for drug development. Novel innovations in microscopy have enhanced experimental throughput by improving spatial resolution and tissue penetration and have overcome physical access issues. This has been achieved by: the development of super-resolution microscopes capable of resolving structures to below the diffraction limit of 200nm; incorporating multi-photon techniques into intravital and fibre-optic microscopy, which allow image collection at greater tissue depths; and the automation of microscopy and image analysis for HTS<sup>1</sup>.

The use of fluorescence is central to the role of cell based imaging in drug discovery. Fluorescent probes label and track 'targets' central to pathological processes; targets mainly include single genes or proteins. Targets can be tagged with fluorescent proteins such as Green Fluorescent Protein (GFP), which auto-fluoresces without substrates or co-factors and allows for real-time analysis of molecular events in living cells. GFP has revolutionised orphan receptor research; endogenous ligands have been identified by imaging GFP-tagged therapeutic proteins<sup>2</sup>. GFP-tagged proteins have been used to determine the site and time course of receptor expression and to relate receptor dynamics with therapeutic outcomes<sup>3</sup>. For example, automated imaging of fluorescent protein reporters has facilitated the interrogation of the Gonadotrophin Releasing Hormone Receptor (GnRHR) signalling to the Raf/MEK/ERK and Ca<sup>2+</sup> /calmodulin/calcineurin/NFAT cascades. This has contributed to the development of cetrorelix, a GnRHR antagonist used to treat hormone sensitive cancers of the prostate and breast<sup>4</sup>.

Direct and indirect immunofluorescence, which involves the conjugation of fluorescently labelled proteins to primary and secondary antibodies, has contributed to the selection, characterisation and target validation process in drug discovery. To illustrate this principle direct and indirect immunofluorescence has been used to characterise neurotransmitter release in multimeric voltage-gated K<sup>+</sup> channels (Kv1); this has pharmacological implications for drug discovery in disorders such as Alzheimer's disease, which are characterised by impaired neurotransmitter release from central Kv1 ion channels<sup>5</sup>.

Fluorescence Resonant Energy Transfer (FRET) microscopy is a HTS cell imaging technique based on the physicochemical property of an excited fluorophore rapidly losing energy to a nearby molecule that is capable of absorbing it. Therefore, FRET is a powerful tool to detect and locate protein interaction sites within live cells and can be used to measure targeted events, such as a pharmacological intervention, which produces changes in the molecular proximity of two proteins<sup>6</sup>. Other similar high-throughput cell-imaging assays include: bimolecular fluorescence complementation; enzyme fragment complementation; and the yeast two-hybrid assay, which can detect protein-protein or protein-DNA interactions. Flow cytometry has contributed to the drug discovery pipeline. For example, flow cytometry has been used for ex vivo analysis of in vivo efficacy of chemotherapeutic agents such as enzastaurin, a protein kinase C inhibitor, on intracellular phosphoprotein signalling in monocytes obtained from cancer patients. These results confirmed the efficacy of enzastaurin by revealing reduced PKC activity following drug administration<sup>7</sup>.

Cell-based reporter assays using luciferase have contributed to HTS and drug development by enabling the assessment of target transcriptional activity. For example miRNA's, which regulate gene expression, have been linked to cancer and viral infections, identifying miRNA's as potential targets for drug discovery. HTS using luciferase reporter assays have facilitated cell-based imaging of miRNA's<sup>8</sup>. However, cell based reporter assays are not ideal for drug discovery as they have a high false positive rate. Furthermore, luciferase reporter assays are unable to confirm whether the positive result is due to the test compound rather than the induction of alternative signalling pathways by the test compound or hydrolysed products of the test compound.

Cell imaging using radio-ligand binding assays, which are low-throughput methods, have facilitated the identification of compounds capable of binding to and either activating or inhibiting target GPCR's. They can also

quantify second messenger responses. Further benefits include generating data to: measure binding affinity by saturation or competition analysis; determine dose-response relationships; and determine the potency and efficacy of novel compounds. Other examples of low throughput cell based imaging techniques that have been used in drug discovery include: conventional and confocal microscopy; and western blotting to detect targets that are phosphorylated.

Cell based imaging techniques have played a key role in assessing the safety of drugs as part of the drug development process. This can be illustrated by use of the In vitro micronucleus assay, which detects micronuclei (damaged pieces of chromosomes), which serve as markers of drug-induced genotoxicity<sup>9</sup>. Pharmaceutical regulatory bodies require the application of tests that screen for genotoxicity prior to drug approval.

Despite the multitude of both high throughput and low throughput cell-based imaging assays currently available the future of cellular imaging in drug discovery may reside with non-invasive imaging. For example, Raman spectroscopy, which is a scattering technique that uses vibrational information specific to chemical bonds and molecular symmetry, will inevitably expose novel approaches to non-invasively identify pharmacological targets whilst being equally or more accurate, predictive and cost-effective relative to current methods.

In summary cell-based imaging assays have proven instrumental in drug discovery. Fine-tuning existing assays coupled with the development of non-invasive 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 unravelling disease processes and identifying new therapeutic targets. This will turn the hope of advancing drug discovery into a more realistic and exciting expectation.

### References:

1. Starkuviene V, Pepperkok R. The potential of high-content high-throughput microscopy in drug discovery. *Br J Pharmacol.* 152 (2007) pp: 62-61.
2. Daly CJ, McGrath JC. Fluorescent ligands, antibodies, and proteins for the study of receptors. *Pharmacol. Ther.* 100 (2003) pp: 101-18.
3. Arun KH, Kaul CL *et al.* Green fluorescent proteins in receptor research: an emerging tool for drug discovery. *J Pharmacol Toxicol Methods.* 51 (2005) pp: 1-23.
4. Reissmann T, Schally AV *et al.*, 2000. The LHRH antagonist cetrorelix: a review. *Hum Reprod Update* 6 (2000) pp: 322-31.
5. Skaper SD. Ion channels on microglia: therapeutic targets for neuroprotection. *CNS Neurol Disord Drug Targets* 10 (2011) pp: 44-56.
6. Viladarga JD. Studying ligand efficacy at G protein-coupled receptors using FRET. *Methods Mol Biol.* 756 (2011) pp: 133-48.
7. Green LJ, Marder P, *et al.* Development and validation of a drug activity biomarker that shows target inhibition in cancer patients receiving enzastaurin, a novel protein kinase C-beta inhibitor. *Clin Cancer Res.* 12 (2006) pp: 3408-3415.
8. Connelly CM, Thomas M *et al.* High-Throughput Luciferase Reporter Assay for Small-Molecule Inhibitors of MicroRNA Function. *J Biomol Screen* (2012) doi: 10.1177/1087057112439606
9. Lang P, Yeow K *et al.* Cellular imaging in drug discovery. *Nature Reviews Drug Discovery* 5 (2006) pp: 343-356.







The World Journal of Medical Education & Research (WJMER) is the online publication of the Doctors Academy Group of Educational Establishments. It aims to promote academia and research amongst all members of the multi-disciplinary healthcare team including doctors, dentists, scientists, and students of these specialties from all parts of the world. The journal intends to encourage the healthy transfer of knowledge, opinions and expertise between those who have the benefit of cutting-edge technology and those who need to innovate within their resource constraints. It is our hope that this interaction will help develop medical knowledge & enhance the possibility of providing optimal clinical care in different settings all over the world.



**WJMER**  
World Journal of Medical Education and Research  
*An Official Publication of the Education and Research Division of Doctors Academy*