3 day practical workshop

Cell based assays for screening

11, 12 & 13 June 2013

European ScreeningPort, Hamburg, Germany

European Pharmaceutical Review and European ScreeningPort are pleased to present a unique practical workshop covering the development of cell based assays for screening.

Course overview

Recent years have witnessed an expansion in the disciplines encompassing drug discovery outside the pharmaceutical industry. This is most notable with a significant number of Universities worldwide now that host infrastructure such as compound libraries and automated screening centres [1-3]. An archetypal small molecule drug discovery project will aim to identify chemical starting points that modify the functions of genes, cells, or biochemical pathways. In some but not all instances, these functions may be linked to disease processes, and an opportunity will exist to further develop the chemical starting points into novel therapeutic agents. In small molecule drug discovery, the ultimate aim is to identify new therapeutics, an activity that for reasons of high risk and cost has historically been conducted within the commercial sectors [4].

The expansion of small molecule drug discovery outside the pharmaceutical industry has coincided with increasing numbers of exploratory molecular targets and mechanisms, both therapeutic and non-therapeutic in origin [5]. Screening using miniaturised microtitre plate formats remains the most widely utilised methodology for identifying novel chemical
starting points that are capable of modulating target function in a meaningful, biologically relevant manner [6]. The first practical steps in drug discovery include the selection of a target (followed by its cloning, expression and purification), development of an assay to monitor the activity of the target, and the synthesis and management of molecular libraries. The second practical steps include the use of the above in screening campaigns to identify Primary Hits, followed with their Validation. In the context of drug discovery projects that make use of biochemical assays with purified targets, the activities of selected Primary Hits would typically be further evaluated in biophysical assays such as surface plasmon resonance and isothermal titration calorimetry. This effort would be expected to lead to the identification of validated Hits with some of these selected for optimisation using multiple criteria including structure activity relationships, selectivity, physicochemical properties and liability [7,8]. The typical workflow described above was arrived at subsequent to the completion of the sequencing of the human genome where a wealth of new targets were identified and considered worthy of exploration for drug discovery purposes.

Despite the successes that have been reported in the literature [6] where the above approaches have led to the identification of potent and selective compounds, their activities often fail to translate in vivo and this may well be due, in part to the target based assays being non-physiological in nature e.g. the target protein being a truncate of the protein and the assay using a substrate that is non-physiological.

We are now witnessing a resurgence of cell based assays including phenotypic assays where a particular change is monitored, in some cases without knowledge of the underlying target/s upon which compounds are acting upon. Some of the successes using these approaches for drug discovery have been reviewed [9]. It is interesting to note that in some cases where efficacious compounds were identified, the target/s they act upon were identified subsequently. However, their efficacy may still be due to a poly-pharmacological effect that includes the effect of compounds upon additional targets that may still be unidentified. Advances in cell based assays are also being made, for example using human induced pluripotent stem (iPS) cell-derived cells that better recapitulate normal human biology compared to transformed cell lines and non-human primary cells.
The practical workshop – cell based assays for screening will be held between 11-13 June 2013 at the European ScreeningPort facility in Hamburg and will be part lecture based with a significant practical component.

Attendee profile

The practical workshop – cell based assays for screening is designed for scientists at all levels (undergraduates, postgraduates and laboratory based scientists within academic and industrial research organisations) engaged in early stage drug discovery and have an interest in the development, validation and utilisation of cell based assays for screening against small molecule libraries. The practical workshop – cell based assays for screening is equally well suited to technically focused staff from core facilities or contract research organisations who may wish to extend their expertise. The evening dinner on the first day will offer the opportunity for the participants to network and establish relationships that would be mutually beneficial.

Learning objectives

The main learning objectives of the practical workshop – cell based assays for screening will be to examine by way of practical sessions and lectures, the design and application of cell based assays for small molecule screening campaigns in drug discovery. All participants will take part in the practical sessions and these will involve the development of screening compatible cell based assays, Primary screening using a small molecule library, and Profiling of compounds in dose-response experiments. Participants in this workshop will discuss and demonstrate practically: (1) the appropriate steps in selecting suitable assays in light of the fact that a multitude of assay technologies are currently available for a given target; (2) how to select an appropriate technology; which criteria should be examined during the early stage drug discovery process; (3) whether a generic, flexible set of assay methodologies or customised solutions should be applied to the targets being investigated; (4) annotation of hits using cell health assays (e.g. cell viability, proliferation, apoptosis, mitochondrial
toxicity) as well as cardiac hypertrophy and neurite outgrowth assays using human iPS cell-derived cardiomyocytes and neurons.

**Workshop topics**

1. **Lecture:** Introduction to drug discovery and the design and development of assays for drug discovery purposes - what can be achieved and learnings from past successes and failures. Screening jargon and terms.

   **The learning objectives from this lecture will be:**
   - Appreciate that a wide range of assays are available for use in pre-clinical drug discovery.
   - Gain an understanding of how to develop appropriate assays for use in pre-clinical drug discovery.
   - Appreciate the limitation of the currently available assays that are used in pre-clinical drug discovery.
   - Gain an understanding of appropriate data processing required to identify Hits from small molecule screening campaigns.
   - Gain an understanding of the processed involved in validated the Hits identify Hits from small molecule screening campaigns.
   - Gain an understanding the commonly used terms in pre-clinical drug discovery.
   - Appreciate that these terms are used in a variety of contexts within different organisations (e.g. the pharmaceutical industry, biotech and academic).
   - Gain an understanding of how to prepare documents and presentations that refer to assay and compound data in universally recognised formats.

2. **Case study:** Phenotypic cell based assay development and screening.

3. **Lecture:** Reagent characterisation and selection of assays which will ensure translation of Hits between formats.

   **The learning objectives from this lecture will be:**
   - Gain an understanding of the advantages of using cell-based assays for screening.
   - Gain an understanding of the disadvantages of using cell-based assays for screening.
   - Appreciate that additional biochemical assays and/or cell based assays are required to progress compounds for drug discovery purposes.

4. **Practical:** General concepts for cell based assays exemplified using luciferase reporter and High Content imaging assays.

5. **Practical:** IC\(_{50}\) determination for inhibitor, signal stability, choice of liquid handling and Z’ calculation.

6. **Practical:** Application of cell health, cardiac hypertrophy, and neurite outgrowth assays using human iPS cell-derived cardiomyocytes and neurons.
7. **Lecture:** Data analysis and reduction - going beyond the Z'. Discuss methods to analysing *in vitro* biological assays data including false positive/negative rates, dose-response curve fitting and correlations.

   The learning objectives from this lecture/practical will be:
   
   - Gain an understanding that the efficient processing of screening data requires suitable software.
   - Appreciate the need to assess both raw and processed screening data.
   - Gain an understanding of the underlying equations used to normalise data and fit dose-response curves.
   - Gain an understanding of the processes that should be followed in order to identify false positives/negatives in screening.

8. **Lecture:** Analysis of images from High Content Screening assays.

   The learning objectives from this lecture will be:
   
   - Gain an understanding of how to use software for analysing images from High Content Screening assays.
   - Appreciate that image analysis is complex and requires specialist training.
   - Gain an understanding that an integrated multi-disciplinary team is required to progress compounds in pre-clinical drug discovery.

9. **Practical:** Screening of cell based assays against a small molecule library (proof-of-concept screen).

10. **Practical:** Application of cell health, cardiac hypertrophy, and neurite outgrowth assays using human iPS cell-derived cardiomyocytes and neurons.

11. **Case study:** Analysis of High Content Screening assay data.

12. **Lecture:** Integrating your research program, design of project critical paths which integrate *in-vitro, in-vivo* and *in-silico* elements.

   The learning objectives from this lecture will be:
   
   - Gain an understanding of how to construct critical pathways for pre-clinical drug discovery projects.
   - Appreciate that a variety of tools are required to ensure the progression of the outputs of screening.
   - Gain an understanding that an integrated multi-disciplinary team is required to progress compounds in pre-clinical drug discovery.

**Expected outcomes from the workshop**

It is envisaged that upon completion of the course, attendees will have gained an insight into the key parameters to be considered when developing cell based assays and performing small molecule screening campaigns, associated data analysis, validation of Hits and their annotation using a variety of cell health/toxicity/liability assays.
Continuing Professional Development (CPD)

Approved by the Society of Biology for purposes of Continuing Professional Development (CPD), this Biochemical Assays Screening workshop may be counted as 72 CPD credits.

Please note that these credits are only valid if attendees are registered on the Society of Biology CPD Scheme.
References


Workshop instructors

Dr Sheraz Gul
Sheraz Gul is Head of Biology at European ScreeningPort, Hamburg, Germany where he manages the assay development and screening of academic targets. Prior to this, he worked for GlaxoSmithKline for 7 years where he developed biochemical and cellular assays for High Throughput Screening as well as Hit characterization. In addition he has worked in academia for 5 years on proteases and kinases. He is the co-authored of the Enzyme Assays: Essential Data Handbook.

Dr Philip Gibbon
Philip Gibbon is responsible for Operations and Science at European Screening Port. He has experience in drug discovery programs across a wide range of target classes acquired while working at Pfizer and GSK. Philip is deeply involved in many European Open Innovation initiatives involving government, PhRMA and academia (e.g. BioPharma, Framework 7 and IMI). His research interests are directed towards maximising the impact of HTS through the use of more biologically relevant assays, minimising artefacts in assays and the development of new screening methods (e.g. label free).

Dr Bernhard Ellinger
Bernhard Ellinger is a Principal Scientist at European ScreeningPort, Hamburg, Germany. He is responsible for assay development and screening in high content and high throughput settings of cellular and biochemical targets.
## Agenda

### Day 1

<table>
<thead>
<tr>
<th>Time</th>
<th>Activity</th>
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<tbody>
<tr>
<td>9:00-9:30</td>
<td>Introductions.</td>
</tr>
<tr>
<td>9:30-10:00</td>
<td>Lecture: Introduction to drug discovery and the design and development of biochemical assays for drug discovery purposes - what can be achieved and learnings from past successes and failures. Screening jargon and terms.</td>
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<tr>
<td>10:00-10:20</td>
<td>Case study: Phenotypic cell based assay development and screening.</td>
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<td>10:20-10:30</td>
<td>Break.</td>
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<tr>
<td>10:30-11:00</td>
<td>Lecture: Reagent characterisation and selection of assays which will ensure translation of Hits between formats.</td>
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| 11:00-12:00 | Overview of practical work for Day 1 and creation of groups for practical work:  
1. General concepts for cell based assays exemplified using luciferase reporter, cell viability, label-free and High Content imaging assays.  
2. IC<sub>50</sub> determination for inhibitor, signal stability, choice of liquid handling and Z' calculation.  
3. Application of cell health, cardiac hypertrophy, and neurite outgrowth assays using human iPS cell-derived cardiomyocytes and neurons. |
| 12:00-12:15 | Talk from Vendor (optional).                                              |
| 13:15-17:00 | Experimental work.                                                        |
| 17:00     | Group Dinner.                                                            |

### Day 2

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<thead>
<tr>
<th>Time</th>
<th>Activity</th>
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<tbody>
<tr>
<td>9:00-10:00</td>
<td>Discuss results from Day 1.</td>
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<tr>
<td>10:00-10:30</td>
<td>Lecture: Data analysis and reduction - going beyond the Z'. Discuss methods to analysing in vitro biological assays data including false positive/negative rates, dose-response curve fitting and correlations.</td>
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<tr>
<td>10:30-11:00</td>
<td>Lecture: Analysis of images from High Content Screening assays.</td>
</tr>
<tr>
<td>11:00-11:15</td>
<td>Break.</td>
</tr>
<tr>
<td>11:15-12:15</td>
<td>Overview of practical work for Day 2:</td>
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</table>
1. Screening of cell based assays against a small molecule library (proof-of-concept screen).
2. Application of cell health, cardiac hypertrophy, and neurite outgrowth assays using human iPS cell-derived cardiomyocytes and neurons.

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<thead>
<tr>
<th>Time</th>
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<tbody>
<tr>
<td>12:15-13:15</td>
<td>Lunch</td>
</tr>
<tr>
<td>13:15-13:30</td>
<td>Talk from Vendor (optional)</td>
</tr>
<tr>
<td>13:30-18:00</td>
<td>Experimental work</td>
</tr>
<tr>
<td>18:00-18:15</td>
<td>Talk from Vendor (optional)</td>
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<tr>
<td>19:00</td>
<td>Free evening</td>
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**Day 3**

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<tr>
<th>Time</th>
<th>Event</th>
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<tbody>
<tr>
<td>9:00-10:00</td>
<td>Discuss results from Day 2.</td>
</tr>
<tr>
<td>10:00-12:00</td>
<td>Case study: Analysis of High Content Screening assay data.</td>
</tr>
<tr>
<td>12:00-12:15</td>
<td>Talk from Vendor (optional)</td>
</tr>
<tr>
<td>12:15-13:15</td>
<td>Lunch</td>
</tr>
<tr>
<td>13:15-13:30</td>
<td>Talk from Vendor (optional)</td>
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<tr>
<td>13:30-14:00</td>
<td>Lecture: Integrating your research program, design of project critical paths which integrate <em>in-vitro, in-vivo</em> and <em>in-silico</em> elements.</td>
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<tr>
<td>14:00-18:00</td>
<td>Each team to compare results and identify learnings from practical course, presentations from each team and wrap up.</td>
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