# Challenges in Transfer of Complex Assay Formats

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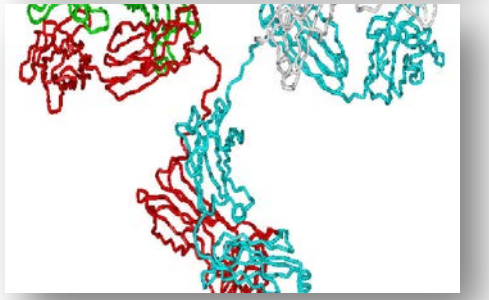
# Overview

- Assay transfer
  - What & Why?
- Common Challenges
- Best Practice to Approach
- Example Transfer for Cell-based assay
  - Immunogenicity
- Summary & Critical Aspects



# Assay Transfer: What & Why?

## Transfer of Analytical Method

### Starting Point Variable

From partially developed non-validated methodology to fully validated robust procedure

### Common Objectives

Demonstration of ‘acceptable’ assay performance at transfer site

## Reasons for Transfer

Transfer from R&D to Regulatory environment

Resource/ technology availability

Leverage cost & efficiency benefits

Accelerate study timelines

Partner with technical expertise

# Common Challenges to Assay Transfers

- Design of effective transfer strategy
- Partnership & communication
- Differences between research sites:
    - Equipment
    - Readout technology
    - Reagents
    - Techniques
    - Interpretation of methodology and/or data
- Aggressive timelines

# Approach to Assay Transfer

## Pre-Transfer

- Early planning
- Understand needs = establish purpose
- Suitability of methods

## Develop Effective Documentation

- Transfer study plan: '*What, How, When*'
  - Agreement between sites
  - Detailed, tailored objectives
  - Pre-determined acceptance criteria

## Lab Phase

- Method optimised as required for purpose
- Discussion and agreement
- In-line with study plan & recommendations

## Reporting

- Final Report of complete study
- Capture solution based approaches
- Demonstrate 'fit for purpose'

**Regular Communication** → **Monitor Constantly** → **Feedback**

# Example Transfer
## Challenges & Identified Solutions

### Cell-Based Assays

- Used in all stages of drug development
- Unique complexity & variability
- Considered most relevant measure of biological mechanism of action

### Neutralising Antibody Assay

- Immunogenicity testing

# Assay Specific Transfer Challenges

## Neutralising Antibody (NAb) Assay

- **Variability**
  - Target expression levels - adequate, stable, specificity
    - Cell growth conditions/ seeding density
    - Cell passage number
- **Limited Dynamic Range**
- **Matrix Interference**
  - Components may enhance/inhibit activity of drug product in assay
- **Choice of signal analysis**
  - Understanding **Mechanism of Action**
    - Overall *in vivo* effect may reflect convergence of multiple signalling pathways
    - *Which one is most relevant to use?*
- **'Fit for purpose'**
  - Suitability for R&D cf. Regulatory environment?
  - Practical, meets requirements of clinical sample analysis?

# Transferred NAb Assay

**Objectives of Transfer Study**:

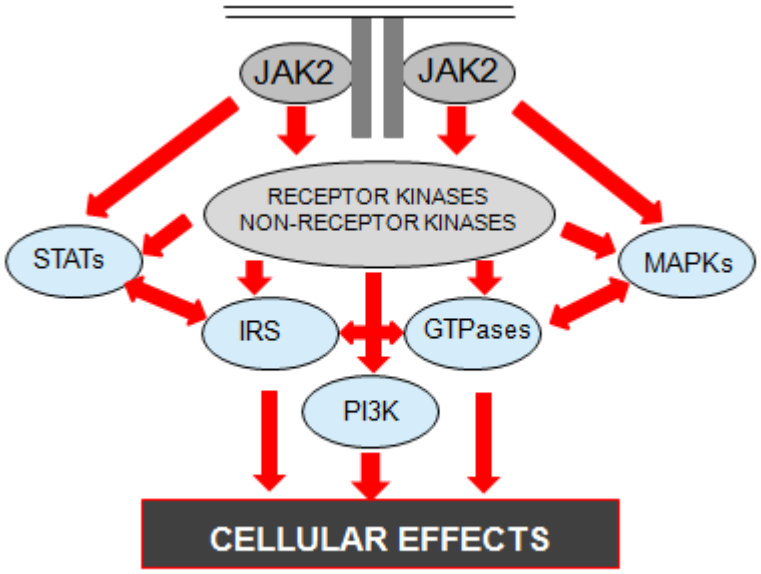
- Confirm suitability of assay for detection of neutralizing antibodies to drug in human serum
- Confirm performance of assays as a pre-requisite for validation
- Demonstrate assay robustness

**Target**
- Hormone Receptor

**Drug type**
- Agonist

**Mechanism of Action**
- Multiple signalling pathways can mediate pleiotropic actions of ligand

**Functional Readout**
- Cell Proliferation = Convergence of many signalling outputs




Adapted from Zhu *et al* Cellular Signalling 2001; 13(9); 559

# Issues with Transfer Assay

## Original Assay

- Day to day variability in raw data
- Effected by cell passage / no. days in culture
- Operator Bias
- Required repeat evaluations

## Revised Approach

- Analysis of raw data compared with 'normalised' data:

✓ Corrected for day to day variability between runs

✓ Allowed main validation criteria to be met

✗ Unacceptable wide acceptance criteria for positive control

## Conclusion

Validation failed due to inadequate robustness

# Agreed Transfer Assay Re-work

- **Modify assay to overcome main obstacles encountered:**

  (1) use of cells in continuous culture

  (2) use of complex downstream readout

(1) Identified alternate cell line

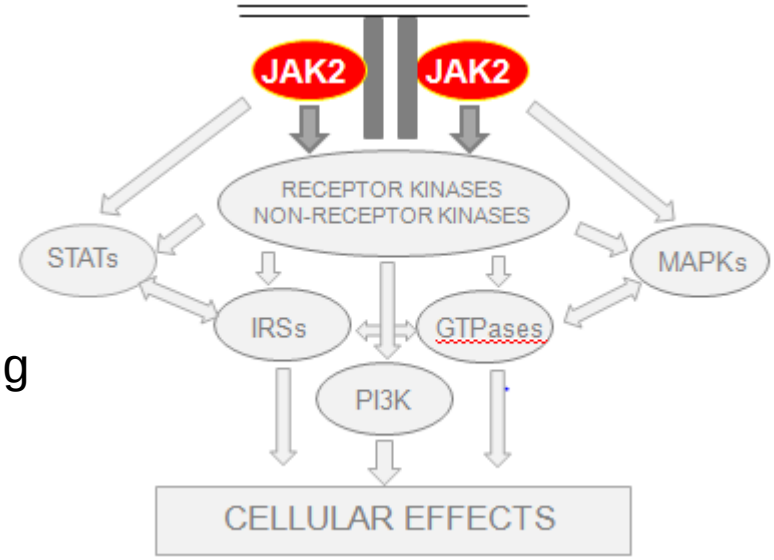
- Engineered, specific responsive cell line
- No sub-culture requirement; immediate use from liquid $N_2$

(2) Functional readout of 'upstream' signaling

Direct, Specific, Less Complex

**JAK2 = Pivotal Role**

Suitable readout for Mechanism of Action

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# Comparison of Methodology

## Continuous sub-culture cells

17 days

**Revive** Cell vial

↓

**Sub-culture** minimum **2 weeks**

↓

**Harvest** cells; spin & re-suspend x2

↓

**Cell count**; volume required

↓

Prepare **off-line plate**;
pre-incubate 60min

↓

Incubate Cells + 'reagents'
**48 hr** (5%$CO_2$/37ºC)

↓

**Detection**

↓

**Read** Luminescence

## Immediate use cells

2 days

**Revive** Cell vial

↓

Resuspend, count**, plate**

↓

Incubate ~**22 hr** at 5%$CO_2$/37ºC

↓

Prepare **off-line plate** 'reagents'

↓

Incubate Cells + 'reagents'
**3 hr** (25ºC)

↓

**Detection**

↓

**Read** Luminescence

# Assays Comparison: Key Parameters

| Selected Validation Parameters | Transfer Assay Cell proliferation | Revised Assay 'Upstream' Signalling |
|---|---|---|
| Sensitivity to Drug Stimulation | ++ | + |
| Minimum Required Dilution | 1% serum + | 2% serum ++ |
| Screening Assay | Results normalised to ratio | Raw results |
| Acceptance criteria | Range ± 30% + | Range ± 20% ++ |
| Sensitivity to Positive Control | ++ | + |
| Inter-assay Precision | + | ++ |
| Short-term Stability | Fail? | Pass |
| Drug tolerance | ++ | + |
| Robustness | Fail | Pass |

# Comparing and Ensuring "Fit for Purpose"

| CRITERIA | **Transfer Assay**: Cell proliferation | **Revised Assay** Signalling |
|---|---|---|
| **Cell line / Target Receptor** | Mouse<br>Mutated /humanised rat | Human<br>Full length human |
| **Sub-culture required** | Minimum 2 week | None |
| **Stability of S/N** | Day to day variability | Stable |
| **Impact of sub-culture duration** | Variability in sensitivity | Sub-culture not required<br>Uniform cell 'age' / condition |
| **Robustness** | Inadequate<br>(likely to cause failure of acceptance criteria to be met in SA) | Acceptance criteria meet for sensitivity & system suitability |
| **Intended Use**<br>▪Start up time<br>▪Planning Required<br><br>▪Data turnaround time | Minimum 2 weeks<br>Advanced- adequate flasks for no. plates needed<br>Within 4 weeks | Immediate<br>Stock of vials in liquid $N_2$<br><br>Within 2 weeks |

# Summary

- Transfer may commence from different starting points
- Many challenges due to large number of variables involved
- Assay specific pitfalls
- Tried & tested solutions

## Critical Factors for Assay Transfer Success

Regular communication & forward planning
Clearly defined objectives & tailored criteria
Continuous monitoring to ensure purpose built approach

# Acknowledgements


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