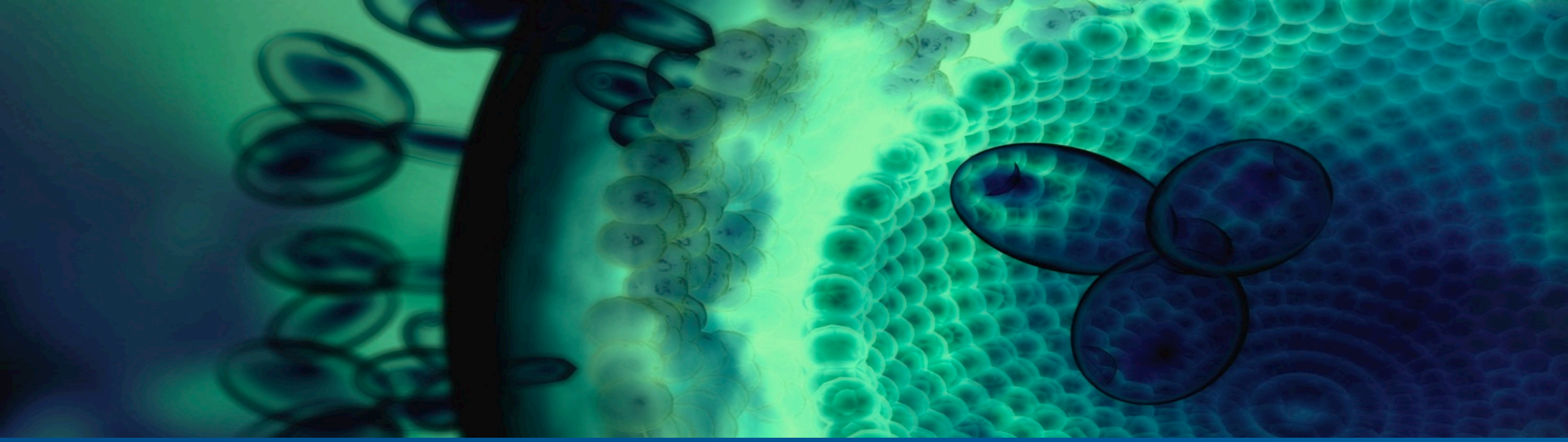


Pharma/CRO alliance: what are the keys of success in transfer of assays

Pictured above: The structure of HIV.

Lieve Dillen – Janssen R&D – Development Bioanalysis
EBF open meeting 17-20 November 2020

Janssen | PHARMACEUTICAL COMPANIES OF Johnson&Johnson

OUTLINE

- Introduction – changing landscape – situation anno 2020
- Internal method development
- Transfer to CROs and method development follow up
  - functions involved @ pharma and @ CRO
  - Information shared
  - Scientific discussions
  - Information exchange – when and how to communicate
- Trouble shooting – examples
- Conclusions: what are the keys?

Changing Landscape over the years

Some standard discovery bioanalytical support (entire process) outsourced to CRO

Preclinical GLP: internal bioanalytical GLP work stopped

preclinical GLP tox programs : outsourced

2010 2012 2014 2016 2018 2020

Clinical studies beyond POC: outsourced

Preclinical GLP: also internal in vivo activities stopped

- Mechanistic studies
- Bioanalytical method development
- **Bioanalytical method validation**
- **GLP Tox**
- **FIH clinical studies**

- Mechanistic studies
- Bioanalytical method development
- **Bioanalytical method validation**
- **FIH clinical studies**

Current model describing bioanalytical phases

Bioanalytical strategy for post candidate selection drugs
in house (green) and at CRO (orange)

Dose-escalation studies (dose selection for GLP tox studies)	Method development Robustness evaluation	Transfer method to CRO
method development and validation	Bioanalysis of GLP studies	Method development for FIH ~~Scientific Validation~~/Regulatory Validation
Bioanalysis of FIH SAD and MAD	Method development (human plasma) Regulatory Validation	Bioanalysis in support of clinical program

Lean internal method development process

- Drug candidate results from internal portfolio
  - Tailored but as much as possible standardized approach
    - Collect physicochemical and stability information of the drug
    - Select optimal IS (analogue vs STIL dependent on availability)
    - Collect info on expected exposure range in studies
    - Species considered in GLP
  - LC-MS/MS optimization (ionization, retention, phospholipids, …)
  - Sample prep, matrix effects, adsorption and stability
  - Robustness run = 1 A&P run (QCs 6 fold including LLOQ)
  - Transfer summary document shared

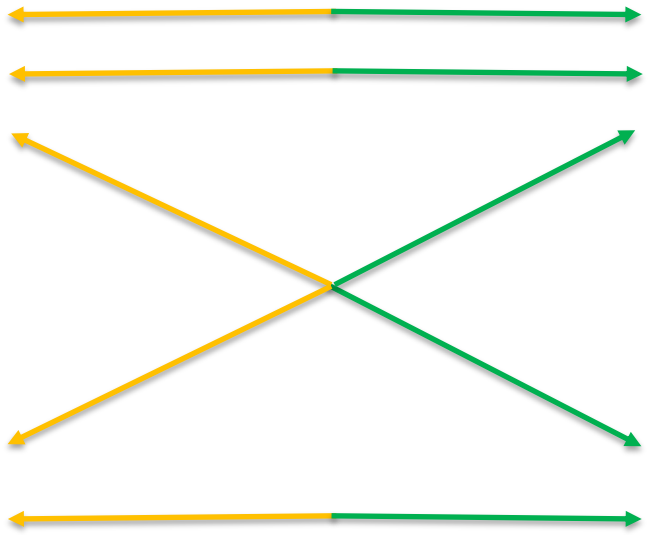
- Drug candidate acquired through in-licensing, acquisitions
  - Method evaluated – decided whether to keep at CRO of partner or switch to preferred CROs
  - Method development in function of troubleshooting.

Transfer of the method: interactions

Sponsor organisation

Operational team
- Lead
- method developers
- analysts

Bioanalytical Project Management



CRO organisation

Method development team
- Lead
- method developers

Operational team
- Lead per project
- analysts

Transfer of the method

- Via email of the sponsor's project manager to SD method development @CRO (and project manager @CRO if assigned).
  - Detailed method description
  - Structure and physicochemical properties
  - Summary of available information on metabolites, stability
- Follow up in TC
  - Detailed information can be disclosed (individual experiments)
  - Discussion on approach at CRO
- Example of method transfer documents shared


SUM_method


SUM_adsorption


SUM_stability


SUM_robustness

- Secured share points accessible for CRO and sponsor to exchange results/protocols
- During method development weekly updates

Considerations for modifications upon transfer of assay

Most frequent changes discussed with CRO:

- chromatographic system (UHPLC or HPLC platform) (driven by availability of # instruments) – caveats: carryover - resolution with a metabolite
- MS platform (eg Sciex 6500 proposed while method was developed on API4000) – risk of saturation at ULOQ
- injection volume (combined with additional dilution of supernatant) – solubility, carryover, signal-to-noise ratio can be impacted
- regression model/weighing factors
- preparation of calibration curves (plasma calibration samples prepared in bulk versus calibration samples spiked freshly from solvent based spiking solutions)

Why do methods not (always) transfer one tot one?

- Even with identical equipment same performance not always realized
- LC-MS tubings/lengths and ID are different
- MS conditions:
  - Electrospray conditions (position of LC outlet versus orifice)
  - Condition of ionization source - maintenance and intensity/type of samples analysed
  - Calibration
  - Resolution/IE settings of the quadrupoles
- Perceived unimportant details are unintentionally not included
- Solvents are from different quality/vendors
- Consumables are different and can impact method performance
- Storage conditions - walk in freezers – exposure to light
- Robust method should be tolerant to small changes

Example: unintentional change/unidentified impact

- Extensive internal experience with assay – challenging project - stability issues
  - Preclinical species validated internally
  - Criticality in the assay seems a small detail
    - Small amounts of organic were not tolerated
    - pH during precipitation critical

- Transfer of assay to CRO – with extensive discussions on the details of the assay
- Assay performance issues during sample analysis
  - assay transferred back to method development team@CRO
- Adapted method instructions were **accidentally not shared with sponsor**
  - New method developer @CRO was not informed on critical aspect
  - New assay successfully validated and applied in GLP studies

- Study selected during sponsor audit – results all within compliance but scientifically incorrect

- Revalidation and re-analysis needed

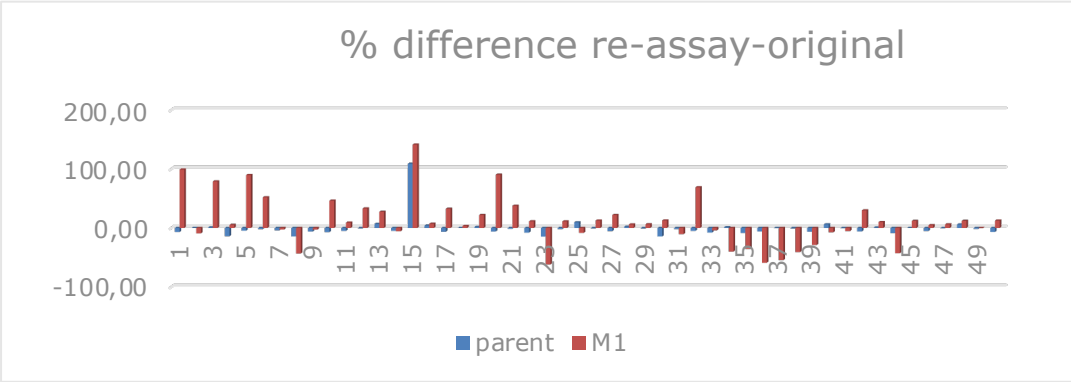
- Continued communication within and between organisations is key

Example: unnotified difference

- Assay for drug candidate validated @ sponsor's lab
- Beyond phase 1: outsourced to CRO
- @CRO: LTS (-20°C) > 1 year failed > -20% bias re-analysis confirmed the observation
- @sponsor: > 2 years LTS proven
- Interaction CRO – sponsor: additional investigation @ sponsor
  - Stress light stability evaluation demonstrated light sensitivity
  - Walk in freezer (daily illumination) @CRO identified as root cause
  - Prolonged storage with intermittent exposure to light responsible for degradation
- Interaction and open discussion between partners is key
  - Shared responsibility

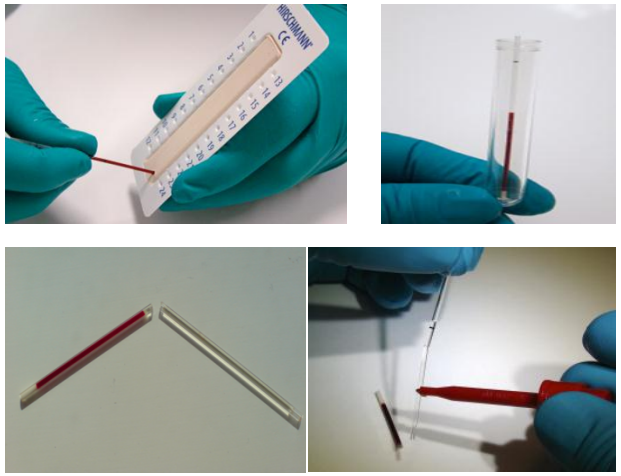
Example : in licensed compound – validated method – failed ISR

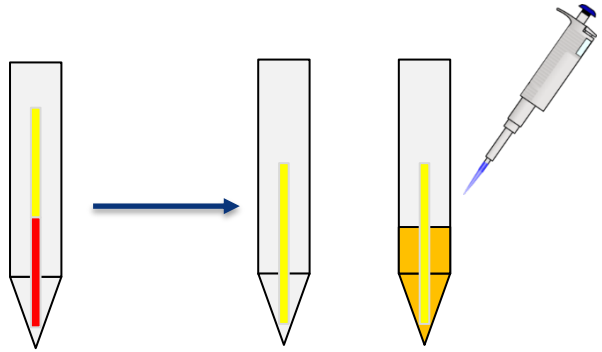
- 2-in-1 assay quantifies parent (R-CO-NH2) & M1 (R-COOH) validated at first CRO (not preferred provider) – no initial internal involvement
- ISR passes for parent but consistently failed for M1 (in clinical and GLP studies)
- Investigation in sponsor's lab:
  - M1 results reproducible after single dosing but not after multiple dosing
  - Investigation revealed study samples contain high levels of M1-glucuronide (up to 80x M1 conc. after RD)
  - Assay uses evaporation step; M1-glucuronide can decompose to M1
  - Assay re-developed without evaporation step; issue resolved
- New Assay conditions transferred to CRO
  - Revalidation
- Not all information is known during initial method development – responsibility of project manager to keep abreast of new information



introducing new approaches: CMS

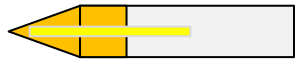
- Sponsor: capillary microsampling introduced as standard sampling technique in rodent GLP studies





10 µL plasma + 100 µL Buffer
E2E capillary (+2% BSA)

Horizontal capillary wash-out



= 110 µL of diluted plasma

Subsample analysed

- Sponsor's experience in validated CMS assays halted with decision to stop internal GLP
- Sponsor built substantial experience in preclinical non GLP studies but experience with validating the assays was limited

- Mutual visits to CRO and sponsor organized to train practical aspects

introducing new approaches: CMS

- Considerations to be discussed upfront method development
  - Study samples diluted in buffer
    - Calibrators in capillaries
    - Calibrators in diluted plasma
    - Calibrators spiked to diluted plasma
    - QCs sampled as study samples
      - Prepare in capillaries – wash out together with study samples
  - ISR samples – diluted plasma samples for re-analysis
  - Additional stability program in diluted plasma
  - Additional burden for method development and bioanalytical lab

Example: Capillary microsampling

- Project: parent drug validated @ CRO - 2 metabolites qualified assay
- In preclinical program: 2 metabolites added to the validated assay
  - Internal standards: parent drug and M1 STIL available; STIL M1 used for M2
- During GLP program switched to CMS -> revalidation @ CRO
  - Combined validated assay for 3 analytes
  - STIL synthesis for M2
  - Calibrators prepared in diluted plasma
  - QCs in capillaries
- Validation: some STS and LTS failed for metabolites
  - Preparation errors due to complexity
  - Building experience with capillaries
  - variability especially for M2
- Mitigation discussed: scrutinize differences in lab practices

LTS for M2 (LQC 30 ng/mL)

Time (days)	Bias(%)	Time (days)	Bias(%)	Time (days)	Bias(%)
52 (-20°C, cap)	-15.3	74 (-20°C, cap)	-16.7	154 (-20°C, cap)	-13.0
52 (-20°C, BSA diluted)	-4.9	74 (-20°C, BSA diluted)	-7.8	154 (-20°C, BSA diluted)	-22.3
52 (-70°C, cap)	-2.8	74 (-70°C, cap)	-2	154 (-70°C, cap)	-8.9
52 (-70°C, BSA diluted)	-10	74 (-70°C, BSA diluted)	-10.4	154 (-70°C, BSA diluted)	-9.7

Example: Capillary microsampling

- Mouse GLP study:
  - Many analytical runs rejected for M2 (QCs outside criteria)
  - ISR for M2 rejected due to QCs out of acceptance criteria
    - ISR results for M2 were within criteria
- Combined CRO and Sponsor investigation
  - Wash out solution slightly different
  - Sponsor used solvent spikes as calibration standards
  - Light sensitivity in solvent for M2 (amber versus foil protection)
- Not trivial to identify root cause but probably related to complexity (combined 3 analytes, smaller sample volumes, building experiences with capillaries)

Keys to successful method transfer

- Majority of assays transfer without problems
- Info sharing – Info sharing - Info sharing
- The devil is in the detail
- Regular/continued communication - transparency/open minded – provide the details (reluctance or only partial disclosure of raw data experienced)
- Install software tools – share points or other secured portals to exchange (raw) data
- Building relationships/partnership at different levels (preferred partners)
  - Understand mutual processes
- Building trust and respect – especially in difficult projects – be constructive
- Consider external lab as an extension of your own lab

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