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Feeding the Pipeline: Optimizing Academic-Industry Collaborations

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MDF Industry Meeting
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TUCSON Innovation CENTER

Agenda

- Academia and Industry Perspectives
- Screening Tree
- Biological Assay
- Translatability
- Quality and Robustness
- Capacity and Cost

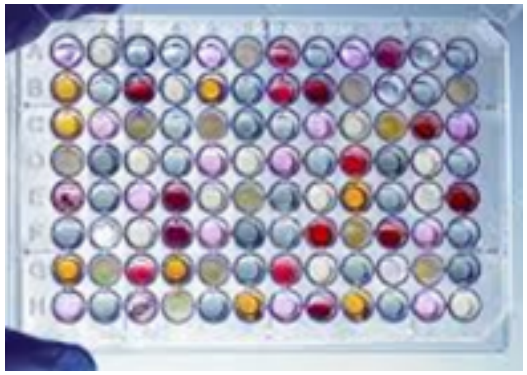
Industry Perspective :
Partnerships With Academia on Drug Discovery

- **Academia :**
  - Hypothesis-driven research to develop a plan
  - Chase results to generate new discoveries and publications
  - Any assay can be converted to screen millions of compounds
  - Plenty of time to sort out the issues
  - You need to have controls

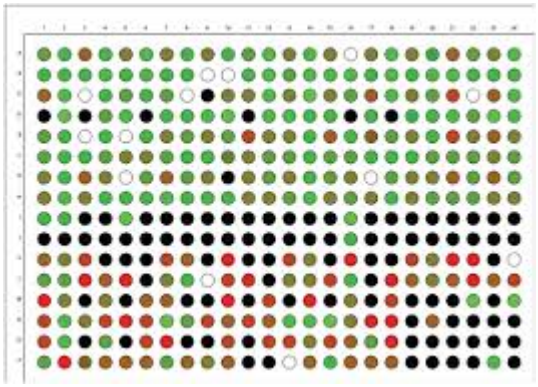
- **Industry:**
  - Execution of the plan on a validated hypothesis
  - Very little time to make changes ; always something else in the wings
  - HTS is challenging and costly !
    - Confirmation, downstream assays and selectivity assays brings many difficulties
    - Don't chase every everything interesting - leave behind compounds that don't fit
  - There are many obstacles
    - Some reagents don't handle well, long waiting times, insoluble compounds perform differently in different buffers and conditions
    - if an assay is too dependent and too responsive to presence of e.g. detergents and temperature gradients, then get ready for headaches
    - You may not have controls

Technology Transfer

- High Throughput Assay Adaptation



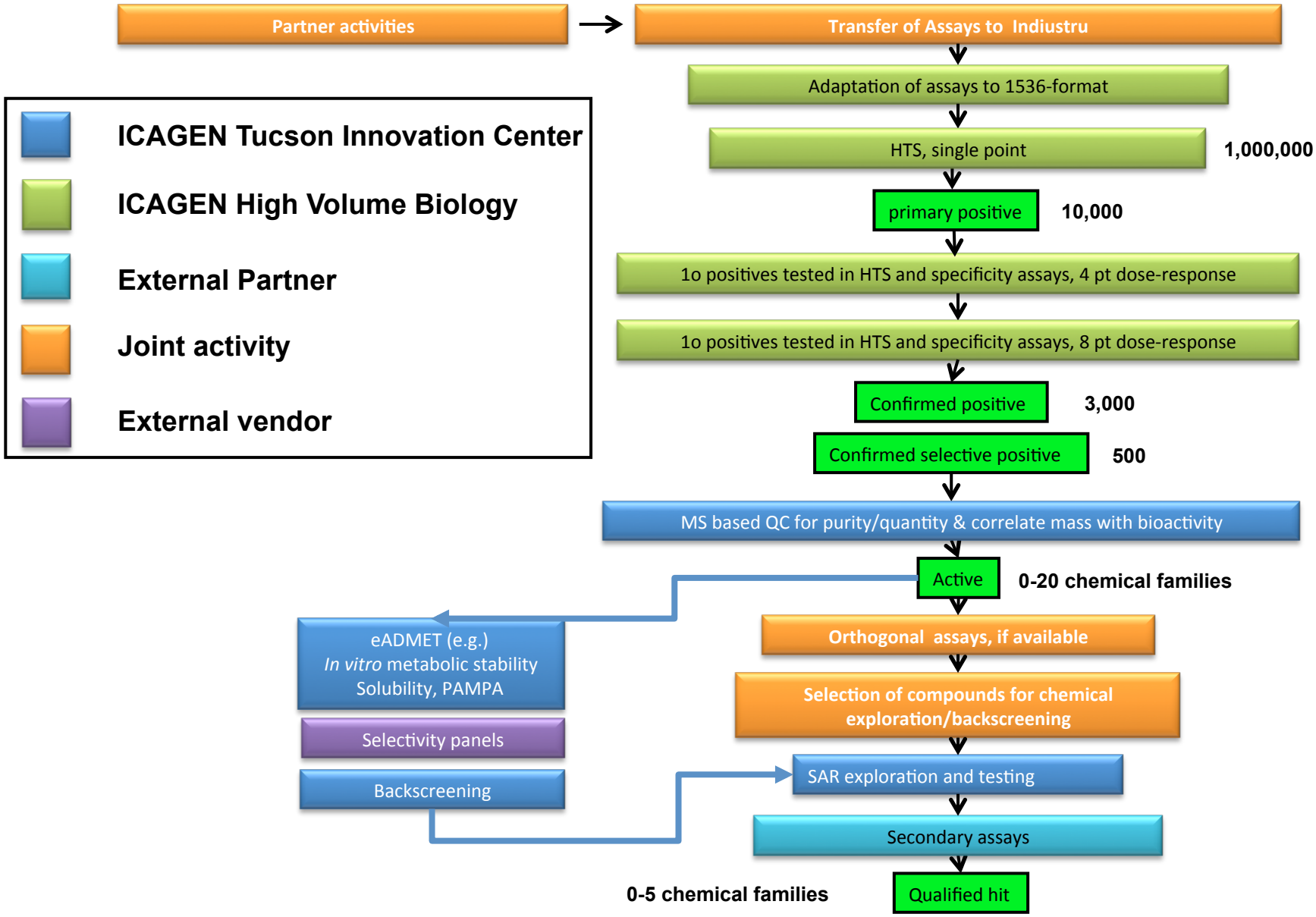
96 Wells



384 Wells

1536 Wells

Collaborative Screening Tree



Screening Tree Overview:

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- Ideally complete DRAFT prior to screening
  - Planning & resource allocation tool
- Project Progression Map
  - Where are we and what's needed next
  - Build filter to select safe efficacious molecules
  - Enables advanced planning and resource allocation
- Living Document
  - Changes as additional data sets are generated
  - Assays brought online – or NOT

Screening Tree

Design:

- Primary Assay
- Orthogonal primary *in vitro* assay
  - Ideally under different experimental conditions
- Selectivity assays
  - monitor related subtypes and/or anti-targets
- *in vitro* functional assay
  - Demonstrates modulation of a phenotyp
  - Relevant to disease in human tissue
- Pharmacodynamic assay
  - Cell based
- *in vivo* pharmacodynamic model
- Animal disease model
  - Demonstrate efficacy with a lead series compound (POC)

*if necessary for credentialing package

Biological Assays:
Drug Discovery Assays Defined

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Type	Purpose
Primary screen and assay	Assay against selected target, pathway or phenotype; to identify initial hits and test potency of all compounds in program. The assay used as the high throughput screen.
Counterscreen	Eliminate unwanted actives from primary screen. Performed in parallel with or after the primary screen. The assay used in the counter-screen is developed to identify compounds that have the potential to interfere with the assay used in the primary screen (the primary assay). Counter-screens can also be used to eliminate compounds that possess undesirable properties, for example, a counter-screen for cytotoxicity
Orthogonal activity assay	An assay performed following (or in parallel to) the primary assay to differentiate between compounds that generate false positives from those compounds that are genuinely active against the target.
Ortholog primary assay	Test activity of compounds against homologous target from species used to conduct in vivo experiments; usually mouse for initial efficacy studies; later rat and dog for safety studies
Selectivity assay	Test selectivity of compounds against closely related targets that could cause safety issues
Functional assay	Cellular assay demonstrating physiological function of target. An assay used to test the activity of compounds found active in the primary screen (and orthogonal assay) using robust assays of relevant biology. Ideally, these are of at least medium-throughput to allow establishment of structure-activity relationships between the primary and secondary assays and establish a biologically plausible mechanism of action.

Biological Assays:

The Future Starts Here

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- **Assays should:**
  - **Be Biologically Relevant**
  - **Demonstrate**
    - **Pharmacology**
      - **Dose response with control compounds**
    - **Sufficient Precision , discrimination (+/-) and assay sensitivity**
      - **Can detect small changes (< 2X)**
      - **discriminate between moleules**
    - **Robustness and reproducible**
      - **Independent of daily fluctuations and well location**
    - **Appropriate Throughput at an appropriate cost**
      - **Total screen for 1M compunds costs less then $100K (10 cents/well)**
  - **Provide rapid and accurate feedback to:**
    - **identify active series or to guide SAR on lead molecules**

Biological Assay:

THE most Important Aspect of Any Project

- Challenges
  - Demonstrate a translatable phenotype relevant to disease state
  - May not be easy to see a relevant phenotype
    - Good: Pompe Disease – Measure glycogen levels
    - Bad: Duchenne Muscular Dystrophy – Utrophin levels
    - Ugly : Sporadic ALS - Motor Neuron Survival

- Opportunities:
  - Patient-derived cells and tissues
    - Appropriate system for translational pharmacology : Tissues
    - Capture unique pathophysiological differences not observed in cell lines
    - Examine specific genetic backgrounds

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Biological Assays: Enhancing the Probability of Success

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- Differences Lab to Lab (associate to associate)
  - Results should be confirmed where possible in another laboratory
  - Follow precise SOP from originating lab (NO Modifications)

- Differences assay to assay
  - Orthogonal assays confirm hypotheses
  - Reveal differentiating aspects of compounds assayed

- Differences from cell to cell
  - May observe unexpected cell specific, compound activities
  - construct in multiple, distinct cell types/backgrounds
  - Related targets to be evaluated in format parallel to primary assay

- Differences species to species
  - Activity of *in vitro* efficacy should be measured in different species
    - Particularly animal model POC

Biological Assays: Compounds & Solvent Effects

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- Small molecule collections stored in DMSO
  - Assays are sensitive to solvents used in the assay
    - Usually less than 0.1 % for robust effects
    - Greater than 1% DMSO very challenging
- Compound screening assays perfomred at a concentration of 1–10 µM compound
  - Evaluate assay performance at 3 & 10 uM
  - Perform Pilot screen

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Translatability of Assays: Pharmacological Relevance

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- Pathophysiology Modulation
  - Pharmacology is predictive of the disease state
  - Identify compounds with the desired MOA
    - Cellular or Target:
- Validation
  - Modulate the Biology: target, pathway or phenotype
  - Tool compound and/or Genetics
    - potent and selective activity
    - Knock out or down : reduce assay signal
    - Complementation:
      - inactive mutant should not rescue the signal
      - active target rescues the signal

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Assay Quality: Overview

- Assay is reproducible
  - Across assay plates, screening runs, and screening days

- Assay performance:
  - Z' on the assay plate and the entire screen
  - Variance of a standard compound (control)
    - Intrawell, intraplate, intrascreen
  - Fail plates if they fall outside predefined criteria
    - Rescreen if possible

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Assay Quality:
Factors Affecting Reproducibility

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- Complexity: KISS
  - Simplicity is better
  - Assays with few steps increases reproducibility !
  - Multiple steps :
    - Flying across the country with multiple stops : More can and will go wrong

- Reagents:
  - Stable reagents/biologicals with limited temperature and humidity effects

- Equipment
  - PMs performed on routine basis and calibrated
  - developing quality control practices for all items of laboratory automation

- Pilot Screens
  - Evaluate the performance of the assay on a compound collection
  - FDA Approved Drug Collection (Optimal set)
    - Hit Rate , False positives, variability of assay performance in HTP

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Assay Quality
Statistical Parameters for Evaluation

- Z' factor (Range: 0 to 1.0)
  - Industry standard to measure assay quality
    - Zhang *et al*., 1999
  - Statistical variance
    - Established by the high & low signals of controls in the assay
  - Z factor > 0.4 is considered appropriate for screening

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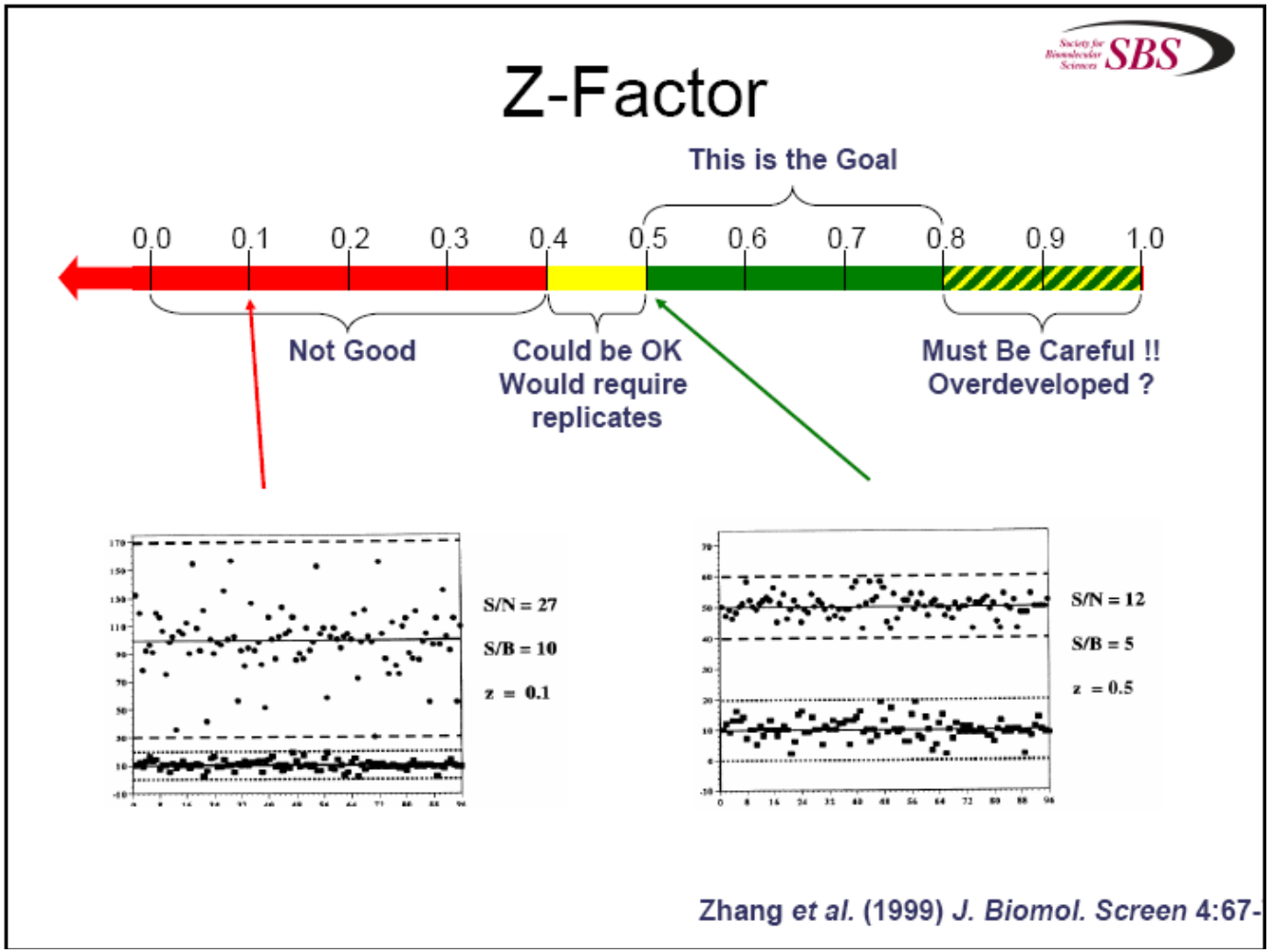
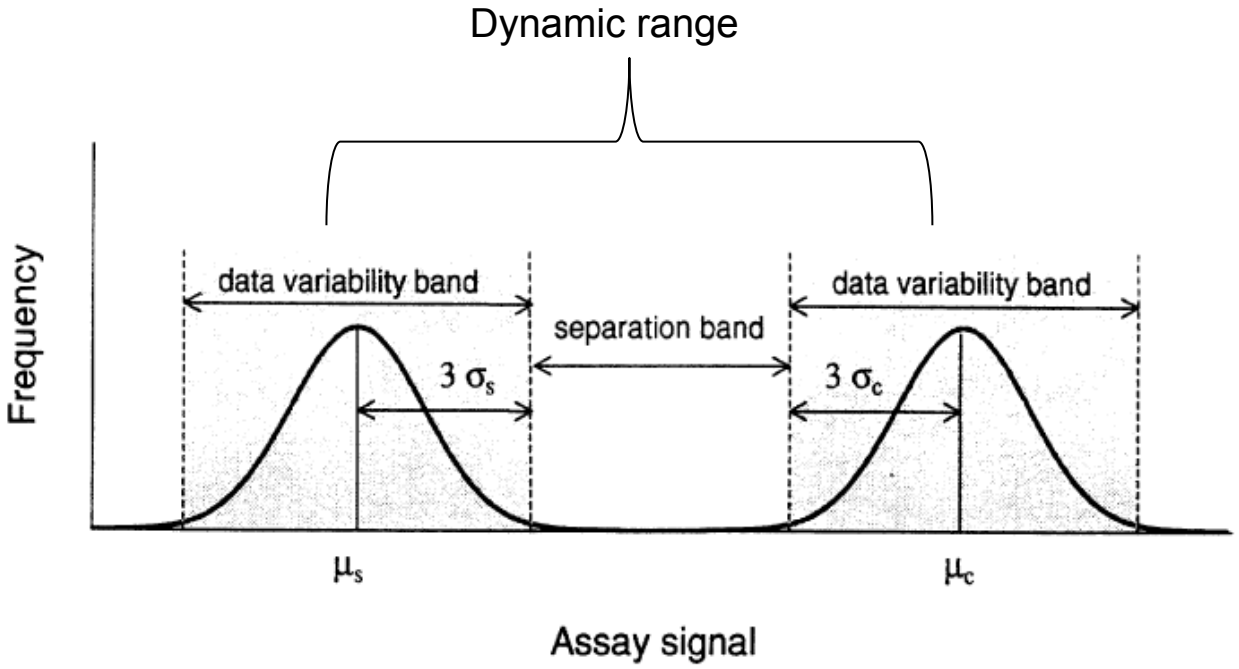
Statistical Parameters for Evaluation
Z-Factor



Assay Quality
Statistical Parameters for Evaluation

- Dynamic Range
  - Screening window coefficient = ratio of the separation band to the signal dynamic range

- Large dynamic range and/or small data variability
  - Improves assay performance and results significance



Zhang et al, 1999

Assay Quality Acceptance Criteria

- **Inter-plate and Inter-day asessment**
  - The normalized average median signal
    - **Not > 2 within days**
    - **Not > 2 across any two days**

- **Intra-plate assessment**
  - Plate CVmax and CVmid ≤ 20%
  - CVmin ≤ 20%
  - Z’ ≥ 0.4

- **Other parameters**
  - No material edge effects
  - No significant drift during performance of the assay
  - No plate spatial effects

Assay
Capacity and Cost Considerations

- Human Resources
  - Significant cost
- Evaluate the cost versus benefit of assays
  - Empirically in pilot screens
- 384-well or 1536-well microtiter plate assay
  - Reagents and assay volumes are optimized to minimize the costs of the assay
  - Ideally less than 0.3 per well or less

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Summary
Key Recommendations

- Partnering : Academia and Industry
  - Align on goals early !
    - In-Depth Meeting
  - Project Progression
    - Meet regularly : at least once per month
    - Establish sharing portal
    - Exchange personnel
  - Share data transparently
    - The good, bad and the ugly
  - Work towards:
    - Robust assay – Complete pilot screen
    - Low Cost – Perform actual calculation
    - High reproducibility & Quality – Do not select data
    - High throughput reality – Scalable solution
    - Validate results in different labs

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Thank-You