EIP
European Immunogenicity Platform

EBF 16th Open Symposium November 15th 2023

OVERVIEW EUROPEAN IMMUNOGENICITY PLATFORM (EIP) CROSS-VALIDATION OF IMMUNOGENICITY ASSAYS

Daniel Kramer (Sanofi), EIP Chairman on behalf of EIP

EIP Overview

- The European Immunogenicity Platform (EIP) was founded as a non-profit organization early 2007 by European experts in the field of immunogenicity
- The European Immunogenicity Platform acts as a central meeting place for European biopharmaceutical companies, institutes, CROs and scientific experts active in the area of immunogenicity
- Its scope is
  - Interaction with authorities regarding immunogenicity guidelines
  - Formulate active recommendations regarding immunogenicity
  - Stimulate research addressing the clinical and non-clinical effects of unwanted immunogenicity
  - Collaboration between academia and pharmaceutical companies
- Through its working-group structure, the EIP can react in a focused way on regulatory and scientific evolutions in the immunogenicity-field

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Current EIP Members

Currently the EIP has 41 full members and two associate members (Amy Rosenberg & Vibha Jawa)

Abzena	Campbell Bunce
Amgen	Dan Mytych
AstraZeneca	Jo Goodman
Bayer	Joanna Grudzinska-Goebel
BioAgilytix	Frank Horling
BioNTech SE	Arno Kromminga
Boehringer Ingelheim	Patrick Garidel
Byondis	Myrthe Rouwette
Celerion	Wibke Lembke
DDS	Deborah McManus
Formycon	Susanne Pippig
Fresenius Kabi SwissBioSim	Martin Ullmann
Genmab	Arnout Gerritsen
GlaxoSmithKline	Erik Meyer
Hansa Biopharma	Yvonne Stenberg
ImmuneSpec	Elise Pepermans
ImmunXperts	Sofie Pattijn
Integrated Biologix	Sebastian Spindeldreher
Labcorp Drug Development	James Munday
Leukocare	tbd
Lonza	Noel Smith
Lundbeck	Mikkel Nors Harndal
Luzsana Biotechnology	David Floch
Merck & Co, Inc.	Linlin Luo
Merck Group	Kyra Cowan
Molecular Partners	Joanna Robinson
Novartis	Lydia Michaut
Novo Nordisk	Karin Weldingh
Pfizer	Sophie Tourdot
Pharming	Simone Talens
QPS	Camille Picq
Roche	Gregor Lotz
Sandoz	Anita Rudy
Sanofi	Daniel Kramer
Sanofi-Gent	Karen Heyninck
Sanquin	Theo Rispens
SciPot Consultancy	Melody Janssen
Svar Life Science France	Michael Tovey
Swedish Orphan Biovitrum	Nina Brenden
Thermo Fisher Scientific	Åsa Marknell DeWitt
UCB Biopharma	Veerle Snoeck

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EIP Governance

- A Board of Directors is the governing body of EIP, to set strategy, drive the organization, and protect the interests of members
  - Daniel Kramer (Sanofi) Chairman of the Board of Directors
  - Barbara Vercruyssen Director Finance and Operations
  - Sophie Tourdot (Pfizer) Director Scientific Affairs

  - Tim Hickling (Roche)
  - Frank Horling (BioAgilytix)
  - Arno Kromminga (BioNTech SE)
  - Lydia Michaut (Novartis)
  - Sofie Pattijn (ImmunXperts)
  - Noel Smith (Lonza)
  - Veerle Snoeck (UCB Biopharma SRL)
  - Sebastian Spindeldreher (Integrated Biologix)

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EIP Working Group Structure

Non-Clinical Immunogenicity Risk Assessment (NCIRA)
Sebastian Spindeldreher & Noel Smith

- **New Modalities**
  Vibha Jawa, tbd
- **Prediction of Impact**
  Chloé Ackaert, Tim Hickling, Samuel Pine
- **Standardization in-silico/in-vitro**
  Noel Smith

Immunogenicity Risk Assessment (IRA) Strategy
Joanna Grudzinska-Goebel, Arno Kromminga & Veerle Snoeck

→ Alignment on the Immunogenicity Risk Assessment strategy performed in pharmaceutical and biotech companies

Immunogenicity Assays
Linlin Luo
Bernd Potthoff

→ Cross-validation of immunogenicity assays

Biosimilars
Martin Ullmann

Particle Characterization
Patrick Garidel

Potential topics to join forces between EBF and EIP

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Cross-Validation of Immunogenicity Assays

Cross-Validation of Immunogenicity Assays

- Cross-validation of Immunogenicity assays should be considered in the following cases:
  - Transfer of immunogenicity assay to new bioanalytical lab within a clinical trial
  - Transfer of immunogenicity assay to a new bioanalytical lab across pivotal clinical trials (if pooling of immunogenicity data across studies is intended to help assess the overall impact of immunogenicity)
  - Changes of the immunogenicity method within a clinical trial or across pivotal clinical trials (e.g. new technology platform if original is discontinued)
- In contrast to PK assays, no regulatory guidance is available describing the cross-validation of Immunogenicity assays
  - Current FDA guidance does only provide high level information under “Reproducibility”:
    - “Reproducibility is an important consideration if an assay will be run by two or more independent laboratories during a study, and a sponsor should establish the comparability of the data produced by each laboratory”
    - “Comparable assay performance, including sensitivity, drug tolerance, and precision, should be established between laboratories”

EBF and EIP surveys indicated the need for harmonization within biopharmaceutical industry

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Need for Cross-Validation

- Is formal immunogenicity cross-validation needed if similar validation parameter between labs/assays are obtained?
    - As immunogenicity assays are quasi-quantitative by nature even similar validation parameter might not guarantee same results (ADA/NAb prevalence/incidence, kinetics, titer) “How similar is similar enough?”
- If a different positive control is used for both labs/assays, cross-validation might still be successful although key assay parameters are significantly different

Method	Positive control	Sensitivity	Precision
ADA assay 1	Human anti-rabbit	120 ng/mL	Precision for all controls: <20%
ADA assay 2	Rabbit monoclonal antibody	16.5 µg/mL	Precision for all controls: <20%

Cross-Validation (incurred samples)
- 98% match for ADA status
- 100% titers within 1 dilution

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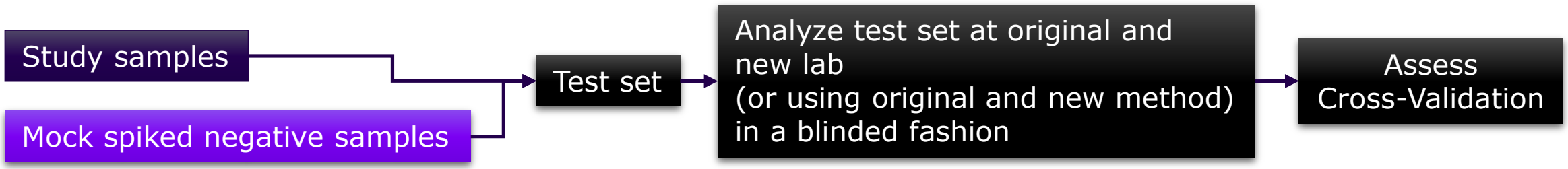
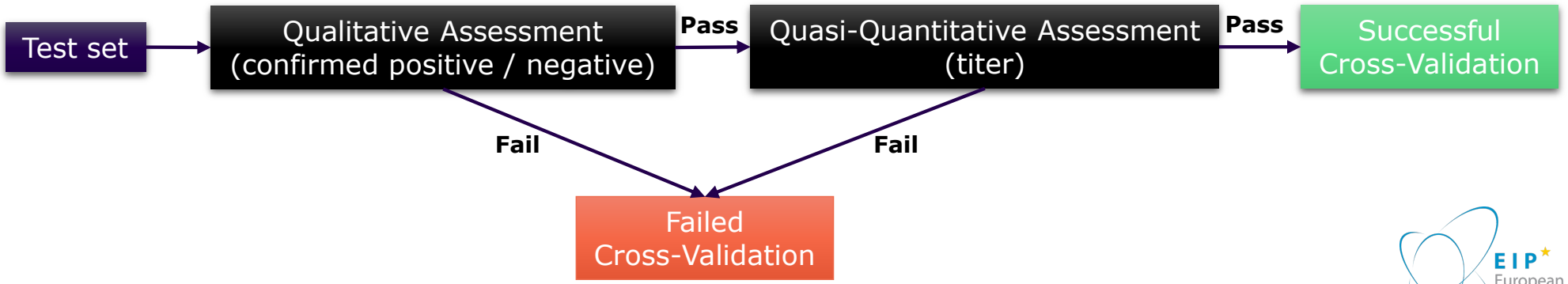
Cross-Validation – Sample Considerations

- Theoretically, it might be best to use real ADA/NAb positive and negative study samples
  - Shortcomings:
    - Proportion of ADA/NAb positive samples is usually significantly smaller than ADA/NAb negatives
    - ADA positive study samples might not cover the full assay range
- A mixture of real study samples and mock spiked negative samples is expected to represent the best compromise
  - Individual negative samples spiked with different concentrations of the positive control can reflect the full range of positivity (borderline to high positive samples)
  - A mixture of study samples and mock spiked samples allows to balance the ADA positive / ADA negative sample ratio



Qualitative- and Quasi-Quantitative Assessment

- The confirmed positive / negative rate of the test set should be compared during the cross-validation between original and new lab (or original and new method)
- It is deemed important to also compare the titer of confirmed positive samples of the test set during cross-validation as the impact of immunogenicity on pharmacokinetics, pharmacodynamics, safety, and efficacy may correlate with ADA titer rather than incidence



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Qualitative Assessment

- The qualitative status (confirmed positive or negative) of how many samples of the test set will need to concur to pass cross-validation?
- Statistical approaches are considered providing an objective criterion
  - Cohen´s Kappa Test is widely used to assess agreement between two categorical variables
    - <0.2: poor agreement
    - 0.21-0.6: moderate agreement
    - 0.61-0.8: substantial agreement
    - 0.81-1: almost perfect agreement

Potential threshold

Lab B	Lab A		
	Positive	**Negative**	**Total**
Positive	P11	P12	**B1**=P11+P12
Negative	P21	P22	**B2**=P21+P22
Total	**A1**=P11+P21	**A2**=P12+P22	**N**

$$\kappa = \frac{p - e(\kappa)}{1 - e(\kappa)}$$

$$p = \underbrace{\frac{P11 + P22}{N}}_{\text{Overall agreement propensity}}$$

$$e(\kappa) = \underbrace{\frac{A1}{N}\frac{B1}{N}}_{\text{Chance that both labs independently classify AB as « Positive »}} + \underbrace{\frac{A2}{N}\frac{B2}{N}}_{\text{Chance that both labs independently classify AB as « Negative »}}$$

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Sample Size of the Test Set

- Number of samples within the test set should also be based on statistical considerations
- Sample size is depending on the selected threshold for the Cohen´s Kappa coefficient
- Using a test set of 90 samples (45 positives / 45 negatives) and a threshold of (at least) 0.80 for the Kappa coefficient, allows to demonstrate that the Kappa coefficient is significantly higher than 0.6 (indicating substantial agreement) with 83% power

Quasi-Quantitative Assessment

- Options to assess titer comparability of the test set
  - X% of titer results need to be within ±2 titer steps
    - Employs the same criterion used to determine a treatment-boosted response
  - X% of titer results need to be within the number of titer steps determined by the minimum significant ratio (MSR) of the original assay (MSR=$10^{2\sqrt{2}\times SD}$)
    - Titrate High Positive Control (1 run, up to 10 titration curves)
    - Determine endpoint titer
    - Standard deviation (SD) of the endpoint titer used to calculate MSR
- Based on internal Sanofi experience, a criterion of 80 % of titer results within ±2 titer steps seems to be a reasonable criterion

	HPC titer
	800
	1600
	800
	1600
	800
	800
	800
	1600
	800
	800
Mean	**1040**
SD	**386**
MSR	**3**

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Summary & Conclusion

- Cross validation of immunogenicity assays should always be considered if methods or labs are changed within a clinical trial or across pivotal clinical trials
- Similarity of major validation parameters is necessary but not sufficient to indicate successful cross-validation
- The test set for cross-validation might consist of real study samples supplemented with mock spiked negative samples
  - The sample size of the test set should be based on statistical considerations
- A tiered approach for cross-validation of ADA assay should be employed consisting of
  - A qualitative (confirmed positive / negative) comparison
  - The comparison of titer values (after successful qualitative comparison)
- Cohen´s Kappa test is offering an objective criterion to assess qualitative cross-validation
- Concordance of titer values might either be assessed using the MSR or the “±2 titer steps” criterion

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THANK YOU!!!!!

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