Reference Standards: Overview and Strategy for Development to Commercialization

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CASSS / WCBP CMC Strategy Forum: Reference Standards For Therapeutic Proteins
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Pfizer
WORLDWIDE RESEARCH & DEVELOPMENT
BioTherapeutics Pharmaceutical Sciences

Topics Covered

- Review of guidance and regulations
- Roadmap (timing, logistics and lessons learned)
- Characterization Techniques
- Reference Standard Monitoring and Recertification

ICH Guidance on Reference Materials

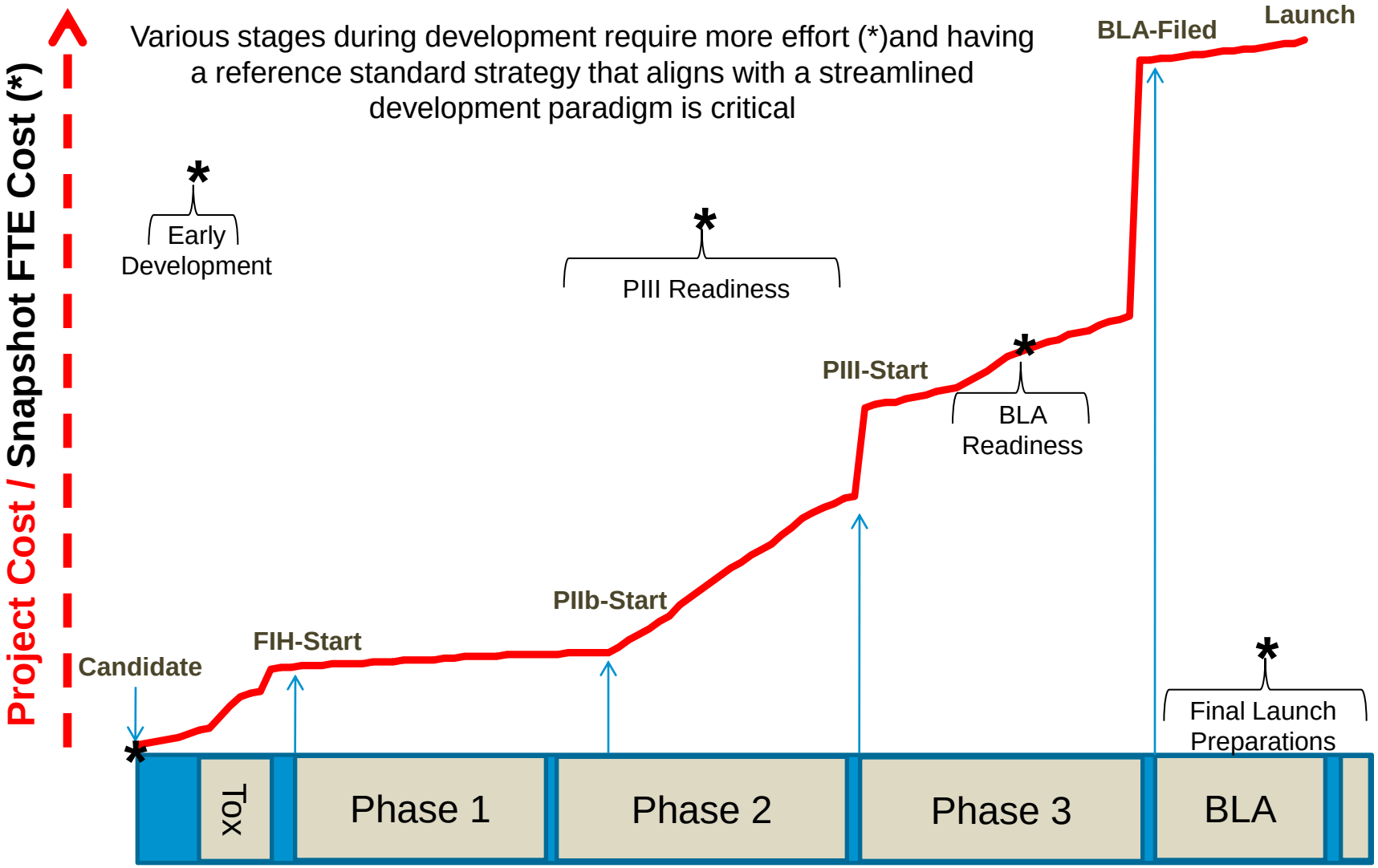
"For drug applications for new molecular entities, it is unlikely that an international or national standard will be available. At the time of submission, the manufacturer should have established an appropriately characterized in-house primary reference material, prepared from lot(s) representative of production and clinical materials. ….which will serve for biological and physicochemical testing of production lots."

"The results of biological assays should be expressed in units of activity calibrated against an international or national reference standard, when available and appropriate for the assay utilized. Where no such reference standard exists, a characterized in-house reference material should be established and assay results of production lots reported as in-house units."

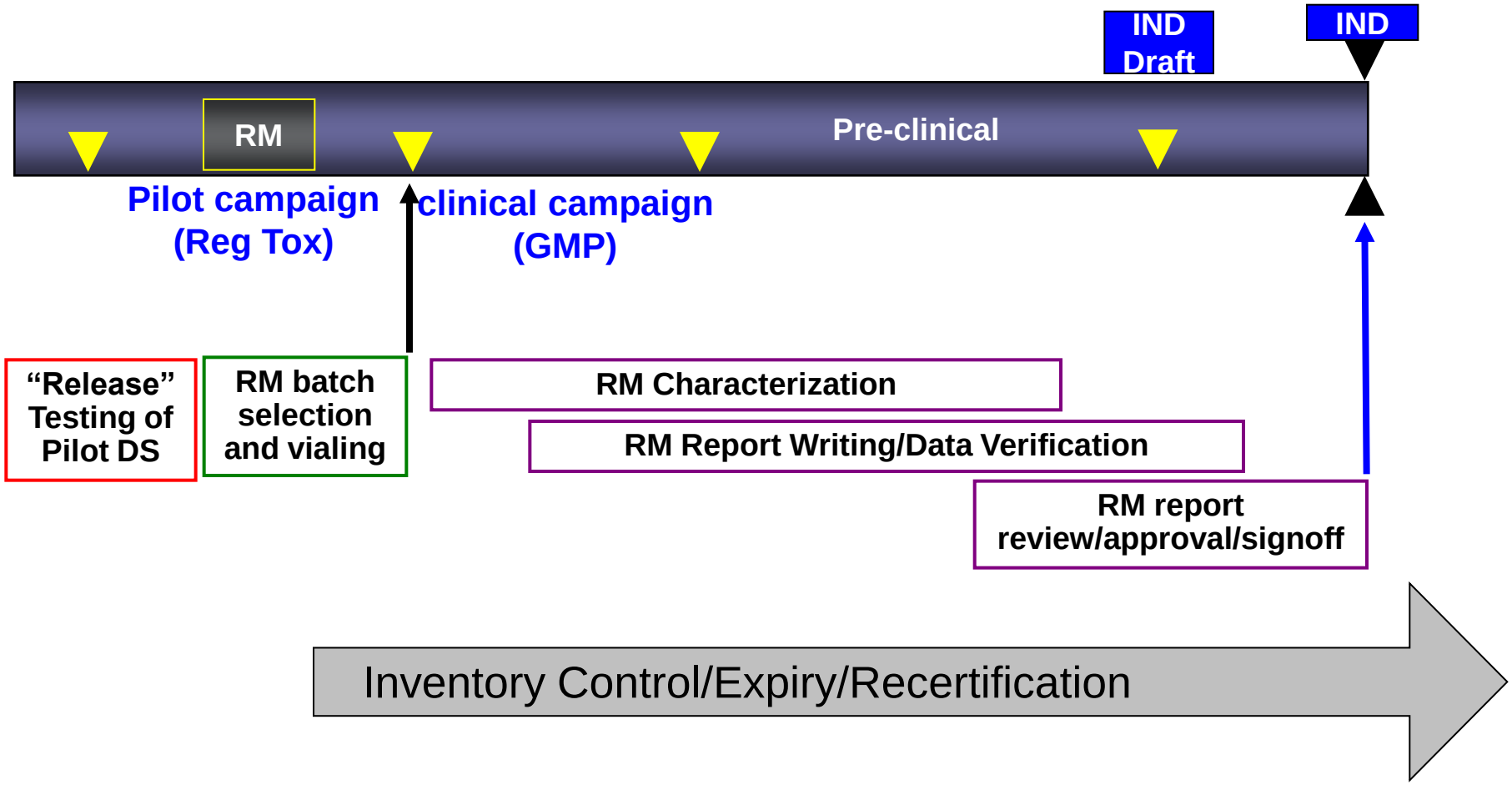
Reference Standard Strategy is a Key Process During Development

- Minimize the number of reference standards during development
- Alignment of cell line and process development is critical
- Maximize the use heightened characterization data of Reference Standard in filings by using it in S.3.1 Elucidation of Structure
- Reference Standards are the bridge back to non-clinical data
- Reference Standard is always used during comparability exercises during development

Alignment of Reference Standards and Development Paradigm



Early Development Roadmap

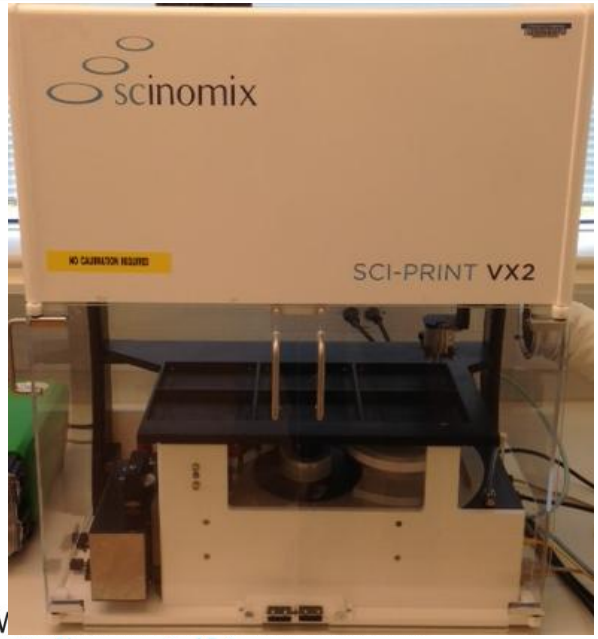


Early Development Project Logistics

- Drug Substance batch(s) used in non-clinical studies is established as the first reference standard
  - Pre-clinical batch(s) use Pre-MCB
  - Platform manufacturing and purification same as GMP batch but smaller scale
- Approximately 10 g of material will be required for reference standard (e.g., 4000 vials at 2.5 mg/vial); actual amount will depend on product (concentration, etc.)
- Reference standard is diluted if the concentration is greater than 50 mg/ml
- Initial reference standard is used to release clinical batches, establishing comparability between Toxicology and Clinical batches
- Data generated during reference standard characterization will be used to populate S.3.1 in regulatory filings

Early Development Process: Filling, Storage and Maintenance

- Reference is filled, stored and maintained and distributed by the QC/Stability group
- Pedigree of Drug Substance batch(s) is maintained for all reference standards
- Filled in PP cryrovials and stored at -70C.
  - Extractable and leachable studies were conducted on vial types to determine suitability.
- Automated or manual filling and labeling is utilized

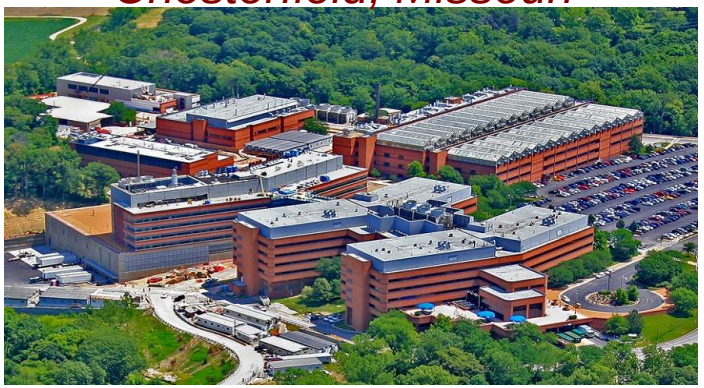


Early Development Process: Filling, Storage and Maintenance

- Homogeneity/Uniformity testing is required on filled material to establish concentration
  - Concentration typically determined by A280 and AAA used to confirm
- Reference Standard is monitored on stability as a special arm / condition or tested for recertification
  - 24 month initial use period is given to reference standards
- Liquid stability is established by leveraging formulation development data
- Minimum of 100 reference standard vials are maintained through out development

- BioTherapeutics Development Reference Standards are managed in Chesterfield, MO
- 40 Reference Standards filled annually
- Approximately101 Active Reference Standards equivalent to ~290K vials

Chesterfield, Missouri



LIMS System is Critical in Managing Development Reference Standards

Development reference standards are managed in PFE LIMS in order to provide a single global repository

Benefits include:

- Enhanced compliance for approvals, inventory control and tracking
- One interface for all ref std tasks:
  - Testing, requests, distribution, inventory, certificates
- Global inventory and ordering
- Auto-notification for standard requests and distribution
- Auto-notification for low inventory levels
- Electronic Signatures for authorizing & activating
- Enhanced ability to query, track and monitor re-evaluation dates

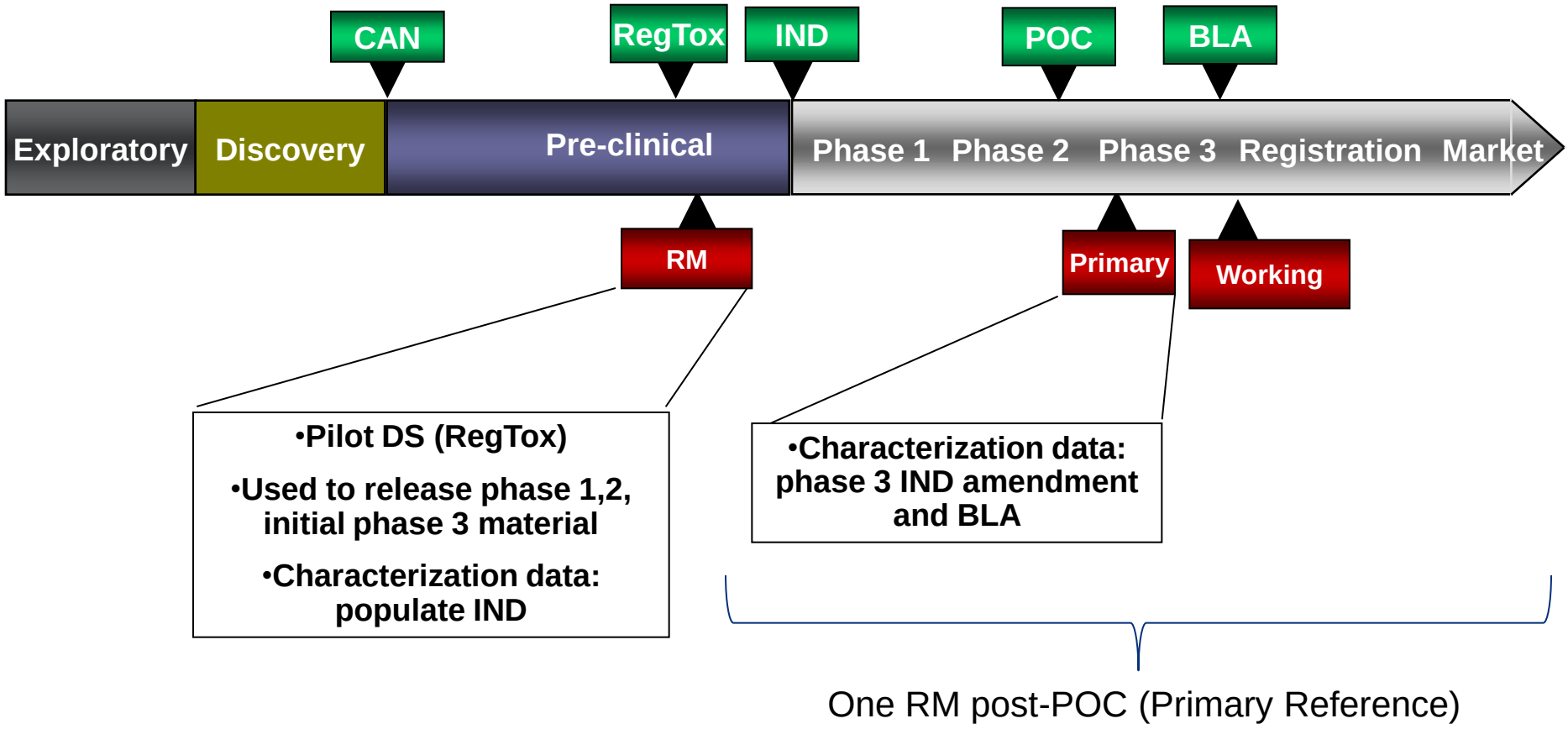
Reference Standard Characterization Data Populates Elucidation of Structure in IND

Attribute	Analytical Approach	Methodology
Primary structure	Confirm primary structure and identify posttranslational modifications at the peptide level	Liquid chromatography/mass spectrometry (LC/MS) – peptide mapping of reduced and alkylated proteolytic fragments
Posttranslational modifications – N-linked oligosaccharides	Confirm N-linked oligosaccharide structures and relative abundances	Hydrophilic interaction liquid chromatography (HILIC) of released, 2-aminobenzamide (2-AB)-labeled N-glycans
Posttranslational modifications – Disulfide bonds	Determine level of unpaired protein sulfhydryls	Ellman's assay
Molecular mass	Confirm primary structure, posttranslational modifications, and multi-chain architecture of the intact molecule, and identify the major and minor product isoforms	Electrospray ionization mass spectrometry (ESI MS)
Molecular charge	Determine charge heterogeneity	Imaged capillary electrophoresis (iCE)
Higher order structure	Assess secondary structure	Far-UV circular dichroism (CD)
Aggregation and fragmentation	Determine levels of monomer and high molecular mass species (HMMS), including aggregates, and characterize molecular form of the HMMS	Size exclusion HPLC (SE-HPLC), confirmed by multi-angle light scattering (MALS) and analytical ultracentrifugation -sedimentation velocity (AUC-SV)
	Determine levels of low molecular mass species (LMMS), including fragments	Capillary gel electrophoresis (CGE)
Biological activity	Assess target binding	Enzyme-linked immunosorbent assay (ELISA) or Cell Base Assay

Post Proof of Concept Reference Standard is the Primary

- Post POC reference standard process is the same as early development process
  - LIMS
  - Automated or manual filling and labeling utilized
  - Stored and managed by Pharmaceutical Science until commercialization
- Post POC is the Primary Reference Standard
- The commercial working reference standard will be compared to primary reference to release commercial supplies

Reference Standard: Timeline



Conclusions

- Pfizer's BioTherapueitics Reference Standard Program complements the development strategy
- Reference Standard management is systems and process based due to number of projects in portfolio
- Maximize the use of heightened characterization data gained on reference standards in filings
- Approach is consistent with industry best practices

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Thank you to the CASS/WCBP Organizing Committee

Abstract

Reference standards play a critical role in characterization, comparability, lot release and confirmation of stability for therapeutic products. Different organizations create and utilize such standards in different ways during development, which are sometimes unique to individual organizations. Pfizer BioTherapeutics Pharmaceutical Sciences has established a reference standard program that meets the current regulatory guidance and expectations. In this presentation I will highlight the current reference standard process utilized during development, point out lesions learned along the way and demonstrate that Pfizer's approach is aligned industry best practices.

The guiding principles critical in developing the current reference standard approach are the following:

- Minimize the number of reference standards during development . Maintenance, support and management of reference standards over a large portfolio takes a large number of FTE's and adds to the overall cost of development.
- Assurance that cell line and process development groups are aligned and able to support the minimal reference standard approach.
- Maximize the use of heighten characterization data gained on reference standards for use in filings.
- Reference standards are the bridge back to non-clinical data and are always used during comparability exercises during development.

Note: BioSimilars and Vaccine, while they follow similar guiding principles, are out of scope for this presentation