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Perspective

Roadmap for Drug Product Development and Manufacturing of Biologics

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ABSTRACT

Therapeutic biology encompasses different modalities, and their manufacturing processes may be vastly different. However, there are many similarities that run across the different modalities during the drug product (DP) development process and manufacturing. Similarities include the need for Quality Target Product Profile (QTTP), analytical development, formulation development, container/closure studies, drug product process development, manufacturing and technical requirements set out by numerous regulatory documents such as the FDA, EMA, and ICH for pharmaceuticals for human use and other country specific requirements. While there is a plethora of knowledge on studies needed for development of a drug product, there is no specific guidance set out in a phase dependent manner delineating what studies should be completed in alignment with the different phases of clinical development from pre-clinical through commercialization. Because of this reason, we assembled a high-level drug product development and manufacturing roadmap. The roadmap is applicable across the different modalities with the intention of providing a unified framework from early phase development to commercialization of biologic drug products.

Introduction

The discovery and development of therapeutic biologics, especially antibodies and engineered antibody modalities, has expanded in recent decades to more than 100 monoclonal antibody (mAb) biologics that are marketed and approximately 1000 investigational molecules are currently in the clinic globally.[1,2] Developing a high quality biologic therapeutic drug from early stages of discovery to a marketed product is a highly expensive endeavor, with the average investment being further compounded by the high attrition rates in clinical development.[3] The clinical and commercial success of a biologic ultimately depends on its safety, efficacy and immunogenicity profile in human patients[4] for which the drug product must be repeatedly produced at consistently high quality, efficiency and reduced cost.[5,6] In this publication, we outline the streamlined approach or a roadmap for the formulation and drug product development and manufacturing from early stage to process validation and commercialization. The roadmap is developed based on experience with approved multiple protein and monoclonal antibody products, however, should be applicable across all biologic therapeutic

Abbreviations: AAV, adeno associated virus; ADC, antibody,drug conjugates; BLA, biologics license application; BPOG, BioPhorum Operations Group; CDMO, contract development and manufacturing organization; CE, capillary electrophoresis; cIEF, capillary iso electric focusing; CMC, chemistry, manufacturing and controls; CPP, critical process parameters; CPV, continued process verification; CQA, critical quality attributes; CSTD, closed system transfer device; DEHP, di,2,ethylhexyl phthalate; DOE, design of experiments; DP, drug product; DS, drug substance; ELISA, enzyme,linked immunosorbent assay; EMA, European medicines agency; FDA, U.S. Food and Drug Administration; FMEA, Failure Mode and Effects Analysis; GMP, good manufacturing practices; GRAS, generally regarded as safe; HCP, host cell protein; IEX, ion,exchange chromatography; ICH, International Council for Harmonization; IND, investigational new drug; IV, intravenous; mAb, monoclonal antibody; PAI, Pre,Approval Inspection; PC, Process Characterization; PFS, Pre filled syringes; PTM, post translational modifications; PVC, polyvinyl chloride; SE HPLC, size exclusion high performance liquid chromatography; PAGE, polyacrylamide gel electrophoresis; PPQ, process performance qualification; QbD, quality by design; QC, quality control; QTPP, quality target product profile; RP, reversed phase; SC, sub, cutaneous; SDS, sodium dodecyl sulfate.

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products. The following sections provide clarification around these concepts, while providing additional information and resources.

Defining the roadmap for drug product development

The aim of biopharmaceutical development is to design a quality product and manufacturing process to consistently deliver the intended performance of the product and meet the needs of the patient.[7] The challenges related to the formulation and DP development can be addressed through properly designed studies outlined in the roadmap as delineated in Table 1 and discussed in more detail in the following sections. The roadmap was designed based on a Quality by Design (QbD)-like approach and experience from developing drug products at the clinical and commercialization stages, regulatory guidance's to industry from worldwide regulatory agencies, and recent reviews on manufacturing and DP development. While the number and types of biologics have significantly diversified over the past two decades encompassing monoclonal antibodies, antibody derivatives, Fc-conjugates, cytokines, bi-specific antibodies, antibody drug conjugates, pegylated enzymes, nucleic acids (e.g. mRNA) often in combination with a drug delivery system (e.g. lipid nanoparticles, virus like particles), viral vectors, and cellular based systems, the essential studies from pre-clinical development to commercialization are similar. Indeed, many of the guidance documents from regulatory authorities are not specific to the modality being studied and are adopted based on the needs of a program.[7–14] In some cases such as gene and cell therapies, additional guidance is available from the FDA, and provides additional clarification.[15–17]

Central to defining the roadmap is a recognition of common challenges encountered during development of the different DP formats, such as liquid and lyophilized drug products in vials and pre-filled syringes. Common issues are highlighted in Table 2 based on the examples of therapeutic proteins. However, those issues are relevant to most drug products, albeit the relevancy and level of understanding will be specific to the molecule being studied. To enable early clinical studies, a platform formulation and manufacturing process, requiring minimal development, may be used in clinical phases I and II.[18] Novel modalities, where little prior-art knowledge and no platform formulation is developed yet, may require more extensive formulation studies for early clinical phases I and II. As product development progresses to the clinical phase III and commercialization stages, additional studies are performed based on alignment with updates to the QTPP to define an optimized formulation for maintenance of drug quality and stability during commercial manufacturing, storage, and clinical administration.[18,19] An important aspect of the DP development studies is drug substance (DS) process and the resulting quality/purity of the DS. The source of the materials for the sequence of studies used for the DP development and characterization are highlighted in Fig. 1 on the example of a mAb. A close relationship between the process development team and the formulation team is essential, to continually understand what modifications to the process may affect DP quality and stability, including modification of both upstream production and downstream purification. Changes to upstream production such as fed-batch to intensified fed-batch to continuous process production for mAbs can all affect impurity profiles, introducing changes in the host cell proteins, such as peptidases and esterases, that can affect the long-term stability of the product. For cell and gene therapy products changing from plasmid-based production to stable cell-line based production, and during scale-up from small batch 2 L fermenters to ≥ 2000 L fermenters, can have large effects on the impurity profiles. Seemingly minor changes to downstream processing related to changes in column suppliers, column purification conditions, changes in filter types, filter material of construction, and chemical suppliers, can all introduce changes in the impurity profile of the host cell protein and chemical impurities, such as metal contamination may affect long term stability. For these reasons, formulations developed with early material should be verified with final process material for the appropriate stage of development by performing bridging studies. During pre-clinical/phase I development, ideally the formulation is confirmed with final process material from the toxicology lot, while development performed during clinical phase II is confirmed with the Pivotal material and during the PPQ stage prior to commercialization.

In the case of accelerated registration, Phase II may not be required and proceeding from phase I to pivotal is common, especially for critical oncology drugs for unmet indications. In such cases, the process and formulation teams can consider combining the studies such as formulation development and robustness studies along with process characterization of the CPPs and worst-case process conditions.

Pre-Clinical/Phase I Development

The early stage of development for a biological drug product is defined by multiple activities with the goal of submitting an Investigational New Drug (IND) application to the regulatory authorities and gaining approval to initiate Phase I clinical trials. These activities include development of a manufacturing process for the drug substance, development of a formulation and design of the drug product, analytical development, pre-clinical toxicology and safety studies in animals, cGMP manufacturing of the DS and DP, writing of the common technical document and submission of the IND application to the regulatory authorities for review and approval to initiate the clinical trials. As outlined in Table 1, the drug product development roadmap encompasses a subset of these activities and is discussed in subsequent sections.

Initial QTPP Development

Prior to initiating development activities, it is critical to define the QTPP. The QTPP is focused squarely on the CMC attributes including the intended use in clinical setting, route of administration, dosage form, delivery systems, dosage strength(s), formulation, storage conditions, container closure system, and drug product quality criteria (e.g., sterility, purity, stability and drug release) appropriate for the intended marketed product.[7] Importantly, the QTPP can be used to help select the modality and the final candidate molecule during the early preclinical phase of development. QTPP can incorporate anticipated commercially important product characteristics, though this is not important during early development, and can enable patient centric design of the commercial DP presentation necessary for market acceptance. Given the numerous tools available for designing, incorporating, and selecting desired characteristics into the molecule, significant problems from post translational modifications, aggregation and viscoelastic problems may be avoided. This will allow for greater flexibility of storage, handling and delivery options identified during clinical development. The QTPP is an important communication tool[20] not only within the program teams, but also between industry and the FDA or other regulatory agencies providing clear definition of CMC aspects for the DP. This document is essential for establishing a QbD based development program[18,21,22] and is used as blueprint for establishing the characteristics of the DP that will enable the clinical trial program. Development of the QTPP[7,20] promotes dialog between the product development team and the clinical team, as the drug product characteristics are defined during formulation development. At times these characteristics may impose limits on the clinical trial design, such as insolubility or unacceptable viscosity at high protein concentrations. The QTPP is considered a living document that is continually updated as new knowledge is gained from product

Table 1
Roadmap for biologics drug product development.

Pre-clinical	Phase I	Phase II	Pivotal studies/Phase III	PPQ/ Commercial

• TPP developed by joint project team
• Develop initial QTPP and potential CQA's for Phase I
• Developability study (CMC stage) and/or pre-formulation studies
• Verify if platform formulation and analytical assays (if available) are suitable
• Early formulation study
• Non-GMP DP batch used for animal toxicology studies (Tox Batch): fill-finish, release testing
	• Lead lot stability for DS and DP from Tox Batch material
	• BDS Freeze-thaw and stability monitoring in representative containers
	• Confirm formulation for Phase I and Phase II clinical trials (fast to clinic/ not commercial ready)
	• Syringe and IV infusion in-use compatibility studies
	• Container/closure selection and compatibility for DS and DP
	• Establish the drug product manufacturing process (BDS to DP)
	• Initial definition of in-process control strategy
	• GMP DP batch: Fill-finish, release and stability monitoring
	• Development of matching placebo
		• Refine QTPP and confirm CQA's for DP (Late phase development and commercialization)
		• Define additional analytical methods based on QTPP
		• Oxidation and deamidation studies
		• Initial photostability under forced deg conditions (support Comm. Form Dev)
		• Define and develop formulation for Phase III and commercial use (liquid/ lyophilized/PFS)
		• Define commercial container/closure system
		• Comparison of Commercial Formulation to Phase I Formulation
		• Transportation/stability studies
		• Formulation Robustness (DOE) studies
		• Process Development of DP production
		• Formulation and concentration
		  • Freezing (rate and Tg')
		  • Thawing
		  • Mixing
		  • Filtration
		  • Filling
		• Update in-use stability to support Phase III
		• Microbial challenge studies to support Phase III (lyophilized/IV/syringe)
		• Device compatibility and definition of combination product pathway
			• Refine QTPP (for commercialization)
			• Establish pivotal study and commercial manufacturing site and ensure alignment of DP production with CDMO processes
			• Refine in-process control strategy
			• DP engineering run including mixing homogeneity study using placebo/active
			• GMP DP batch for pivotal clinical studies: Fill-finish, release and stability monitoring
			• Process risk assessment (Hazards or FMEA approach)
			• Process characterization studies
			• Photostability under ICH guidelines and confirmatory use conditions – Single Batch
			• Extractable and leachable risk assessment and studies
			• Expanded syringe and IV infusion compatibility studies
			• Transportation studies for product impact (IQ/OQ studies)
				• Finalize CQAs
				• Ensure alignment of DP characteristics with commercialization target
				• Manufacturing site risk assessment (FMEA) for commercial phase
				• Risk mitigation studies
				• Validation plan finalization
				• Finalize control strategy for DP process
				• Commercial manufacturing site readiness
				• Supply chain and raw material risk assessment and risk mitigation
				• Facilities and equipment qualification
				• DP Process Validation cGMP studies
				• DP PPQ runs at manufacturing site
				• DP Stability – 3 batches with at least one batch in secondary packaging
				  • Continuous process verification
				  • PPQ lot stability monitoring
				• Transport validation using actual shipping lanes
				• Microbial challenge studies
				• Preparation of BLA and Pre-Approval Inspection

Table 2
Common issues during development for different biologics DP presentations.

Liquid formulations	Lyophilized formulations	Pre-Filled Syringes related issues (In addition to issues for liquid formulations)
Formulation and excipient related issues • Design space not defined • Conversion from frozen liquid to a refrigerated liquid • pH shift during freezing • Low concentration formulation (< 200 μg/mL formulation): • adsorption and analytical challenges • High concentration formulations: increased viscosity, higher rates of aggregation/self association, opalescence, liquid-liquid phase separation, increased color, . . . • Polysorbate instability due to esterase activity resulting in subvisible and visible particle formation • Metal contamination (His/Phosphate/Citrate), excipient degradation (polysorbates, His), nanoparticles (sucrose, trehalose), frozen state crystallization (sorbitol, trehalose), protein modification (citrate, succinate) • Inconsistent raw materials impurities **Instabilities of the molecule: example protein** • Increased aggregation, subvisible and/or visible particle formation during FBDS storage and/or freeze-thawing • Aggregation, subvisible or visible particle formation during DP manufacturing (stirring, tubing/pump compatibility), transportation, long-term storage, freezing during storage, In-use stability • Post-translational modifications during storage, leading to decrease in potency, aggregation/particle formation, ADA response, reduced half-life, . . . • Clipping/backbone hydrolysis – enzymatic and non-enzymatic • Photosensitivity **Container closure and device-related issues** • Container incompatibility – glass lamellae formation and delamination due to formulation • Extractables and leachables from container closure system • Device compatibility and new surface interactions **Issues related to application to the patient** • Loss or aggregation during in-use stability studies, IV administration • Injection site pain – switching from IV to SubQ (citrate present, pH) • Reduced solubility at neutral pH (injection site precipitation)	**Formulation and excipient related issues** • pH changes during freezing – formulation issue due to poor choice of buffer salts such as sodium phosphate • Cold denaturation • Exposure of molecule to ice-liquid interphase • Fogging of vials • Less choice of excipients: volatile buffers (e.g. acetate) and excipients that are reducing the Tg' and Tg (e.g. sorbitol) not suitable for lyophilization • Unwanted crystallization of excipients during lyophilization and/or storage (e.g. sucrose) **Instabilities of the molecule** • Loss of activity due to unfolding during freezing and/or lyophilization process • Increased instability (e.g. protein unfolding, aggregation and particle formation after lyophilization and reconstitution and after mechanical stress of lyophilized cakes) **Lyophilization (process) related issues** • Long freeze-drying cycle time for formulation with low Tg' /Tc • Cake collapse, either macro or micro-collapse if Tg'/Tc are exceeded during the process • Non-optimal lyophilization cycle resulting cake appearance issues • Cracking of cakes after lyophilization, during storage and/or shipping • High moisture content leading to increased rates of degradation such as aggregation, oxidation, hydrolysis, . . . • Too low moisture content (overdrying) can result in instability as well • Heterogeneity of crystallinity in the cake including formation of mannitol hydrate resulting in moisture & reconstitution issue • Long reconstitution time especially for high concentration protein formulations • Foaming after reconstitution • Nanobubbles after reconstitution	**Formulation and excipient related issues** • Polysorbate level is too low to prevent protein from binding the silicone oil droplets resulting in an increase in subvisible and visible particles • Polysorbate level is too high resulting in emulsification of the silicone oil and polysorbate resulting in an increased silicone oil droplets level **Components of PFS that can induce instability** • Silicone-oil layer and droplets • Tungsten remaining in the glass neck and potentially reacting with the protein to induce chemical changes, and form subvisible or visible particles • Incomplete curing of the glue can result in PTM's of the protein, such as increased oxidation or adduct formation, for e.g. related to formation of methacrylate • Needle shield or plunger components **Issues related to PFS functionality** • Air bubble size set during vacuum or mechanical stoppering • Leakage of the solution around the plunger due to movement of the stopper/plunger rod during air transportation from reduced air pressure - breaking sterility • Leakage of solution around the plunger due to freezing occurring during transportation • Changes in break loose and glide force during storage - limit glide force to 20 N • Sterilization related changes in surface chemistry of the syringes

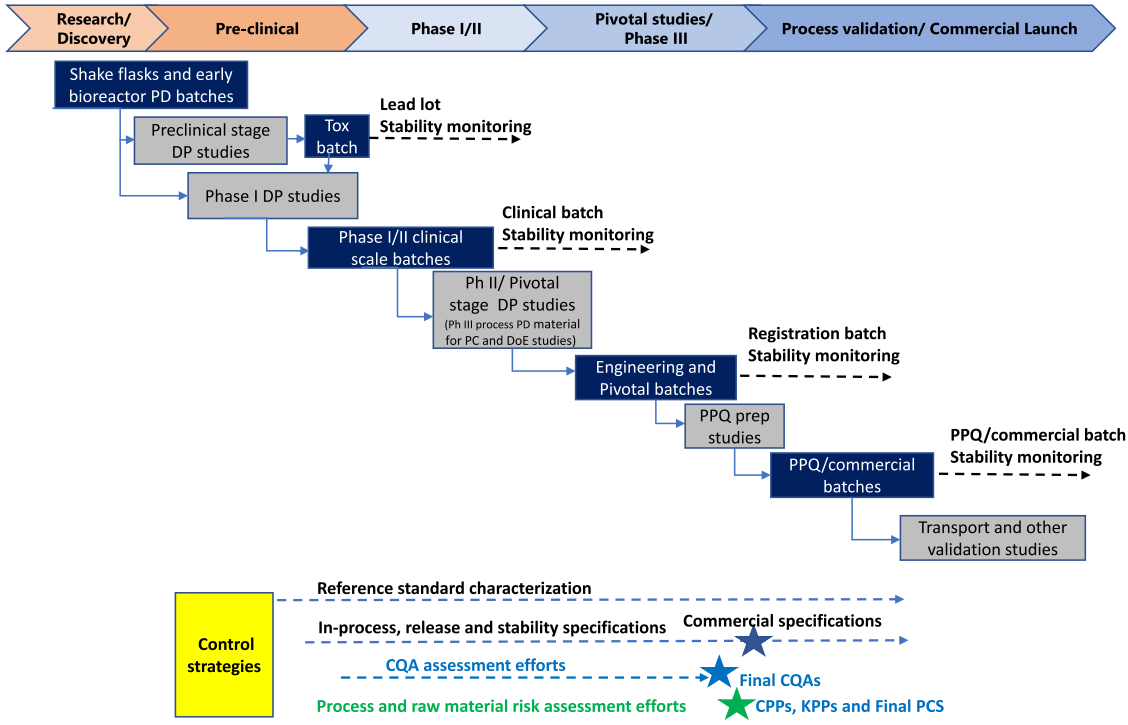

Fig. 1. Timeline and material for biologics drug product development, characterization and qualification studies. Solid Arrows point to sequence of material flow and activities.

understanding during development and clinical trials. An example of a QTPP for a mAb and how it changes between early and late-stage development is shown in Table 3.

Analytical Development

Establishment of the analytical assays for defining the degradation and impurity profile of the DP is often done in parallel with both establishment of the manufacturing process and development of the formulation. The analytical methods themselves are typically defined as those for quality, safety, activity, quantity, and purity including process and product related, and can be subdivided into both product specific and compendial assays. The product specific assays are just as the name suggests and are generally concerned with active pharmaceutical ingredient product quality and activity or potency, including SE-HPLC analysis for protein aggregates,[23–26] analytical ultracentrifugation for empty and full AAV capsids,[27,28] CE-SDS or SDS-PAGE for protein fragments[25,29] and for vaccines and viral vectors,[30] IEX or cIEF for charge heterogeneity related to post-translational modifications[31–33] and for AAV,[34] binding assays for establishing early activity[35–37] followed by functional assays to establish potency.[38–40]

Many companies develop analytical platforms for molecular entities. In case no platform assays are yet established, molecule specific analytical methods need to be developed. Analytical platforms allow for evaluation of new products using established analytical assays. Examples include SE-HPLC,[41,42] reduced and non-reduced CE-SDS,[43,44] and cIEF.[45–47] While binding assays are target specific, the assay itself can be established within a company as a platform such as ELISA or Biosensor platforms[48–50]. At this stage the assays are developed as “fit for purpose” and qualified by the analytical development group then transferred to the quality control group to be used for DS and DP GMP manufacturing, release, and stability studies. Because formulation development will be in parallel with analytical development, a strategy should be in place to demonstrate comparability of the stability using the early analytical assays and qualified assays later in development.

Non-product specific assays which aim at product quality are typically compendial assays and are established by the US Pharmacopeia, the European and Japanese Pharmacopeias and others as appropriate. Assays in this category include color, clarity, visible particulates, subvisible particulates[51,52] pH, osmolality, protein concentration container closure integrity, sterility and bacterial endotoxin and extractable volume. While these assays are not developed as product specific, assays such as sterility, endotoxin and bioburden need to be verified for the product matrix. For some molecules, product specific analytical methods can also be found in the Pharmacopoeias (e.g., RP-HPLC for insulin, polysorbate). Non-compendial, non-product specific assays include polysorbate concentration.

It is important for the formulation development team to use the same platform assays as those being used by the analytical team and demonstrate that the platform assays appropriately detect the intended degradation products.

Formulation Development

Ideally, formulation development is performed in parallel with the development of a manufacturing process, characterization of the molecular entity and establishment of the analytical assays. From the beginning it is recommended that excipient, raw materials, and consumable sources be verified to meet pharmacopeial standards, and that it can be sourced at a suitable grade for later manufacturing. Excipient types suitable for use in formulations can be found in the Generally Regarded as Safe (GRAS) directory maintained by the FDA,[53] the FDA inactive ingredient guide,[54] and the Excipients Browser from UCSF[55] which maintains extensive information on excipients in the FDA databases. Many researchers will try novel excipients not yet approved in commercial products. While these may provide desired DP characteristics the formulation team needs to be aware of the additional development time, cost and regulatory efforts

Table 3
Quality target product profile (QTPP) for biologics drug product development.

Product Attribute	Phase I/II	Phase III/Commercial
Indication	Arthritis	Arthritis
Molecule type	IgG1	IgG1
Route of administration	Intravenous/subcutaneous	Subcutaneous
Dosage form	Liquid, single use	Liquid, single use
Dose	0.1–1.5 mg/kg variable dose	150 mg fixed dose
Protein content per container	100 mg extractable	150 mg deliverable
Biocompatibility	Acceptable toleration on infusion or injection	Acceptable toleration on injection
Drug product presentation	25- 50 mg/mL	150 mg/mL
Primary container	2R type 1 borosilicate glass vials, aminated stopper	1 mL long staked needle glass type 1 pre-filled syringe
Target shelf life	≥2 years at 2 to 8 °C	≥3 years at 2 to 8 °C
Compatibility with Manufacturing Process	Minimum 7 days at 25 °C plus ≥2 years at 2 to 8 °C Okay with 150 mg/mL during UF/DF	1 month at 25 °C plus ≥3 years at 2 to 8 °C Okay with 175 mg/mL during UF/DF
Viscosity properties	≤ 10 cP	< 15 cP, No Injectability issues
Degradants and impurities	Below safety threshold and cleared by process	Below safety threshold and cleared by process
Pharmacopeial compliance	Meets pharmacopoeial requirements, colorless to slightly yellow, practically free of visible particles and meets USP<787> criteria for subvisible particles	Meets pharmacopoeial requirements, colorless to slightly yellow, practically free of visible particles and meets USP<787> criteria for subvisible particles
Aggregate	<5 %	< 4%
Fragments	<5 %	< 3%
Acidic and basic charge variants	<50 %	<40 %
Glycosylation profile	>80 % complex fucosylated; low mannose	>80 % complex fucosylated; <10 % mannose forms
Host cell proteins	<100 ppm	<50 ppm
Osmolality	250 to 380 mOsm/kg range	260 to 340 mOsm/kg range

that may be required, such as extensive animal tox studies, to bring novel excipients to commercialization. The goal at this stage is to get to the clinic as quickly as possible, while providing the clinical team enough flexibility for Phase I safety studies and adapt to Phase II dose finding studies. Because of this the product characteristics will continue to change as the impurity profile of the product is improved with the development of the manufacturing process. Impurities include post translational modifications (PTMs), aggregates,[56,57] process residuals such as protein A from affinity column, host cell DNA,[58–60] plasmid DNA for gene therapy products, as well as host cell proteins[61–63] for protein therapeutics and gene/cell therapy products. Understanding the impurity profile of the product being used for development at any one time is important as these may lead to effects on the stability,[64–66] particle formation,[67] and immunogenic potential,[68] albeit recent analysis of HCPs in mAb preparations suggests the immunogenic risk is low[69] of the drug product. On the other hand, HCPs with lipase/esterase function will hydrolyze polysorbate, which can result in the formation of fatty acid (sub-)visible particles and a loss of functional polysorbate.[70,71] Formulation platforms are also typically used for products during early phase development. For mAbs a typical liquid platform formulation may be 25–100 mg/mL mAb, histidine buffer, polysorbate 80, and isotonic sucrose at pH 6.[72–74] The advantage of a platform formulation at this stage is speed to the clinic with verification of the stability in the platform formulation through a minimal set of studies including early shipping studies[75,76] or agitation studies,[77–79] freeze/thaw study,[80–82] and a frozen (−80 °C and −20 °C) and liquid (2–8 °C, 25 °C and 40 °C) storage study. Storage at −80 °C provides not only reference control, but also preliminary data for drug substance storage. Long term frozen storage of DP significantly reduces the risk of PTMs or shipping induced aggregation. Many companies use development platforms where the formulations are stored frozen at −20 °C or −80 °C, or lyophilized to avoid PTMs during storage, aggregation/particle formation during storage at refrigerated temperature and during shipping of the product to storage depots and clinical sites. However, for many antibody-based therapeutics, platform liquid formulation at 2–8 °C storage from Phase I stage is also becoming prevalent and provides the advantage of gaining clinical experience with this DP presentation from early stages of development.

If formulation development studies are carried out with early process development material and shown to be stable, then production of the toxicology batch may be performed in parallel with long term formulation studies, rather than sequentially, significantly reducing development time.[83] For either new, or significantly modified molecular entities such as Fc-conjugates, mAb-protein/peptide conjugates, or new protein biotherapeutics a more traditional formulation development pathway will need to be followed and in general consists of a pH and excipient screening study, acute studies to understand degradation pathways, solubility and aggregation propensity, freeze/thaw, shipping/agitation studies and frozen/liquid storage studies.[84–88]

In the case of formulation and process development of antibody-drug conjugates (ADCs) the three components making up such a molecule should be considered, namely the antibody DS material, linker drug DS material and final DP material. Stability profiles of the antibody can be different once the linker drug is conjugated. Heterogeneity of the conjugation can result in issues such as increased aggregates and loss or hyperactivity of the molecule. Formulation aspects for cell therapy are not that evolved and involves the cell storage solution which has different amounts of dimethyl sulfoxide (DMSO). Formulation aspects for gene therapy follow the principles of the mAb formulations. Grossen et al.[89] provide an excellent review of formulation and development for recombinant AAV.

Consideration should also be given to establishing a particle profile early in development and continue to characterize the profile through to commercialization due to their potential impact on immunogenicity.[171] While microscopy and light obscuration are the compendial methods for defining numbers and sizes of particles, collection of data by orthogonal techniques such as flow imaging and background membrane imaging are encouraged by the FDA early on in development and can provide information on the root cause for the particles. Reasons for rejects during DP fill and finish operations are cosmetic, vial/stopper/crimp defects, and intrinsic and extrinsic

particulates in the container. Characterizing the propensity of the molecule to form insoluble aggregate or particles, conditions that facilitate their formation, and the particle characteristics may lead to identification and mitigation strategies.

To proceed through development faster, ensure compatibility of the formulation with the storage container and reduce DP manufacturing costs, companies can use a pre-defined container/closure system, which is compatible with and previously qualified for use with fill lines at the intended CDMO or DP manufacturing site. The most common vials are ISO 2R, 6R and 10R glass type I with either 13 mm or 20 mm fluoropolymer coated elastomeric stoppers. The vials can be obtained in tubs pre-washed and depyrogenated, ensuring they have undergone the same treatment as would be used for the DP manufacturing process. Stoppers can be obtained pre-washed and ready to sterilize prior to use, again ensuring that a company only needs to minimally process the components prior to use and mimicking the manufacturing process.

In-use compatibility studies

Depending on the intended target organs and delivery route the Phase I clinical trials may be initiated with IV, intraocular, intrathecal, subcutaneous or other appropriate route if feasible at this stage and as defined by the QTPP. Prior to using DP in the clinic, in-use compatibility, and stability studies with the appropriate delivery system such as IV diluents and delivery systems, closed system transfer devices (CSTD), and syringe components are necessary to ensure the safety and delivery of the active pharmaceutical ingredient. While there is only minimal regulatory guidance, two publications from industry working groups recently published industry approaches to in-use stability and compatibility of biological products covering both clinical development and commercial use.[90,91] For IV delivery, the steps necessary for preparation of the diluted DP in the IV bag, types of IV bags, acceptable storage time and temperature, priming of the IV line, types of in-line filters and delivery time are described in the Pharmacy Manual. At the early stage of development IV infusion compatibility studies should be minimal and demonstrate compatibility with either saline and/or dextrose with a single representative IV bag/infusion set. In rare cases, an additional diluent stabilizer solution will need to be developed to reduce adsorption. PVC is the most common material of construction for an IV bag, albeit non-PVC bags are also being used. CSTDs are increasingly being used in pharmacies in the US for preparation of product in the IV bag, because of USP<800> Hazardous drugs —handling in healthcare settings. Biologics (even if nontoxic) are falling under the category of USP<800>, if they are antineoplastic drugs. While CSTDs from different manufacturers have common component categories, their design and materials of construction are highly variable and may result in numerous problems including shedding of particles, aggregation, interfacial stress, adsorption, and large hold-up volume.[92–94] If a CSTD is to be used during Phase I, it will be necessary to work with the individual pharmacy or clinical sites to define the CSTD used at the site, assess compatibility of product with that CSTD, and if deemed necessary test the CSTD with the DP. Because of the variability in CSTDs from manufacturer to manufacturer, it is not possible to generalize testing of the DP against materials of construction as is done for IV delivery systems. Syringe compatibility studies are also needed to ensure stability prior to delivery to the patient. It should be emphasized that following preparation of the IV bag, they should not be transported by pneumatic tubes within a hospital or clinic as this has been shown to cause extensive particle formation for some products in IV bags.[95] Additionally, if the pharmacy is separate from the clinic, product transport studies of the prepared product may be needed to ensure stability from dose solution preparation to delivery to the patient bedside. If microbial challenge data is not available, the storage time in IV bags or syringes will be limited to 4 h at room temperature,[96] although the time may be longer depending on the country and regulatory authority. It is also possible to leverage microbial challenge studies with formulation platforms allowing for a single study to be used across different development programs to support a longer IV bag hold period.[172]

GMP DP Manufacturing and Stability Monitoring

At this stage the DP should be designed so that no special process requirements are needed to fill the DS into the primary packaging material. The easiest pathway here is to make formulated bulk DS directly into formulated bulk DP by the following process: formulated bulk DS, mixing, bioburden filtration, transfer hold in a different vessel, sterilizing filtration to produce formulated bulk DP, filling, stoppering, capping, and visual inspection. Most CDMO's have specific container/closure systems qualified on their lines and by aligning with them during development an engineering run is usually not necessary. If needed, a dilution step can be incorporated so that the more concentrated DS can be used for Phase Ib or Phase II. The control of critical steps is important to ensure quality of the final manufactured DP. Typical testing for material being manufactured for early phase studies are bioburden control, protein concentration after mixing or dilution, integrity of the sterilizing filters, fill weight checks to ensure adequate extractable volume, appearance testing after DP manufacture, sterility, and container closure integrity testing to ensure sterility of the DP during storage.

The toxicology batch can also serve as the lead lot for stability of the Phase I supplying GMP batch if there are no meaningful changes in formulation and process from the toxicology to GMP manufacturing process. To extend the retest date so that it is sufficiently far ahead of the DP used in the clinical trials, a non-GMP lead lot such as a toxicology batch made using the same formulation and process as the GMP lot and stability monitored using the same qualified assays as those for the GMP can be used. Using the non-GMP lot from the tox material should provide for at least an additional 3 months of stability ahead of the GMP lot and align release and stability of the tox lot with that for the GMP lot. In such a scenario, the IND can be filed with at least 1 month of stability from the GMP DP lot and leverage the longer stability period of the toxicology lot as supporting lot information for extending the shelf-life of the GMP lot during the clinical studies.

Phase II Development

Phase I process and DP format can be used for Phase II studies. Late-stage DP development for a biologic is defined by multiple activities divided across three phases being Phase II, pivotal studies/Phase III, and PPQ/Commercial launch as defined in Table 1 and Fig. 1. The activities are much more labor and material intensive than that for early-stage development due to multiple factors including redesign of the formulation to ensure quality, potency and safety while satisfying multiple demands for patient comfort and compliance, manufacturing, distribution, and regulatory requirements. Ultimately, the studies executed, and the information obtained is designed to provide a comprehensive data and information package for DP sections of the BLA.

QTPP and Analytical Methods

The commercial DP, being the formulated DP in its final container closure and delivery device, is introduced with the initiation of the Pivotal Phase III clinical trial (Fig. 1). For this to occur the DP requirements outlined in the QTPP need to be aligned with updated characteristics for commercialization including the final design of the DP, handling

and delivery and marketing and sales expectations as early during Phase II as possible. For a home administered product patient compliance and comfort can be significantly enhanced by use of a PFS/autoinjector combination or other on-body delivery device, along with an increase of the room temperature stability which allows for ease of handling by the patient. These types of patient centered considerations are the basis for patient centric design which is necessary for a successful product. For example, if the disease is RA, then a convenient PFS will be required. For oncology, in contrast, IV may be acceptable. Anticipation of potential characteristics enabling the commercial formulation and container/closure system should be finalized by the mid-point of the Phase II trials to coincide with development of the final downstream process allowing for sufficient long term stability data to be collected prior to DP engineering and clinical batch production. This places a burden on the DP team to begin development of the commercial formulation during the Phase II dose finding clinical studies without full knowledge of the commercial target. However, information from similar products already on the market, the Phase II study design, animal modeling studies and information from the marketing team can be used to design boundaries for the formulation, as well as define the target container/closure strategy. An example of this is shown in the Quality TPP of Table 3 demonstrating the transition from early-stage product requirements to late-stage requirements of a liquid DP for a mAb. Notably, the dosing has moved from weight based to fixed dose and the target dose has been set at 150 mg. For a home administered product by subcutaneous injection this translates to a concentration of 150 mg/mL setting the new concentration target for the product with the ability to increase the product concentration to 175 mg/mL or greater allowing for overconcentration and recovery of material from the process. Coincidental with this is greater knowledge of process capabilities such that quality attribute targets can be tightened leading to a safer product. Delivery devices are important to define at this time, i.e., a PFS, a PFS/autoinjector or an on-body device among others, as these will introduce new materials of construction leading to new contact surfaces for the DS and possibly modification of the formulation excipients. Additionally, these examples are considered combination products which require additional regulatory guidance.

Coincident with defining attributes for the commercial DP, it is important to refine critical quality attributes (CQAs) to both measure and control them as possible by the formulation design. Depending on the commercial needs of the product such as increased storage time at room temperature, quality attributes may become CQA's if their quantities increase and affect the potency or safety of the product. Based on the CQA's, information from the Phase I stability studies, and the commercial container/closure system additional or new analytical methods may require development, such as changes in the break loose and glide force of the plunger in a PFS, changes in surfactant stability over time, silicone oil microdroplets in PFS, or post-translational modifications leading to changes in potency such as oxidation, isoaspartate formation or deamidation. While isoaspartate formation and deamidation are intrinsic to the molecule structure and affected primarily by sequence context and pH,[97,98] oxidation can come from a variety of sources including vapor phase hydrogen peroxide used for sterilization of isolators and fill/finish equipment,[99] reactive oxygen species formation due to metal contamination and reaction with polysorbate,[100,101] and reaction of various excipients with oxygen.[102,103] These should be assessed as early as possible. Additional assays developed for the formulation development studies should be done in cooperation with the analytical development team since validation will eventually be required in a QC environment. If assays used for formulation development deviate from those developed for the QC labs, plans should be designed for demonstrating comparability of the methods to avoid changes in the stability profiles of the development and clinical lots.

Formulation Development

The formulation developed during the pre-clinical/ Phase I stage was designed for speed, the formulation developed during the Phase II stage is designed for use in the pivotal or phase III clinical studies and commercialization of the product making the requirements for stability much more stringent, and patient centric design much more important. Except for cell-based therapeutics and some viral vectors, the expectation for most biologics is storage at 2–8 °C for a minimum of 2 years with additional stability for up to a month at room temperature. This translates to a much greater possibility of subvisible and visible particle formation and increases in post-translational modifications which may lead to reduced potency or formation of particles. Interactions with new surfaces from containers and delivery devices will also need to be accounted for. Ideally, the product is in its final container closure with a delivery device such as an autoinjector for a PFS or an on-body device in conjunction with a vial. The reality is that the dose will not be known, subcutaneous vs intravenous may not be known, and the use of a delivery device will likewise be undecided at this point. If any of these are known, the information can be incorporated into the formulation and DP design; if not, then the formulator will need flexibility and patience. For design of formulations the reader is referred to several good reviews for proteins,[87,104–108] high concentration mAbs,[109,110] cell based therapies,[111,112] Lipid Nanoparticles[113] and viral vector based therapies[81,89,114] so will not be described here. Recent publications summarizing excipients and their classes found in formulations,[115,116] excipients used for mAbs and high concentration mAbs,[73,74] publications on quality by design concepts,[117–119] and drug product process development to ensure GMP scale manufacturing readiness[99,120–123] may be of use as well. For mAbs the most common formulation redesign is an increase in concentration to accommodate higher doses, and use of a pre-filled syringe with an autoinjector for self-administration. The increased doses are driving concentrations towards 200 mg/mL and greater and pushing the previous limits of subcutaneous administration from 1.5 mL towards 2 mL and greater which can necessitate the use of non-standard PFS or on-body injectors for patient convenience. All of which can increase the development time in multiple ways and will need to be accounted for in the development timelines. Development of a PFS will require additional studies including stability in the presence of silicone oil, additional surface contact interactions, bubble size, etc. .[124–128] Newer PFS systems eliminate the use of silicone oil or use a baked on or crosslinked silicone oil system and should be explored during development along with the evaluation of material supply chain and availability of fill/finish CDMO's which can accommodate these newer systems. As the new Phase III/commercial formulation is developed it is important to compare those formulation to that used during the Phase I clinical studies. Significant changes in the stability profile may lead to changes in the clinical safety signals and this should be avoided. If differences are unavoidable depending on the concentration, time/temperature profile, container/closure system, etc. these should be fully understood and discussed with the clinical and regulatory teams. Depending on the degree of change to the formulation from that used in the Phase I/II studies, a cross-over or bioequivalence clinical study may be needed prior to the start of Phase III clinical trials.

Additional Studies Supporting Formulation Development

A variety of other studies may also be needed in addition to the reformulation including a transportation study, a photostability study, an updated in-use stability study, additional container/closure system studies, and possibly a device compatibility study. Development of a matching placebo may need to be included as well, depending on the DP characteristics, and planned clinical protocol. While preliminary

transportation or simulation studies may have been performed it is important during development of the commercial formulation to incorporate a generic transportation study with defined vibration and shock to simulate real world shipping lanes and ensure definition of a formulation which can maintain DP stability.[129] Here it is important to either have a group within the company that has a validated shipping protocol or to work with a company that has a validated shipping simulation process with experience on the impact of different stresses encountered during shipping on product quality of biopharmaceuticals. These studies, combined with freeze/thaw and long-term stability, should be used together to define the final commercial formulation. Scaled-down photostability studies based on DP manufacturing conditions and expected in-use exposure should be carried out at the beginning of the commercial formulation development to understand if any residues are particularly susceptible to photodegradation and if excipient such as peroxide scavengers, hydroxyl radical scavengers, or metal chelators are necessary to reduce photodegradation of the DP. This is particularly important to protect the safety of patients using home injectables, which may inadvertently be exposed to UV light[130,131] or longer-term exposure to visible light which recent studies are demonstrating also leads to degradation.[132]

Along with transportation and photostability an update to the in-use stability studies may be necessary to support Phase III and commercialization. At this stage these should be done on a case-by-case basis. The early in-use studies should be evaluated to ensure the materials of construction from earlier in-use compatibility studies support the current design, country requirements and availability of IV bags. While a final microbial challenge study would not be carried out until the beginning of the PPQ/commercial launch preparations, if the product requires reconstitution, it may be advantageous to carry out microbial challenge studies to facilitate ease of handling at the Phase III clinical sites or during home administration depending on the individual circumstances. This, and other circumstances, should be decided on a case-by-case basis in consultation with the clinical teams and pharmacies.

Container-Closure Systems

Primary container-closure systems have evolved over the last two decades to include new vial types (glass/plastic/hybrid), multiple PFS configurations (Silicone oil, baked on or crosslinked Silicone oil, plastic, and new Glass PFS with Gore stoppers and no silicone oil). These need to be considered during pharmaceutical development based on product needs. The commercial container-closure system can greatly influence the stability of the DP. While it is common to use a perfluoropolymer coated rubber stopper to prevent curing and other compounds from leaching into the DP solution, the container contact surface and gas permeability can affect DP performance. Plastic vials have a much higher gas exchange rate than glass leading to higher rates of oxidation. Formulation components including buffer, pH and excipients can lead to hydrolytic attack and delamination of glass vials resulting in release of metals and glass lamellae formation. High surface areas and low protein concentrations can lead to product loss and leachates from the glue holding the needle of staked needle syringe, tungsten oxides from PFSs and silicone oil in PFSs can all affect product quality leading to aggregation, subvisible and visible particle formation and oxidation. Various types of coatings and configurations are available including silicone oil free syringes,[133] plastic vials and syringes with multi-layer barriers to prevent gas permeation, glass vials with pure silicone dioxide layers and other configurations which can overcome problems for a particular drug product during commercialization. If a combination product will be developed for Phase III clinical studies and commercial launch, then DP compatibility will be necessary during development of the Phase III/commercial formulation. Combination products include use of a PFS, PFS/autoinjector combination, and cartridge with an on-body delivery device among other combinations. Parameters such as ejection rate, needle length, needle diameter, device temperature, hold time, and device leachates may all affect the quality, potency and ultimately safety of the drug product.

Initial Formulation Robustness Studies

Robustness studies by use of design of experiments (DoE)[134,135] will demonstrate the relationship between factors affecting a process and the output of the process. The timing of the full DoE study should be as early as possible once the formulation is developed, and representative Phase III process material is available. That will enable definition of the excipient and other formulation ranges prior to setting the specifications for Phase III and commercial batches. The manufacturing process and with it the resulting formulation composition, will have some inherent variability due to different excipient manufacturers and lots, buffer preparation, API concentration, etc. To account for this variability limits are placed on the important parameters. For example, pH may target pH 6 ± 0.25 and polysorbate may target $0.02\% \pm 50\%$ (w/v). The limits on each of the excipients, biologic concentration, temperature range, and storage time need to be fully understood and their effects on product quality and stability defined often by using a DOE approach.[136–138] These studies should be carefully planned, and the output evaluated to ensure the proper analytics are in place. The output of the analytics is meaningful and can be used to provide limits on manufacturing parameters and predict the impurity limits and inherent variability expected from the stability program so that product profile limits can be defined which provide for quality, potency and safety without forcing rejection of a lot or unduly shortening product storage stability.[139] A design should be chosen that ensures parameters which affect quality, potency and safety of the product which can be enhanced with additional parameters later in development.

DP Process Development

During development of the formulation, it is essential to understand the stresses the product will encounter during the DP manufacturing process and adjust the formulation to account for these stresses as they can lead to aggregation, particle formation, oxidation, leachate impurities, bioburden problems, sterility issues, and incomplete mixing following thawing or dilution. While the unit operations are well defined for most large biopharma manufacturers, the recent increases in virtual companies and small biopharmaceutical companies outsourcing the formulation and manufacturing to different CDMOs can make this part of the process challenging. It therefore requires a close collaboration of the team working on the formulation with the company who will carry out the DP manufacture. During early development select scale-down studies based on platform filters, mixing and tubing/pumping compatibility will suffice to understand if any major issues need addressing. Freeze/thaw at scale should be carried out either with placebo or a protein solution such as bovine serum albumin (BSA) to define the thawing parameters. During later stages of development, the potential and cost of failure increases dramatically, and potential formulation fixes may lead to additional clinical studies and timeline delays, thereby making this a critical part of the DP development process. Again, this work should be done in close collaboration with the intended DP manufacturer due to the numerous configurations of stirrers, filters, transfer containers, pump type, pump speed, tubing/pumping configurations and tubing sterilization processes that can all affect the quality of the final product. For lyophilized products, the development of a lyophilization cycle, including a design space for the lyophilization process and considering scale-up and transfer is important. These

studies, together with formulation robustness will form the basis for defining CQAs, and an in-process control strategy to ensure a consistent and high-quality product. The recent reviews by Das et al.[173] and Liebner et al.[174] provide an excellent summary on this topic.

Phase III Development

Stages of Process Validation

The activities related to this stage are geared towards further developing and finalizing the process, formulation, and analytical methods for both the pivotal clinical study stage and commercial launch of the DP. At this stage, the project teams need to initiate efforts to define the strategies for process validation and BLA submission. The guidance from the FDA on process validation is useful here.[140] The regulatory agency defines process validation as the collection and evaluation of data, from the process design stage through commercial production, which establishes scientific evidence that a process is capable of consistently delivering quality product. Process validation involves a series of activities taking place over the lifecycle of the product and process. The guidance from FDA describes process validation activities in three stages and a version of it is represented in Fig. 2.

a. Stage 1 – Process Design: The commercial manufacturing process is defined during this stage based on knowledge gained through development and scale-up activities. This is the summation of all activities including process development for pivotal /commercial material followed by process characterization leading to the finalization of commercial process, formulation, and analytical methods.
b. Stage 2 – Process Qualification: During this stage, the process design is evaluated to determine if the process is capable of reproducible commercial manufacturing. Typically, the requirement is to demonstrate reproducibility using three successful batches using the commercial process. However, for DP presentations with multiple Stock Keeping Units (SKUs) and presentations, the number of batches that need to be conducted depends on the validation parameters. It is important to ensure a streamlined technology transfer from development site to manufacturing site or from one manufacturing site to the other to ensure a robust and reproducible manufacturing process while considering technical and operational aspects.[141–144]
c. Stage 3 – Continued Process Verification: Ongoing assurance is gained during routine production that the process remains in a state of control. The purpose of the verification is to ensure that there is no process drift beyond the established qualified process range.

Refine QTPP (for commercialization) materials

Based on the results of the Phase II clinical studies the Clinical and Commercialization Teams will have a refined TPP and product label for pivotal studies and potentially for commercial launch. This information along with revised CQAs will be used for refining the QTPP with updates to the target biologic concentration, stability profile of Phase I and II clinical lots, clinical outcomes and impact to parameters that are linked to safety, efficacy, and immunogenicity, product presentation, container/closure, and impurity profiles.

As shown in Table 3, the QTPP may look different from the initial QTPP based on the input from the marketing/commercialization teams and the molecular candidate profile. This is an important iteration of the QTPP, as well as the CQAs as it sets the outline for drug substance and drug product process and presentations for pivotal supply, which should not deviate much from what is intended to be commercialized. The revised QTPP will provide the framework of related analytical methodologies, (e.g., Break-Loose Extrusion and glide forces measurements for PFS development), process requirements and control strategies that need to be implemented to ensure commercialization success.

Internal Manufacturing or CDMO Site Selection

It is necessary to establish the pivotal study and commercial manufacturing site and ensure alignment of DP production with the CDMO or internal manufacturing processes as early as possible, once it is determined that a pipeline candidate will proceed to Phase III/ pivotal studies. It is crucial to have a well-planned selection criterion for CDMO.[145,146] The QTPP should be used as a guide for establishing

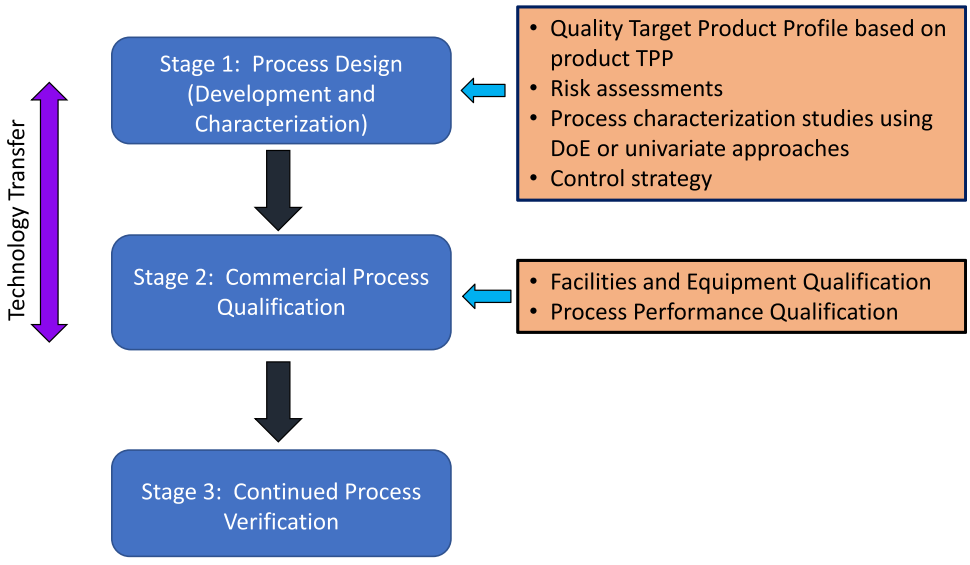

Fig. 2. Lifecycle of process validation for biologics.
Simplified outline based on regulatory guidance140.

the CDMO site for the pivotal material and commercial supply. The facility fit that matches the QTPP requirements is the major criteria for the site selection apart from the facility fit according to the technical requirements, manufacturing capacity to address commercial needs, Cost of Goods, quality, and regulatory profile of the site. Material for Phase III/pivotal clinical studies should ideally be produced at the same manufacturing site as that planned for commercial launch due to comparability considerations.[147-149] Each manufacturing site or CDMO may have differences in details of the unit operations from that used for the Phase I material for DS and DP manufacturing and comparability needs to be demonstrated for DP from different sites. It is therefore essential that planning of the pivotal DP manufacturing studies be tightly coordinated with the DP CDMO site. Based on any differences from unit operations used during process and formulation development additional small-scale studies may be necessary to ensure success of the engineering and Pivotal study DP manufacturing campaigns. If the product is intended for use with a PFS or other special device, it is important to incorporate those requirements for CDMO site selection.

Photostability Under ICH Guidelines and Confirmatory Use Conditions

The exposure of proteins to light and the ensuing chemical and physical degradation has been reviewed in the literature.[131,150] Detailed photostability studies following both ICH guidelines and intended manufacturing and product use are designed and carried out at this stage.[131,150–152] Because most biologics will have some level of light sensitivity, the photostability studies based on ICH guidelines will elucidate the degradation extremes and should be used to ensure that analytics are in place to detect possible degradants during the confirmatory use conditions. Additionally, the studies should demonstrate the effectiveness of secondary packaging for protecting the product. Because the final design of the secondary packaging is usually not ready at this time, the photostability studies may not be complete and additional confirmatory studies will be needed later once the packaging is finalized. In contrast to the ICH based study design, the confirmatory use studies are based on a detailed understanding of the total light exposure during the entire DP processing, manufacturing and intended product distribution.[153] Ideally, the internal manufacturing facility or CDMO would already have the 'light mapping' information for a particular facility, but timelines should be built to gather this information in the likely event it is not readily available. The light mapping study can then be used to design the confirmatory study with the worst-case scenario of exposure during manufacturing and showing that the process control strategy can minimize the light related damage to the therapeutics.[153] If not already demonstrated, intended distribution corridors and product use profiles may elucidate additional product liability that require additional mitigation strategies by proper product packaging especially using secondary packaging.

In-Use Compatibility Studies

Based on the use case of the investigational product and QTPP requirements, delivery devices such as syringes, IV bag and tubing, and infusion systems will need to be re-evaluated and expanded to account for use of the commercial formulation and the different materials of construction that may be encountered by a product during production and use. The list of commodities commonly used by the intended Phase III sites may be useful to obtain to evaluate through the studies. These may be different types of syringes, PVC and DEHP-free IV bags, polyolefin IV bags, different in-line IV infusion filters, CSTDs, and materials encountered for different infusion systems such as ambulatory pumps and on-body devices. The intercompany perspective is useful in this context to review the appropriate conditions to evaluate.[91] On-body devices have many more requirements and related tests than systems listed here and are not covered in this roadmap.

Refine In-Process Control Strategy

In-process control strategies should be designed and implemented based on an assessment of CQAs, the QTPP and a detailed understanding of the manufacturing process at the manufacturing site, according to the regulatory guidance on the topic.[154] The control strategy may include process tests and/or controls such as pre and post filtration bioburden and endotoxin testing, density measurement, protein concentration measurement post-mixing, post filtration sterilizing filter integrity testing, 100 % fill weight check, 100 % DP container inspection, subvisible particle analysis by light obscuration and flow imaging microscopy to ensure process consistency, filter flush, fill line & needle flush, etc. depending on the individual product. The in-process controls are put in place to ensure manufacture of a product with consistent quality, potency, and safety prior to use by the patient. It is highly recommended that the process control strategy is captured through a technology transfer report that is provided to the DP site for initiation of large-scale batches so that the manufacturing site is fully aware of the requirements.

DP Small Scale and Engineering Run

A DP manufacturing run including process development for thawing, transfer and filtering, dilution, mixing homogeneity, sterilizing filtration, container filling, freeze-drying cycle where applicable, stoppering, sealing and inspection should be carried out using the final process, or similar, pivotal material or a suitable placebo depending on what is available either at small scale or closer to manufacturing scale through engineering or demonstration batches. These studies are primarily to qualify the unit operations and validate that the expected quality metrics can be met.[18] The engineering batch can also be used to determine the mixing parameters range including mixing speed range corresponding to different batch volumes to proceed to pivotal batches and later for PPQ batches. Water or placebo can be used if the active product has viscosities below 3–5cP. Most DP sites use qualitative mixing speed such as 'slight to moderate vortex' for mixing of the early-stage clinical batches. The engineering batch if performed using water or placebo can be used to test the full range of commercial batch size to determine what is the appropriate mixing speed in RPM that matches the qualitative parameter and whether the homogeneity of the solution is assured through the minimum to maximum proposed batch size. Additionally, the engineering batch can be used to determine the acceptable parameters for freeze drying cycle for freeze dried products, nitrogen, or inert gas overlay to control the % oxygen overlay in vials for oxidation sensitive products, the maximum hold period post bioburden in the transfer vessel prior to sterile filtration and whether filter and line flushes/rejects are appropriate. Most CDMOs utilize the engineering batch to set up the filling line and capping parameters prior to proceeding to the GMP batch. It is suggested that the release and if needed, stability of the engineering batch is evaluated before locking the process for the GMP batch, so that a process control strategy can be fine-tuned based on the product quality results. The results of the engineering batch can be summarized in the form of a technical report and used to finalize the process outline and process control strategy in the technology transfer report prior to pivotal study GMP batches.

GMP DP Batch for Pivotal Clinical Studies

After the DP team evaluates the results of the process control and product quality results from the engineering run DP batch, the team will be ready to initiate the GMP DP batch using the pivotal DS material at the intended commercial CDMO following cGMP practices. All analytical testing including in-process controls, batch inspection, DP release and DP stability should be done using qualified methods that are intended for PPQ and commercial production. It is ideal to complete analytical method validation prior to pivotal material release to ensure that the methods do not vary much between release of pivotal material to PPQ material. However, if that is not feasible then care should be taken not to make major changes to the analytical methods that will result in differences of the product quality profiles between the pivotal and PPQ/commercial lots. If changes in analytical methods and/or new methods are introduced post pivotal material lot release, then appropriate bridging studies need to be performed using pivotal lots to demonstrate that such methods are indeed useful for monitoring CQAs and are stability indicating prior to method validation. It is suggested that for at least the first two batches that the product quality results of one batch is available prior to initiating the second batch so that additional refinement of process steps and control strategy is performed to ensure increased technical and regulatory success. The release and stability data from the pivotal supply batches are typically included in the IND amendment for initiating the pivotal studies. The pivotal supply batches are typically used also as registration batches for stability and therefore it is important to follow the ICH guidelines for release and stability testing.

Process Risk Assessment

Once the pivotal studies are initiated, the process teams need to be ready to initiate the process characterization and subsequent PPQ stage. At this time, it is important to perform the process risk assessment of the final process used for pivotal material supply and assess the risk relative to the commercial launch. The process risk assessment is typically performed using a simplified hazards analysis (i.e. severity and occurrence relative to causing a hazard) approach or using FMEA (Failure Mode and Effects Analysis) approach.[22,155] However, the process team may decide to use any other risk assessment tool in the QbD toolbox that is appropriate.[22,155,156] In DP manufacturing the protein DS, in a suitable final formulation, is combined with the desired primary packaging (e.g., syringe, cartridge, or vial) that guarantees product integrity and enables transportation, storage, handling and clinical administration.[99] The protein DP is exposed to several stress conditions during each of the unit operations in DP manufacturing, some of which can be detrimental to product quality.[99] Risk assessment should consider evaluation of all the potential stress factors during manufacturing. FMEA is a structured approach used to identify potential failure modes, their causes, and their effects in a manufacturing process.[156] FMEA is used here to identify potential failures in the process that could impact the safety, efficacy, immunogencity and quality of the product. This may involve identifying potential failures in areas such as raw material sourcing, manufacturing equipment, environmental conditions, process steps, and packaging. By analyzing each potential failure mode, its root cause, and its potential impact on the product, the FMEA can help manufacturers identify and prioritize actions to mitigate or eliminate the risks associated with the identified failure modes.[156] FMEA analysis should be conducted jointly with the manufacturing, analytical, quality, and drug product teams. The identified risks and mitigations should be fed back to the process control strategy. This risk assessment is further used to perform the criticality assessment and to identify or designate the critical process parameters (CPPs) based on effect on CQAs, key process parameters (KPPs) based on effect on process performance such as product yield or non-key process parameters (nKPP) not affecting CQAs and process performance.[157] This is then used to develop the process control strategy including the in-process tests and in-process controls, specifications, and process characterization range based on the criticality of the process step. The process risk assessment report is a living document that needs to be updated once all risks have been mitigated through process characterization studies or team decides to tolerate those residual risks before proceeding to PPQ stage.

Process Characterization Studies

Process characterization for the DP manufacturing is typically carried out using pivotal process material or representative process material from engineering batch to investigate and characterize the effects of manufacturing parameters for the unit operations highlighted through process risk assessment. Scale-down models may need to be used considering the material constraints, if the at-scale effects can be reproduced at the small scale based on prior knowledge and qualification specific to the molecule.[18,158] Once a scale down model qualification is performed or is in progress based on a prospective protocol, process characterization studies can be initiated using such models. Qualified analytical methods need to be used, in addition to characterization methods. The analytical methods need to be aligned with those to be validated for release and stability to evaluate the effects of process parameters on quality, potency, and safety. Process parameters investigated may include pH, temperature, mixing time, mixing speed, pumping speed, filtration speed and time, hold time, freeze-drying cycle steps for lyophilized products, fill speed, total process time and air gaps for PFS, contact materials, etc. and should identify critical control parameters for which an in-process controls strategy is designed and includes specifications for critical process parameters, critical material attributes and acceptance criteria to ensure release and stability specifications are met. Process characterization DoE studies can be performed in conjunction with formulation robustness DoE studies with appropriate controls to ensure best use of material and to cover worst case conditions. The process characterization studies should consider all process steps including lyophilization cycle parameters, headspace (if inert air/nitrogen is used for oxygen sensitive products), temperature cycling to support accidental freezing of the products, etc. The results of the process characterization studies need to clearly identify the proven acceptable range (PAR) where the product quality of the product is expected to meet specifications and the normal operating range (NOR) within the PAR range that will be used for routine manufacturing and included in batch records.[18,157] The process control strategy document which outlines the list of in-process tests, in-process controls, specifications, etc. needs to be prepared based on the results of the process characterization studies and is considered a living document which needs to be finalized after PPQ batches are completed.

Sterile Filter Validation

Sterile filter validation needs to be initiated as soon as there is an indication that team needs to start preparing for process performance qualification (PPQ). Sterile filter validation may take 4 to 12 months based on the validation parameters which includes product specific bubble point determination, physicochemical, microbiological including microbial challenge study, and E&L testing.[159,160] The validated filter and process conditions sets the basis for defining the sterile filtration unit operation process for PPQ batches.

Extractable and Leachable Risk Assessment and Studies

An extractable and leachable risk assessment is conducted identifying all equipment, single-use parts and container-closure components which may lead to changes in the impurities profile of the drug product.[161–163] Typical surfaces include bulk DS containers, stainless steel hold tanks, transfer tubing and connectors, mixers, filters, vials, stoppers, and other materials of construction that come into contact with the DP during manufacture and storage. Extractable studies may be used to define impurities that are monitored, as well as set limits if appropriate for leachates entering the drug product during manufacture or storage. Guidance from BPOG[164] provides the risk assessment and sequence of steps to be taken for addressing the regulatory requirements for extractables and leachables.

Transportation Studies for Product Impact

Ideally transportation studies are conducted during commercial formulation development to ensure that proper levels and type of excipients are used to protect the product from expected vibration and shock during shipping. For various reasons this may not be possible, in which case a transportation study should be conducted with material provided from the pivotal process. Potential problems may become evident such as inadequate surfactant concentration leading to unacceptable levels of subvisible or visible particles. If this occurs and it is necessary to change the formulation delays to the program may occur due to redevelopment of analytical techniques or in extreme cases the need to conduct clinical crossover studies to implement the new formulation at the end of the Phase III studies. The initial transportation studies should include shock and drop, truck and air transport simulation using representative biologics material to evaluate if the transportation stress poses any risks to product quality. Numerous studies have demonstrated timely evaluation of such stress is needed so that corresponding formulation and/or primary and secondary packaging solutions are implemented in a timely manner prior to commercialization.[75,122,165]

PPQ/Commercial

Finalize CQAs

The batch release results, stability trends of the early development batches and pivotal batches, and process characterization studies should be used to determine the product quality and stability profile of the molecules and if there is a correlation of observed levels of purity or heterogeneity to potency of the molecule. Also, forced degradation studies and characterization of the purified fractions of the variants can be used to determine if the variants have any effect on potency of the molecule, and to finalize the list of CQAs.

Commercial Manufacturing Site

Typically, the commercial manufacturing site selected is the same as the pivotal manufacturing site to avoid the hurdles of establishing process and analytical comparability between the two sites. However, there may be instances where additional sites may be needed to address the capacity requirements of the DP based on commercial demand. In such cases, the additional sites need to be qualified as well, and comparability of process and product quality of the lots manufactured relative to the pivotal study lots need to be demonstrated.

Manufacturing Site Risk Assessment (FMEA) for Commercial Phase

FMEA based risk assessment is typically performed by the manufacturing site based on the facilities fit of the manufacturing process, qualification range of the equipment, utilities, etc., and to determine if there is risk of the manufacturing process prior to proceeding with process performance qualification runs. The risk assessment is used by the CDMO to determine if risk mitigation measures related to equipment, utilities, and facility fit need to be conducted prior to proceeding with PPQ batches. Outcome of the Manufacturing FMEA needs to be completed well in advance prior to PPQ. The risk assessment needs to be updated delineating whether risks will or have been mitigated or will be tolerated through the PPQ stage. Similar to the process risk assessment and process control strategy document, the manufacture site risk assessment document is a living document that is updated throughout the course of PPQ preparation and execution.

Raw Material Risk Assessment

Excipient, raw material, and consumable sources should be verified to meet pharmacopeial standards, at a minimum. The raw materials need to be qualified to ensure high purity, low extractables and leachables, low supply chain issues and to meet use case standards as outlined in the QTPP.

In preparation for process qualification runs, the raw material risks are evaluated, and suitable control measures need to be in place to ensure there are no quality and supply chain risk measures. For PFS, it is typical to set incoming commodity specifications based on functionality by this stage. The control strategy for raw materials can include second sourcing (e.g., excipients. vials, stoppers and PFS), performing required vendor and in-house testing to ensure quality etc. If second sourcing of materials are required to mitigate supply chain constraints, it is ideal to address those through PPQ strategy or otherwise qualification through additional lots and regulatory approval may be required which can result in delay. Different batches of raw material may need to be qualified through the PPQ batches prior to commercial launch.

Additional Requirements for Combination Products

During this stage, the design considerations of primary and secondary packaging along with additional requirements of the combination product need to be addressed based on inputs from the marketing team. For example, if PFS or patch injector systems are required for commercial launch, it is important to consider the combination product timeline as early as possible starting in Phase III/pivotal study stage, ideally as soon as the QTPP for the Phase III stage is established. Developing a PFS presentation for biologics is complex compared to a vial presentation due to components, processing, and combination product pathway. The design control process needs to be completed with customer and user information. The combination product development will require a Design Development Plan, user needs and use cases study, stakeholder needs study, device design and user FMEA (risk assessments) and multi-stage human factors studies. Development of combination products for biologics needs to address technical and regulatory requirements for biologic product and device through an integrated approach.[166]

Validation Plan, Activities and Process Control Strategy

A master validation plan specific to the project is prepared to outline the Stage 1, Stage 2 and Stage 3 efforts of the product as outlined in Fig. 2. The validation project plan should consider validation of equipment, utilities and facility validation, sterile filter validation,

transportation validation and cleaning validation. The validation plan activities should carefully consider the sterility assurance requirements including Container Closure operational qualification, qualification of the seal integrity of the primary container closure system, glass vial washing and depyrogenation, stopper washing and sterilization, autoclave equipment sterilization, aseptic process simulations including media fills. Subsequently, the validation project plan and PPQ protocols are authored to address the validation parameters and specifications. These should consider the risk assessments performed, PC studies, CQA, process parameter classification and process control strategy. The validation project plan and PPQ protocols including sampling plans need to be finalized in advance of PPQ batches. The analytical assays for release and stability are validated at this point. The facilities and the equipment need to be in place and qualified prior to PPQ runs. FDA guidance regarding periodic qualification of sterilization and capping equipment is crucial to ensure microbial control. The final process control strategy (PCS) is prepared based on the final CQAs and the CPPs to be qualified through the PPQ batches. The components of the PCS include in-process controls, in-process tests, release and stability specifications, characterization, and comparability studies.

Process Performance Qualification Batches

According to general regulatory expectations, the DP PPQ runs are performed at the commercial manufacturing site. DP stability is expected for 3 batches with at least one batch in secondary packaging. However, the number of PPQ batches required can be determined by other factors.[167]

Second sourcing of primary containers is always a good thing to consider at this stage and incorporate during the PPQ batches to avoid supply constraint issues later post-commercialization.

Process validation studies such as sterile filter validation are typically performed prior to PPQ whereas solution holds for microbial growth are performed in conjunction with PPQ batches. The rationale for the number of PPQ batches needs to be justified based on the batch size, validation parameters including mixing speed and time, solution homogeneity, fill homogeneity, sterile filtration parameters etc. If the PPQ batches are not planned to be commercialized, it is suggested that at least one of the PPQ batches is subjected through limited or full final secondary packaging configuration to ensure availability of containers for photostability studies and to ensure product quality is not affected through the secondary packaging process step. The packaged active vials or PFS can furthermore be used for shipping lane validation studies.

PPQ Lot Stability Monitoring

The PPQ lots are expected to be placed on recommended and accelerated ICH storage conditions apart from stressed conditions including ICH Photostability conditions. The stability profile should be demonstrated to be similar to the registration batches to support the shelf life of the final commercial DP. Forced degradation conditions and thermal cycling of the product from recommended to frozen or high temperature conditions and back to recommended temperatures needs to be performed as supportive studies to demonstrate the robustness of drug product stability and to support temperature or other excursions that can happen during the shipping and storage of the drug product throughout its life cycle.

Transport Validation Using Actual Shipping Lanes

Transport shipping validation for DP should typically include simulated and real time shipment studies. Simulated shipping studies include shock-drop, truck, air transport, etc., components that simulate actual shipping conditions that the DP may go through in unpalletized or palletized packaging configuration to determined impact on product quality of the biologics. Performance qualification of the drug product in primary containers is performed using the actual shipping lanes that will be used by the product from the time it leaves the DP manufacturing site through central distribution centers to final storage warehouses. The purpose of this qualification exercise which may involve three or more batches is to demonstrate that the primary and secondary packaging remains intact and that the temperature storage conditions for the product are not impacted during actual shipment conditions during summer and winter seasons. Shippers for distribution of the final DP to patients is also expected to be qualified.

Physicochemical in-use compatibility Studies and Microbial Challenge In-Use Studies to Support Label Claim (Lyophilized/IV/Syringe)

The final IV bag/line and in-use stability needs to be performed using the broader commercial IV bags and line compatibility studies that may be used at the clinical sites in the intended market. This information can be gathered by requesting the Phase III/pivotal study sites to record the IV bags, lines, CSTD devices, etc., that are mostly used or requested by the clinical sites across the intended markets, and then performing a gap analysis to determine if additional components need to be evaluated. Once the gap analysis is performed, the team needs to ensure high risk components that are mostly likely to be used across the intended markets are tested and deemed compatible prior to commercialization. If any compatibility issues are observed, it is important to include those in the prescribing information (PI) that supports the label use. Studies should be performed to support microbial challenge to support >4 h storage following dissolution of lyophilized material or dilution of DP solution in IV bags and lines during IV administration.[91,168] Some products are administered through infusion or ambulatory pumps for 24–48 h and so the microbial challenge are expected to be conducted at twice the intended storage and administration time by some of the regulatory agencies.

Continuous Process Verification (CPV)

Continuous Process Verification is the step of process validation to verify on a continuous basis that the validated drug product process is in the state of control during routine commercial manufacturing. The CPV program determines opportunities to adjust the process limits to reflect established process capability and identify parameters and attributes that exhibit greater than predicted variability. This includes monitoring the CQAs, CPPs and other process controls of all the batches that are being manufactured, access process capability and ensure everything is within the validated range and there is no process drift outside the validated range over time. A properly designed CPV program enables the assessment of opportunities to discontinue in-process testing based on established process controls.[169,170] For trending analysis, upper and lower control limits of the process need to be established and the results need to be routinely evaluated against agreed upon Nelson Rules for trend analysis. The CPV step includes the internal manufacturing unit or CDMO compiling the data and providing as part of the annual product quality review report that needs to be filed with the regulatory agencies.

Preparation of BLA and Pre-Approval Inspection

In preparation for BLA submission, the process teams need to ensure that all deviations and out of specifications (OOS), out of trend (OOT) observed for clinical supply, registrational, stability and PPQ batches are notified to the regulatory agencies and addressed in a scientifically robust manner, if not already completed. All CAPAs and

change control procedures need to be appropriately addressed in a timely manner and procedures; policies need to be in place to ensure operational success for launch, especially if gaps were observed. Checklist of activities in preparation for BLA and PAI inspections includes data integrity check, lab notebooks check, traceability, mock audits to ensure PAI readiness, etc., all ensure integrity and credibility with the regulatory inspection teams. These aspects determine whether the company is ready to manufacture batches that are not adulterated and all lots to be manufactured are in a good state of control to ensure safety, efficacy, and potency of the therapeutic drug batches.

Conclusions

As outlined in the DP development roadmap, the development of a biologic DP requires multiple steps, that need to be planned and coordinated between the different expert teams involved, including Process Team, Commercial/Marketing Team, and Clinical Team. The key to success is implementing a capable project management structure and assure that information and know-how exchange between the different project teams and development phases is working properly. The basis for the development should be a thorough understanding of the properties and stability profile of the biologic itself, as well as the involved analytical characterization methods and processes used for manufacturing. All involved project teams should know the TPP and QTPP and jointly strive towards developing a DP presentation, including formulation and container closure system that stabilizes the biologic during manufacturing, storage, shipment, handling, and administration to the patient. Modern drug product development should make use of advancements in science and technology, be it from instrument or data analysis/science perspective, and after critical evaluation integrate novel approaches during drug product development. It is essential to consider sustainability of the DP development as an additional factor, besides time and cost. At the end, all efforts are made to provide safe and efficacious drugs at high quality to the patients, to prevent and treat diseases and improve quality of life.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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