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REVIEW ARTICLE

Perspectives on clinical drug development of Long-Acting Injectables

Long-Acting Injectable Aqueous Suspensions—Summary From an AAPS Workshop

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Abstract

Through many years of clinical application of long-acting injectables, there is clear proof that this type of formulation does not just provide the patient with convenience, but more importantly a more effective treatment of the medication provided. The formulation approach therefore contains huge untapped potential to improve the quality of life of many patients with a variety of different diseases. This review provides a summary of some of the central talks provided at the workshop with focus on aqueous suspensions and their use as a long-acting injectable. Elements as formulation, manufacturing, *in vitro* dissolution methods, *in vitro* and in vivo correlation, in silico modelling provide an insight into some of the current understandings, learnings, and not least gaps in the field.

Keywords aqueous suspensions · *in vitro* dissolution · long-acting injectables · manufacturing · PBPK modelling

Introduction

Long-acting injectables (LAIs) are pharmaceutical products intended for weekly, monthly, or with an even lower administration frequency, which provides a continued drug release and thereby a steady exposure of the delivered compound (1, 2). LAIs are typically administered intramuscularly (IM) or subcutaneously (SC) as oil solutions, forming gels, microspheres, implants, micro- or nanosuspensions (3). SC dosing is well suited for self-administration and are normally perceived as less painful injections, however, for higher injection volumes (2–5 mL) IM injection is needed and it is the administration route used for most commercially available LAIs (3). For individuals suffering from dysphagia or chronic disease where limited patient adherence may have a negative outcome on the treatment efficacy, LAIs can provide a huge benefit in disease treatment. The incidence of chronic illnesses and of dysphagia is significantly growing in our ageing population. For this reason, many disease areas may benefit from the constant drug delivery that a LAIs formulation may offer (3, 4).

LAIs have, with huge clinical success, been applied in the treatment of antipsychotic conditions and the area contains some of the most successful LAI formulations seen from a therapeutic perspective. In the 1960s, oil-based parenteral depot formulations, containing antipsychotics, were introduced in the clinical practice. In 2003 a LAI suspension of risperidone (Risperdal Consta®, Janssen Pharmaceutics) entered the market, which increased the use of LAI formulations significantly. Before this period, there was

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some reluctance to employ parenteral depot formulation of antipsychotics owing to concerns over side effects, tolerance, and pain upon injection (3, 5). These issues were much lower with the new formulation approach applied in Risperdal Consta®. Since then, the micro- and nanosuspension technology has significantly advanced and many of the more recent LAIs are nano-or microsuspensions. Hence, this is currently one of the important technologies in the field of LAIs. Remenar (6) recently reviewed the LAI formulations in the antipsychotic area, where trends in formulation platforms for US approved drug products over the years are outlined, due to the longer focus on the specific indication (see Supporting Information). Positive clinical results have clearly shown the benefits of LAIs (see below) and clinicians increasingly appraise the platform for its benefits (5, 7, 8). To further explore the advantages of this enabling platform and its opportunities in different therapeutic areas, a workshop on 'Patient-Centric Design of Long-Acting Injectable Drug Products' was organised in March 2022 by scientific colleagues and hosted as an online conference by the American Association of Pharmaceutical Scientists (AAPS). This review will provide a summary of the topics discussed at this workshop with focus on LAI suspensions, highlighting their formulation and manufacturing, their *in vitro* dissolution, pharmacokinetic modelling, answers to all questions raised at the workshop, and a scientific gap analysis based upon all the above.

Why is There a Need for a Long-Acting Injectable?

Where the orally administered molecule has a time window of release equal to the time it takes the dosage form to transit through the gastrointestinal tract, an injected product can be designed to release its drug load over weeks to months, *i.e.,* a LAI. This generally indicates that the blood plasma concentration profile, following administration with a LAI formulation, may have less fluctuations over time (1, 9), see Supporting Information (Figure 2S). Another evident advantage for the patient is the longer exposure, which improves the patient compliance (2, 10).

The development of effective, reliable and safe LAIs has the potential to aid many disease areas, but the technology will most likely have the most important effect/impact on chronic conditions or conditions that require a treatment for 4 to 6 months, such as tuberculosis and leprosy. Patients with chronic conditions/illnesses are required to take their medicine for a longer duration—if not life-long. Multiple reviews and studies have demonstrated that compliance from oral medication during both short- and long-term medication may be below 80 percent when one tablet is prescribed per day and even less in the case of multiple tablets per day (11-16), see Fig. 1.

LAIs would provide the patient with the benefit of convenience, as the daily intake of a tablet might confront the patient with his/her disease on a continuous basis. As mentioned above, most of the clinical experience with LAIs have been obtained with antipsychotics, where the compliance has been described critical for the clinical outcome. Weiden *et al.* (17), investigated the California Medicaid data in the period from 1999 to 2001 with the purpose of evaluating the association between partial compliance and hospitalization. Weiden and co-workers (17) included patients with schizophrenia, *i.e.,* patients with an ICD-9-CM code of 295.xx, which had at least two dispensing events for antipsychotic medications during a six-month period. Qualifying prescription claims included claim for all approved oral antipsychotic medications, including antipsychotics available before 2000. The outcome of the investigation clearly demonstrated how poor patient compliance, in this patient population, could significantly increase the risk of rehospitalization (see Fig. 2).

This combined with studies that have demonstrated how increased compliance means a better functionality for patients treated with antipsychotics (18-20) provide LAIs with a therapeutical benefit relative to orally administered medication. A recent extensive review of clinical evidence in the field have provided statistical support of this perception, *i.e.,* that LAIs provide a better treatment to patients suffering of schizophrenia when compared to a similar oral treatment. Okili *et al.* (21) analysed clinical studies, where

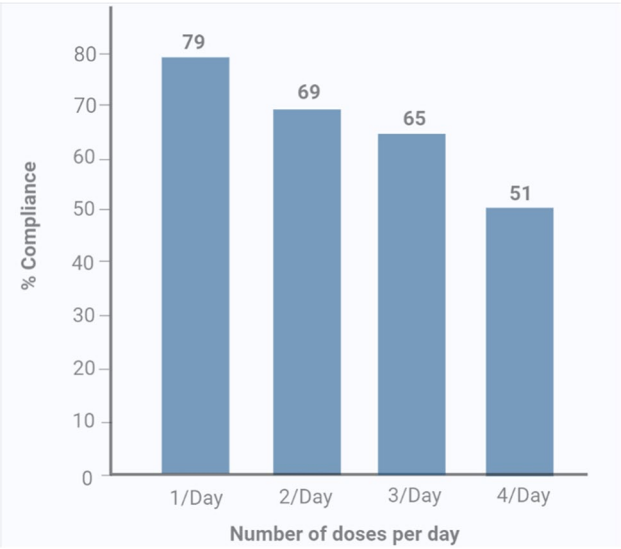

Fig. 1 Mean rate of compliance based upon a review of 76 trials using electronic monitoring in chronic medical conditions (pulmonary, ophthalmology, hypertension, etc.), (modified from Coleman *et al.*, (12))

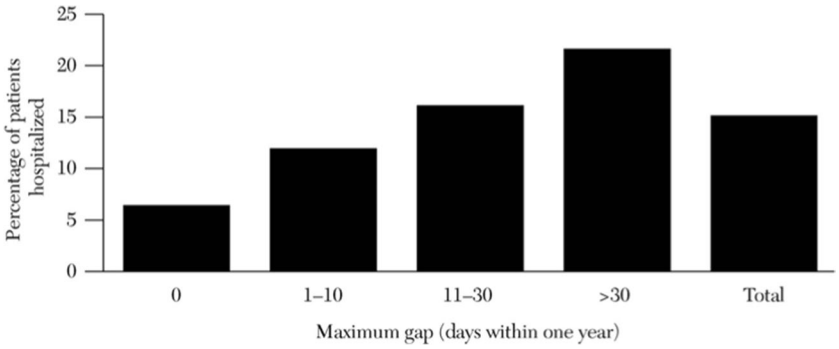

Fig. 2 Percentage of patients with schizophrenia rehospitalized, by maximum gap in therapy (figure reproduced from Weiden *et al.* (17) with permission)

the relative change in the Positive and Negative Syndrome Scale (PANSS) was evaluated for oral *versus* LAI for an antipsychotic treatment (see Fig. 3). Nine studies were identified that fulfilled the inclusion criteria in the analysis. Of these, two studies reported a better performance on the PANSS scale following oral administration relative to the LAI administration, where one had no difference included in the 95% interval. The other seven studies, where three studies had no difference in their 95% confident interval, concluded that the patients had more benefit being treated with the LAI formulation relative to the oral treatment.

The analysis by Okoli and co-workers (21) and the functionality studies mentioned above clearly demonstrate that LAIs do not only offer convenience for the patient, but more importantly also a better treatment with a better clinical outcome. Though most of the knowledge in the field originate from antipsychotics, LAIs are also explored in a number of other therapeutical areas, *e.g.,* HIV, which might be further broadened to other unexplored disease areas as well. Hence, there seems to be a huge untapped potential for patients and humanity in the advantages LAIs offer.

Formulation Classes of Long-Acting Injectables

In the 1950s, the Food and Drug Administration (FDA) authorized the first LAI products, formulation's based on oil solutions and aqueous suspensions. Some LAI products have been approved since, but from year 2000 and onwards, an increasing number of LAI products have been approved in a variety of therapeutic areas. LAIs are especially important in conditions that need long-term or chronic therapy, such as schizophrenia, hormone replacement treatments, HIV, and tuberculosis (22-24). In the literature, there is no well-established consensus on the categorization of the various LAI technologies; nonetheless, they may be generally classified into four formulation classes, as depicted in Fig. 4, with certain exceptional systems not falling into any of these categories.

Study name	Std diff in means	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Macfadden et al., 2010	-0.091	0.107	0.011	-0.301	0.119	-0.849	0.396
Bai et al., 2007	0.251	0.284	0.081	-0.305	0.808	0.885	0.376
Keks et al., 2007	-0.126	0.124	0.015	-0.369	0.117	-1.017	0.309
Emsley et al., 2008	-0.540	0.207	0.043	-0.946	-0.135	-2.612	0.009
Stankovic et al., 2019	-0.803	0.292	0.085	-1.376	-0.231	-2.749	0.006
Rosenheck et al., 2011	-0.645	0.107	0.011	-0.854	-0.436	-6.038	0.000
Barrio et al., 2013	-1.186	0.301	0.090	-1.776	-0.597	-3.945	0.000
Kim et al., 2008	-0.189	0.286	0.082	-0.749	0.371	-0.662	0.508
Petric et al., 2019	1.024	0.355	0.126	0.329	1.719	2.889	0.004
	-0.281	0.152	0.023	-0.579	0.016	-1.854	0.064

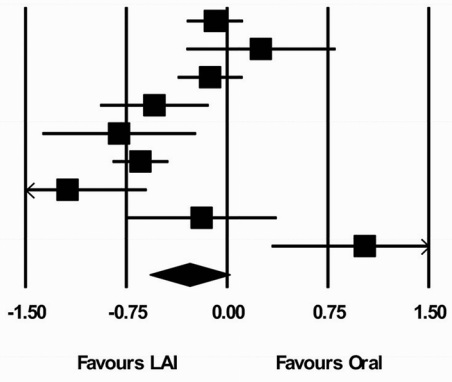

Fig. 3 Forest plot of studies assessing the differences in patient outcome between long-acting injectable (LAI) and oral antipsychotics using the Positive and Negative Syndrome Scale (PANSS) (Figure reproduced from Okoli *et al.*, (21) with permission)

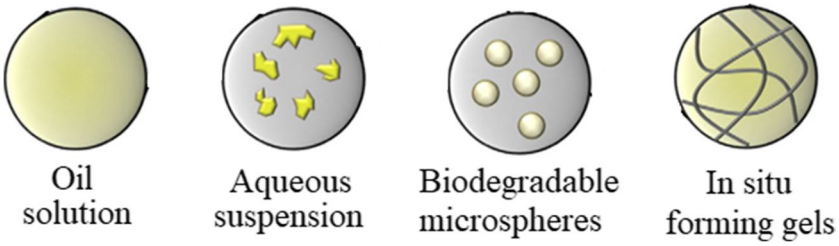

Fig. 4 Graphical representation of the main formulation types used as long-acting injectables. Figure modified from Owens and Rannard (9)

There are commercial products in all the four classes of LAI technologies presented in Fig. 4. As mentioned above, the oil solution was among the first LAI formulation that entered the market, a formulation where the compound is solubilised in the oil and where the diffusion of the compound out of the vehicle combined with the vehicle "digestion" is driving the compound release. The formulation approach is most usable for highly lipophilic compounds. The formulation type is relatively robust and can in some cases be thermally heat sterilised, making it a very attractive formulation approach. Different lipids have been used in commercial products including castor oil, cottonseed oil and sesame oil. In principle a lipid suspension can be used, but this approach is less applied and investigated.

The aqueous suspension, which is the focus of the present review, is the most dominant formulation type in the field of LAIs. A list of FDA approved LAI suspensions is provided in Table I. This formulation class will be further discussed in the sections that follows. The third class of formulations is the biodegradable microspheres, where the compound is encapsulated into a biodegradable matrix, which erodes as a function of time whereby the encapsulated compound is released. Poly(lactic-co-glycolic acid) (PLGA) is a frequently used polymer in this formulation type, which is well suited for highly aqueous soluble compounds. Manufacturing of the microspheres can be complicated as formulation, process and equipment parameters may influence the final product. Nevertheless, formulations have been approved demonstrating the feasibility of the technology.

The last type of LAI formulation is *in situ* forming gels, which are a formulation system where all the components are solubilised in an organic solvent, *i.e.,* a polymer/gelator and the active pharmaceutical ingredient (API). Through the solvent displacement principle, the organic solvent diffuses away from the injection site leaving the polymer and the API. The water present in the tissue will induce the polymer to form a gel, in which the active ingredient is captured. This gel can be formulated with polymers that slowly erode, thereby controlling the release rate of the entrapped compound. The systems work well for compounds that are soluble in a biocompatible organic solvent. However, some aqueous solubility is required so the compounds do not precipitate upon the diffusion of the organic solvent. An overview of the four different main classes of LAI formulations and relative attributes can be found in Table II, with the reservation that other technologies exist, which is not described in the table.

As discussed above, the field of LAIs is rapidly growing across the pharmaceutical industry for the treatment of chronic conditions, as it offers i) the opportunity to reduce administration frequency, ii) to improve patient's adherence, iii) to improve patient's quality of life, and iv) to reduce costs for the healthcare system. A recent example on the impact of FDA approved LAIs is the approval of Cabenuva, consisting of Cabotegravir and Rilpivirine for the treatment of HIV (25), *i.e.,* a combination product from ViiV/GSK and Janssen. Other key cross-pharma collaborations (*e.g.,* Gilead and Merck) highlight the intense activity in the area (26). However, the path for development of LAIs is still very exploratory. The aim of the workshop, discussed in this review, was to gather experts in the field across the pharmaceutical industry, regulatory agencies, and academic groups to outline current gaps and define strategies to accelerate the development of this game-changing class of drug products.

Suspensions as Long-Acting Injectables

Nanosuspensions obtained through milling have meet a significant market need for delivery of poorly water-soluble drugs and the technology is applicable to virtually all routes of administration. One of the first patents in the field was filed on the 25th of January 1991 and entitled Surface Modified Drug Nanoparticles (27). The intellectual property claims in the patent were very broad, including as claim number one: "Particles consisting essentially of 99.9 – 10% by weight of a crystalline drug substance having a solubility in water of less than 10 mg/mL, said drug substance having a non-crosslinked surface modifier adsorbed on the surface thereof in an amount of 0.1 – 90% by weight and sufficient to maintain an effective average particle size of less than about 400 nm." The principle in the technology forms the scientific basic of multiple commercial LAI formulations including Invega Sustenna® (paliperidone palmitate), though for Sustenna®, the particle size is larger than the claims in the mentioned patent. The mean particle size has been reported to be 785 nm (28).

Table I FDA Approved LAI Suspensions (IM: Intramuscular; IA: Intraarticular; SC: Subcutaneous; IB: Intrabursal; PA: Periarticular; IV: Intravenous)

Trade name	Active ingredient	Product presentation	Route of administration	Year of FDA approval	Maximum dose	Dosing interval	Indication	Excipients in formulation
Abilify Maintena®	Aripiprazole	Lyophilised powder	IM	2013	400 mg (vial)	Monthly	Schizophrenia	Carboxymethyl cellulose sodium, mannitol, sodium phosphate monobasic monohydrate, sodium hydroxide
Aristada®	Aripiprazole lauroxil	Solid crystalline	IM	2015	1064 mg (syringe)	Two months	Schizophrenia	Sorbitan monolaurate, polysorbate 20, sodium chloride, sodium phosphate dibasic anhydrous, sodium phosphate monobasic
Aristada Initio® [1)]	Aripiprazole Lauroxil	Solid crystalline	IM	2018	675 mg (syringe)	Initial dosing	Schizophrenia	Polysorbate 20, sodium chloride, sodium citrate dihydrate, sodium phosphate dibasic anhydrous, sodium phosphate monobasic dihydrate
Bicillin® L-A	Penicillin G benzathine	Solid crystalline	IM	1950	1, 2 and 4 mL (0,6, 1,2 and 2,4 M units	Two weeks	Syphilis, prophylaxis	Sodium citrate buffer and, as w/v, approximately 0.5% lecithin, 0.6% carboxymethylcellulose, 0.6% povidone, 0.1% methylparaben, and 0.01% propylparaben
Depo-Medrol®	Methylprednisolone acetate	Solid crystalline	IM, IB, PA	1959	80 mg/mL (1 mL vial)	Single dose	Arthritis	Polyethylene glycol 3350, myristyl-gamma-picolinium chloride, sodium chloride
Depo-Provera®	Medroxyprogesterone acetate	Solid crystalline	IM	1960	400 mg/mL (2.5 mL vial)	Weekly	Metastatic endometrial or renal carcinoma	Polyethylene glycol 3350, sodium sulfate anhydrous, myristyl-gamma-picolinium chloride
Depo-subQ Provera 104®	Medroxyprogesterone acetate	Solid crystalline	SC	2004	104 mg (syringe)	Three months	Birth control	Methylparaben, propylparaben, sodium chloride, polyethylene glycol, polysorbate 80, monobasic sodium phosphate dibasic sodium phosphate and methionine povidone
Invega Sustenna®	Paliperidone palmitate	Solid crystalline	IM	2009	234 mg (syringe)	Monthly	Schizophrenia	Polysorbate 20, polyethylene glycol 4000, citric acid monohydrate, sodium hydroxide, sodium dihydrogen phosphate monohydrate, disodium hydrogen phosphate anhydrous
Invega Trinza®	Paliperidone palmitate	Solid crystalline	IM	2015	819 mg (syringe)	Three months	Schizophrenia	Polysorbate 20, polyethylene glycol 4000, citric acid monohydrate, sodium hydroxide, sodium dihydrogen phosphate monohydrate, disodium hydrogen phosphate anhydrous

Table I (continued)

Trade name	Active ingredient	Product presentation	Route of administration	Year of FDA approval	Maximum dose	Dosing interval	Indication	Excipients in formulation
Invega Hafyera™	Paliperidone palmitate	Solid crystalline	IM	2021	1560 mg (syringe)	Six months	Schizophrenia	Polysorbate 20, polyethylene glycol 4000, citric acid monohydrate, sodium hydroxide, sodium dihydrogen phosphate monohydrate, disodium hydrogen phosphate anhydrous
Ryanodex® [2)]	Dantrolene sodium	Lyophilized powder	IV	2014	250 mg (vial)	Prior to surgery	Malignant hyperthermia	Mannitol, polysorbate 80, povidone K12, sodium hydroxide or hydrochloric acid
Kenalog®-40	Triamcinolone acetonide	Solid crystalline	IM or IA	1958	40 mg/mL (10 mL vial)	Several weeks	Anti-inflammatory	Sodium chloride, benzyl alcohol, carboxymethylcellulose sodium, polysorbate 80
Zyprexa Relprevv®	Olanzapine pamoate	Solid crystalline	IM	2009	405 mg (vial)	Monthly	Schizophrenia	Carboxymethylcellulose sodium, mannitol, polysorbate 80, sodium hydroxide and/or hydrochloric acid
Vocabria®	Cabotegravir	Solid crystalline	IM	2021	600 mg (vial)	Monthly	HIV-1 infection	Mannitol, polysorbate 20, polyethylene glycol (PEG) 3350
Rekambys®	Rilpivirine	Solid crystalline	IM	2021	900 mg	Monthly	HIV-1 infection	Citric acid monohydrate, glucose monohydrate, poloxamer 338, sodium dihydrogen phosphate monohydrate, sodium hydroxide

[1)] The formulation releases the API over an extended period, but it is only intended for one injection at the start of the treatment with Aristada® or if a dose of Aristada® was missed

[2)] Ryanodex is an immediate release formulation for intravenous administration and is therefore not an LAI. It was included into the table despite this as it is an aqueous nanosuspension, why the formulation composition may still be of interest

There are also examples of application of particle size reduction in other fields than LAIs. Poor water solubility remains a common issue in pharmaceutical development, where approximately 40% of marketed drugs and as many as 90% of the APIs in the discovery pipeline are poorly water-soluble (29). A considerable number of the top 200 drugs exhibit clinical or pharmacokinetic limitations that arise from their poor water solubility. Nano-milling offers drug developers an option to enhance the dissolution rate and potentially also the solubility of these poorly water-soluble APIs, to facilitate an increased bioavailability. Since 2000, a growing number of BCS class II and IV APIs have been commercialized using this versatile technology, including both oral solid dose (OSD) and parenteral formulations (see Table 1S in the Supporting Information). As mentioned above, the use of API suspensions for LAI formulations has a long history, where the formulation strategy is to engineer the particle size that fits the release rate desired to obtain a defined plasma-concentration profile of the specific compound. This drug delivery approach may have some advantages for a given compound over the alternative technologies, such as oil solutions, *in situ* forming gels and PLGA microspheres, as also discussed above (see Table II). However, it should be stressed that one size does not fit all so it is beneficial for the pharmaceutical developer to have an open mind to all options and use the best suited technology for the specific API and target product profile, just here the focus will be on the suspension technology.

Stability Considerations for Suspensions for Long-Acting Applications

One of the key attributes that should be kept in mind during formulation development and manufacturing of a LAI, is the formulations long term-stability, which is affected by the unfavourable thermodynamics of suspensions (2). As the particle size defines the safety and efficacy of the suspensions, the formulator must adequately handle potential physical stability challenges, such as agglomeration, sedimentation, Ostwald ripening, and secondary nucleation (30). The small particle size of micro- and nanosuspensions results in a high surface area increasing the system's Gibbs free energy (Eq. 1), which is fundamentally energetically unfavourable for the formulation and linked to the surface tension (10):

$$\Delta G = \gamma_{s/l}.\Delta A \tag{1}$$

where ΔG refers to the system's Gibbs free energy increase, $\gamma_{s/l}$ refers to the solid–liquid interfacial tension, and ΔA refers to the surface increase of this solid–liquid interface (10).

A decrease in Gibbs free energy drives these unstable systems to a thermodynamically more stable state, which means that the systems will shrink their interfacial surface area by agglomeration leading to particle size growth (31). To prevent this phenomenon to occur, the formulator has to include excipients in the formulation with stabilizing properties, such as surfactant(s) and/or polymer(s), which may stabilize the system through, i) the reduction of the interfacial tension *via* wetting properties, ii) steric hindrance (steric stabilisation), iii) the electrostatic repulsion of (surface-) charged individual particles (electrostatic stabilisation), or iv) the combined steric and electrostatic stabilisation. (2, 10, 30, 32). In addition to their influence on short- and long-term storage, the stabilizers also aid in the creation of submicron particles during manufacturing (30).

Sedimentation is another naturally occurring phenomenon that must be addressed. As long as the resuspendability is sufficient, simple shaking will homogenize the system, nonetheless, the formulator must typically take this into account as hard sediments may form over time (2). According to Stokes' law, the pace of this process depends on particle size, medium viscosity, and the density difference between the medium and API (30). Since smaller particles will compensate for this sedimentation process through Brownian motion, particle size reduction is the most often used approach to prevent severe particle settling. Additionally, the inclusion of viscosity enhancers, such as carboxymethylcellulose, may facilitate this process (2).

Due to secondary nucleation and Ostwald ripening, the shelf life of nano- and microsuspensions can be significantly shortened (see Figure 3S in the Supporting Information for a graphical description of the process). Secondary nucleation is the natural crystallization of a supersaturated system, wherein medicine can dissolve and crystallise from a seeded supersaturated matrix (2). Ostwald ripening, on the other hand, only occurs in polydisperse systems in which large particles grow at the expense of smaller particles. Smaller particles will have a greater saturation solubility than their bigger counterparts and will dissolve more readily, resulting in a high local concentration of API. This local variation in API concentration will push a flow of dissolved API molecules to bigger particles, where they will crystallize and trigger particle growth (2, 30, 33). A narrow particle size distribution may decrease the disparities in saturation solubilities, hence preventing Ostwald ripening. Stabilisers may also decrease the interfacial tension and adjust the Ostwald ripening process provided that they do not increase the solubility of the compound. In this regard, several experts claim that an excess of stabilizer may accelerate Ostwald ripening (30, 33). Overall, current understanding describes Ostwald ripening as a multi-stage process, where surfactant concentration may be a critical formulation attribute (34). Other stability phenomena, such as digestive ripening and intraparticle ripening, can sometimes occur in inorganic materials (35). Recent literature suggests that these rare phenomena,

Table II Overview of the Main Technologies for Long-Acting Injectable Formulations

Attributes	Oil solution/suspension (e.g. Fluanxol® Depot)	Nano-/microcrystals(e.g. Abilify Maintena®)	PLGA microspheres (e.g. Risperdal Consta®)	In situ forming systems (e.g. Eligard®)
Diluent	Oil	Aqueous	Aqueous	Non-aqueous
Major ingredients	API + oil	API + stabilizing excipients: water-soluble surfactants and/or hydrophilic polymers (e.g. polysorbates and poloxamers)	API + biodegradable polymer (e.g. PLGA)	API + organic solvents (e.g. NMP or DMSO) + biodegradable polymer (e.g. PLGA)
Drug loading	Highly API depended;	≤350 mg/mL	≤100 mg/mL	≤250 mg/mL
Volume of injection	Typically 1–2 mL	Typically 1–5 mL	Typically large (≥1 mL)	Typically smaller (<1 mL)
Route of administration	i.m. or s.c. (i.m. > s.c.)	i.m. or s.c. (i.m. > s.c.)	Usually i.m	i.m. or s.c. (s.c. > i.m.)
Viscosity of active formulations	Moderately viscous (~30–35 cP at 25 °C)	Non-viscous suspension	Non-viscous suspension	Viscous (~100 cP at 25 °C) gel containing API in suspensed or dissolved state
Needle size required	21—25 G	21—25 G	20—23 G	23—27 G (highly depending on system viscosity)
Delivery control	Partition out of oil phase and oil digestion Delivery for more than 4–6 weeks difficult	Usually no burst release; delayed T_{max}; terminal $T_{1/2}$ sporadically independent of particle size	Initial lag time or (burst) release can be difficult to control	Controlled C_{MAX}/C_{MIN}; near zero-order in best case
Sterilization	Aseptic manufacturing or thermal heat sterilization	Aseptic manufacturing or gamma-irradiation	Aseptic manufacturing	Aseptic manufacturing or gamma-irradiation
Product configuration	Often ampules	Often prefilled syringe (room temperature or refrigerated); powders for reconstitution	Powders for reconstitution; often refrigerated	Refilled syringe possible; cold supply chain might be required
Manufacturability	Scalable and transferrable	Scalable and transferrable, but not straightforward	Can be difficult to scale-up and transfer reproducibly	Scalable and transferrable
Regulatory	History of acceptance; Early LAI for CNS diseases oil depot, newer developments more limited	Long history of acceptance (≥10 marketed drug products—i.e. incl. microsuspensions)	Long history of acceptance (≥10 marketed drug products)	Limited history of acceptance; few marketed drug products

where the suspensions particle size distribution narrows or even decreases during stability, might occur in organic suspensions as well (36-38).

Currently, the selection of an appropriate stabilizer and stabilizer concentration is solely dependent on trial and error. Few researchers have attempted to establish a more logical strategy or forecasting methodologies (32). Overall, current research seems to be focused on streamlining high-throughput procedures to save time and resources (33), more details to be provided below.

Formulation Development of Suspensions for Long-Acting Injectables

Milling is one of the most common pharmaceutical unit operations to reduce drug particle size and increase surface area. Nanomilling refers here to the process of reducing particle size below 1000 nm by wet media milling using an agitator bead mill, high-pressure homogenisation, or high-pressure micro-fluidisation. The conversion to nanocrystals increases the surface area-to-volume ratio of the API, allowing for greater interaction with water, which in turn increases the APIs dissolution rate. The smaller API particles generated by nanomilling can dissolve more readily, with the rate of dissolution being inversely proportional to the diameter of the particle (39). Additionally, nanomilling improves the homogeneity of a drug product and therefore content and dose uniformity.

Nanoparticulate suspensions should be formulated to avoid aggregation, as mentioned above. The hydrophilic polymers, surfactants and ionic molecules used to stabilize the colloidal dispersions can be found in marketed products and are Generally Recognized as Safe (GRAS) materials. The applicability of this technology is therefore primarily defined by the APIs aqueous solubility and its other physicochemical properties and not by the therapeutic category or chemical structure. A successful approach to formulating poorly water-soluble APIs for IM administration can be both micron- and nano-sized suspensions. There are commercially micro-sized suspensions available, such as Bicillin® C-R 900/300 (penicillin G benzathine and penicillin G procaine suspension) and Depo-Provera® Contraceptive Injection. These micro-sized suspensions can only be administered IM or SC injection, not intravenous. Particle sizes in the intermediate range have been successfully employed in the case of Invega Sustenna® and Abilify Maintena® and in the smaller range as in the case of Aristada®.

The particle size is usually perceived as the determining factor for the release rate of nano- and microsuspensions, but also parameters as solid-state characteristics of the API and formulation may affect the pharmacokinetics. An example of the effect of API particle size on the maximum plasma concentration (C_{max}) can be found in the Supporting Information (Figure 4S), where C_{max} is inversely proportional to the particle size. Such a correlation between the LAI formulation and the pharmacokinetic profile is not universal and it needs to be determined for the specific API and the specific formulation. To the best of our knowledge, there is no theoretical methodology that can provide guidance on the optimal particle size range, *i.e.,* the particle size has to be defined experimentally from compound to compound. The influence of the stabiliser on the release rate is also not well characterised, though Kesteleyn and co-workers (40) demonstrated small differences in the plasma concentration profile after injection of compound 11 from suspensions stabilised with poloxamer, vitamin E-TPGS and polysorbate 20. Some of the development journeys described in the literature may provide some insight into the biopharmaceutical link to LAI suspensions, however, it is in general a field where scarce information is available in public domain.

Suspensions—both nano- and microparticulate consist of API, water, a stabilizer(s) and other possible excipients. In the case of nanoparticulate suspensions, a high-energy media milling process is used to wet mill the slurry and create a colloidal dispersion with particles in the size range typically of 100 to 400 nm in diameter. These nanoparticulate suspensions do not aggregate due to the non-covalent adsorption of stabilizing polymers onto the surface of the particle, which decreases the particles' surface-free energy. The polymers that are used to stabilize the colloidal dispersions are typically hydrophilic in nature and can be found in marketed products. These polymeric stabilizers are GRAS materials. The coarse (microparticulate) suspensions (particles in the micron size range) will settle over time and the key to their viability is that their resuspendable which is often induced with gentle agitation in the clinical setting. This may present a significant challenge but can be potentially overcome *via* appropriate formulation or particle engineering approaches. Depending on the particle size of the coarse suspensions, micronization of the bulk API may suffice or one may have to employ a wet milling or alternative particle size reduction process (such as microfluidization). The formulator must also understand and be able to control the solid-state characteristics of the API. Milling is a high energy process which may induce polymorphic transformations. If there is a possibility that this may occur, it is important to start with the appropriate polymorph and track the API's solid state during milling as well as storage.

Additional excipients can be added, as required, to enhance product quality and provide a pharmaceutically elegant final product. These can include ingredients found in solution-based parenteral formulations such as anti-oxidants, antimicrobial preservatives, buffers/pH adjusters, reconstitution or bulking agents for lyophilized formulations, and tonicity modifiers. The key difference

between solution and suspension formulations is (are) the stabilizer(s). The stabilizer is an essential component that provides physical stability by keeping the particles discrete and separated. The stabilizer may also i) aid in redispersibility upon storage in the case of coarse suspensions, ii) aid reconstitution in the case of a lyophilized formulation and (iii) influence pharmacokinetics in conjunction with particle size. The stabilizer can be either non-ionic or ionic and can be either polymeric or a small molecule. Examples of polymeric stabilizers for parenteral administration include lower molecular weight polyvinylpyrrolidones (Kollidon® 12 PF and Kollidon® 17 PF), Pluronic® F-68, Vitamin E TPGS, and carboxymethylcellulose. Examples of non-ionic and ionic stabilizers include Tween-20, Tween-80, sodium deoxycholate, and sodium dioctylsulfosuccinate. Analogous to other non-solution dosage forms (such as nanoemulsions, lipid nanoparticles, and polymeric nanoparticles), the formulation and the manufacturing process are entwined to the successful end-product.

For parenteral suspension products, there are two possible final dosage forms: a ready-to-use suspension or a lyophilized suspension. Lyophilisation is a technical solution to stabilise a suspension that is physical instable, *i.e.*, to prevent particle size growth during storage. If a lyophilized suspension is required, the key quality attribute is to be able to reconstitute the lyophilized formulation to a particle size that is comparable to that of the pre-lyophilized formulation. Primary packaging of these drug products includes vials for both ready-to-use suspensions and lyophilized suspensions and as lyophilized powder in dual chamber syringes.

Manufacturing Considerations of Suspensions for Application as a Long-Acting Injectables

Sterilization

The most important aspect of any parenteral product is its sterility. This requirement imposes significant challenges in the development of sterile nanoparticulate and microparticulate suspension formulations. However, these challenges are not insurmountable. For all suspension formulations, the developer needs to track physical and chemical stability, homogeneity/content uniformity, syringeability and solid-state properties (morphology, particle morphology and surface characteristics).

Nanoparticulate Suspensions

In the case of ready-to-use nanoparticulate suspensions, there are three major methods of sterilization that are evaluated. These are terminal sterilization (heat, gamma radiation, or e-beam), sterile filtration, and aseptic processing. The use of terminal sterilization is desirable both from an ease of manufacturing and a regulatory perspective. However, only a small fraction of nanoparticulate suspension formulations is amenable to terminal sterilization. One example is N1177, an iodinated diagnostic-imaging agent. This particular nanoparticulate suspension formulation has a pre-autoclaved mean particle size of about 170 nm. After autoclaving (118 °C for a 15-spore log reduction) the N1177 nanoparticulate suspension formulation, the mean particle size grew to about 200 nm. Only a small fraction of nanoparticulate suspension formulations is capable of being milled to sufficiently small particle sizes (mean of less than 90 nm) to allow for sterile filtration. The ability to sterile filter nanoparticulate suspension formulations is not only a function of particle size, but also particle morphology, concentration of API, and viscosity. These parameters affect the extent of hydraulic packing and subsequent binding of the filter. In the case of aseptic processing, sterile API is required or alternatively, the formulation can be sterilized by heating the pre-milled slurry. The stabilizer and any added excipients can be dissolved in water and sterile filtered as it is charged to the recirculation vessel. Once all of the sterile formulation components are in the recirculation vessel, the milling process can be conducted in a pre-sterilized, closed milling system. After the targeted particle size is achieved, the sterile nanoparticulate suspension formulation can then be subjected to further aseptic processing, such as filling and lyophilization.

There are two principal methods for sterilization of lyophilized nanoparticulate suspension formulations: aseptic processing and terminal sterilization using gamma-irradiation or e-beam exposure. In the case of aseptic processing, there are three possible routes:

- Sterile filtration of the nanoparticulate suspension formulation, followed by aseptic filling, and lyophilization
- Heat sterilization of the pre-milled slurry, followed by aseptic processing, and lyophilization
- Use of sterile API in combination with aseptic compounding, processing, filling, and lyophilization

In the lyophilized state, the majority of the APIs that have been evaluated have withstood gamma-irradiation (typical dose is 25 kGray).

Coarse Suspensions

In the case of ready-to-use coarse suspension formulations, the same considerations are taken into account as in the case of nanoparticulate suspensions. The difference is that sterile filtration is not possible so there are only two methods

of sterilization to evaluate. These are terminal sterilization and aseptic processing. The developer will always evaluate terminal sterilization but can resort to aseptic manufacturing, if necessary. One point is that aseptic manufacturing is almost always an option; however, this may be a more complex and expensive approach. These well-established technical solutions mean that there is a high probability of developing a sterile product. Many of the APIs evaluated for nanomilling are amenable to bulk sterilization using gamma irradiation or e-beam. In the worst case, sterile filtration and aseptic crystallization of the API is likely an option.

Manufacturing Process of Suspensions

Nanoparticulate and some coarse suspensions are manufactured using a nanomilling process. The process consists of recirculating a slurry comprised of API, stabilizers, and water through the high-energy media mill. This results in the comminution and dispersion of the API crystals from initial size of 10–100 µm to a final mean diameter down to about 100 to 300 nm for nanoparticulate suspensions and high nanometer to low micrometer sizes for coarse suspensions. Typically, polymeric milling media is used during the nanomilling process to impart mechanical and hydraulic shearing plus impact forces within the media mill chamber. Alternatively, ceramic media may also be used. The API crystals are fractured and dispersed as they recirculate through the mill and are sterically and/or electrostatically stabilized by non-covalent adsorption of the stabilizers onto their surface. If the particle size of the coarse suspension is sufficiently large, then micronization (jet milling) may provide an acceptable, ready-to-formulate particle size.

Scale Up

Upon successful pre-clinical formulation and process development, the next step is scale up. When developing a strategy for scale up, you need to consider the anticipated peak commercial volumes which dictate the size of the media mill the developer will need. Important factors to consider are:

- the residence time (*i.e.,* the time the API slurry is in the high energy agitated bed chamber and where the particle size reduction occurs) to achieve the target particle size
- if the final drug product is amenable to terminal sterilization or if aseptic manufacturing is required
- if the final drug product will be a ready-to-use liquid suspension or lyophilized
- if the API is highly potent then containment and other engineering controls will be required to safely handle and contain the product during manufacture

There will always be a risk of some contamination introduced into the drug product during the milling process including attrition from the milling equipment and milling media. This being the case, it is advantageous to mill at the highest API concentration possible in order to both minimize the contamination levels (as a function of API concentration) and maximize the milling efficacy (quantity of API processed as a function of energy input). The milling impurities are minimal and controllable with the above-mentioned measures.

A wide range of commercially available agitator bead mills are available (41, 42). The nano-milling process is directly scalable—from lab scale (10 s of mg API) to full commercial scale (500 kg API). This has been demonstrated for multiple APIs including those used in marketed products (for 505(b)(2) development programs) and New Chemical Entities (NCEs). The primary parameter that measures scalability is replicating the particle size distribution of the suspensions over the range of the mill sizes (Fig. 5). The excellent process scalability is obtained through precise control of the critical process parameters in the nano-milling process, including the consistency of the polymeric milling media. Commercial vendors have developed agitator bead mills that are suitable for aseptic manufacturing. Lubrizol Life Science Health (LLS Health) has also designed and built a high energy media milling system (SteriMill™ Technology) specifically intended for aseptic nano-milling.

An essential component of the nano-milling process is the polymeric milling media such as Purolite's PuroMill™, which is a pharmaceutical grade advanced technology milling media. The original polymeric milling media for pharmaceutical applications was developed by Eastman Kodak. It was called PolyMill™ Polymeric Milling Media. PolyMill™ was a highly crosslinked copolymer of styrene and divinylbenzene. It was specifically designed to be chemically and biologically inert and possesses exceptional wear resistance.

Microbiological Testing

Sterility testing of suspensions and nanosuspensions is challenging as the formulations in most cases are unsuitable for the USP < 71 > membrane filtration method. This is why the direct transfer method often needs to be applied for suspensions. This may also be challenging since the opacity of the suspension make it difficult to examine the media visually and observation of microbial growth may be obscured. Transfer of portions to fresh media during the test period may be necessary to dilute out the opacity and allow for visualization of microbial contamination.

A word of caution for bacterial endotoxins test with suspensions. Endotoxins can be incorporated into bulk API crystals during recrystallization. In one formulation, we observed an increase in endotoxins that was inversely

proportional to particle size of the drug crystals. This was ultimately traced to water used during the recrystallization step.

In Vitro Release of LAI Aqueous Suspensions

LAI suspensions involve technologies such as microencapsulation, Atrigel, nano-crystal technology, and drug-device combination products (*e.g.*, prefilled syringes). LAI suspensions are intended to maintain the efficacy of therapeutic agents for a couple of weeks to several months following IM, SC or other local injections (*e.g.*, intra-articular). According to USP < 1151 > *pharmaceutical dosage forms*, a suspension is a biphasic preparation that consists of solid particles dispersed throughout the liquid phase. Although the final forms share the same term "suspensions," these can be oil-based crystalline or amorphous drug particles, aqueous-based crystalline or amorphous drug particles, and polymer-based (*e.g.*, Lupron Depot, a PLGA microsphere product; Perseris, a PLGA in situ forming implant) depending on the suspending media and the composition of the solid particles. Based on different manufacturing process and stability considerations, some are formulated as ready-to-use suspensions, and others are formulated as lyophilized powders which require reconstitution prior to injection (termed as "for suspension"). Specifically, LAI aqueous suspensions are generally categorized into nanosuspensions, and microsuspensions according to the drug particle size. These formulations achieve their long-acting effect through different release mechanisms such as polymer degradation, slow drug dissolution and combinations thereof. This work focuses on LAI aqueous suspensions, as also mentioned above.

Most of the LAI aqueous suspensions approved by the US FDA were approved after 2009. With the exceptions of Depo Provera CI, 150 mg/mL (a medroxyprogesterone acetate (MPA) suspension) and Invega Sustenna (a paliperidone palmitate suspension), there has been no generic equivalent versions of the LAI aqueous suspension products. This may be due to the complexity of these formulations as well as the cost of the associated clinical trials. To facilitate the development and approval of generic LAI aqueous suspensions, alternative bioequivalence approaches such as *in vitro-in vivo* correlation (IVIVC) present a great potential. IVIVC development is not required for new drug applications (NDAs) or abbreviated new drug applications (ANDAs). However, it can tremendously reduce the cost since a successful Level A IVIVC may serve as a surrogate for *in vivo* bioequivalence. To the best of our knowledge, only Invega Sustenna has IVIVC development in the NDA package, among all the commercially available LAI aqueous suspension products (44).

Methods to Test the *In Vitro* Release of LAI Aqueous Suspensions

In the NDA and ANDA processes, *in vitro* release testing serves as a quality control (QC) tool to set release specifications and identify any out-of-specification products. The *in vitro* release testing method should be capable of evaluating batch-to-batch consistency and stability of the formulations. Ideally, an *in vitro* release testing method should also enable successful establishment of IVIVCs to reduce the need for clinical studies. U.S. FDA recommended compendial methods for LAI aqueous suspensions include USP apparatus 2 (paddle) and 4 (flow-through). However, the duration (maximum 2 days) of these release testing methods is much shorter than the *in vivo* efficacy duration (weeks to months), which makes IVIVC development barely possible. Therefore, attempts have been made to increase the duration of the *in vitro* release testing for LAI aqueous suspensions using Depo SubQ Provera 104 (an MPA suspension) as the reference listed drug (RLD) (45).

To enable cross-lab comparison and global harmonization of the release data, compendial methods (USP apparatus 2 and 4) with minor modifications were utilized here to perform *in vitro* release testing. To test the discriminatory ability of the method, the RLD and one in-house formulation (F1) were used. Three USP apparatus 2 methods with different devices (Float-A-Lyzers, enhancer cells and in-house designed devices) and one USP apparatus 4 method with semi-solid adapters were used for the release testing of the MPA suspensions.

The USP apparatus 2 with Float-A-Lyzers method was able to extend the release duration to 5 months. However, this method was not able to differentiate the RLD and F1 formulations. The limited discriminatory ability was considered to be due to the violation of sink condition inside the dialysis bags. The knowledge gained from this testing method was that higher pore size of membrane may be helpful to provide better discriminatory ability. Since there are no commercially available Float-A-Lyzers with a higher molecular weight than 1000 kD, fiber glass filter membranes (with a pore size of 2.7 μm) were selected to further develop the method. The USP apparatus 2 with the enhancer cells method was initially developed to investigate the release testing of semisolid dosage forms such as ointments (46, 47). This method showed a prolonged release duration of ~ 2 weeks with good discriminatory ability and acceptable reproducibility for the MPA suspensions. However, issues with the enhancer cells, such as membrane wrinkling during assembly, can cause sample leakage and/or poor contact of the formulation with the membrane, leading to poor reproducibility. To simplify sample loading, an in-house designed device was manufactured and tested. The method extended the duration of release to approximately

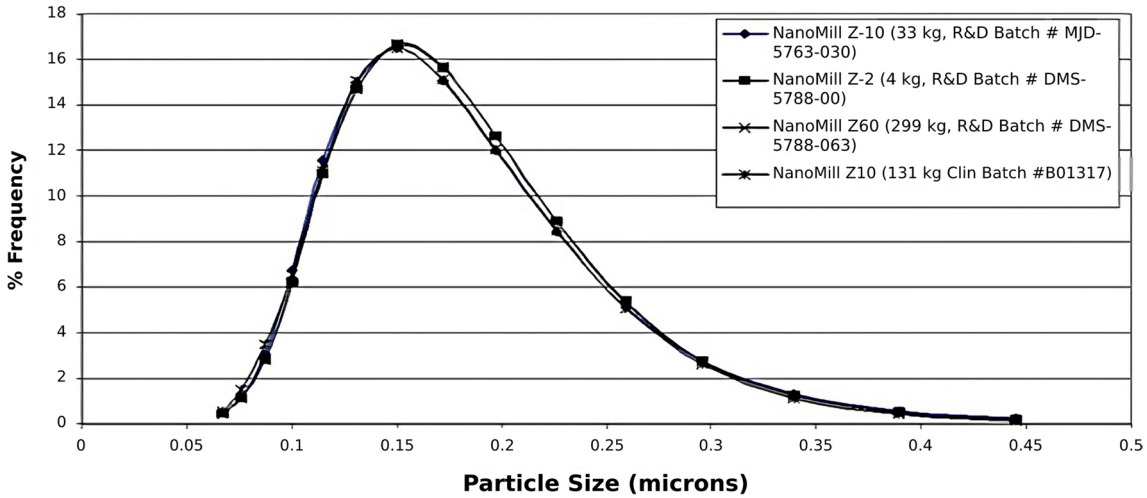

Fig. 5 Particle size distributions produced in lab, pilot, and full commercial scale media mills (43)

1 month with good discriminatory ability. However, large variations in the release profiles were associated with this method, which was considered to be due to the need for further manufacturing design optimization. Accordingly, of the USP apparatus 2 methods tested, the enhancer cells method appeared to be superior for this application.

The USP apparatus 4 with semi-solid adapters method was originally developed for release testing of semi-solid dosage forms (48). The adapters were used here as a sample holder and no membranes were applied. This method extended the duration of release to approximately 1 week and showed good reproducibility and discriminatory ability. The flow rate did not affect the release rate of the MPA suspensions. Among the methods tested, the USP apparatus 2 with enhancer cells and the USP apparatus 4 with semi-solid adapters were the most promising methods for IVIVC development of the MPA suspensions. Both methods were able to differentiate formulations with different particle size and source of excipients.

In Vitro/In Vivo Correlation Development of the Long-Acting Injectable Aqueous Suspensions

To establish IVIVCs of MPA suspensions, *in vivo* release testing of five selected MPA suspensions (four Q1/Q2 equivalent MPA suspensions and the RLD) were performed using a rabbit model. The preclinical *in vivo* data was compared to the *in vitro* release data obtained using the two promising release testing methods (USP apparatus 2 with enhancer cells and USP apparatus 4 with semi-solid adapters) (49). The *in vitro* and *in vivo* data showed the same rank order for all the tested formulations except one Q1/Q2 equivalent suspension with the smallest particle size. This exception was ascribed to the particle size growth over time (Ostwald ripening effect) as well as particle aggregation and recrystallization at the injection site. Accordingly, this formulation was not included since "same rank order" is the minimum requirement for IVIVC development. The data analysis was performed using Phoenix WinNonlin software with an IVIVC toolkit. First, the *in vitro* data obtained from USP apparatus 2 with the enhancer cells method was used to compare with the *in vivo* release data. Unfortunately, a Level A IVIVC was not successfully established due to the external prediction error percentage (%PE) of the C_{max} (− 17.5%) was over the 10% criteria, despite %PE for the area under the plasma curve (AUC) (− 4.2%) being within the criteria. Second, the *in vitro* data obtained from the USP apparatus 4 with the semi-solid adapters method was used to establish an IVIVC. A level A IVIVCs were successfully developed using either three or four formulations, with an external %PE for both AUC and C_{max} (less than the 10% criteria) despite inconclusive internal predictability. To sum up, the USP apparatus 4 with the semi-solid adapter method showed superiority to the USP apparatus 2 with the enhancer cell method. Therefore, for the purposes of product quality control and IVIVC development, the USP apparatus 4 method may be prioritized during *in vitro* release testing method development for LAI aqueous suspensions.

The RLD (Depo SubQ Provera 104) selected in the above studies is a ready-to-use micro-sized suspensions in a prefilled syringe. The developed methods may or may not be suitable for the other types of LAI aqueous suspensions such as nanosuspensions, dual chamber or vial package suspensions, *i.e.*, with dry powder and diluent in separate vials or dual chambers, with Abilify Maintena being a commercial example. Some modifications to these existing methods or newer designs may be required to obtain optimal *in vitro* release profiles of the LAI aqueous suspensions. The *in vitro* methods as well as IVIVC development using a rabbit

model will lay a solid foundation for the other LAI aqueous suspensions. For IVIVC development, it is relatively easier for the AUC to reach the %PE criteria set by the FDA guidance. However, it appears to be more challenging for the C_{max} to meet the set %PE criteria. This phenomenon has also been observed in the preclinical IVIVC development of other types of LAIs such as PLGA-based microspheres (50-53). In conventional IVIVC modeling, it is difficult to incorporate any formulations with unexpected *in vitro* release characteristics as compared to *in vivo*, such as the MPA formulation with the smallest particle size. To have a better understanding of the underlying reasons for these aspects, mechanistic IVIVC modeling such as physiological based pharmacokinetics (PBPK) model should be pursued. PBPK models enable prediction of drug distribution and clearance at specific tissues and given physiological environments. Accordingly, these models may be more promising to extrapolate the animal IVIVCs to human IVIVCs despite the challenges.

Opportunities for Improvement/ Acceleration of LAI DP Development *via* Modeling

Although LAI products are marketed for different therapeutic areas, their development is complicated by various challenges related to manufacturability and stability, dosing, tolerability, control over the release kinetics and the design of lengthy clinical trials. In silico modelling of the pharmacokinetics (PK) can support the drug development process of LAIs from discovery to clinical development stages, *e.g.,* by reducing the number of animal studies, costs and overall timelines, guiding formulation design and understanding the mechanisms of the underlying processes (54). A recent review article by Dubbelboer *et al.* (55) highlighted the increased activity over the past decade in developing in silico models for LAI applications. On one hand, empirical models (*e.g.,* population PK (pop-PK) or PKPD models) describe the PK by one/two/or multi-compartmental models mostly based on available preclinical or clinical datasets. On the other hand, more mechanistic in silico models such as PBPK models, the focus of this section, apply a bottom-up approach to simulate the PK based on drug product and physiological parameters and can therefore enhance the understanding of the underlying mechanisms. This section summarizes the status and challenges of in silico modeling for LAIs as discussed during the AAPS workshop. Two specific examples are given of; i) the application of in silico models for early compound and platform screening, and ii) the development of PBPK models for clinical translation.

Physiological-Based Pharmacokinetic (PBPK) Modeling

PBPK models present an opportunity of incorporating specific mechanisms that might impact the drug release from the formulation and the rate of appearance in the systemic circulation, including the impact of physiological response at the site of injection (56). The rate of dissolved drug uptake from the injection site into the systemic circulation can be predicted from drug properties (tissue/plasma partition coefficient) and the blood flow through the injection site—a process that is well established for PBPK models (57). To make the PBPK models useful for the LAI formulation development, processes impacting the rate and extent of the drug dissolution/release from the formulation at the injection site need to be characterized quantitatively. The presentation by Lukacova focused on evaluating the impact of several of these processes on the drug dissolution from crystalline suspensions of low solubility compounds. Temporal changes in the volume of depot compartment (*i.e.,* injection site) due to inflammation after cabotegravir IM injection in rat (58) allowed to explain paliperidone exposure after IM injection of paliperidone palmitate suspension in rat (56). However, a change in the onset of inflammation was required to correctly capture the shape of plasma concentration–time profile of paliperidone. Even though it has been shown that different compounds cause different inflammation response (56, 59), it is unclear what was the reason for the differences in this case: difference in inflammation response due to different compound, hydrolysis of paliperidone palmitate to paliperidone, or possibly the delay compensates for another, yet unidentified, process. Simulations of SC and IM injections of cabotegravir crystalline suspension in human suggested possible aggregation of injected particles and large effective diffusion layer surrounding the dissolving particles/aggregate resulting in much slower *in vivo* dissolution than would be expected based on particle size and solubility of the compound. In order to reproduce the clinical plasma exposures (60), a completely static environment and significant aggregation (with the aggregate radius for IM injection being 10–15-times larger than for SC injection) were required. These simple adjustments allowed to reproduce the initial rate of drug appearance in systemic circulation and overall exposure, but a mismatch in the shape of the observed and simulated plasma-concentration time profiles (Fig. 6) suggests additional processes might be playing a role.

These two examples explored separately the potential impact of inflammation and particle aggregation/static environment, but multiple processes are likely to contribute to the *in vivo* behavior of the injected suspension. This was

explored in the third case study evaluating the *in vivo* performance of five formulations of low solubility drug with differing drug particle sizes injected SC to rabbits. Accounting for all previously identified processes (temporal changes in injection compartment volume due to inflammation, particle aggregation, and larger diffusion layer thickness around dissolving particles/aggregate) were required to correctly capture the shape of the plasma concentration profiles. Detailed information about the drug particle size distribution in each formulation was available for this case study and the relationship between the measured and effective *in vivo* particle size distribution was explored. One of the formulations was used to determine a methodology for scaling the measured particle size distribution into the effective *in vivo* particle size distribution. The same methodology was subsequently used to predict the effective particle size distributions and the *in vivo* exposures for the remaining four formulations. The prediction errors were less than 20% for most of the predicted C_{max} and AUCs. Only two values (C_{max} for one formulation and $AUC_{0\text{-inf}}$ for one formulation) had prediction error more than 20%, but all predicted pharmacokinetic parameters were within 35% of the observed data. The simulated plasma concentration–time profiles also matched the shapes of the observed profiles well.

Modeling Challenges for Long-Acting Injectables and Improvement/Acceleration of Drug Product Development

Future implementation of state-of-the-art in silico approaches will facilitate the LAI development process from discovery to clinical development stages. However, the AAPS workshop also highlighted the existence of several challenges for the successful development of PBPK models for LAI applications:

- PBPK models rely on a detailed understanding of the role of formulation and physiological parameters. These are still underexplored for LAIs. For aqueous suspensions, the release is governed by a complex and dynamic interplay between API, formulation and physiology (57). As a result, PBPK models are mostly semi-mechanistic and often rely on partial fitting for accurate PK simulations. More research is needed to increase the accu-

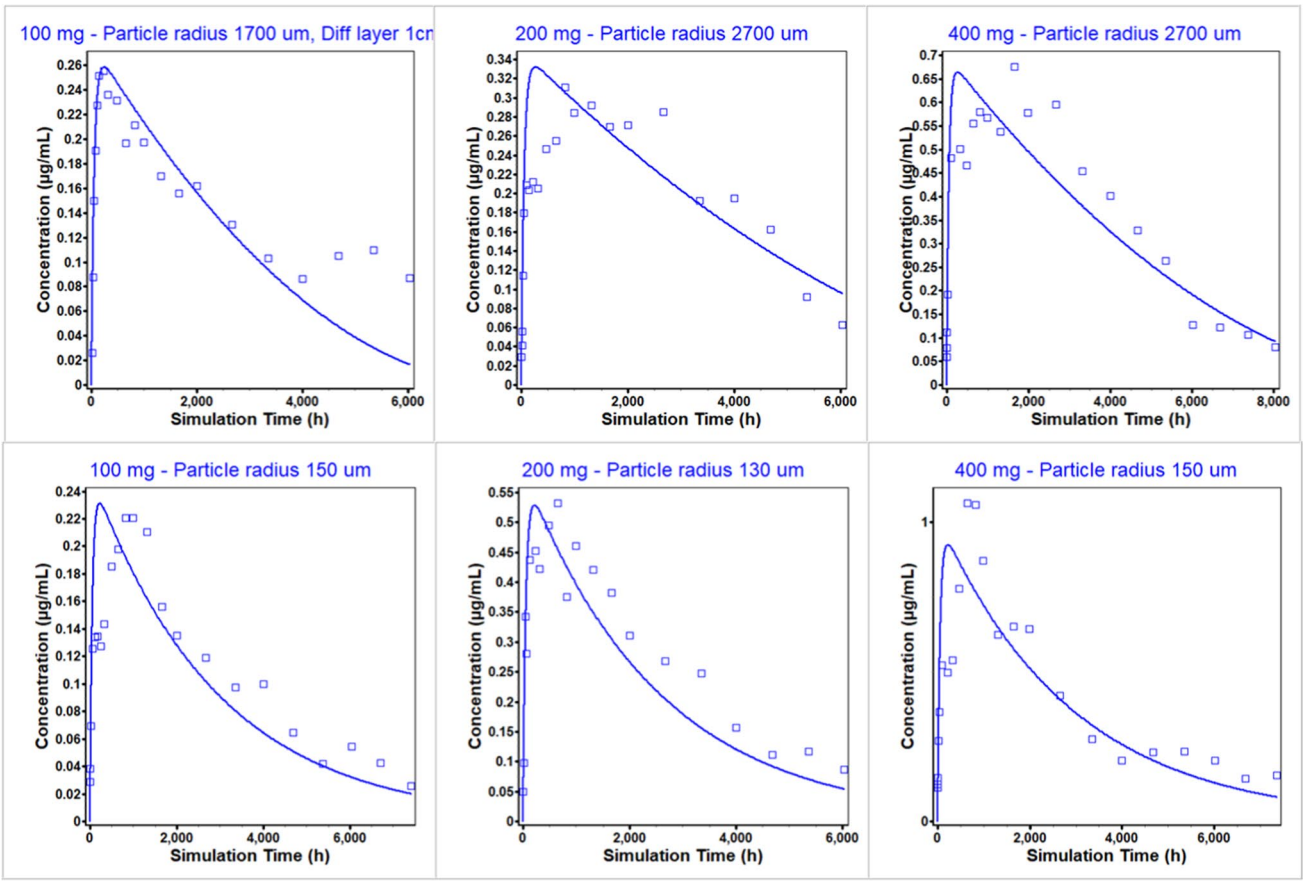

Fig. 6 Simulated (lines) and observed (points) plasma concentration–time profiles after 100 mg (left), 200 mg (center), and 400 mg (right) IM (top) and SC (bottom) injection of cabotegravir suspension in healthy volunteers. Observed data from (60)

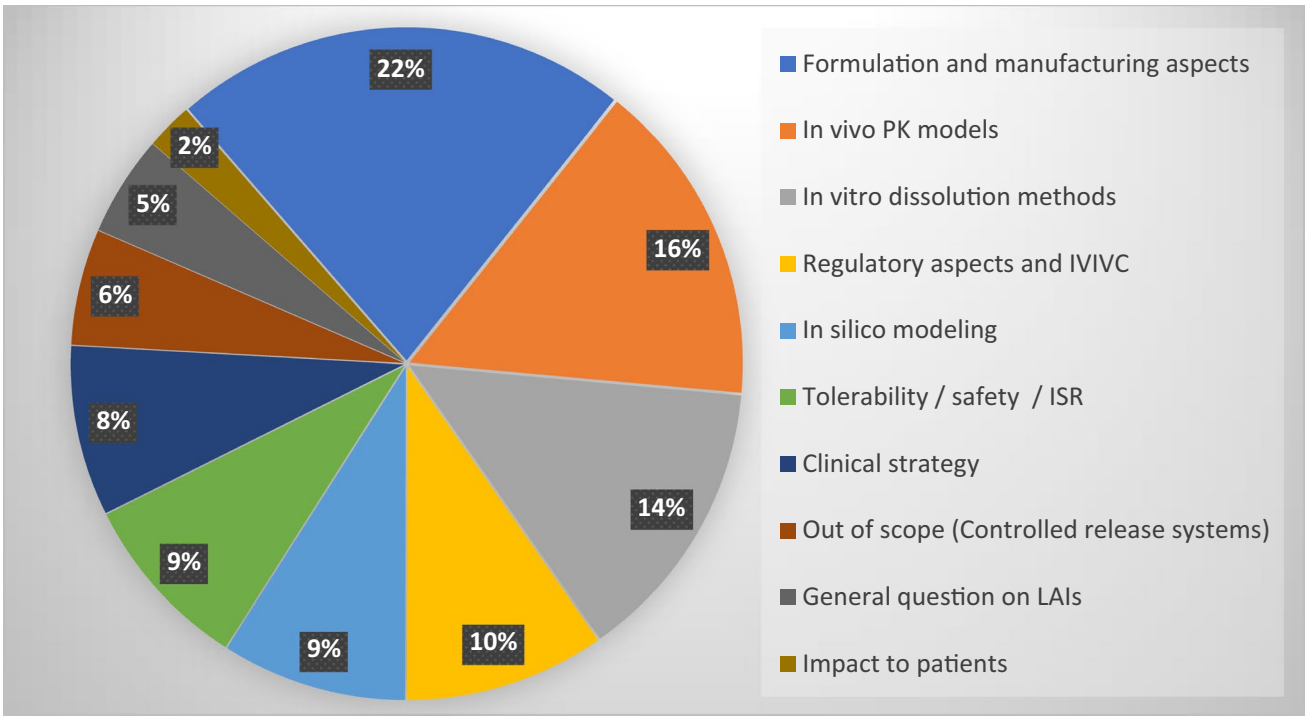

Fig. 7 Overview of the main areas raised questions at the workshop fell within

racy as well as predictiveness of such models. For this reason, PBPK models are also useful in exploring and identifying possible mechanisms impacting drug release from LAI formulations. This helps to guide experiments needed to quantitatively characterize these mechanisms and improve simulations in the future.

- The translation of preclinical PK to humans remains challenging as well. If assuming depot distribution, release, injection site physiology and inflammatory response are similar, PBPK models validated in preclinical species could in theory be translated to humans. However, quantitative translation of *in vivo* behavior to humans requires a better understanding of injection site differences. Furthermore, the applicability of a certain animal model together with dosing conditions depends on the question to be addressed. For preliminary formulation screenings, PK studies in rodents can be utilized. The administration and evaluation of clinically relevant injection volumes requires non-rodent studies. Extensive screenings in such species are not preferred for ethical reasons.
- Mechanistic PBPK models require adequate *in vitro* input to describe the release kinetics. As discussed in the section above, the development of biorelevant release methods for LAIs is complex. Adapted USP 2 and USP 4 methods (45) have been successful in *in vitro* discrimination between aqueous suspensions with different particle size. Discrimination towards other formulation parameters such as stabilizing excipients remains challenging.
- IVIVCs, as discussed in the previous section.
- Clinical studies of LAI products are, by definition, time-intensive to obtain a complete read-out, complicating study designs and extensive clinical evaluation of formulation parameters. In addition, for new drug substances, clinical phase 1 safety studies require an immediate release formulation to evaluate safety and tolerability, postponing the clinical evaluation of LAI formulations to a later stage. PBPK and empirical in silico models may complement each other in overcoming some of these challenges and accelerating the future LAI development process.

Questions and Answers

During the AAPS workshop, a high number of questions were raised by the audience after each talk, but also after the panel discussions held. The main themes for the raised questions have been identified and scored for frequency, as depictured in the pie chart in Fig. 7.

The key themes where questions were raised distributed widely from a scientific perspective, with formulation and manufacturing elements being the most frequent topic. The questions raised were partly a reflection of the topics brought up by the presenters and partly the topics brought to the agenda by the participants. Most of the questions were at the intersection between two or more key themes (*e.g.,* how

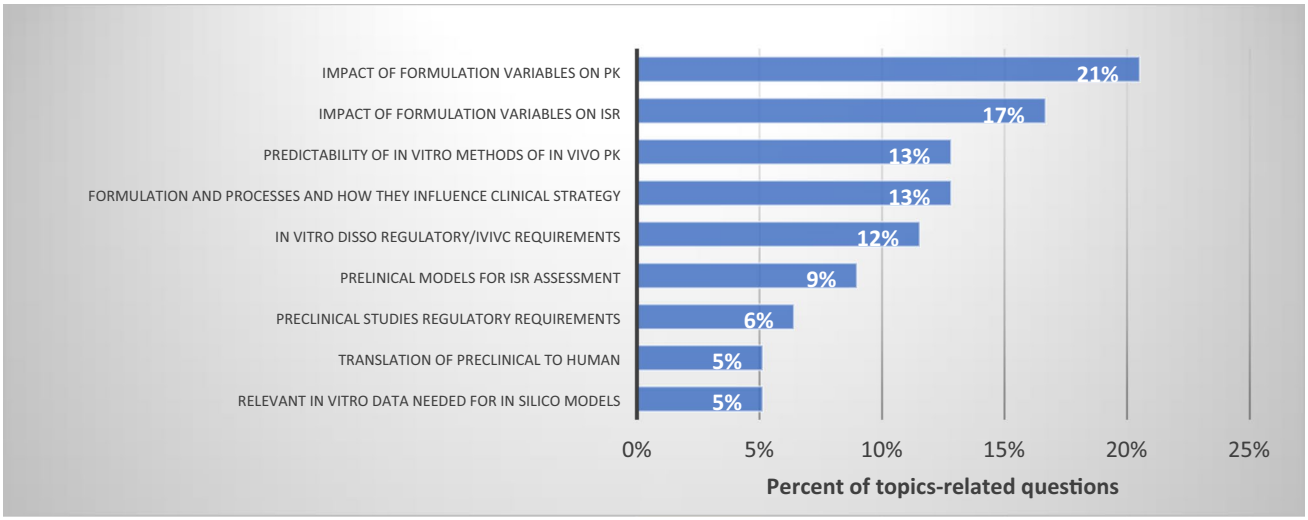

Fig. 8 The distribution of the specific topics of interested raised by the audience (ISR; injection safety and tolerability)

particle size and formulation attributes contribute to ISRs and PK performance). Therefore, a different analysis of the topics of interest from the audience, highlighting the intersection across themes, is reported in Fig. 8, providing a different framing of the questions.

There may be many reasons to why the questions distribute as they do, however, it points towards some of the scientific gaps that may currently exist. A full list of the raised questions at the workshop is provided in Table 2S in the Supporting Information. The response and the respondent are provided in the Supporting Information, if no written response was provided the question is still included.

Concluding Remarks and Gap Analysis

LAI drug products greatly improve patient's compliance, convenience, and life quality, as clinically demonstrated. LAIs are therefore a formulation approach that could be relevant in many disease areas with longer treatment periods. A clearly defined Target Product Profile (TPP) helps to guide the entire LAI development and should early be defined in the development process with a patient centric focus, as each LAI technology has its suitability, limitations, and complexity–where complexity may mean longer development timeline and hence higher cost.

Specifically for this workshop aqueous suspensions applied as LAIs were in focus. In this context nanomilling was discussed as it may provide a platform for rapid and efficient development and commercialization of long-acting injectables products. The milling process technology is highly developed and commercial products already exists. Process scale-up, aseptic manufacturing process, and sourcing may be challenging, but with the many commercial products available both a technical and regulatory path have been demonstrated. A high number of scientific gaps exists within both formulation sciences and manufacturing of suspension. More public information on suspension formulations, their stability and pharmacokinetic profile may start filling some of these gaps. Within manufacturing science, the gaps have not been strongly discussed in the present summary; however, defining the milling conditions, time, and scale-up processes is an area where modern engineering could have a beneficial impact to remove some of the complexity, generating predictive models which may support the definition of the process and keep it in control.

Our biopharmaceutics understanding on LAIs still has gaps and requires further research: systematic *in vivo* studies linked with PBPK modelling would be beneficial to identify critical drug product parameters and biological elements and their impact on the pharmacokinetic performance. Generating additional dissolution examples with bio-relevant dissolution approaches for LAIs could support the needed academic discussion of the sciences around dissolution to progress the field. Altogether, while it is clearly demonstrated that LAIs have a huge potential in the treatment of multiple diseases and commercial products exists, it is also clear that there are many elements of the science around LAIs we do still not fully understand. The scope of workshops like the one summarised here is to continue generating awareness and encourage scientist from the entire pharmaceutical ecosystem to share and present their findings, so the field can progress fast for the benefit of the patients we all serve.

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Declarations

Conflict of Interest R,L., J.G., and N.D. are employees of Lubrizol Life Science, V.L. is an employee and holds stock of Simulations Plus and S.A. is an employee of GlaxoSmithKline. The authors report no other conflicts of interest in in this work.

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