EXPERT OPINION

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Neonatal medicines research: challenges and opportunities

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Introduction: The key feature of the newborn is its fast age-dependent maturation, resulting in extensive variability in pharmacokinetics and -dynamics, further aggravated by newly emerging covariates like treatment modalities, environmental issues or pharmacogenetics. This makes clinical research in neonates relevant and needed, but also challenging.

Areas covered: To improve this knowledge, tailoring research tools as well as building research networks and clinical research skills for neonates are urgently needed. Tailoring of research tools is illustrated using the development of dried blood spot techniques and the introduction of micro-dosing and -tracer methodology in neonatal drug studies. Both techniques can be combined with sparse sampling techniques through population modeling. Building research networks and clinical research skills is illustrated by the initiatives of agencies to build and integrate knowledge on neonatal pharmacotherapy through dedicated working groups.

Expert opinion: Challenges relating to neonatal medicine research can largely be overcome. Tailored tools and legal initiatives, combined with clever trial design will result in more robust information on neonatal pharmacotherapy. This necessitates collaborative efforts between clinical researchers, sponsors, regulatory authorities, and last but not least patient representatives and society.

Keywords: agency initiatives, clinical pharmacology, covariates, dried blood spot, mechanism based models, microdosing, microtracer, neonatal networks, newborn

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1. Introduction

Drug development depends on clinical pharmacology which intends to predict drug effects based on pharmacokinetics (PK) and pharmacodynamics (PD). These principles also apply to children, including neonates. Neonates are the group of children from birth up to and including the age of 27 days or the equivalent maturational age (44 weeks postmenstrual age) and include term and preterm neonates. They represent a particularly vulnerable subgroup of the pediatric population. Fast developmental changes during the neonatal period result in extensive PK/PD variability, making pharmacotherapy (e.g., appropriate formulation, dose) and research in this subpopulation even more specific [1-3]. Moreover, only a limited number of subjects is available in every individual unit, since studies are performed in neonates suffering from a particular disease. Besides this logistical issue, there are also ethical, economical and practical obstacles. Consequence and despite the legal initiatives, newborns remain one of the last therapeutic orphans [4,5].

1.1 From challenges to opportunities: limited drug labeling changes despite legal initiatives

The federal US legislation and European initiatives resulted in a notable increase in pediatric studies and in pediatric labeling changes [6]. Unfortunately, very few were relevant to neonates. In a recent analysis on 406 pediatric labeling changes under the

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Article highlights.

- Despite the legal initiatives taken in different parts of the world to stimulate pediatric drug research, the available knowledge on neonatal clinical pharmacology is still lagging behind.
- The key feature of neonatal physiology is fast maturation, resulting in extensive variability in PK and PD, further aggravated by other covariates.
- To improve both the quantity and quality of the knowledge on neonatal pharmacotherapy, tailored research tools as well as building research networks and clinical research skills are needed.
- We illustrate the feasibility to tailor research tools to neonates by the clinical use of dried spot blood sampling strategies, micro-dosing and -tracers in neonatal studies. Population pharmacokinetic approaches are based on estimations of mean and variances through merging of information from all observations.
- Both FDA and European Medicines Agency (EMA) aim to address the lack of sufficient labeling information for neonates, either through a Neonatal Subcommittee of the Pediatric Advisory Committee or by introducing expertise of neonatologists. EnprEMA (European Network of Paediatric Research at the European Medicines Agency) is also considering establishing a working group on neonatal pharmacotherapy.

This box summarizes key points contained in the article.

Federal Legislation, only 6% of these label changes included neonates. These changes involved 23 drugs for 11 indications (e.g., linezolid, rocuronium, remifentanyl, sevoflurane, stavudine, nevirapine) [4,5].

A recent analysis of the number of neonates planned for enrollment in clinical development studies over a 3-year period suggested that only 33,200 neonates were planned annually (2011 – 2013, Evaluate Pharma, query tool that pulls data mainly from clinicaltrials.gov). The majority (> 31000 cases) related to studies initiated by non-industry (academic) sponsors, while only about 4 – 5% were industry requested, suggesting that there is limited research activity that will lead to labeling changes [Pam Simpkins, pers. commun.].

In this paper, we aim to describe challenges and influences related to neonatal medicines research. This includes newly emerging tools to facilitate studies in neonates (e.g., dried spot blood (DSB), micro-tracer and -sampling studies, different PK/PD models), but also burdens related to the effective organization of studies, covering the need to build networks and research capacity. These topics will be preceded by an introduction on PK and PD in neonates to illustrate the need to for tailored dosing, and neonatal formulations.

1.2 Principles of neonatal pharmacokinetics and -dynamics

Newborns differ from children and adults in their drug response. These differences may be caused by changes in PK

and/or PD. The PK processes considered are absorption, distribution, metabolism and excretion (ADME), while PD comprises the physiological and biological response to administered drug and represent both efficacy and safety. Maturation changes in neonatal period can significantly affect all PK processes, as well as PD [1-3]. Besides maturational changes, newly emerging covariates like treatment modalities (e.g., hypothermia, extracorporeal circulation), environmental issues (microbiome, critical illness, co-medication) or pharmacogenetics further intensify the PK/PD variability in early infancy [7].

The absorption of drugs is affected by age, formulation, dose or route of administration as well as by food and other drugs that interact. For example, in neonates intra-gastric pH is elevated (> 4) which may increase bioavailability of acid-labile compounds (penicillin G) and decrease bioavailability of weak acids (phenobarbital) when given orally [1]. Additionally, gastric emptying in neonates is delayed, which means that also duodenal absorption of drugs (e.g., paracetamol) is slower in neonates [8]. Characteristics that affect absorption and unpredictable PK of drugs administered intramuscularly include decreased muscle mass, decreased contractility and reduced overall muscular perfusion [1]. A specific issue in neonates is the unanticipated higher absorption of drugs administered transdermally due to proportionally higher body surface area and a more permeable skin. These differences are even more pronounced in premature neonates. For example, topical anesthetics may result in methemoglobinemia because of higher absorption and reduced capacity to metabolize these compounds [9]. Finally, rectal absorption is generally increased in neonate, but also variable due to the difference in rectal venous drainage systems [8].

Differences in distribution of the drug relate to body composition, systemic and regional blood flow, plasma protein binding, as well as permeability of membranes. The body composition in early infancy changes rapidly, resulting in an age-dependent proportion of body water and fat. Hydrophilic drugs like aminoglycosides have a larger volume of distribution in neonates that can be explained by larger extracellular fluid (40 – 45% of the body weight) compared to 25 – 30% at the end of infancy and to 20% in adults [10,11]. On the other side, neonates have a decreased drug protein binding capacity. Clinical implications of alterations in drug protein binding are most relevant for drugs which are highly protein bound and have a narrow therapeutic index [12]. To illustrate this, both differences in protein binding characteristics and their covariates within neonates as well as compared to other populations have recently been described for cefazolin [13,14]. Both absorption and distribution is in part also guided by active transport, but data on the ontogeny of transporters and its covariates are almost absent.

In general, drug metabolism is low in neonates and phenotypic, age-driven activity of drug-metabolizing enzymes is considered as a main contributor to the PK differences between neonates, children and adults. However, this does

not exclude extensive inter-individual variability within the neonatal population [15,16]. In addition, renal function and liver flow are influenced by physiological changes depending on age, for example, the glomerular filtration rate (GFR) in full term neonates is 35% of the adult values [17,18]. Term neonates have a rapid increase in GFR during the first 2 weeks of life and reach adult values by the first year of life [17,18]. Premature infants show similar trends, with a slower initial rise in GFR due to nephrogenesis not being completed until 34 weeks of gestation [18,19]. Active tubular secretion and tubular reabsorption are also immature at birth (20 – 30% of adult values), reaching adult values by 7 – 12 months of life [20].

Differences in neonatal physiology can also affect PD, resulting in differences in drug efficacy or toxicity. Age-related developmental changes in the functionality or expression of receptors, and differences in disease status may alter the pharmacological response to drugs [1,21,22]. For example, inotropic agents in neonatal myocardium act in a more limited fashion than in children or adults. This is due to a lower ratio of active myofilaments to non-contractile elements, greater stiffness of the ventricle, underdeveloped cardiac sympathetic nerves and higher cardiac output per unit surface area [23]. Furthermore, immaturity can result in an altered risk of drug toxicity, even a decreased risk. To illustrate this, neonates appear to be less susceptible to renal toxicity induced by aminoglycosides compared to children and adults, due to reduced intracellular accumulation of these compounds in the renal tubular cells [24,25]. Similar to the expansion of biomarker research in human (adult) medicine, there is an active search for robust biomarkers to evaluate interventions in neonates and infants. A biomarker is a characteristic or quantitative indicator that reflects either normal biologic or pathological processes, or pharmacological responses or (side)-effects. This includes imaging techniques, analysis of samples (blood, urine, saliva) but also covers questionnaires or long-term neurodevelopmental outcome assessment. However, the introduction of these biomarkers first needs robust validation in this population. This has recently been illustrated, for example, plasma creatinine or cystatin C as biomarkers of renal function in early infancy [26,27]. The emerging field of metabolomics in plasma, urine or maternal milk may provide unique insight into the host's dynamic behavior in different neonatal conditions, including drug exposure and its effects [28]. Although promising, the metabolomics markers have to undergo prospective validation before such markers can be integrated in clinical care or clinical research.

The issue of maturational PK and PD is not limited to the active compounds, but should also consider excipients. The use of potentially toxic excipients in medicines given to neonates is not rare, as they are present in many commonly used drug products [29,30]. Recent observational studies confirmed the almost uniform exposure to relevant excipients (e.g., propylene glycol, ethanol, benzyl alcohol) in the UK and Estonian cohorts of neonates [31,32]. In order to establish safe limits for excipients, the European and US Pediatric

Formulation Initiatives have developed a Safety and Toxicity of Excipients for Pediatrics (STEP) database, aiming to centralize any available excipient safety and toxicity data [33]. Similarly, the European Study of Neonatal Excipient Exposure project aims to assess neonatal exposure to potentially toxic excipients [32]. PK and PD data for specific excipients (propylene glycol, methyl- and propyl-parabens) have recently been reported [34–36].

In summary, a large number of variables influence the disposition of active ingredients and excipients in neonates. The overall effect of these variables is very difficult to predict so that it is necessary to conduct PK studies in neonates, if only to validate predictions from carefully constructed predictive models (cf *infra*, 2.3). It may be necessary to use age-specific formulations since excipients that are safe in other age groups may be harmful in neonates, while highly concentrated formulation may result in additional unexplained variability in drug exposure. To illustrate this, we refer to our observation on the impact of the introduction of a pediatric vial (50 mg/ml) instead of an adult vial (250 mg/ml) on amikacin clearance predictability and dose accuracy [37].

2. Opportunities and influences to improve neonatal medicines research

We aim to describe opportunities and influences related to neonatal medicines research. This includes newly emerging tools, tailored to conduct studies in newborns (e.g., DSB, micro-tracer and -sampling studies, different PK/PD models), but also burdens related to the effective organization of studies, covering the need to build networks and research capacity.

2.1 DSB sampling strategies

Collection of blood samples to estimate PK or PK/PD in neonates is hampered by ethical issues as well as technical challenges, since PK studies in general rely on repeated quantification of drug levels in the blood compartment. The EMA guideline recommends (not evidence based and legally not binding, but strong commitment) for blood sampling in neonates that 'trial-related blood loss [...] should not exceed 3% of the total blood volume during a period of four weeks and should not exceed 1% at any single time' [38]. This means that maximum volume of total blood (75 – 80 ml/kg) that can be safely removed from a 500 g preterm for trial purposes is 1.2 ml over 4 weeks and 0.4 ml/sample. For a term newborn (3.5 kg), this is 6 ml and 2 ml, respectively. Consequently, PK studies in neonates should be designed and performed within these blood sampling limitations that cover both PK and safety labs. Methods to limit blood sampling volume and frequency are encouraged to be used and developed as well as alternative methods to using blood [38]. DSB samples, combined with population PK modeling techniques is one potential method to facilitate PK studies in neonates [39,40]. Interestingly, the same rationale can be built for preclinical PK studies in small animals.

DSB refers to a blood sampling technique where small volumes of blood are spotted on an appropriate filter paper, dried and subsequently shipped for analysis. The technique itself has been introduced already a long time ago in neonatal care to screen for phenylketonuria, and became well established for applications such as the broader neonatal screening for inborn (metabolic) diseases. The same methodological approach has more recently experienced a novel surge of interest in the context of drug development, that is, toxicokinetic and PK studies, and of clinical pharmacotherapy, that is, therapeutic drug monitoring (TDM) [35,41-50].

The specific area where DBS is complicated is the bioanalysis. Although the preparation of plasma from whole blood can also be considered a pre-analysis sample preparation, the use of DSB as material is different. Extracting analytes of interest (e.g., mother compound, metabolites, excipients, different drugs) that are trapped in denatured protein matrix on a filter paper requires careful optimization and standardization. Among other issues, the optimization of the extraction and the selection of the card type are specific questions that should be considered [44,47]. Obviously and in contrast to serum or plasma analysis, the underlying DBS matrix is whole blood. Variability in hematocrit was the single most important factor that affected assay accuracy. Neonates exhibit considerable inter and intra-individual variation in hematocrit values during the first 28 days of life, in part explained by age or disease characteristics. Using dexamethasone as illustration, Patel *et al.* evaluated different factors, including blood volume, blood hematocrit and spotting device that may affect drug quantification measurements based on DBS [44]. These authors hereby documented that the assay accuracy and precision values remained within the 15% variability limit with fluctuations in hematocrit of 5%. Variations in the volume of blood spotted did not appear to affect the performance of the developed assay. Similar observations were made regarding the spotting device used.

Because of the low volumes, and low amounts, sensitive and specific tools like liquid chromatography with single quadrupole mass spectrometry (LC-MS) and more often tandem mass spectrometry (LC-MS/MS) quantification techniques are commonly used to develop measurement methods. The development of such methods to measure a range of environmental toxicants, excipients or drugs in DBS has been a more recent goal. The approach has been proved to be effective in the field of neonatal pharmacology, based on compound-specific reports, for example, caffeine, diphantoine or fenytoine, topiramate, canrenoate, propranolol, linezolid, metronidazole or dexamethasone [39-44,49,51].

Interestingly, others also reported on the simultaneous measurement of different compounds (e.g., rifampicin, clarithromycin and its metabolites) in the same sample [48], or used the DBS technique to quantify prenatal ethanol exposure (i.c. phosphatidylethanol) [45], to quantify temporal trends in exposure to environmental chemicals (i.c. polychlorinated biphenyls and organochlorine pesticides) [46]. Finally, the

same approach has been used to quantify excipient exposure (i.c. methyl- and propyl-parabens) in neonates [35]. In contrast, we quantified propylene glycol exposure based on plasma samples [34], but were unable to retrieve this compound in DBS. We hypothesize that this is due to evaporation during the drying procedure following sample collection. Similar, benzyl penicillin and other β -lactam antibiotics may be too unstable for DSB analysis.

2.2 Microdosing and microtracers

Quantitative assessment of metabolites in early phase of clinical product development remains an analytical challenge when commonly used methods, standards and assays like HPLC or similar are not yet available. The use of tracers through radioisotopic labeling, commonly with ^{14}C , is the preferred method to explore and quantify the absolute yields of different metabolic routes in the absence a priori knowledge of the human metabolism [51,52]. In a 'microdose clinical trial', candidate compounds are administered in limited doses estimated at not having pharmacological or toxicological effects, aimed at candidate selection for proceeding to clinical development starting from Phase I. Such a dose is defined as 1% of either the No Observed Adverse Effect Level (NOAEL), or the predicted pharmacological dose based on animal data, or 100 mg of the new compound [51,52]. Dose linearity between the microdose and the (assumed) therapeutic dose is a prerequisite to extrapolate initial findings to dosing guidelines.

Because of the very low doses, microdosing only became feasible due to the development of high-performance analytical technologies, such as AMS (accelerator mass spectrometry) and LC/MS/MS (liquid chromatography/mass spectrometry/mass spectrometry). This is because these technologies enable determination and quantification of compound (mother compound, metabolites) at very low concentration or amount in samples collected following administration of a very limited dose. Based on these technology and study approach, data on the metabolism of a drug candidate can be obtained in early development of the product by administering a ^{14}C -drug to adults volunteers or patients, and comparing the plasma concentration-time curves for total ^{14}C and unchanged parent compound. Full metabolic profiles can be generated as an early indication of the drug's metabolism in humans or absolute bio-availability can be estimated [51,52]. More recently, the practice of microdosing has extended to other areas of drug development, including *in vivo* drug-drug interactions in human adults, but also explorative studies in special populations, including newborns [53-55]. This practice builds further on clinical experience with the use of stable isotopes in neonates to quantify carbohydrate, fat and amino acid metabolism or synthesis of specific peptides or proteins [56]. In this way, the use of isotopes to describe drug disposition is an additional application of a methodology already in practice in pediatric clinical research and

further builds on the collected experience, including aspects of feasibility and safety [56].

PK studies in neonates are commonly limited by the small volume of blood that can be collected. The high sensitivity of the above-mentioned analytic technologies (AMS, LC/MS/MS) facilitates PK studies by reduction of the sample volumes needed. To the very best of our knowledge, there are at present two compounds (^{14}C -ursodiol and paracetamol) to illustrate at least the feasibility to apply such study methodology (microdosing, microtracer) in neonates [53-55]. Gordi *et al.* reported on the repeated administration of ^{14}C -ursodiol microdosing in 5 non-cholestatic and 3 cholestatic newborns, together with 40 mg/kg of non-labeled ursodiol [54].

Besides low sampling volumes, quantifying exogenous doses against the endogenous background was feasible by the ^{14}C tracer molecule. Two consortia evaluated ^{14}C -paracetamol metabolism using AMS technology in infants. Mooij *et al.* reported on 10 newborns, infants and toddlers (0.3 – 83.1 months) who were simultaneously exposed to an oral ^{14}C microdose (3.3 ng/kg) in addition to a regular intravenous paracetamol dose (15 mg/kg, q6h) (microtracer approach). The authors hereby documented paracetamol disposition in their cohort. Using a similar approach, a multi-centre clinical study to evaluate the use of a microtrace dose of ^{14}C -labelled Paracetamol and AMS bio-analysis (PAMS study) as new tools in drug development to determine PK in 60 preterm and term neonates, infants and toddlers was recently finalized [53,55]. These studies also used 'microdoses' where the ^{14}C label was administered separately from a therapeutic dose. Interestingly, DBS analysis was not possible in these studies because the background rates of ^{14}C in filter paper were too high and non-cellulose based papers were not useful.

At least, the available data provide a proof of concept and feasibility that microdosing and microtracer study approaches are indeed possible in neonates. It hereby still needs to be proven that the dose linearity between the microdose and the therapeutic dose also exist in early infancy.

2.3 Population modeling in early infancy

About 30 years after its introduction, PK population approaches became the reference approach for drug modeling in pediatrics and early infancy. *In silico* prediction of PK behavior in pediatric patients is not intended to replace clinical studies. However, it provides a valuable aid to decision-making with regard to first-time dosing in children and study design. The clinical study then becomes 'confirmatory' rather than 'exploratory' [57-62]. Regulatory bodies increasingly encourage the use of modeling and simulation methods for all pediatric drug development programs [63] which also is reflected in a number of regulatory guidance documents [64]. This is because the main limitations in this specific population is the blood volume to be collected and – related to this – sampling frequency, and the number of patients to be recruited. PK population approaches using sparse sampling may resolve both issues. Population PK

approaches are based on estimations of mean and variances through merging of information from all observations (concentration/time, concentration/effect) in all individual patients. Therefore, every effort should be made to simultaneously collect data on covariates to unveil indicators related to maturational changes in PK or PD. Consequently, it is advisable to include data on covariates like gestational age, postnatal age, postmenstrual age, birth weight, body weight, renal function, albumin, concomitant medication or co-morbidity in PK/PD studies [57-62]. Guidance is generated through, for example, the EMA modeling and simulation work group and workshops are organized on this topic [65,66].

Drug modeling can be considered to be a success when such an exercise can support a decision, for example, initial dose to be studied in a study, tailored individualized dosing in the clinical setting, or anticipate the extent of drug-drug interactions. Depending on the underlying distribution of parameters or covariates, both parametric and non-parametric estimation methods can be used. Datasets limited to or including preterm and term newborns hereby present the biggest challenge, since such datasets display an extensive range in addition to mean or median estimates. The rapid developmental changes in ADME, combined with all possible disease processes that interfere with these developmental changes ('ontogeny'), necessitate a study design that is sufficiently powered to estimate mean values but also to quantify the impact of covariates.

There are in essence two different approaches, either physiologically based pharmacokinetics (PBPK) or empiric, exploratory model building. PBPK models aim to translate anatomical, physiological, physical and/or chemical descriptions of all phenomena involved in the complex drug disposition process to predict concentration/time or concentration/effect patterns. PBPK modeling offers a unique method to incorporate different levels of information (drug physico-chemical properties, preclinical data, clinical data) to estimate age-specific PK [60,62]. Although a degree of simplification and empiricism is still present in such PBPK models, they have an extended domain of applicability compared to that of classical, empirical based models. The main advantage of PBPK models is that prediction becomes possible, while empirical-based models need preliminary observations, although also PBPK models are most informative when supported or improved by empirical data [10,59,62]. Any population PK approach can account for covariates within a given pediatric population, but they generally do not account for real-time growth and maturation of individuals through the time course of drug exposure [63]. Integration of time-varying system parameters ('re'sampling) during prolonged studies to correct for subject maturation has been reported to provide more robust predictions. Obviously, re-sampling was most frequent in early neonatal life, reflecting the fast maturational changes in this subgroup [63].

Unfortunately, the available knowledge on developmental physiology in early infancy is still too limited, particularly in

preterm neonates. This is at present also reflected in the fact that the most commonly used PBPK modeling tools (i.e. Symcyp, PK-Sim) do not yet contain robust information on preterm neonates [54,67]. This includes, but is not limited to the physiological parameters driving oral absorption throughout early infancy. To illustrate this, we would like to refer to a very recently published paper on PBPK-based modeling using both software packages mentioned to predict oral drug exposure over the entire pediatric age range. Using sotalol as a model drug, both PBPK software packages evaluated reflected properly the age-related PK changes and predicted adequately the oral sotalol exposure in children of different ages, except in neonates where an above twofold error was observed [67]. These differences were likely explained by controversies and knowledge gaps on neonatal development of the gastrointestinal tract and functions [68].

The same limitations also hold true for other reported examples, including a PBPK model for voriconazole (no estimates below 2 years) [69], theophylline and midazolam (term neonates) [70], caffeine and theophylline (term neonates) [71]. Based on prediction of clearance of 11 drugs (including midazolam, caffeine, cisapride, omeprazole, gentamicin, vancomycin) and its associated variability in neonates, infants and children, the accuracy of the physiologically based model incorporated in the Simcyp software was superior to that of simple allometry, especially in children < 2 years old [72]. Still, only 70% of the predicted median clearance values were within the twofold of the observed values, compared to 89 – 100% in the other pediatric age categories. To the best of our knowledge, PBPK examples in preterm neonates have only been reported as abstracts (aminoglycosides, paracetamol) [73,74].

Translation of the already available knowledge, either implicitly hidden in mechanism-based models or explicitly integrated in physiologically based population PK/PD models hold the promise to facilitate drug evaluation in (pre)term neonates, to explore the impact of covariates and to design studies in the most effective manner [59,63]. This combined use of both modeling approaches ('semi-physiological models') is of relevance, since we do not have (yet) sufficient data on the developmental physiology in early life. The maturational patterns described and the extent of the impact of covariates can subsequently be applied to predict *in vivo* concentration-time profiles for compounds that undergo similar routes of elimination. This concept has been applied for developmental glucuronidation and developmental GFR [10,75].

In this way, such *in vivo* observations can also be used as a "bottom-up" approach to learn more about the maturational patterns, and to guide research on gaps in the knowledge on developmental anatomy and physiology. PK/PD models do not only hold promise 'top-down' to design studies, but may also serve as indicators to guide research on developmental physiology as 'confirmatory' instead of 'exploratory'. Improved knowledge on developmental pharmacology hereby does not only support the individualized drug prescription,

but can also improve the knowledge on aspects of developmental biology that are not yet fully understood, or difficult to explore in the human newborn (e.g., drug-drug interaction, transporter activity).

2.4 Legislation driven initiatives

The federal US legislation and consecutively, the European Paediatric Regulation resulted in a notable increase in pediatric studies and in a relevant increase in labeling changes. Unfortunately, only few of these changes included drug labeling changes for neonates [4,5] and an EMA survey confirmed that (pre)term newborns are the age group with the highest 'off-label' use reported in Europe [76]. This induced in both agencies initiatives to stimulate neonatal medicines research. FDA and EMA encourage a coordinated collaborative approach to product development, including formulations, as an important step towards more effective product development for neonates.

The FDA is working to address the lack of sufficient labeling information for neonates. This is achieved through establishing a Neonatal Subcommittee of the Pediatric Advisory Committee and by introducing expertise of neonatologists to assist FDA in establishing priorities and scientific pathways for neonatal product development [77]. This initiative is part of the recently revised Food and Drug Administration Safety and Innovation Act (FDASIA, 2012) and may be an important driver to stimulate neonatal pharmacological research.

According to EU legislation companies have to obtain the approval, from the European Medicines Agency (EMA), of the studies to be done with neonates or children well in advance of their application for marketing authorization in adults. The Paediatric Investigation Plan to be agreed with the Paediatric Committee at the EMA (PDCO/EMA) needs to discuss the development program, specifically including neonates, describing the development of neonatal age-appropriate formulation as well as the specific non-clinical and clinical development required (if any) for all pediatric age subsets. PDCO from its beginning placed great emphasis to ensure that the neonatal population is adequately covered. This significantly increased the percentage of PIPs including neonatal studies, compared to the initial proposal of pharmaceutical companies: from 15 to 28% in January–October 2008 [78], and from 24 to 32% in March–December 2011 [76]. This is not reflected in any changes of authorization as the completion of most neonatal studies has been deferred, that is, can take place after the adult development. In order foster and focus on the drug development for this age group, the PDCO created in 2013 a Neonatal Working Group comprised of pediatricians including neonatologists and pediatric intensive care specialists as well as formulation and methodological specialists. The main area is to provide support for the review of product specific PIPs with regard to neonatal issues. This group collaborates closely with Enpr-EMA (European Network of Paediatric Research at the European Medicines Agency), which also has established a working group with

specific emphasis on neonatal pharmacotherapy. Enpr-EMA develops a network of networks, investigators and centers with specific expertise in the performance of studies in the pediatric population. The mission hereby is to facilitate drug trials in order to improve the availability of labeled, authorized medical products. This is obviously not limited to, but includes neonatology. The aims of this working group are to provide further expertise to the Paediatric Committee (PDCO), for example, with respect to the review and update of the EMA guideline on the investigation of medicinal products in term and preterm neonates [38], the inventory on therapeutic needs in neonates and the impact of 10 years of the Paediatric Regulation on data generated in (pre)term neonates. Last but not least, the working group aims to collaborate with learned societies on, for example, trial design, the validity of extrapolation, the use of biomarkers, or the assessment of long-term outcomes. The inclusion of the youngest pediatric age-groups also has led to an increase of numbers of juvenile animal studies [79]. Further research is needed regarding the impact of the use of preclinical models and toxicity studies specifically for the neonatal drug development. Regulatory tools and methods (e.g., qualification of methodologies, scientific advice and protocol assistance, innovative task force activities) should be considered as support from the regulators. In the end, these methods will have to be used when evaluating and assessing novel medicines for neonates [80].

2.5 Caregivers and trials in neonates

Obviously, the general regulatory guidelines on best practice in research should also be applied when conducting a study in newborns [76,77]. However, there are some issues that are worth considering because of the specific contextual factors relevant to studies in neonates. Before starting a study, caregivers of a given neonatal unit should be aware that the needs to conduct a given study in line with the guidelines may disrupt or intervene with local practices or routine procedures [81]. Thorough process mapping is crucial, taking both into account the specific aspects of the unit (e.g., protocols, referral, safety procedures) and the requirements of a given study trial (type of patients, duration of the study, data to be collected). No study can be implemented by the study team alone and a study will impact on all caregivers in the unit. Trials that recruit shortly after birth, benefit from prenatal information on the ongoing trials, while formal consent is best obtained after delivery [81].

As described above, most of the currently conducted studies were initiated by non-industry, academic sponsors. Clinicians commonly use a pragmatic, randomized trial design, while more sophisticated, advanced trial designs as well as a broader 'product development approach' should be considered to make most use of the high quality data. Issues not commonly considered include, for example, product stability, compatibility, dose selection, or the concept of adverse events versus adverse effects.

For example, several large-scale trials of inhaled nitric oxide for extremely preterm infants have been conducted in the absence of evidence relating to the optimal dosage regimen or inclusion criteria [82]. Initiatives in Europe and the US have funded research into off-patent medicines that have demonstrated that a structured drug development program is possible in neonates [81]. Neonatologists should insist on incorporation of evidence-based treatment sufficient to support product labeling in neonates [7,81,83-85]. Journal publication and expert opinion are in general not sufficient for labeling.

Issues or problems related to clinical investigators as mentioned by the FDA during the webinar are the insufficient documentation of parental permission, insufficient documentation of the institutional review board (IRB), problems related to the reproducibility of laboratory tests, inability to validate documentation, poor quality of data or inability to enroll or follow patients [86]. Further issues relate to having and document sufficiently safety data, including appropriate long-term (efficacy and safety) data specifically for neonates. Be aware: for a monitor or a regulatory body: 'what has not been documented and stored and consequently, cannot be verified, never happened'. Clinicians may be content with data that are sufficient for publication because they have the ability to monitor their patients after a medicine is administered. If imperfect data led to inaccurate results then clinicians can adapt to the consequences in real time. Regulators cannot monitor individual patients and so have to be assured of the quality of the data before grant a marketing authorization or license. This does not mean that 'off label is equal to off knowledge' in an individual newborn, but the final aim should be marketing authorization and labeling.

Both issues, that is, center-specific skills and problems related to recruitment can be addressed by building and – even more relevant – maintaining research networks. This is because the cost of building and taking down individual networks for each product development plan is prohibitive [86]. While agencies can try to act as catalysts to stimulate such network building, it is up to the individual caregivers and neonatal units to integrate the need to contribute to neonatal medicines research and product development as part of their mission statement [81,84-86]. At least, the output and the impact of pediatric pharmacotherapy of either topical networks [European Cystic Fibrosis Society, Clinical Trials Network for cystic fibrosis (ECFS-CTN) [87], Pediatric Rheumatology International Trials Organization (PRINTO) [88]] or national networks (e.g., Medicines for Children Research Network) illustrates the potentials of network building [89]. The StaR initiative is such an attempt to try to translate the experience built through the Medicines of Children Research Network to address the paucity and shortcomings of pediatric trials, including in neonates to a global level. This initiative involves methodologists, clinicians, patient advocacy groups and policy makers dedicated to developing practical, evidence-based standards for enhancing the reliability and relevance of pediatric clinical research [90]. The Global Research

Framework on study design

- **Maximise use of existing data**
 - Extrapolation
 - Modeling
 - Systematic review or meta-analysis
- **Minimise burden for the individual patient**
 - Opportunistic samples
 - Scavenged samples
- **Minimise number of recruits**
 - Adaptive designs

Figure 1. Framework on study design for neonatal medicines research.

in Pediatrics (GRIP) Network of Excellence is developing methods and case studies to support an infrastructure for the development of medicines in children [6].

At present, a search in the Enpr-EMA database on preterm and/or term newborn results in 30 hits, so there is for sure potential to build such networks. Similarly, at the latest European Academy of Pediatric Societies (EAPS) meeting in Barcelona (October 2014), about 60 neonatologists attended a dedicated workshop on neonatal medicines research. About half of the attendees had experience with FP7 projects, reflecting the fact that translation from temporary, project-related research facilities to permanent research facilities should be feasible [83].

3. Conclusion

Despite the legal initiatives taken, the knowledge on neonatal clinical pharmacology remains suboptimal. The key feature of neonatal physiology is its fast maturation. As a consequence, there is extensive variability in PK and PD in newborns. This makes clinical research in neonates relevant, but also more difficult. To improve the knowledge on neonatal pharmacotherapy, tailoring of research tools as well as building research networks and clinical research skills are needed.

We illustrate the feasibility to tailor research tools to neonates by DSB sampling strategies and by the first reports on micro-dosing and -tracers in neonates. Population pharmacokinetic approaches are based on estimations of mean and variances through merging of information. Datasets in (pre) term newborns hereby present the biggest challenge, since such datasets display an extensive range. Legal initiatives of both FDA and EMA aim to address the lack of sufficient labeling information for neonates through establishing dedicated working groups.

4. Expert opinion

Newborns remain one of the last therapeutic orphans. This is because the legal initiatives taken in different parts of the

world resulted in an increase in pediatric drug labeling changes, but only few of these related to neonates [4,5]. Among different challenges, only a limited number of subjects is available in every individual unit, since studies are performed in neonates suffering from a particular disease. Besides this logistical issue, there are also ethical, economical and practical obstacles [85]. In Figure 1, we suggest a framework on how neonatal medicines research should evolve.

Driven by an identified therapeutic need, a first step should be to collect already existing data and maximize their use. We refer the interested reader to the EMA concept paper on extrapolation of efficacy and safety in medicine development [91]. The approach taken on excipients mentioned in the introduction (STEP, ESNEE) are illustrations of such a structured approach [32,33]. Besides extrapolation and other modeling approaches, there is also value in meta-analysis. The use of 'individual patient data' instead of 'aggregate data' hereby allows exploration of effect modifications at the level of patient characteristics. It also allows multivariate analysis of the collected covariates, correcting for possible confounding factors. Similar to the network approach as a tool to improve neonatal medicines research, collaboration between different research groups is hereby crucial. Such an approach has been reported, for example, ventilation modes [92], or for inhaled nitric oxide in preterm neonates [82,93].

In the subsequent study design, the available information, including (partial) extrapolation from, for example, children for efficacy of antibiotics, can be used to minimize the burden for the individual patient. There are numerous examples on the use of TDM data to develop and subsequently validate PK models [10] or to illustrate its limitations related to, for example, creatinine assays or vancomycin assays used [94]. Opportunistic sampling, that is, sample collection at times where there is a clinical indication to collect blood, or scavenged samples (i.e., based on remnants initially collected for clinical use) can be used. However, both approaches do need a detailed registration of sampling times, dosing and covariates. A PK model is sufficiently sophisticated to explore the explained variability, but the extent of the unexplained variability largely depends on the quality of data collection ('rubbish in = rubbish out'). Finally, one should realize that PK modeling can also be used to develop the most informative sampling schemes, but that such schemes may result in conflicts between clinical needs and study related needs [58,81].

Finally, one should try to minimize the number of patients to be recruited while still being able to provide the answers needed. A smaller study is not only cheaper, but also sooner finalized, and data to base clinical decisions on will become available sooner. Adaptive designs or effective dose (ED₅₀ or ED₉₀) studies have been reported, for example, patent ductus arteriosus and ibuprofen (continual reassessment method) [95] or endotracheal intubation and propofol (ED₅₀ for endotracheal intubation) [96].

Besides tailoring research tools, another framework (Figure 2) focuses on building networks of stakeholders active

Figure 2. Collaborative model with multiple partners needed to facilitate neonatal medicines research.

in the field of neonatal medicines research. Such networks should be interdisciplinary. This is because interaction throughout product development is crucial. The society, and in our population, especially young parents, should be aware of the concepts and relevance of clinical research and the consent procedure. In essence, they should be the advocates of the adagio that ‘neonates should be protected through, not from research’. The fact that parents or patient groups are represented in, for example, IRB’s or at the PDCO is an asset, that should be further explored. Caregivers should be involved in trial design, including feasibility, but also when priorities on therapeutic needs are considered (e.g., priority list pediatric off patent medicines). There may be a benefit for building networks between sponsors (multi-drug and multi-company studies) and support the clinical research networks. This is

because costs to build and close down individual networks for each product development program are prohibitive.

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Declaration of interest

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