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THEMED ISSUE REVIEW

Current knowledge, challenges and innovations in developmental pharmacology: A combined conect4children Expert Group and European Society for Developmental, Perinatal and Paediatric Pharmacology White Paper

Anne Smits[1,2] | Pieter Annaert[3] | Giacomo Cavallaro[4] | Pieter A. J. G. De Cock[5,6,7] | Saskia N. de Wildt[8,9] | Jenny M. Kindblom[10] | Florian B. Lagler[11] | Carmen Moreno[12] | Paula Pokorna[8,13,14,15] | Michiel F. Schreuder[16] | Joseph F. Standing[17,18,19] | Mark A. Turner[20] | Benedetto Vitiello[21] | Wei Zhao[22,23,24] | Annelie-Martina Weingberg[25] | Raffaella Willmann[26] | John van den Anker[8,27,28] | Karel Allegaert[1,29,30]

[1]Department of Development and Regeneration, KU Leuven, Leuven, Belgium

[2]Neonatal intensive Care unit, University Hospitals Leuven, Leuven, Belgium

[3]Drug Delivery and Disposition, Department of Pharmaceutical and Pharmacological Sciences, KU Leuven, Leuven, Belgium

[4]Neonatal intensive care unit, Fondazione IRCCS Ca' Grande Ospedale Maggiore Policlinico, Milan, Italy

[5]Department of Pediatric Intensive Care, Ghent University Hospital, Ghent, Belgium

[6]Heymans Institute of Pharmacology, Ghent University, Ghent, Belgium

[7]Department of Pharmacy, Ghent University Hospital, Ghent, Belgium

[8]Intensive Care and Pediatric Surgery, Erasmus MC-Sophia Children's Hospital, Rotterdam, the Netherlands

[9]Department of Pharmacology and Toxicology, Radboud Institute Health Sciences, Radboud University Medical Center, Nijmegen, the Netherlands

[10]Pediatric Clinical Research Center, Queen Silvia Children's Hospital, Sahlgrenska University Hospital, Gothenburg, Sweden

[11]Institute for Inherited Metabolic Diseases and Department of Pediatrics, Paracelsus Medical University, Clinical Research Center Salzburg, Salzburg, Austria

[12]Institute of Psychiatry and Mental Health, Child and Adolescent Psychiatry Department, Hospital General Universitario Gregorio Marañón, School of Medicine, Universidad Complutense, IiSGM, CIBERSAM, Madrid, Spain

[13]Department of Pharmacology, First Faculty of Medicine, Charles University and General University Hospital in Prague, Prague, Czech Republic

[14]Department of Paediatrics and Inherited Metabolic Disorders, First Faculty of Medicine, Charles University and General University Hospital in Prague, Prague, Czech Republic

[15]Department of Physiology and Pharmacology, Karolinska Institutet and Karolinska University Hospital, Stockholm, Sweden

[16]Department of Pediatric Nephrology, Radboud University Medical Center, Radboud Institute for Molecular Life Sciences, Amalia Children's Hospital, Nijmegen, the Netherlands

[17]UCL Great Ormond Street Institute of Child Health, London, UK

[18]Great Ormond Street Hospital for Children NHS Foundation Trust, London, UK

[19]Institute for Infection and Immunity, St George's, University of London, London, UK

[20]Department of Women's and Children's Health, Institute of Life Course and Medical Sciences, University of Liverpool, Liverpool Health Partners, Liverpool, UK

[21]Division of Child and Adolescent Neuropsychiatry, Department of Public Health and Pediatrics, University of Torino, Torino, Italy

On behalf of the Innovative Medicines Initiative conect4children (IMI c4c) Expert group on Developmental Pharmacology and the European Society for Developmental, Perinatal and Paediatric Pharmacology (ESDPPP)

[22]Department of Clinical Pharmacy, Key Laboratory of Chemical Biology (Ministry of Education), School of Pharmaceutical Sciences, Cheeloo College of Medicine, Shandong University, China

[23]Department of Pharmacy, The First Affiliated Hospital of Shandong First Medical University & Shandong Provincial Qianfoshan Hospital, Jinan, China

[24]Clinical Research Centre, The First Affiliated Hospital of Shandong First Medical University & Shandong Provincial Qianfoshan Hospital, Jinan, China

[25]Department of Orthopedics and Traumatology, Medical University of Graz, Graz, Austria

[26]Biozentrum, University of Basel, Basel, Switzerland

[27]Paediatric Pharmacology and Pharmacometrics, University Children's Hospital Basel (UKBB), University of Basel, Basel, Switzerland

[28]Division of Clinical Pharmacology, Children's National Hospital, Washington, DC, USA

[29]Department of Pharmaceutical and Pharmacological Sciences, KU Leuven, Leuven, Belgium

[30]Department of Hospital Pharmacy, Erasmus MC University Medical Center, Rotterdam, the Netherlands

Correspondence
Anne Smits, MD, PhD, Neonatal intensive care unit, University Hospitals Leuven, Herestraat 49, 3000 Leuven, Belgium.
Email: anne.smits@uzleuven.be.

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Developmental pharmacology describes the impact of maturation on drug disposition (pharmacokinetics, PK) and drug effects (pharmacodynamics, PD) throughout the paediatric age range. This paper, written by a multidisciplinary group of experts, summarizes current knowledge, and provides suggestions to pharmaceutical companies, regulatory agencies and academicians on how to incorporate the latest knowledge regarding developmental pharmacology and innovative techniques into neonatal and paediatric drug development.

Biological aspects of drug absorption, distribution, metabolism and excretion throughout development are summarized. Although this area made enormous progress during the last two decades, remaining knowledge gaps were identified. Minimal risk and burden designs allow for optimally informative but minimally invasive PK sampling, while concomitant profiling of drug metabolites may provide additional insight in the unique PK behaviour in children. Furthermore, developmental PD needs to be considered during drug development, which is illustrated by disease- and/or target organ-specific examples. Identifying and testing PD targets and effects in special populations, and application of age- and/or population-specific assessment tools are discussed. Drug development plans also need to incorporate innovative techniques such as preclinical models to study therapeutic strategies, and shift from sequential enrolment of subgroups, to more rational designs.

To stimulate appropriate research plans, illustrations of specific PK/PD-related as well as drug safety-related challenges during drug development are provided. The suggestions made in this joint paper of the Innovative Medicines Initiative conect4children Expert group on Developmental Pharmacology and the European Society for Developmental, Perinatal and Paediatric Pharmacology, should facilitate all those involved in drug development.

KEYWORDS
developmental pharmacology, drug development, paediatrics

1 | DEVELOPMENTAL PHARMACOLOGY

Pharmacotherapy is a powerful tool in preventive and curative medicine, with shifts in practices based on perceived needs. In children, an increase in outpatient prescription drug use for asthma, attention-deficit/hyperactivity disorder (ADHD) and contraception is reported, but a decrease in antihistamines, antibiotics, and upper respiratory combinations drugs.[1,2] Based on a survey in 2010, amoxicillin was the most frequently prescribed drug in children up to 11 years, and methylphenidate in adolescents.[2] Besides drug utilization, drug disposition and effects may differ between populations. General clinical pharmacology principles apply to all populations and refer to the processes

involved in drug disposition (pharmacokinetics, PK) and drug effects (pharmacodynamics, PD). PK is determined by drug absorption and distribution, followed by metabolism of most compounds and excretion of the compound and/or its metabolites. PD in part relates to characteristics of drug receptors or targets as well as postreceptor effects. For the paediatric population (i.e. from early neonatal life through adolescence), developmental changes in biological processes involved in PK and PD need to be considered (developmental pharmacology).[3,4] Due to rapid physiological changes (e.g. maturation of liver and kidney function, organ-specific growth), developmental impact on PK and PD is most remarkable in the neonatal period and first year of life. However, PK and/or PD may also differ from adults during childhood and adolescence.[3,4] As a consequence, safe and effective therapy for an individual child cannot be ensured by linear extrapolation of adult dosing recommendations. Knowledge of ontogeny of biological processes involved improves PK and PD predictions, and supports individualized and targeted drug therapy.[5] Multidisciplinary collaboration between basic scientists, paediatric subspecialists, pharmacists, (clinical) pharmacologists, pharmacometricians, biomedical engineers, veterinarians and other experts expands our understanding rapidly. Besides ontogeny, nonmaturational factors such as environment, disease states, or co-treatment can also influence PK/PD.[6] The goals of this paper are: (i) to summarize current knowledge; (ii) to provide illustrations for pharmaceutical companies, regulatory agencies and academicians about how to incorporate the latest knowledge regarding developmental pharmacology; and (iii) to underline why a comprehensive approach for such incorporation should be considered. In addition, some innovative techniques for paediatric drug development are summarized. The scope of this paper includes pharmacology (PK and PD), while other aspects of drug development are not covered. This paper reflects a collaboration between researchers from the Innovative Medicines Initiative conect4children (IMI c4c) Expert group on Developmental Pharmacology, and the European Society for Developmental, Perinatal and Paediatric Pharmacology (ESDPPP).[7,8]

2 | DEVELOPMENTAL PK

In the outpatient setting, drugs are commonly administered orally. In contrast, in an intensive care setting the parenteral (mainly intravenous) route is used in 71.3, 60.7 and 68.7% of drug administrations in adult, paediatric and neonatal intensive care unit patients, respectively.[9] For most drugs, children are sufficiently different from adults to require dosing adaptations. Understanding the fate of a compound in the body is essential to select the optimal dosing strategy for each individual. Size and age-related changes in drug absorption, distribution, metabolism and excretion pathways have been extensively studied.[3,10–12] Although knowledge of developmental PK has increased, knowledge gaps still remain. Within the developmental PK section, we will provide current evidence on maturational aspects of absorption, distribution, metabolism and excretion (ADME) processes. Neonates with intrauterine growth restriction (IUGR) and adolescents were selected as special paediatric populations to further illustrate these concepts. Furthermore, we will discuss how including metabolite concentrations in bioanalytical approaches can provide useful data for paediatric predictive PK models, and how improvements can be made towards less invasive sampling methods to gather data on drug disposition in children.

2.1 | Developmental biological processes involved in drug disposition

2.1.1 | Absorption

Absorption determines how concentration changes after extravascular administration, including absorption rate (*how rapid?)* and bioavailability (*how much?)*, both impacted by growth and maturation. Enteral (buccal, rectal, oral) or nonenteral (nasal, ophthalmic, dermal, inhalational, intramuscular, intrathecal) routes are relevant.

For enteral administration, maturation includes gastric and intestinal pH and motility, pancreatic lipase activity, bile acid secretion, and presystemic (first pass) drug metabolism and transport in the intestinal wall. The gastric fluid composition (bile acids, osmolarity, pH) displays age-dependent changes, further affected by feeding frequency and type.[13] The capacity of the newborn to produce gastric acid appears similar to older populations, despite the higher pH in their gastric fluid.[13,14] This discrepancy is probably explained by the impact of frequency and volume of milk feeds, alkalinizing the gastric content. Consequently, plasma concentrations of the acid-labile antibiotic penicillin reached 5–6 times higher values in neonates compared to older children.[15] Gastric emptying is another example of the merged effect of maturation and feeding practices. In a meta-analysis of data from preterm neonates to adults, not age but meal type (aqueous>breast milk>formula milk>semi-solid, solid) was the main determinant of gastric emptying, with collinearity between age and meal type.[16] Pancreatic lipase activity is typically low at birth and evolves during infancy, somewhat compensated by lipase activity (bile salt-stimulated lipase) in fresh human milk.[17] The bile acid pool size, bile flow and its ileal reabsorption also display maturation, resulting in lower duodenal bile acid concentrations in neonates.[10] The activity of intestinal drug-metabolizing enzymes (DMEs) and drug transporters (DTs) co-determine age-dependent drug bioavailability.[18,19] Intestinal drug metabolism matures slowly, as exemplified by midazolam's decreasing bioavailability over the paediatric age range (reflecting increasing first pass metabolism in the intestine and liver), although nonmaturational factors also determine a significant part of the variability.[20] For example, Blake *et al.* illustrated that dextromethorphan and caffeine metabolism in infants were affected by the type of nutrition (human milk *vs.* formula).[21]

For nonenteral administration routes that display an absorption phase, absorption kinetics relates to the drug diffusion rate as well as tissue perfusion rate (i.e. regional blood flow), with possible bypass of the first-pass effect. Absorption through the skin can be more rapid and/or more extensive in children (e.g. topical timolol for infantile

haemangioma in preterm neonates, or minoxidil for paediatric hair disorders)[22,23] prompting attention to possible unintended (systemic) side-effects after local application.

2.1.2 | Distribution

Following absorption, most drugs will gain access from the systemic circulation to other body compartments. Distribution profiles depend on drug (e.g. size, lipophilicity), patient (e.g. blood flow, biological barrier characteristics including DT expression, membrane composition), or both (e.g. plasma/tissue protein binding). The apparent volume of distribution (Vd, L or L/kg) reflects the degree to which a drug is distributed in body tissue relative to the plasma. Weight is the obvious main driver of the absolute volume of distribution (L), while age-related changes in drug protein binding and body composition in part explain developmental differences in distribution, when expressed as relative volume (L/kg). The major drug binding protein albumin (primarily binds neutral to acidic drugs) increases in concentration during the first 1–3 years.[24] Data on α-1-acid glycoprotein (binds basic or cationic drugs) are sparse, but paediatric reference ranges indicate an increase up to 5 years of life.[25] Besides proteins abundance, binding of competitors at protein binding sites can alter binding affinity and influence unbound, pharmacologically active, drug fractions. The unbound fraction of albumin-bound drugs, cefazolin, flucloxacillin, vancomycin, cefoperazone and phenobarbital, is higher in neonates compared to older populations.[26–30] Concerning body composition, the total body water fraction (>70%) is higher in neonates compared to children and adolescents (around 60%).[31,32] With maturation the fraction of total body water declines, while the fraction of fat mass increases. One study in nonobese children (age 7–14 years) demonstrated a mean percentage body fat of 20% in boys and 25% in girls,[33] but the obesity epidemic in children, adolescents and adults has resulted in large variations in fat mass.[34] Variation in body composition may require a different drug dosing.[35]

Multiple brain-specific factors impact drug distribution to the brain, and their influence differs throughout development. Blood–brain (BBB) and choroidal blood–cerebrospinal fluid (BCSFB) barrier function is still immature in children. Efflux transporters acting on amphiphilic drugs are likely to be more efficient around birth at the BCSFB, while at the BBB, some transporter expression is down-regulated compared to adult levels.[36] Verscheijden *et al.* recently described location and transporter-specific maturation of ATP-binding cassette transporters in both human BBB and BCSFB.[37] Besides, cerebral blood flow, drug binding to and uptake by neural cells, drug metabolism, volume of cerebral fluid compartments and rate of cerebrospinal fluid secretion contribute to cerebral drug bioavailability.[36]

In addition to development, nonmaturational factors (e.g. diseases, co-treatment) affects drug distribution. It has been documented that sepsis alters drug distribution, resulting in lower amoxicillin concentrations and a longer half-life in neonates as compared to nonsystemic disease conditions.[38] Also, body composition may be altered in conditions such as severe illness or eating disorders.[32,39] Extracorporeal membrane oxygenation (ECMO) in neonates leads to a larger Vd for both lipophilic and hydrophilic drugs.[40] During paediatric ECMO the Vd (L/kg) of e.g. analgo-sedatives also increases, showing the importance of including infants and children treated with ECMO in PK studies for drugs relevant to this population.[41]

2.1.3 | Metabolism

Drug metabolism takes place in the liver and a variety of other organs, and is highly variable. The impact of maturation is highest in the first years of life. Drugs can be metabolized by Phase I and II DMEs, which show enzyme- and organ-specific maturational patterns. Recent proteomics analyses have quickly increased understanding of these patterns.[42,43] Three patterns can be identified with: (i) most DMEs being low at birth and increasing to adult levels during the first months of life; (ii) high at birth and decreasing thereafter and; iii) stable expression. Most DMEs (e.g. CYP1A2, 2A6, 2D6; 2E1, 3A4) follow the first pattern, with birth and external factors contributing to rapid maturation. Based on *in vitro* data, adult values of CYP1A2 activity and protein expression are reached at age 5–15 and 1–5 years of age, respectively. For CYP2E1 activity is low in foetuses and reaches 50% of adult values in infants. Its foetal protein expression is variable and adult values are reached at 1–5 years of age.[44] *In vivo* data display additional variability.[45] For the CYP3A family, protein expression is 65–80% in foetuses but remains relatively constant at later ages. Interestingly, CYP3A4 enzyme activity hereby increases after birth (reaching 30–40% of adult values at 1 month, 50% at 6–12 months and 100% at age 1–5 y), while CYP3A7 is highly active in foetuses and early neonatal life, with a subsequent decrease in activity during the first weeks of life.[44,46,47] This shift between CYP3A7 and 3A4 occurs immediately after birth.[46] A detailed overview on multilevel ontogeny patterns of hepatic DT and DMEs is recently compiled by van Groen *et al.*[44] When doses are linearly extrapolated, immature drug metabolism can lead to toxicity. Exceptions may occur such as for paracetamol, where immature uridine 5′-diphospho-glucuronosyltransferase (UGT) activity is compensated by high sulfation activity directly after birth.[48] For many metabolized drugs, clearance is increased in toddlers and young infants resulting in subtherapeutic exposure, which is often not due to increased DME activity per se, but overall increased metabolic capacity as a result of a relatively large liver size at this age.[49] The interplay of maturation with other factors further adds variability to paediatric drug metabolism. Inflammation/sepsis leads to decreased CYP3A4 mediated midazolam clearance,[50] while genetic variation further affects omeprazole and tacrolimus disposition.[51,52] Moreover, treatment modalities may lead to variable disposition, e.g. during therapeutic hypothermia.[53]

2.1.4 | Excretion

Excretion occasionally occurs through the hepato-biliary or pulmonary route, but most commonly by the kidneys (glomerular filtration rate

[GFR], tubular secretion and tubular reabsorption). Ontogeny of biliary excretion appears to be fast, attaining adult activity within the first weeks to months.[54] Elimination kinetics in the lung is determined by alveolar ventilation, functional residual capacity and cardiac output.[55]

Based on aminoglycoside and vancomycin datasets, GFR maturation has been described, using a bodyweight-dependent exponent and postnatal age (PNA).[56] By pooling of GFR estimates (polyfructose, chrome-ethylenediaminetetraacetic acid, mannitol or iohexol) GFR has been shown to reach half the mature value based on allometric body size by 48 weeks postmenstrual age (PMA), with full maturity being attained before 2 years of age.[57] In a recent analysis on differences in GFR between preterm and term neonates, Salem *et al*. documented that both postnatal and gestational age (GA) are relevant in GFR development until 1.25 years, as PMA ignores birth as a pivotal event.[58] Compared to glomerular filtration, tubular functions appear to mature more slowly.[59,60] Our knowledge is limited, but recent kidney drug transporter expression data show different rates and patterns of maturation, which appears aligned with PK data of corresponding substrates.[61,62]

In addition to maturation, GFR variability is further affected by nonmaturational covariates. To illustrate both ends of the spectrum, critically ill children may display enhanced kidney perfusion and glomerular hyperfiltration, resulting in *augmented renal clearance*.[63] In contrast, chronic kidney disease (CKD) and acute kidney injury are associated with reduced clearance. The impact of CKD is not limited to decreased excretion, as it may lead to modifications in absorption, distribution, transport, and/or metabolism.[64]

2.1.5 | Special populations

As mentioned earlier, two specific paediatric populations will be discussed, namely neonates with IUGR and adolescents, to further stress the relevance and diversity in developmental PK. IUGR is the result of foetal growth failure caused by various factors, displaying foetal and postnatal features of malnutrition, while small for GA (SGA) is based on any birth weight <10th percentile for a given GA and may reflect genetic influences on size.[65,66] Although definitions differ, both terms are often used interchangeably, as illustrated below. Sparse data are available on the impact of IUGR/SGA on PK. Hepatic DME and DT expression are affected by IUGR.[67,68] To illustrate its relevance, S-ibuprofen clearance was 3.11-fold higher in SGA compared to appropriate for GA preterms.[69] IUGR also results in decreased renal clearance due to impaired renal developmental programming, decreased nephron number and GFR, leading to CKD.[70] Examples are reduced vancomycin, gentamicin or amikacin clearance in SGA compared to appropriate for GA neonates.[71–73] Finally, the impact of IUGR on PK probably remains relevant throughout paediatric life as e.g. GFR remains lower.[74]

At the other end of the paediatric spectrum, adolescents are commonly regarded as similar to adults with respect to PK. However, some organ systems undergo intense development during adolescence (e.g. skeleton, reproductive tissue, central nervous system, etc.), with PK implications. Activity of several DME change throughout physical and sexual maturation, with largest variability in infants and adolescents. As an example, alterations in sex and growth hormones appear to reduce DME (CYP1A2) activity, except progesterone which appears to increase CYP3A activity.[75,76] To illustrate altered PK, for the oral combined (nomegestrol acetate + oestradiol) contraceptive pill Zoely (Theramex Ireland, Dublin, Ltd), lower exposure of oestradiol (−36%) was observed in adolescents compared to adults.[77] Another study evaluating the effect of lixisenatide observed shorter time to maximal serum concentration (C_{max}), and lower absolute C_{max} in adolescents compared to adults.[78] Finally, a recent systematic review showed differences regarding dose-related concentrations in children and adolescents compared to adults for 14 of 26 neuroactive/psychoactive drugs.[79] These examples of age-dependent PK differences underscore (i) the need to involve paediatric pharmacologists to evaluate when detailed studies on drug efficacy and safety are needed in paediatric subgroups, and (ii) that developmental age rather than administrative (chronological) age needs to be considered when assessing PK(/PD) in adolescents.[76] Approved dosing for adults and adolescents seems highly equivalent, allowing allometric scaling with subsequent limited PK studies on a case-by-case approach.[80,81] Nevertheless, the unique developmental setting of adolescence often requests population-specific PK(/PD) attention.[76]

2.2 | Utility of measuring drug metabolite levels to support PK studies in children

About a decade ago, Anderson and Holford already pointed towards knowledge and expertise gaps in paediatric pharmacotherapy: the poorly studied impact of metabolites on effect was 1 of these factors.[82] Indeed, while metabolism generally leads to loss of activity, several drugs that are also used in neonates and children are known to generate metabolites that are pharmacologically active. The fact that this level of clinically relevant detail on metabolite disposition remains unknown for many drugs used in paediatric populations underscores the need for increased efforts to measure drug metabolites. The advances in the performance of bioanalytical methods in the past decade can facilitate this ambition. Indeed, recently developed methods have pushed the limits to new heights, not only in terms of analytical sensitivity (lower quantification limits) and sample volume requirements (often below 10 μL or even less), but also in terms of the ability for concomitant measurement of parent and multiple metabolites in a single injection for liquid chromatography with tandem mass spectrometry (LC–MS/MS) analysis.[83,84] Also, the use of a [^{14}C]-microtracer with accelerated mass spectrometry for metabolite profiling has been reported.[85] In the current section, we aim to illustrate the possible utility of measuring drug metabolite concentrations to support PK studies in neonates and children. While metabolite levels will not always provide additional insights regarding the disposition of a given drug, in several cases metabolite profiles will support: (i) enhancing the knowledge of *in vivo* impact of ontogeny of elimination pathways; and (ii) improved understanding of the

PKPD profiles of drugs generating pharmacologically/toxicologically active metabolites. As described in detail below, morphine and paracetamol generate a pharmacologically and a toxicologically relevant metabolite, respectively. In addition, metabolites may contribute to unravel developmental toxicity mechanisms like for ifosfamide[86] or valproic acid.[87]

2.2.1 | Morphine and its metabolites

Morphine undergoes biotransformation to morphine-3-glucuronide and morphine-6-glucuronide (M6G). Only M6G has a μ-opioid agonism. The PK-related effect of morphine in neonates and children thus depends not only on ontogeny of the morphine elimination process, but also on specific ontogeny profiles of M6G formation and elimination, justifying quantification of M6G next to morphine also in paediatric morphine PK studies. A meta-analysis reported moderate (2-fold) to high (10-fold) interindividual variability in morphine clearance (and in relative M6G levels) depending on the paediatric age group, but especially in critically ill patients.[88] Moreover, the highest interpatient variability in dose–exposure relationship was observed in neonates and infants. Specifically in neonates morphine elimination is driven by hepatic maturation, while renal maturation determines its glucuronides excretion. As illustrated in a meta-analysis by Knosgaard *et al*.,[84] PMA and PNA provide the best elimination predictions of morphine and its glucuronides, respectively. By additionally taking into account size-related parameters, the authors constructed a model to describe morphine and metabolites PK in (pre)term neonates.

2.2.2 | Paracetamol and its metabolites

Several independent studies have addressed the maturation of elimination of paracetamol and its phase-2 metabolites (paracetamol glucuronide, paracetamol sulfate).[89,90] Whereas glucuronidation matures during the first 2 years of life, sulfation shows a different developmental pattern with higher expression of sulfotransferase (SULT)1A1 and SULT2A1 in infants and young children compared to adults.[42] In addition, a population PK study demonstrated that the oxidative paracetamol biotransformation pathway (eventually yielding a.o. mercapturic acid metabolites) was subject to only small maturational changes.[91] Consistently, in very preterm neonates, paracetamol glucuronidation was very low, whereas other pathways (sulfation, glutathione conjugation) were of relatively higher importance compared to adults.[92] Interestingly, the clinical relevance of UGT1A9 polymorphism, the glucuronosyltransferase isoform involved in paracetamol glucuronidation, resulted in up to 42% decreased glucuronide formation clearance in some neonates. This finding illustrates that the impact of polymorphisms on top of maturational covariates should not be ignored *a priori* in neonatal pharmacology.[93] In addition, measuring paracetamol glucuronide concentrations thus contributed to a more accurate identification of the factors (polymorphism and CL maturation) determining variability in neonatal paracetamol exposure. A similar concept was recently presented for omeprazole. Based on a population PK model in infants for omeprazole and 2 metabolites, the complex interplay of both ontogeny and polymorphism of CYP2C19 was illustrated.[52] For older paediatric age groups, knowledge gaps exist regarding paracetamol PK, as illustrated in a review on paracetamol use in overweight children and adolescents.[94] It remains uncertain whether the documented increased clearance in obese adults also applies to obese adolescents. This urges to determine PK of paracetamol and its (hepatoxicity-related) metabolites in this population, which can then support acceptable dosing recommendations in children and adolescents.

2.3 | Minimal risk and burden designs for PK in vulnerable populations

The study of PK in infants and children has historically been challenging due to sample size, heterogeneity of study population and patient burden (blood sampling).[95] Optimal PK sampling strategy must consider both timing and number of samples per patient, and also minimizing sample volume using innovative bioanalytical methods. Trial designs need to minimize risk and burden for participants and their families. This covers not only sampling, but broader trial-related and ethical considerations.[96] The child's interest should always prevail over that of science and society.[96]

2.3.1 | Timing and number of samples per patient

Each sample is precious and should only be collected if it is predicted to provide optimal and needed information. From a PK modelling perspective, optimal sampling design allows parameter estimation with maximal precision. Modelling and simulation are strongly recommended in paediatric investigation plans[97] and methods for optimally designing sampling schedules and to derive the required number of study participants[98] are now available. From previously developed PK models in adults or older children, it is possible to derive optimal sampling times with the highest chance of estimating PK parameters most precisely in the population of interest. Opportunistic PK sampling design could mitigate the challenges associated with PK studies in children. However, it should consider the density and quality of sampling, as well as the stability of the drug.[99] Inappropriate design will result in biased[100] or imprecise[101] PK model estimates. Judicious use of optimal design[102] or simulation estimation[103] can, however, mean that with a few samples precise estimates can be derived making studies minimally invasive and maximally informative. Mathematically optimal sampling design might not be clinically optimal in terms of number and timing of samples. It is therefore important to take the practicalities of sampling and ethical aspects into consideration when designing PK studies.[96] Furthermore, reliability of extrapolation methods may differ between paediatric subpopulations and should be considered to limit the number of samples as much as possible.[104]

2.3.2 | Low volume sampling

Microsampling, which is the collection of smaller-than-normal plasma samples for bio-analysis, may provide a solution and includes mainly dried blood spots (DBS), dry plasma spots, volumetric absorptive microsampling or capillary microsampling techniques. In DBS or dry plasma spots, a small volume of blood or plasma is applied on an absorbent paper which is dried after saturation has occurred. In the laboratory, the blood or plasma is eluted out of the paper and analysed. The advantage of DBS is that analytes have higher stability in ambient conditions for several days or months.[105] Application of DBS and rapid LC–MS/MS-based methods have allowed a deepening knowledge of propranolol PK in (pre)term neonates.[106] Cohen-Wolkowiez *et al*. reported on the feasibility of using DBS concentrations in combination with plasma samples for PK model building of piperacillin and tazobactam in infants.[101] A quite similar, optimized technique is volumetric absorptive microsampling, in which a fixed blood volume is absorbed by the porous, hydrophilic tip of the device. After the tip is dried, it is sent to the lab for drug extraction and bio-analysis. In capillary microsampling, blood is collected in a capillary tube and subsequently centrifuged in this tube before bio-analysis. Overall, microsampling seems promising as it would allow a significant reduction in the blood volume required.

Besides minimally invasive blood sampling, approaches for sampling in other matrices are developed/under development. Microneedle technology is known as a (transdermal) drug delivery system.[107] Nevertheless, as it creates (a) transient channel(s) across the skin, it can also be used for sampling. Microneedle-based interstitial fluid (ISF) sampling can complement conventional blood or urine sampling. In adults, microneedle-based ISF from human skin was collected to quantify selected biomarkers, and was well tolerated.[108] To illustrate its applicability, caffeine PK in adults and glucose response after a meal and insulin administration in diabetic children were reported to be similar in plasma and ISF.[108] Although this technique is mainly applied in research settings, it is promising as microneedle insertion was less painful compared to subcutaneous catheters in diabetic children and adolescents.[109]

A noninvasive technique for measuring drug exposure is using saliva as matrix. The advantages of saliva monitoring in paediatric PK trials are acknowledged by the Food and Drug Administration (FDA) as it reduces blood sampling, is easy to collect and causes minimal discomfort.[110] The usefulness of saliva for therapeutic drug monitoring has been studied for e.g. anti-epileptics, antiretrovirals, antipsychotics, antibiotics and antifungals, but is dependent on the physicochemical properties of the drug.[111–114]

2.3.3 | Microdosing

Microdosing is another promising method to minimize patient burden while studying PK. The dose is 1/100th of the no observed adverse effect level. A microdose can be quantified using the most sensitive ultra-performance LC techniques or when labelled with [^{14}C], as microtracer quantified by accelerator mass spectrometry. A prerequisite is dose linearity from the microdose up to therapeutic ranges.[115] In children, it has shown feasible to perform such studies which can also be used to study age-related variation in drug and metabolite PK. Studies characterising paracetamol, midazolam and ursochol PK are published.[20,116–118] The recent FDA guidance on neonatal drug studies suggests to consider microdosing to explore ontogeny.[119] Moreover, a recent proof-of-concept study showed the feasibility to safely use a [^{14}C] microtracer to elucidate paediatric metabolite pathways, which may address the challenges discussed above.[85]

3 | DEVELOPMENTAL PD

There is limited progress in knowledge on how growth and development impact PD.[4] To determine the effects of growth and development on PD, the greatest challenge is probably the robust and direct quantification of drug effects. Receptors for most drugs are not in the vascular space, but in the tissues. Because of technical and ethical constraints, such tissue *compartments* cannot be easily accessed repeatedly in children.[120] Therefore, PD evaluation usually depends on indirect measurements of drug action. To mitigate these challenges, researchers started to explore the use of (functional) biomarkers as tools for assessing developmental PD.[121,122] Biomarkers of PD as clinical substitutes for defining PD in paediatric patients are currently still limited.[123]

Developmental PD investigates the age-related maturation of biological systems and how this affects drug response (i.e. potency, efficacy or therapeutic range). We aim to illustrate that developmental aspects can be considered in drug targets and in PD endpoints, using 2 groups of conditions as examples (section 3.1 and 3.2), and in disease-specific PD assessment (section 3.3). As these sections are based on illustrations, no generalizable conclusions can be drawn.

3.1 | Identifying and testing treatment targets and effects in rare diseases

Basic science has elucidated the pathogenetic mechanisms of disorders that often present in the paediatric population, such as spinal muscular atrophy (SMA), Pompe's disease and fragile X syndrome.[124–126] This knowledge is relevant to paediatric drug development because it provides the opportunity to identify new drug targets (Table 1). Mechanism-targeted drug development may rather lead to disease-modifying therapies than the traditional symptom-targeted approaches, at least in conditions with relatively simple mechanisms.[126,127] Nevertheless, for e.g. fragile X syndrome, specific treatment hypotheses were formulated following understanding of the imbalance between excitatory glutamatergic transmission and inhibitory GABAergic system, but clinical trials have not yet provided evidence of efficacy for the proposed treatments.[125] However, even in this setting, mechanism-targeted drug development may serve to guide research. A specific challenge for paediatric drug development

TABLE 1 Examples that developmental pharmacodynamics (PD) needs to be considered during drug development

Developmental PD topic	Category	Examples
Type of drug target ***Where the drug acts on?***	Mechanism-targeted therapy	- neuromuscular diseases: Building on the understanding of SMA genetics, novel compounds that specifically address the basic deficit, as nusinersen, were developed and approved for clinical use.[127] - metabolic diseases: For Pompe's disease, caused by an enzyme deficiency, a targeted therapy is developed (enzyme replacement therapy with recombinant human α-glucosidase from CHO cells (alglucosidase alfa).[126]
Developmental changes in drug target ***Why is drug action different?***	Molecular	- receptor structure: The GABA receptor subunit composition changes with age, and this explains paradoxical seizures in preterm infants and neonates when benzodiazepines are administered.[128,129] - receptor density: Increased expression of the μ-opioid receptor postnatally in rats is documented. Human studies are lacking, but an altered expression might explain why neonates are more sensitive to morphine.[128] - receptor affinity: High concentrations of high-affinity receptors for insulin are present on mononuclear leukocytes in neonatal cord blood (receptor affinity $5.9 \times 10^8\ M^{-1}$ *vs.* $2.9 \times 10^8 M^{-1}$ in neonates *vs.* adults)[130]
	Biochemical	-vancomycin AUC/MIC target: As only unbound drug can have pharmacological effect, this target should be based on unbound vancomycin. Maturational changes in vancomycin protein binding (i.e. median fraction unbound of 0.9 *vs.* 0.6 in neonates *vs.* adults) suggest altered AUC/MIC target throughout development.[131]
	Physiological	- ontogeny of target organ system: Maturation of the adaptive immune system (CD4 T cell count)[132] and the coagulation system (vitamin K-dependent coagulation factors)[133] explain differences in drug action with age. - sensitivity of target organ system: The potency of the immunosuppressant cyclosporine at inhibiting peripheral blood monocytes proliferation and interleukin-2 expression was 2-fold and 7-fold lower in infants (0–1 years) compared to older children. This might indicate altered target concentrations and dosing.[134]
Age-and disease-specific PD endpoints and assessment tools ***Which drug effect to assess?*** ***How to assess drug effect?***	Endpoint category	- vital signs: Blood pressure in children, and consequently the diagnostic thresholds for degree of hypertension, vary with age, height and sex.[135]
	Assessment tools	- for efficacy of psychoactive drugs (e.g. symptom assessment): Hyperactivity is the most apparent symptom of ADHD in young children, but this gradually attenuates with age while inattention, impulsivity, and deficits in organization and planning become evident features in adolescence.[136] - for safety of psychoactive drugs (e.g. ADR assessment): Higher incidence of sedation reported as ADR of antipsychotics and antidepressants in children compared to adults, related to the underlying ADR mechanism (off-target drug effect and sensitivity of paediatric subgroups to a specific ADR).[137]
Therapeutic window of opportunity ***When to achieve optimal drug effect?***	Neurologic system	- maturation of glutamatergic/GABAergic balance: Given the rapid changes in brain structure and function due to synaptogenesis and pruning during the first years of life, a treatment addressing the glutamatergic/GABAergic imbalance could have an effect at a younger age (e.g. until 36 months) but not if administered later.[128,129]

SMA: spinal muscular atrophy, CHO: Chinese hamster ovary, GABA: γ-aminobutyric acid, AUC: area under the concentration-time curve, MIC: minimal inhibitory concentration, ADHD: attention deficit hyperactivity disorder, ADR: adverse drug reaction.

is identifying if and how a treatment response is dependent on the developmental stage of the child. Possible windows of opportunity for the treatment of disorders that often present in the paediatric population, adds to the complexity of paediatric pharmacology (Table 1). Even more challenging is drug development for conditions such as autism spectrum disorder, whose pathogenesis has not been clarified yet and is probably heterogeneous. In this area, there are ongoing trials based on hypotheses of neuronal transmission abnormalities.[138,139]

The evaluation of PD effects and the impact of growth and development are challenging to study in rare inherited diseases. The variable clinical phenotypes and time points of diagnosis, and the low incidence make it difficult to evaluate PD and separate the impact of maturation and natural disease course from other factors. Moreover, the drug of interest is usually the only treatment option, making placebo-controlled designs ethically critical. Innovative methods can overcome these challenges. Clinical trial endpoints can be optimized by modelling natural history data (registries),[140] fallback tests for co-primary endpoints,[141] and goal attainment scaling.[142] Confirmatory adaptive designs can minimize the number of needed patients and improve the significance of results.[143] The dilemma of placebo control in untreatable diseases can be addressed by delayed start randomization.[144] Furthermore, registry-based studies can support risk–benefit analysis.[145]

3.2 | Developmental PD: The adaptive immune system as illustration

In the early days of human immunodeficiency virus (HIV) therapy it was thought that CD4 T cell count was an unreliable biomarker of disease progression in children younger than 5 years.[146] Despite having *normal* CD4 counts, infants and young children often had uncontrolled viral loads and poor clinical outcomes. This was due to the fact that thymic output displays age-related activity, resulting in infants and young children having up to 5-fold higher normal CD4 counts than adolescents.[132,147] By leveraging biological prior data on thymic output inferred from T-cell receptor excision circles and T-cell turnover inferred from Ki67 expression,[148] one can scale PD models for expected changes in T-cell production and turnover. PD models using this scaling can be developed and applied in paediatric studies.[149] For the immunosuppressant cyclosporine, in vitro age-dependent PD has been reported (Table 1).[134]

3.3 | Developmental PD: Neurological and psychiatric disorders as illustration

The structural and functional changes in the brain throughout infancy, childhood, adolescence and young adulthood are reflected in continuous modifications in the phenotypic manifestations of psychopathology (e.g. ADHD, Table 1).[136] It is important that symptom rating scales take this developmental trajectory into account. Developmentally appropriate assessment tools should be sufficiently sensitive and valid to treatment-related effects in children with neurological disorders for use in clinical trials. In addition to disease-specific core symptoms, also comorbidities may require their specific assessment tools. Besides efficacy, safety profiles can differ between children and adults. For antipsychotics and antidepressants, a significantly higher incidence and a different profile of adverse drug reactions (ADRs) was reported in children (including adolescents) compared to adults (Table 1).[137] Central nervous system changes including synaptogenesis, connectivity, and differential maturation of neurotransmitter systems continue throughout adolescence and can even take 3 decades.[150,151] This can also contribute to differential response to pharmacotherapy between adolescents and adults.[128]

4 | INNOVATIVE TOOLS FOR PAEDIATRIC DRUG DEVELOPMENT

Drug development plans not only need to incorporate the latest advances on developmental PK (section 2) and PD (section 3), but also innovative techniques. While some innovative approaches mentioned earlier (e.g. microsampling section 2.3) are already more established, the current section provides illustrations of experimental tools. Multidisciplinary collaboration creates opportunities to model pathophysiological processes, identify molecular targets and test pharmacotherapy. Table 2 gives examples of innovative tools available in the developmental pharmacology field.

For understanding the ontogeny of the drug target organ system, *in vitro* research, and preclinical (animal) models can be implemented. For the latter, species-specific differences in organ maturation, logistical disadvantages and imbalance between disease model and drug development are limitations to consider.[166] Finding preclinical (animal) models that display physiology and development comparable to human models is challenging.[167] Differences often relate to ADME characteristics, size or receptors. Although underexplored, juvenile pig models are of interest to investigate disease mechanisms (for neonates several [e.g. asphyxia,[168] necrotizing enterocolitis,[169] IUGR[170] and resuscitation[171]] are described), and subsequently to assess PK/PD. Disposition of dexmedetomidine during hypothermia, and an approved paediatric investigation plan for 2-iminobiotin, both in perinatal asphyxia pig models illustrate the potential and limitations of juvenile animal models.[172] Such data can be used to develop a PBPK framework, and allow to distinguish the impact of asphyxia *vs*. hypothermia on PK, which is not feasible in a clinical setting.[160]

In section 4.1, we discuss opportunities and challenges of examples of preclinical cellular systems and additional animal disease models. Innovative methodology is also being developed to increase understanding of the ADME ontogeny. This will be illustrated in section 4.2 by selected endogenous biomarkers reflecting ADME relevant protein activity: the applicability of this work in paediatrics is limited, and this is considered as a research tool.

TABLE 2 Examples of innovative tools for paediatric drug development

Tool	Aim	Example
Patient derived inducible pluripotent stem cells	To model cellular phenotypes of inherited diseases for drug discovery and characterization of pharmacodynamics	Duchenne muscular dystrophy[152] Spinal muscular atrophy[153]
Organoids	To study molecular pathophysiology mechanisms, drug disposition and drug effects	Nephrotic syndrome[154] Genetic kidney diseases, including cystinosis, ADPKD[155] Cystic fibrosis[156,157] Biliary atresia[158]
Juvenile animal models	To develop a preclinical model for paediatric drug research and a juvenile PBPK model: Maturational, or disease-related	Juvenile Göttingen minipig model - for physiology data (maturational)[159] - for perinatal asphyxia (disease)[160]
	To study therapeutic strategies for developmental lung diseases	Juvenile rabbit model for BPD[161]
	To identify genes important in human traits and disorders	Zebrafish mutant model for ASD[162]
Endogenous biomarkers	To assess endogenous metabolic activity reflecting drug metabolic activity	4β–OHC and 6β-OHF as surrogates for in vivo CYP3A4 activity[160,163–165]

ADPKD: autosomal dominant polycystic kidney disease; PBPK: physiology-based pharmacokinetics, BPD: bronchopulmonary dysplasia, ASD: autism spectrum disorder, 4β–OHC: 4-β-hydroxycholesterol, 6β-OHF: 6-β-hydroxycortisol.

4.1 | Examples of experimental and preclinical models for paediatric drug development

4.1.1 | Ex vivo 3-dimensional organotypic bone culture model

The growth plate (physis) is a hierarchic organized complex system and can be described as an organ. It is the site where elongation of the long bones occurs in longitudinal growth during development. Particularly in paediatrics and the growing bone, it is paramount to investigate (adverse) and/or osteogenic effects of drugs and biomaterial-based devices on the physis and the growing skeleton in the biological growth mimicking environment.[173,174] Unfortunately, investigating growth at the physis is challenging since an optimal model is currently lacking and inconsistencies between in vitro and in vivo studies exist. The current in vitro models focus on the behaviour of developing osteoblast progenitors, osteoclasts or endothelial cells following drug administration. However, the events occurring in the growth plate during development are not mirrored in these in vitro models.[174,175] New approaches can be found in use of ex vivo 3-dimensional organotypic cultures.[176] An ex vivo bone growth model is based on bone slices from femurs of early postnatal rats, cultured for several weeks, and can further be implemented in preclinical, toxicological and therapeutic investigations.[176]

4.1.2 | Mouse models for preclinical efficacy assessment

Mouse models can be used to study disease mechanisms in association with development and growth. While requirements are defined for toxicity assessment of new drugs, no regulation is available for preclinical pharmacology data used to support human trials.[177] Irrespective, an animal model can be useful for validation of target engagement (proof-of-concept studies). Clinically oriented preclinical studies can support assessment of efficacy also at young ages, support toxicology and dose-finding studies. However, translation of the efficacy profile from mice to humans remains challenging, due to differences in (patho)physiology, the issue of age-matching, presence of different genetic modifiers in mice and humans, and finally by the quality of preclinical data.[178] The absence of requirements for reporting standards in scientific publications has led to efforts in compiling facultative ARRIVE guidelines,[179] but the implementation of these guidelines is still very limited.[180] In the neuromuscular community, a (facultative) advisory board for clinical trials uncovered the challenges of applying results from poorly designed preclinical studies to human trials to improve translatability.[181] Scientific and clinical communities, and industry need to be aware of the risk of using inadequately validated mice model (or other animal model) data for premature translation to a human trial.

4.2 | Endogenous biomarkers reflecting ADME relevant protein activity

Although their relevance is still controversial, endogenous biomarkers have been suggested as possible surrogates for enzymatic activity in adults.[182] Examples are plasma 4β-hydroxycholesterol (4β–OHC, with 4β-OHC/cholesterol ratio) and urinary 6β-hydroxycortisol (6β-OHF, with 6β-OHF/cortisol ratio) for CYP3A4 activity. It is important to know that paediatric data on these markers are only explorative.

Developmental patterns are described: at birth, neonates have lower 4β-OHC, but the level increased significantly between birth and age 4 months to reach adult values. However, a median 4β-OHC/cholesterol ratio at birth (0.19) was already comparable to adults, and

this ratio did not change until 4 months.[163] In children (age 1–17 y) with epilepsy, carbamazepine induces CYP3A4/5, with subsequent 4β-OHC increase.[183] Takaki *et al.* demonstrated that several oxysterols in urine and plasma undergo developmental changes and may be promising candidates for becoming biomarkers for paediatric liver disease.[184] Also 6β-OHF levels display age-dependency, with maximum values at 14–20 years for both sexes.[164] Concerning 6β-OHF/cortisol ratios, higher values are reported in neonates (PNA 1–15 days) compared to infants (30–359 days),[185] while others found different results.[186] A correlation between this ratio and GA and birth weight is described.[186] The ratio at birth in preterm infants was lower than term cases and remained stable the first 14 days, while in term neonates the ratio decreased after birth to preterm levels at PNA 5 days. This ratio further declines with age and remains stable from 21–25 years onwards.[164]

Following (i) confirmation of the patterns observed and (ii) demonstration of the correlation with DME activity, these data might inform future PBPK models for drugs undergoing metabolic elimination. They may contribute to define changes in DME activity due to strong inducers/inhibitors.

Based on a recent systems biology analysis,[187] several endogenous compounds were listed as substrates for human *drug* transporters of the SLC (solute carrier) family. These compounds might be candidates for future biomarkers of *in vivo* activities of various SLC isoforms in children. Finally, testotsterone glucuronide normalized by androsterone glucuronide seems a promising urinary UGT2B17 biomarker in children. In line with the developmental pattern of UGT2B17 expression, this marker was significantly associated with sex, age and copy number variation.[188]

5 | REFLECTIONS ON HOW KNOWLEDGE REGARDING DEVELOPMENTAL PHARMACOLOGY SHOULD BE IMPLEMENTED IN THE PAEDIATRIC DRUG DEVELOPMENT PATHWAY

Due to the anticipated relevance of developmental PK and PD on the design of paediatric clinical trials, there is regulatory guidance and a framework to support paediatric drug development.[189] This has been converted in a paediatric decision tree (Figure 1).[190]

The use of this pathway depends on the availability of an underlying rationale as to what can or cannot be extrapolated from adult to paediatric data and between different paediatric age groups. Bridging can be done by extrapolation, modelling and simulation and has been used for regulatory evaluation.[190,191] Based on assumptions on (dis)similarities in disease and concentration–response profiles, PK, PD and safety data are collected to design the paediatric drug development programme. Data sources may include adult studies, paediatric studies, preclinical (animal) models,

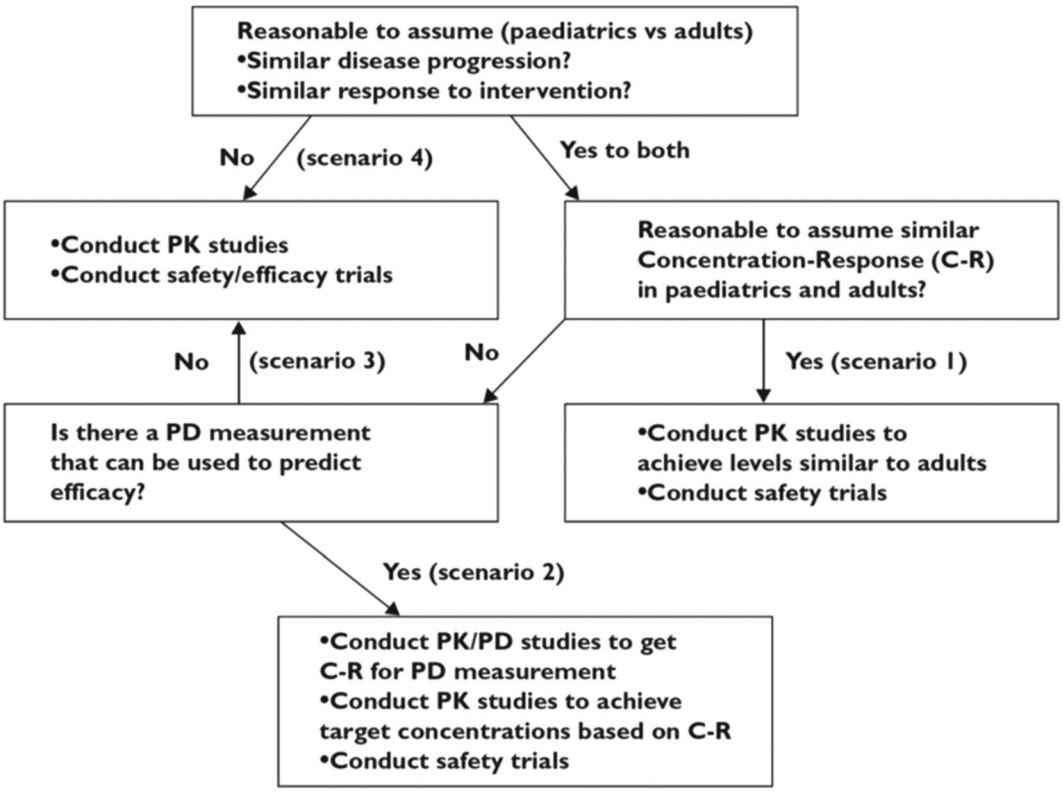

FIGURE 1 Paediatric study decision tree with identified scenarios (from Manolis and Pons, Br J Clin Pharmacol 2009,[190] with permission from Rightslink, John Wiley and Sons and Copyright Clearance Center). PK, pharmacokinetics; PD pharmacodynamics

in vitro and *in silico* models.[192] Ollivier *et al*. described the shift that occurred within paediatric pharmacotherapy from *implicit* (subjective, driven by practical experience, resulting in eminence-based off-label use), to *explicit* (based on and driven by scientific rationale) extrapolation.[104] This shift on how to approach off-label drug use is also reflected in the joint policy statement of the European Academy of Paediatrics and the ESDPPP.[193]

The studies used to construct the paediatric programme need to be planned early in the adult programme and may be included in the adult programme (e.g. identifying dose-exposure-response relationships). Understanding developmental pharmacology is needed to plan and interpret these studies to deploy appropriate methods and existent data.

The goal of developmental pharmacology is to have sufficient evidence about the disposition and effects of drugs to inform and support authorization and accurate pharmacotherapy in children. Large, *traditional* clinical trials are tools to gather data and knowledge, but are not always the best approach to fill these knowledge gaps. First, in the presence of a well-characterized condition and strong evidence for similar drug effects across age groups, well-justified extrapolation supported by modern tools can generate a similar strength of dosing and efficacy evidence as large clinical trials. Modern tools include pharmacometrics, PBPK, and clinical biomarkers. Extrapolation requires fewer patients than required for large trials, as illustrated for HIV or partial-onset seizures,[194] and can also reduce the number of samples per patient. Second, when efficacy trials are done, integrating trial-derived and pharmacometrics-derived results can reduce the uncertainties that the trials address, e.g. refinement towards more targeted and efficient designs. The international paediatric multiple sclerosis study group recommends reconsidering paediatric study designs (including studying safety instead of efficacy and the ethical considerations of using placebo for a highly active disease), as disease mechanisms are shared across the age span.[195] This study group illustrates that knowledge regarding developmental pharmacology can be combined with drug information to justify recruitment of adolescents (or children) to *adult* studies as long as sufficient information on drug and target population (including *ontogeny*) is present. This approach is endorsed in FDA recommendations about study eligibility criteria that suggest increasing diversity in patient enrolment, including children, pregnant or lactating women in e.g. confirmatory clinical trials.[196] However, crucial to such an *inclusive* approach is that developmental PK and PD characterization are considered from the earliest drug development stages. Similarly, sequential enrolment of paediatric age groups, moving to younger age groups only when studies in an older age group have demonstrated safety and efficacy should no longer be taken for granted, unless justified and based on clear scientific rationale such as potential developmental safety concern. Arbitrary sequential enrolment of paediatric subgroups (from older, going to younger ages) in drug development has been questioned in both EMA and FDA, as this may lead to delays in data availability, especially in the younger ages.[96,196] This may result in prolonged off-label practices in these subgroups, with subsequent difficulties to conduct relevant trials in these groups, once the drug is marketed.

This is because sufficient information to justify starting studies in multiple age-groups can emerge from modelling and simulation.[196] Because of the more limited information on ontogeny and pathophysiology, Ollivier *et al*. stated that extrapolation remains most difficult in (pre)term neonates.[104,194] Depending on the neonatal drug development programme, each of the earlier mentioned data sources (adult studies, paediatric studies, preclinical (animal) models, *in vitro* and *in silico* models) can be considered to varying degrees and levels of confidence to guide dosing. Using an illustrative approach, neonatal programmes on meropenem (disease similar to children or adults), clopidogrel or thyroid hormone (diseases related but not similar) or caffeine and surfactants (diseases unique to neonates) have been described.[192]

5.1 | Paediatric drug development pathway, challenges related to PK and PD

PK data are crucial to paediatric drug development as exposure will determine (side-) effects (Figure 1). However, this is not unique to children, so that sufficient adult—or rarely—preclinical (animal) data (section 4.1, Table 2) should be gathered to understand the concentration–time profile (PK) and optimal concentration–response (PD) profile.[192] Repurposing is an important setting, as another indication may require new dose-findings studies, like ibuprofen for closure of a patent ductus arteriosus, or sildenafil for treatment of pulmonary hypertension.[197,198] The earlier mentioned modelling and simulation or PBPK modelling should provide supportive guidance on conducting and designing PK studies, to reduce individual burden (e.g. number and volume of samples, sample technique, opportunistic or scavenged, time window, maximize flexible sampling approach). This should be based on a priori simulations that justify the approach taken and maximize the information used as the source for extrapolation.

Characterizing the impact of development on subsequent PD is still challenging. Paediatric biomarkers should be sufficiently sensitive to discriminate time-dependent changes from medicine- or intervention-related effects to enable modelling of drug–response relationships across the paediatric spectrum.[4] Again, modelling and simulation can support trial design and conduct, while strategies related to enrichment (e.g. treatments for SMA targeting type I *vs*. II *vs*. III, or cystic fibrosis drug modulator therapies, targeted to a specific mutation) can also be useful.[196] Robust modelling efforts necessitate rich data on e.g. the natural disease course and functional outcome variables while considering impact of maturation/development (e.g. sharing of clinical research data on SMA, or natural trend data of lung function tests in cystic fibrosis), most relevant in the field of rare diseases.[199,200] We should focus on patient (and when relevant parent/family)-centred outcome variables, using approaches to include children and young people's opinions.[201,202]

5.2 | Paediatric drug development pathway, challenges related to safety

Drug safety remains 1 of the key elements of the benefit/risk balance assessment by regulatory authorities, health care providers and the public. Standardization of pharmacovigilance and safety is key to facilitate comparison and is embedded in guidance documents. This resulted in regulatory requirements to assess seriousness, causality and severity, irrespective of the population considered, but all have their issues when applied in paediatrics.

Serious adverse event outcomes are clearly defined as including: death, life-threatening events, inpatient hospitalization or prolongation of hospitalization, persistent or significant incapacity or disruption of daily life functions, or a congenital anomaly. However, it may be difficult to disentangle prolonged hospitalization in specific subpopulations such as preterm neonates.[203] *Causality assessment* is another issue, as e.g. the Naranjo algorithm or Uppsala causality tool are not tailored to all paediatric subpopulations.[204] To stress the relevance of availability of these tools, the inter-rater reliability for different tools when applied to suspected ADRs in neonates remained *fair* ($\kappa \sim 0.3$).[205] Similar issues exist for *severity assessment*, subdivided into mild, moderate, severe, life threatening or death (grade 1–5). To limit inter-rater variability, consensus documents related to e.g. vaccine trials (Division of Acquired Immune Deficiency Syndrome—table, adults, paediatrics), oncology (Common Terminology Criteria for Adverse Events, adults and paediatrics) or, recently, neonatology have been reported.[206–209] To illustrate their relevance, grading in part depends on the impact on *age-appropriate instrumental activities of daily living*. To limit variability and standardize event assessment, the impact on *daily living* should be translated to a population-specific setting, such as reported by the generic severity criteria for the neonatal ADR scale.[206]

It is important that safety biomarkers used in drug development programmes, also display age- and non–age-related differences in patterns or quantification. QT(c) times are commonly used biomarkers to prevent cardiac arrhythmias, but these markers display maturational and nonmaturational changes.[210] Recognition and quantification of adverse events of the kidney necessitate age-appropriate reference intervals of kidney function and injury markers.[211,212] Besides these 'general' safety biomarkers, growth, pubertal development (and reproduction) or neurocognitive and behavioural development are of specific relevance to this population.

6 | GENERAL SUGGESTIONS AND CONCLUSION

Knowledge on developmental pharmacology is rapidly expanding, and should be captured in paediatric drug development to achieve safe, effective and individualized pharmacotherapy. A few general suggestions can be made:

- To reduce knowledge gaps in developmental PK, profiling drug metabolites can support mechanistic PK studies and provide insight into *in vivo* impact of ontogeny of elimination pathways, while minimal risk and burden designs allow for optimally informative but minimally invasive PK sampling. In addition, integration of innovative methodology to better understand ADME ontogeny, can provide paediatric drug development initiatives with more profound data on drug disposition in paediatric subgroups. However, limitations of currently underexplored preclinical models and still explorative biomarkers need to be considered. Furthermore, linking exposure (PK) to effect (PD) remains difficult. Clearly establishing robust dose–response relationships is important before exposure-based individualized dosing can be pursued. We are aware that this paper focusses on maturational factors impacting PK/PD. Nonmaturational factors further contribute to inter- and intrapatient PK/PD variability. As impact of both types of factors cannot always be distinguished from each other in clinical trials, preclinical models might offer add-on benefit (e.g. to separate the impact of asphyxia *vs*. hypothermia on PK).[160]
- In addition to developmental PK, PD data are even more relevant, but difficult to collect, as drug efficacy and safety are often considered as final endpoints of pharmacotherapy. Therefore, real world paediatric patient data (e.g. laboratory results from routine clinical care, registries, electronic health files) are useful to support paediatric PD endpoints.[213] Based on disease- and target organ-specific examples, we illustrated that developmental PD should be considered during drug development. However, many age- and disease-specific, validated markers and references values covering the paediatric range are currently lacking for a reliable PD assessment. Finally, when short-term efficacy and safety data become more evident, focus on long-term clinical data on paediatric pharmacotherapy is appropriate.
- The Göttingen minipig is the reference pig strain for PK studies in drug development, but its use remains limited.[166] Regulatory guidelines recommend to use the same species in juvenile and adult toxicity studies. However, species selection for adult studies is not clearly defined and lacks consistency. Optimal selection criteria would probably increase Göttingen minipig use for adult toxicity studies. Also for drug indications limited to children, this strain is the preferred model for preclinical PK and toxicity studies.[166] Further ADME research and PBPK models for this species are needed.[214]
- PK and PD characterization in paediatric subgroups should be considered from the earliest drug development stages. To uniform trial-related approaches, the use of recent instruments e.g. the paediatric decision tree for extrapolation (Figure 1), or assessments of seriousness, causality and severity of adverse effects in paediatric drug development is suggested.
- Continued improvements on targeted and efficient clinical trial designs should become standard in paediatric drug development.[189,206] As highlighted in section 5, some of these reflections are supported by scientific societies on developmental pharmacology (e.g. European Academy of Paediatrics, ESDPPP) and/or

regulatory agencies (FDA, European Medicines Agency). In addition, developmental pharmacology expertise, as part of strategic feasibility—such as the advice offered by the European public-private conect4children consortium—should be integrated in paediatric drug development. We hereby also want to stress the importance of shifting from an arbitrary sequential enrolment of paediatric subgroups to more rational study designs.

In conclusion, this paper summarizes current evidence of developmental PK and PD, and provides scientific insights and suggestions to incorporate the latest knowledge on developmental pharmacology and innovative techniques into paediatric drug development. The developmental pharmacology research field should be driven by developmental (patho)physiology, and multidisciplinary collaboration between academia, industry, regulatory agencies, health-care workers, patients and parents. As an illustration on how to put this call to action, conect4children project (www.conect4children.org) initiated a strategic feasibility advice service to build the bridge between all these partners involved. The aim is to provide innovative methodology advice, including on developmental pharmacology and sharing the latest knowledge, e.g. through this White Paper.

DISCLAIMER

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[Correction added on 8 August 2021, after first online publication: Disclaimer has been added in this current version.]

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COMPETING INTERESTS

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CONTRIBUTORS

A.S. conceptualized and drafted the paper. All co-authors (A.S., P.A., G.C., P.D.C., S.d.W., J.M.K., F.B.L., C.M., P.P., M.F.S., J.S., M.A.T., B.V., W.Z., A.M.W., R.W., J.v.d.A. and K.A.) contributed to writing and revision of the paper. K.A. provided intermittent review. A.S., R.W., S.d.W., J.v.d.A., M.A.T. and K.A. critically revised the prefinal version. All co-authors (A.S., P.A., G.C., P.D.C., S.d.W., J.M.K., F.B.L., C.M., P.P., M.F.S., J.S., M.A.T., B.V., W.Z., A.M.W., R.W., J.v.d.A. and K.A.) revised the paper draft and approved the final text version.

ORCID

Anne Smits https://orcid.org/0000-0002-0710-6698
Giacomo Cavallaro https://orcid.org/0000-0002-4921-1437
Joseph F. Standing https://orcid.org/0000-0002-4561-7173
Mark A. Turner https://orcid.org/0000-0002-5299-8656
Wei Zhao https://orcid.org/0000-0002-1830-338X

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