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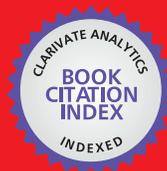
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Changes in Research and Development of Medicinal Products since the Paediatric Regulation

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1. Introduction

The lack or incompleteness of evidence of the efficacy and safety of drugs used in children has been a growing concern in the recent past. The great majority of drugs prescribed to children are often given either on an unlicensed or an "off label" basis simply by extrapolating data for adults and without conducting any paediatric clinical, pharmacokinetics, dose finding, or formulation studies in the paediatric population. The paediatric pharmaceutical repertoire therefore comprised pills too large to swallow and extemporaneous formulations containing excipients unsafe or unpalatable to children.

Diseases in children, however, are often different from their adult equivalents, and the processes underlying growth and development might lead to a different effect or an adverse drug reaction unseen in adults.

The health and, therefore, quality of life of the children in Europe suffer from a lack of testing and authorisation of medicines for their use. It means that children are "orphans" of appropriate medicinal products and children continue to be exposed to risks, and at the same time miss out on therapeutic advances.

This is particularly ironic considering that our modern system of medicines regulation, that ensures the high standards of safety, quality and efficacy of medicinal products for use in adults, was developed primarily in response to therapeutic disasters, or "drug catastrophes", that occurred in children in the past (such as the numerous cases of icterus induced by sulphanilamide which occurred in 1937 and the well-known phocomelia caused by thalidomide in the 1960s).

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For these reasons, the development of regulations and the adoption of stringent criteria on quality, safety and efficacy medicinal products used in the paediatric population have been enforced and led in January 2007 to the introduction in the European Union (EU) of the "Paediatric Regulation" (European Parliament and the Council of the European Union, 2006a, 2006b) governing the development and authorisation of medicines for use in children aged 0 to less than 18 years. The Paediatric Regulation is the latest in a number of incremental regulatory steps to improve public health for children through increasing research, information and availability of medicines. It brings in many new tasks and responsibilities for the European Medicines Agency (EMA), chief of which is the creation and operation of an *ad hoc* Paediatric Committee (PDCO) within the EMA to provide objective scientific opinions on any development plan for medicines for use in children.

Since the entry into force of the Paediatric Regulation, the regulatory environment for paediatric medicines has dramatically changed, a marked "paediatric revolution" aimed at improving the health of children in the EU took place and pharmaceutical companies have been faced with a number of measures, obligations and incentives.

2. The Paediatric Regulation

2.1 Objectives

The objective of the Paediatric Regulation is to improve the health of children in Europe by increasing and facilitating the development and availability of medicines for children aged 0 to less than 18 years, that means changing the way in which medicines are developed. Increasing the development of medicines for children is to be reached by ensuring that those medicines are subject of high quality, ethically researched and authorised appropriately in the relevant population subsets including the neonates, to avoid, at the same time, unnecessary clinical trials in children and not delaying the authorization of medicines for the adult population.

The new key element of the Paediatric Regulation is the early involvement of a pharmaceutical company in the research and development programme of a medicinal product by the requirement to consider the needs of the paediatric population, also in terms of age-appropriate formulations, according to a Paediatric Investigation Plan (PIP) that describes the paediatric development (quality, non clinical and clinical aspects) and all adopted measures necessary to investigate the medicine in the paediatric population. The PIP has to be agreed with an *ad hoc* committee of experts, namely the Paediatric Committee. In case that a) the specific medicinal product or class of medicinal products is likely to be ineffective or unsafe in part or all of the paediatric population, b) the disease or condition for which the specific medicinal product or class is intended occurs only in adult populations, or c) the specific medicinal product does not represent a significant therapeutic benefit over existing treatments, it is possible to apply for a waiver. The Paediatric Regulation also foresees the opportunity to request a deferral thus avoiding the delay of the authorisation for other populations. A deferral allows the postponement of the initiation or completion of some or all of the measures set out in PIP, such as the completion of trials, but not the delay in the submission of the PIP itself.

2.2 Obligations and incentives

The Paediatric Regulation is the first piece of European legislation that requires the pharmaceutical industry to develop medicines for use in children through a system of obligations and incentives, depending on the type of medicinal product concerned.

For *medicinal products not yet authorised* in the EU, according to article 7 of the Regulation, there is an obligation to include the results of studies (conducted in compliance with an agreed PIP), unless a waiver or a deferral was granted by the PDCO, for the validation of a marketing authorisation application for adult and paediatric medicines.

For *authorised products protected by a supplementary protection certificate, or by a patent eligible for a supplementary protection certificate*, according to article 8 of the Regulation, there is an obligation to include either the results of studies (conducted in compliance with an agreed PIP), unless a waiver or a deferral was granted by the PDCO, in applications for variations or extensions (of an existing marketing authorisation) concerning a new indication, pharmaceutical form or route of administration.

The compliance with such obligations allows pharmaceutical companies to benefit incentives and rewards, aimed at encouraging the development of paediatric medicines. Once authorisation is obtained in all EU Member States and study results (whether negative or positive) are incorporated into the product information (SPC), the pharmaceutical company grants rewards and incentives.

In details when an agreed PIP is completed and all the information has been submitted to the regulatory authorities, the medicinal product, falling under article 7 or 8 of the Regulation, will be granted an extra 6 months patent protection (extension of the duration of its Supplementary Protection Certificate [SPC]). This extension will be granted whether or not the data support a paediatric indication.

For orphan medicinal products the incentive takes the form of an extra two years market exclusivity.

The Regulation also establishes a new type of marketing authorisation, called the paediatric use marketing authorisation (PUMA), intended to stimulate the development of off-patent products for use in the paediatric population. The PUMA is the specific instrument created by the Regulation aimed at stimulation the development of off patent drugs which represent a still unmet need in paediatrics, since most of these compounds widely used daily in children of all age groups, have not been adequately tested in the paediatric population. The Paediatric Regulation includes provisions for funding of research into off-patent medicines (Community Framework Programmes for Research, Technological Development and Demonstration Activities or any other Community initiatives for the funding of research). Public funding is necessary as off-patent medicines are of little commercial interest for pharmaceutical companies.

2.3 The Paediatric Investigation plan

As described above, the central key instrument of the Paediatric Regulation is the agreement of the PIP, defined as a research and development programme aimed at ensuring that the necessary data are generated to determine the conditions in which a medicinal product may be authorised to treat the paediatric population.

The PIP details the planned development in terms of efficacy, safety and quality (age-appropriate formulation) and timelines for children from birth to 18 years. In other words, the PIP contains a full proposal of all the studies necessary to support the paediatric use of an individual product, intended for diagnosis, prevention or treatment of a condition.

The paediatric population is in fact composed of a number of population subsets, thus the PIP has to specify which population subsets need to be studied, by what means and by when.

In addition, PIP must describe any measures to adapt the formulation of the medicinal product to make its use more acceptable, easier, safer or more effective for the different subsets of the paediatric population.

The introduction of the PIP in the legal framework of medicines has finally ensured, or at least is expected to ensure, the availability of paediatric data and results, to cover the needs of all age groups of paediatrics, from birth to adolescence. All the regulatory and scientific strategy for developing a product is changing and the development of medicinal products for potential use in the paediatric population becomes also an integral part of the development of medicines for adults.

The PIP has to be submitted to the Paediatric Committee early during the product development before marketing authorisation applications are submitted. Particularly applications for a PIP, including a deferral if relevant, and/or for a waiver should be submitted no later than the completion of the relevant human pharmacokinetic studies in adults, unless justified. This time point was chosen to ensure that the paediatric development of the product is considered at a very early stage of the overall product development rather than as an afterthought.

An *ad hoc* procedure is followed by the PDCO to evaluate and finally agree the PIP. In the specific the evaluation of a PIP is conducted over two periods of 60 days each—maximum. EMA scientific coordinators and two members of the PDCO contribute to the PIP evaluation in a written report (PDCO Summary Report), which is then discussed by the PDCO. In most cases, the Paediatric Committee requests modifications of the proposal at the end of the first 60 days period. The request for modification identifies all necessary changes to study non clinical and/or clinical protocols and/or formulations. At this point it is expected a clock stop at day 60 to allow companies to respond to requests for modification of the plan (approximately 3 months) and once evaluated, PDCO positive or a negative opinions on PIPs and waivers are transformed into binding EMA decisions.

Notably the PIP is legally binding for the pharmaceutical companies willing to seek marketing authorisation since the results generated by the agreed PIP are then assessed by the Competent Authority granting the marketing authorisation (the Committee for Medicinal Products for Human Use for the centralised procedure) or the variations.

2.4 The Paediatric Committee

The Paediatric Regulation brings the creation and operation of a new scientific committee of experts at the EMA, the Paediatric Committee, set up in July 2007 and primarily responsible for reviewing and agreeing applications for paediatric investigation plans including deferrals, and/or waivers.

This committee aims to ensure expertise and competence in paediatric medicines and to provide scientific opinions on any development plan for medicines for use in children.

The PDCO membership includes five members of the Committee on Human Medicinal Products (CHMP) plus their alternates. These five members will provide an important link between the two committees. Member States, that have no representatives as CHMP members in the Paediatric Committee, appoint a member and an alternate. In addition, the European Commission appoints three members plus alternates, to represent health professionals, and three members plus alternates to represent patient associations, following a public call for expressions of interest and after consulting the European Parliament. The EMA and the Commission are expected to cooperate to ensure that the final composition of the Committee, including members and alternates, covers those scientific areas relevant to

paediatric medicines. The members, alternates and experts must not have any financial or other interests in the pharmaceutical industry that could affect their impartiality.

The Paediatric Committee has a number of important responsibilities. Its most important task is to assess the content of PIPs (which may also include requests for waiver and/or deferrals) for medicinal products and to release opinions on them. The Committee may itself impose a waiver from the requirement to provide data from a PIP, if it considers that the product may be unsafe or ineffective in the paediatric population or that the product will not provide any significant therapeutic benefit. In fact, when assessing a Paediatric Investigation Plan, the Paediatric Committee must consider whether or not any proposed studies can be expected to be of significant therapeutic benefit to and/or fulfil a therapeutic need of the paediatric population.

Other specific tasks include establishing an inventory of specific needs for paediatric medicinal products (still under preparation) and giving scientific input in the development of any documents related to achieving the Regulation's objectives. The Committee should avoid requirements for studies in children causing any delay in the marketing authorisation of the medicine for other populations. This is to ensure that medicines are developed for children based on the therapeutic needs of children rather than just on the basis of when the paediatric market may be profitable or incentives might be financially attractive. The Committee also has an advisory role for the EMA and for the European Commission on any question relating to the paediatric use of medicines. This includes giving advice on communication issues relating to conducting research in the paediatric population, on the compliance of an application for marketing authorisation with an agreed PIP or on the safety, quality and efficacy of a medicinal product for paediatric use.

2.5 How to increase paediatric research

One of the above described objectives of the Paediatric Regulation is to increase paediatric research. Clinical trials in the paediatric population require specific expertise, in some cases specific methodology and specific facilities, and should be carried out by appropriately trained investigators. Thus in order to facilitate this aspect the Paediatric Regulation established the creation of an European Network of Paediatric Research and research funding.

The EMA is responsible for establishing a network of existing networks, centres and investigators of paediatric research, which was set up in 2011 and whose objectives are to coordinate studies relating to paediatric medicinal products, to build up the necessary scientific and administrative competences at European level, avoiding duplication of studies and testing in children. The EU network is expected to serve as a tool for industry to perform trials with children in keeping with the PIP. Both technical and/or administrative competences in the performance of paediatric clinical trials through effective collaboration are expected benefits, which will allow to avoid duplication of work and efforts, making the use of facilities more efficient and profitable, to develop common methods of working with special attention to quality assurance. Facilitation of recruitment of patients and avoiding unnecessary studies in children are expected.

2.6 Transparency and information

Transparency and information are other key words of the Paediatric Regulation. Through increased availability of information, the safe and effective use of medicinal products for

children can be increased so promoting public health, preventing the duplication of studies in children and avoiding unnecessary studies. One of the measures is making all paediatric trials included in the European database (EudraCT) accessible to the public both for protocol and results-related information. The increase in paediatric clinical trials transparency beginning from the planning and recruiting of patients to the on-going and finalised studies, is another target of the Regulation. All decisions of the EMA on PIPs, deferrals or waivers of the paediatric development are made public and routinely published on EMA website. Moreover where authorisation is granted, the results of all those paediatric studies, any waivers or deferral, are included in the Summary of Product Characteristic and, if appropriate, in the Patient Leaflet of the medicinal product, whether or not all the paediatric indications concerned were approved by the competent authority.

3. What is changing with the Paediatric Regulation

The Paediatric Regulation is a remarkable step forward, because for the first time in Europe it is a regulation that is provided by law and provides direct economic support for paediatric clinical trials and indirect support for pharmaceutical industries.

Since its establishment in July 2007 the PDCO has assessed a large numbers of procedures: by August 2011, 1087 validated PIPs/waivers have been submitted by pharmaceutical companies covering nearly 1516 indications., and of which 259 have been requests for a full waiver.

Of the 1087 validated applications:

- 74% referred to medicinal products not yet authorised in the EU at the time of the entry into force of the Regulation (so called "Article 7 applications").
- 24% referred to products already authorised, still under patent or supplementary protection certificate, in view to submitting a variation/extension for a new indication, pharmaceutical form or route of administration (so called "Article 8 applications").
- 4% referred to an off-patent product developed specifically for children with an age-appropriate formulation (so called "Article 30 applications").

In the first year of the implementation of the Regulation, most of the applications were "Article 8 applications". After about a year, the balance changed towards a higher proportion of "Article 7 applications". This change is confirmed in 2010 and 2011. For "Article 30 applications", the number of applications submitted is still very low.

In 2011, Olski et al. published the first analysis of the general impact of the Paediatric Regulation on the development of medicinal products in Europe. Three years after its implementation an increase in the availability of medicines with age-appropriate information in the next years is shown at least as reported by the high number of PIPs despite the still modest number of clinical trials performed (Olski TM et al., 2011; Davies et al., 2010; Rocchi et al., 2010).

Most of the paediatric developments will be performed in therapeutic areas, such as endocrinology, oncology, infectious diseases and cardiovascular diseases, which relates to the economical importance in the adult market, while other areas such as pain management still remains less studied.

A high number of full waiver requests are reported for more prevalent adult-only conditions, such as atherosclerosis.

On the light of these results and from a qualitative approach, the Paediatric Regulation appears to fulfil its core goals. The quality of the plans submitted has improved also due to the PDCO demanding better methodological approaches. The PDCO's intervention has also

increased the number of medicines that will be studied in neonates, the most neglected subset to date. A high proportion of agreed PIPs require a specific age-appropriate formulation, more than originally proposed, and all this should help meet the clinical needs of children and health professionals.

The agreed-upon PIPs will provide important short- and long-term safety data.

Some paediatric development plans have been also preliminary discussed during scientific advice procedure, a free access granted by the Paediatric Regulation for any request containing questions on the paediatric development. The advice is provided by the *ad hoc* SAW /Scientific Advice Working Party) of the Committee for Medicinal Products for Human Use (CHMP) and is adopted by the CHMP. For the paediatric requests, members of the PDCO are routinely involved in the procedure as experts. Notably, since the entry into force of the Paediatric Regulation, the number of scientific advice on questions related only to paediatric development has increased steadily, with a total of 32 procedures in 2010. A high number of so-called "mixed" scientific advice/protocol assistance requests, i.e. covering both adult and paediatric development, have also been submitted for which members of the PDCO are generally involved. Compared to 2009 where 35 procedures were submitted, the figure increased to 48 in 2010 (European Medicines Agency [EMA], 2011, thereafter EMA, 2011).

Indeed, there is still a need for additional paediatric information on off-patent medicines and to date, in June, 2011, only a PUMA has been granted for the medicinal product Buccolam (midazolam) intended for the treatment of prolonged, acute, convulsive seizures in paediatric patients from the age of 3 months to 18 years. The funds for the research of off patent drugs granted by the European Commission are currently in stand by and there is hope that next calls of the EC Framework programs will take into account the paediatric population.

One thing is for certain, the whole R&D process from pharmaceutical, non –clinical and clinical to post-marketing studies is continuously evolving and changing starting from *quality* (need for age appropriate formulation specifically addressed to paediatrics), to *non clinical* (need for studies on juvenile animals to evaluate the toxic potential before any administration to paediatrics), *clinical* (need for non conventional approaches in terms of study design and size of the population, choice of adequate endpoints), and *post-marketing* issues (need for *ad hoc* pharmacovigilance measures).

In the following sections, a short overview of how R&D process is improving in the light of new Paediatric Regulation's requirements is provided.

4. Quality: Need for age appropriate formulation specifically addressed to paediatrics

The development of ad hoc formulations intended for the paediatric population is a crucial issue and unfortunately, nowadays there is still limited scientific and regulatory experience. Regulatory initiatives have been undertaken to provide guidance in developing ad hoc medicine. As mentioned above, the adoption of the Paediatric Regulation plus the consequent demand for paediatric studies have started to change the context by finally strengthening the focus on better formulations for children.

The current legislation establishes that pharmaceutical companies need to develop a specific age-appropriate paediatric formulation together with an adequate packaging, user instruction and where relevant dosing device and/or administration device prior to

performing clinical studies in the paediatric population. All these pharmaceutical development aspects may be fundamentally different to those of the existing adult product.

This new regulatory environment stimulates the rational development and testing of age-appropriate medicinal products intended for the paediatric population. Part of this legislation (Recital 9, 10 ; Art. 15) requires that applicants for medicinal products for use in the paediatric population should submit their plans for the development of such products in the form of a PIP for approval, or a waiver, or a deferral.

The spirit of the legislation was to i) to encourage companies to develop specific, 'age-appropriate' paediatric formulations and ii) to develop relevant and acceptable formulations with convenient and precise dosing characteristics, on an industrial scale suitable for marketing. (i.e. the spirit is not to place the preparation of paediatric medicines in a context which is ad hoc, extemporaneous, or magistral preparations).

It is now well accepted that children are not simply small adults and their treatment with pharmaceutical medicines poses problems which are not seen to the same extent in adults. For example, the lower age group subsets of the paediatric population are simply unable to swallow conventionally sized tablets; they may be particularly sensitive to certain excipients that are otherwise acceptable in adult formulations, or there may be compliance problems since they often need to be persuaded to take their medicines, and so on.

Few European Medicines Agency (EMA) documents have been released with the aim of providing guidance on the principles that should be taken into account in the development and the assessment of pharmaceutical aspects of medicinal products for paediatric use.

In the regulatory framework, the starting point was the "Concept paper on the development of a quality guideline on pharmaceutical development of medicines for paediatric use" (Committee for Medicinal Products for Human use [CHMP] et al., 2008, thereafter CHMP et al., 2008) which was the first step to a scientific and harmonised approach to the development of a guideline that provides adequate tools for responsible development of a medicinal product for use in the different subsets of the paediatric population. Information sharing with authorities from other regions (e.g. the FDA) would further support global development.

This guidance however, was preceded by other guidelines published by the EMA, such as:

- "Excipients in the Dossier for Application for Marketing Authorization of a Medicinal Product" (CHMP, 2007a): a key guideline on the quality of excipients, into effect on January 2008.
- "Excipients in the Label and Package leaflet of Medicinal Products for Human Use" (European Commission [EC], 2003, thereafter EC, 2003): one of the most relevant guidelines in the context of quality related to safety, even if established safety profiles and warning statements are based mostly on data in adults, and apply to the adult population. However, it acknowledges that some excipients are not entirely inert but may have side effects.

One of the issues to consider is that the benefits of a medicinal product for paediatric use should outweigh the potential risks associated with its use by the different subsets of the paediatric population as defined in the "International Conference on Harmonisation (ICH) Topic E 11 Note For Guidance On Clinical Investigation Of Medicinal Products in the Paediatric Population" (Committee for Proprietary Medicinal Products [CPMP], 2003, thereafter CPMP, 2003), namely:

- Preterm newborn infants.
- Term newborn infants (0-27 days).
- Infants and toddlers (1 month to 23 months).

- Children (2 – 11 years).
- Adolescents (12 – 16 or 18 years).

The pharmaceutical development aspects should be chosen with the care for each subset of the paediatric population. This classification does not match pharmacological stages. Often, the different subsets (from preterm newborn infants to adolescents) require different approaches. As a consequence, there might be a need to develop more than a single `formulation` which would be appropriate for all ages. On July 2006, the "Reflection paper on formulations of choice for the paediatric patient" (CHMP, 2006) was finalised by the EMA with the aim of providing a comprehensive summary of the physiological and pharmaceutical issues. These could be taken into account in the development of paediatric medicines, looking at the acceptability of different dosage forms, administration volumes, size of unit dosage, taste and the acceptability and safety of excipients in relation to the age and development status of the child.

Afterwards, on January 2010 the "Guideline on the investigation of Medicinal Product in the Term and Preterm Neonate" (CHMP & Paediatric Committee [PDCO], 2009, thereafter CHMP & PDCO, 2009) was effective and specifically addressed to the investigation of medicines intended for neonates.

An additional consolidated guidance on the pharmaceutical aspects of medicinal products for paediatric use was anticipated to be finalised before the end of 2009 in the concept paper. However, "the Draft of Guideline on Pharmaceutical Development of Medicines for Paediatric Use" (CHMP, 2011) has been released for consultation in May 2011. As the deadline for comments is December 2011, the guideline will likely to be published in 2012 as an outcome of a collaborative work between the CHMP, Safety Working Party (SWP), Paediatric Committee, and external experts. This guideline aims to provide additional tools for the rationale pharmaceutical development of medicines for children between birth and 18 years of age.

4.1 Points to consider in developing an age appropriate formulation

Paediatric formulations that permit accurate dosing and enhance adherence (i.e., simple dosing regimen, better palatability) are required for paediatric clinical pharmacology studies and an age-appropriate dosage form must be made available for children.

The goal should ideally be to develop relevant and acceptable safe formulations, which have convenient and precise dosing characteristics for the intended population, made on an industrial scale suitable for marketing.

Basically, the critical points of the paediatric formulations to be considered are related to:

- Routes of administration.
- Appropriate dosage forms: taking into account a formulation that the child can take (size, volume, taste, ease of administration, dosing regimen).
- Dosing accuracy (strength, dose criticality, administration device).
- Treatment duration and setting.
- Appropriate safety considering administration device, handling, excipients such as preservatives, antioxidants, colorants.
- Acceptable organoleptic properties including taste, after taste, smell, colour, texture.
- Minimal impact on life style, ease of administration, good acceptability by children and parents/carers.
- Cost-effectiveness.

4.2 Development of paediatric formulations for paediatric subsets
4.2.1 Preterms and neonates

As above cited, the guidance (CHMP & PDCO, 2009) was released by the EMA and entered into force on January 2010 on the investigation of medicinal products in the term and preterm neonates. This guideline addresses the considerations, requirements for the design and conduct of clinical trials in premature and term neonates. It includes background information on the maturation of organs and of body functions. Formulations and route of administration are also mentioned.

Age-appropriate formulations and strengths using appropriate excipients must be developed to avoid extemporaneous preparations, even more so for neonates. In neonatal practice medication errors are commonly due to use of inappropriate formulations or strengths which require complex calculations and measurement of very small volumes or multiple dilutions. Special care should be given to extremely low birth weight (birth weight <1000 g) and very low birth weight (birth weight <1500 g) newborns.

Notably, attention must be focused on the excipients which can be used on adults and older children, may be toxic in neonates because of their immature and rapidly changing metabolic & elimination system (e.g. less predictable absorption, different volume of distribution, immature clearance mechanism). The salt of the active ingredient and the chemical nature of the preparation must be carefully considered to avoid administration of excessive amounts of electrolytes.

The intravenous (IV) route is normally used in clinically unstable term and preterm neonates. The volume of IV infusions (blood products, total parenteral nutrition (TPN), other IV medication) contributes critically to daily fluid intake. Therefore careful thought must be given to the volume of injection not to exceed the daily fluid allowance. Osmolarity of solutions, pH and infusion rates must be carefully considered. A suitable strength of an IV formulation with an appropriate solvent should enable to administer appropriate volume.

Oral administration should be used when possible and appropriate in the neonatal population. Preparations for oral administration are most likely to be liquid dosage forms, keeping the volume to be administered as small as possible. However, as liquid preparations more often contains excipients like preservatives and antioxidants, special care should be taken regarding the choice of excipients as some may have toxic effects. Sterile and/or single use oral dosage forms may be considered in order to avoid the use of preservatives and to avoid or to reduce antioxidants. When preservatives are required, the concentration should be at the minimum level and a thorough justification for the choice of the preservative should be provided.

Neonates have special needs. If the product is likely to be administered via an enteral (e.g. nasogastric, nasojejunal) tube, issues such as viscosity of formulation (to permit flow of the product through small tubes [e.g. 6FR/8FR] and avoid blockage), size of particles, adsorption to commonly used enteral tubes and interaction with common formula/breast milk should be investigated.

For local or systemic effect a topical administration may be suitable, taking into account skin immaturity, especially in preterm neonates, and the large and more permeable and moisturised surface area.

On the contrary, rectal and intramuscular (IM) administration are not commonly used in this age group, due to erratic absorption and in case of IM also to painful injections that may cause tissue damage. Adverse effects such as muscle contraction and abscess development

can be seen after IM administration. In premature neonates, inefficient muscle contractions and vasomotor activity may alter pharmacokinetics of the drug. In neonates, decreased blood flow may cause variability in drug delivery and absorption.

4.2.2 Infants

The rapid maturation, immune system development and total body growth should be carefully taken into consideration in developing relevant and acceptable formulations for infants. The developmental pattern may occur at a variable level between individuals.

While the rate of oral absorption is slower in neonates than in older paediatric patients, absorption by IM route may be greater by the rich supply of capillaries. In early infancy, the low concentration of bile salts may decrease the absorption of lipid-soluble drugs. Above 1 year of age, infants metabolic activity increases, dose adjustments should be made accordingly. Infant skin is mature; their epidermis is hydrated to a greater extent than adults. During the first years of childhood, the surface area to body weight ratio in infants may be twice that of adults and subject to a change.

The choice of the excipients should be properly justified in PIP in relation to age, treatment duration, severity of disease, rational use, due to infants' juvenile metabolizing capacity. Risk-benefit analysis should support proposed excipient selection. Some excipients are not entirely inert, therefore may present safety problem. Even the so-called child-friendly excipients should be kept to a minimum. When only intended for aesthetic purposes, excipient use should be avoided. It would be a better approach to use marking or embossing instead of inclusion of colouring agent into formulation composition.

As a result of lack of cognitive maturity and coordination, infants are similar to neonates in terms of being fully dependent on caregivers. Appropriate dose delivery and administration devices have to be used. The physiological capability of the infant should be considered. For instance, dry powder inhalers are not appropriate for infants as they cannot generate sufficient air flow. Pressurized metered dose inhalers may be applied to infants from birth if in combination with a specific spacer system and face mask. Administration devices providing accurate dose delivery should be considered. Validated droppers are convenient for infants. Concomitant administration with TPN using the same IV access is discouraged, as TPN formulations are highly variable and may induce oxidation.

4.2.3 Children

This period is much more mature compared to newborn and infants. However, children constitute a subset of paediatric population, with a distinct age range situated at different stages of their physiological and cognitive development. The magnitude of doses varies throughout childhood. Hence, major difficulty would be to choose the optimum formulation applicable for all age range. In this respect, the points to be considered are as follows; younger children are still under psychomotor development, school age children are subject to skeletal growth, weight gain and switch from home life to school life, the onset of puberty can occur as early as 9 years of age. Therefore, stratification by age within this category may be needed for the decision on drug delivery system. Considering the child's cognitive ability, further subdivision of this age group into pre-school children (2-5 years) and school children (6-11 years) would be helpful. The formulation strategy should be chosen on a case by case depending on the drug's physicochemical characteristics, dose and patient's disease status and age. Accurate dosing must be ensured with an appropriate packaging and

administration device. For instance, if the incorrect dosing risk is high, it would be a sound approach to use unit dose, pre-filled oral syringes or cups for the single use . The criticality of the dose should be established to determine the choice of the drug delivery system.

Oral solid unit dosage forms which are intended to be ingested whole may be acceptable for older children. The compelling question is at what age children can safely swallow conventional tablets and capsules. Anecdotal evidence suggest that with support and training, children aged < 6 years can learn to take solid dosage forms but little information on acceptable size of tablets/capsules is available. Formulations providing dose flexibility, such as liquids and multiparticulates (e.g. sprinkles, minitablets, pellets, granules) can be used across different age groups of children. Opening the capsules and mixing with food/beverage facilitate the administration to young children. However, this process should not cause any inconvenience for the child and parent/caregiver. It must be ensured the bioavailability of the formulation would not be affected and handling would not cause any harm to parent/caregiver (e.g. exposure to irritating or cytotoxic drugs must be avoided). Minitablet is suitable for young children. Orodispersible formulations (e.g. orodispersible films and tablets) are encouraging due to their ease of administration, lack of necessity to use water and thereby, adaptability to different settings such as developing countries depending on the feasibility of pharmaceutical manufacturing. Liquid formulations would be alternative if the medication can be administered at an appropriate volume (< 5 ml for children under 5 years and < 10 ml for children of 5 years and older). Furthermore, formulation should taste pleasant to avoid noncompliance.

The usability of the device by the paediatric patient is essential. For instance, inhaling devices should not be too complicated for the pre-school children or children with lack of coordination. Educational training programmes would be helpful to increase adherence to inhalers. Children should benefit from advances in drug delivery. Innovative formulations such as sipping straw (Clarosip® Straw) and dispensing spoon (comprising preloaded drug that on exposure to water turns into a pudding like texture) (Parvulet®) are promising approaches facilitating the administration of dose in an appealing way to paediatric patient. Nevertheless, still few innovative formulations are for prescription drugs on the market. The main limitation of available innovative formulations is the delivery of a single dose. For older children who are able to swallow solid oral dosage forms, multiple dose delivery approaches exist. For example, the rectangular tablet with multiple fraction bars and deep score lines on both sides has been a smart design allowing flexible drug dosing in function of the body weight of paediatric HIV patients. Modified release formulations would be an easy option to omit administration of doses during school hours.

The place and pattern of treatment should be taken into account in paediatric formulation development. The administration setting affects the selection of dosage form. Application of different formulations would be feasible in hospital, while it is not in community. The practicality of using different drug delivery systems in different clinical settings, particularly in paediatric intensive care setting should be considered. The condition (acute/chronic) and severity of the disease plays a role on the selection of appropriate dosage form. During an acute illness, the ease of application becomes an critical parameter. Because the child is more fractious, uncooperative and the medication is relatively unfamiliar each time it is used. Formulation used for a chronic condition may cause repeated cumulative exposure to excipients. Therefore, the acceptable daily intake and safety limits of excipients for children must be checked.

Acceptability on entire age group should be considered. In the matrix given in "Reflection paper on formulations of choice for the paediatric patient "(CHMP, 2006), 'children' have been further divided into pre-school children (2-5 years) and school children (6-11 years) because of the significant changes in the ability to handle some dosage forms between 2-12 years of age. The pre-school period is particularly challenging: neither passive nor active compliance, can be expected. Patient should be kept at the forefront of any evaluation. Conducting research directly with children across various defined age subsets is necessary to gain insight, in particular, to understand acceptability and applicability of different dosage forms.

Palatability is critical to compliance. Many drugs taste bitter or irritate the oral cavity. Undesirable taste and aftertaste have to be improved to provide acceptability of the formulation . Taste masking may rely on sweeteners but often requires multiple approaches. The taste has been started to be seen as a major component of pharmaceutical quality aspects. Additionally, texture (grittiness), smell and ease of swallowing need to be evaluated. Different types of flavours may be more acceptable in one region than another, due to cultural differences.

Topical administration of drugs is also feasible. However, it should be considered that young children have a larger surface area to weight ratio than adults which may result in profound systemic effects.

As alternative promising drug delivery systems, transdermal formulations improve patient acceptability, ideally suit to needlephobes, provide controlled delivery to relevant skin layers and systemic circulation, reduce dose requirement and bring advantage of ease of application and parent drug management. Needle-free drug delivery systems (e.g. microneedles) can mitigate against the pain that can be associated with the parenteral route of administration.

4.2.4 Adolescents

Adolescence period is subject to physical and cognitive changes. The effect of pubertal growth spurt and action of hormones are needed to be to considered during formulation development for adolescents. The pattern of growth should not be altered by the medicinal products.

Adolescents constitute a challenging age subset, especially in terms of noncompliance, due to being in a period of psychological and social transition between childhood and adulthood. Although the ability to cope with dosage form increases, adolescents may reject to take the medication under the effect of peer pressure, emotional change or as they find the medication too childish. As adolescents are responsible of taking their own medication, acceptability is vital for their coordination and compliance. The matrix given in "Reflection paper on formulations of choice for the paediatric patient" (CHMP, 2006) indicated adolescents` dosage form of choice is mainly peroral and topical/transdermal formulations; rectal formulations are accepted under reserve. However, this statement does not reflect an evidence-based finding. Given the limited experience with acceptability of different dosage forms, further comprehensive research should be performed.

4.3 Conclusive remarks

Four years have elapsed since the entry into force of the Paediatric Regulation which has stimulated the conduct of high-quality research with the increase in paediatric trials.

According to "Report to the European Commission on companies and products that have benefited from any of the rewards and incentives in the Paediatric Regulation and on the companies that have failed to comply with any of the obligations in this Regulation, covering the year 2010" (EMA, 2011), the number of companies which have benefited from an extension of the Supplementary Protection Certificate in some Member States is increasing but this number may still be considered limited. The implementation of the paediatric regulation resulted in a large number of PIPs including measures for the development of age-appropriate formulations and progress should continue incrementally. Developing high quality appropriate formulations for a sensitive population with individual needs is a challenging task. Therefore, engagement between key stakeholder including companies, regulatory authorities, health professionals and society is needed. Concerted effort will pave the way for a PIP with a strongly written quality section and thereby, increase the commercial viability of medicines intended for children. Paediatric formulations should not be as an add-on to adult formulations; conversely, it should be seen as an integrated part of the overall drug development programme, unless a waiver is appropriate. The "Guideline on Pharmaceutical Development of Medicines for Paediatric Use" (CHMP, 2011) will provide additional tools for the rationale paediatric formulation development and highlight the main aspects for justification of strategy. PIP should ensure that every study contributes to the paediatric formulation development pathway. Often, problems related to formulation are identified late during the paediatric clinical trial and led to delays and complications in the conduct of the trial. For early scrutiny of pharmaceutical quality-related issues, timely submission and subsequent evaluation of potential formulations would facilitate to investigate the intended marketed product in paediatric clinical trials and would let PIP amendments related to formulation aspects. Research into novel dosage forms and administration devices along with effective utilization of existing drug delivery technologies will be pivotal to develop acceptable, safe, feasible and age-appropriate formulations for children.

5. Non clinical: The need of studies on juvenile animals in order to evaluate the toxic potential before any administration to paediatric population

The 20th century history of drug research and regulation shows that dramatic tragedies such as sulfanilamide (Wax, 1995), thalidomide (Choonara & Rieder, 2002) and TGN 1412 (Suntharalingam et al., 2006) cases facilitated the passage of stronger laws. The elixir of sulfanilamide case can be considered one of the earliest example of disaster in paediatric medicine therapy. After its safety use in adults in fact, an elixir of sulfanilamide was developed to enable administration to children, using diethylene glycol as the solvent because it was odourless, sweet, and syrupy, but without investigate its toxicological properties. Since diethylene glycol is highly toxic causing gastrointestinal, metabolic, renal and hepatic failure, more than one hundred Americans, many of them children, died and this incident was the main cause of passage, in 1938, of the USA federal Food, Drug, and Cosmetic Act.

Other incidents, since then, have induced serious adverse reactions in children (Choonara & Rieder, 2002) but in parallel multiplied efforts have been made from Regulatory agencies in the world, with the commitment of many organizations, including academy and paediatrics, to ensure that paediatric drug therapies are developed with the same level of scientific and clinical rigor as adult therapeutic agents. These efforts culminated in USA and Europe (EU)

paediatric initiatives. In Europe, the Paediatric Regulation was preceded by the ICH Guidance for conducting studies in the paediatric subjects (International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use [ICH], 2000, thereafter ICH, 2000; CPMP, 2001) and it was also accompanied by guidance documents on various themes in the same field, including non clinical investigation of products in the neonatal population. In November 2008 a specific Nonclinical Working Group was created by the PDCO in order to assure consistency of paediatric investigational plan (PIP) evaluation (Silva-Lima et al., 2010). In the following paragraphs it is specifically discussed the role of juvenile animal studies in developing drugs for paediatric use.

5.1 Regulatory situation
5.1.1 Legal rules
The rules governing the clinical development of drugs and their placing on the market are described in the EU Regulations and Directives. The first are the most important legal acts after the treaties. They create the same rules for all citizens of EU Member States (MS), are uniformly valid in all MS of the Community, have direct effect, and should not be transposed into national law. Taking into account the lack of adapted children medicine in Europe, the European parliament put in place the Paediatric Regulation bringing profound changes as described above in the dedicated section of this chapter.

The Directives are binding legislative acts, directed to MS. They provide the criteria and the principles according to which individual MS govern, by means their own acts, matters for which harmonization/convergence of disciplines, in different EU countries, are necessary. Directives always needed a period in order to be transposed into national law. To simplify and harmonize the rules and administrative provisions governing clinical trials in Europe, Directive 2001/20/EC (European Parliament and the Council of the European Union, 2001), also known as Clinical Trials Directive, was enacted; its scope is wide and includes every clinical trial with every medicine on any human subject within any of the 27 MS including paediatric investigation. The Clinical Trials Directive is concretized further by Directive 2005/28/EC (Commission of the European Communities, 2005).

The Directive requires that researches/investigators and sponsors ensure ethical review, the authorization by competent national authorities before enrolling participants, the drug manufacture in compliance with Good Manufacturing Practice (GMP), and rigorous observance of the Good Clinical Practice (GCP) principles during the conduction of the trial. Furthermore, the Directive requires that any changes related to the execution of the clinical study, and its final results, be reported to the supervising authorities. The Directive recognizes that non-commercial clinical trials conducted by researchers (without the participation of the pharmaceutical industry) may be of great benefit to patients. However, after May 2004 no intervention research may be initiated without a "sponsor," defined in the Directive as "an individual, company, institution or organization which takes responsibility for the initiation, management and/or financing of a clinical trial." This means that investigators who wish to perform clinical trials without commercial backing must themselves become the study sponsors. The administrative responsibilities will be exactly the same as those of the pharmaceutical industry for commercial trials. There has been much commentary on this issue (Editorial, 2003; Meunier & Lacombe, 2003; Clumeck & Katlama, 2004). The paper of Welzing et al., 2007 discusses the consequences and implications of the

2001/20/EC Directive for independent ("no profit") investigators involved in paediatric clinical trials in relation to the first paediatric investigator-initiated trial at the University Hospital of Cologne. The authors of the paper agree that the new rule can improve quality of clinical trials, but underline that clinicians and academic researchers cannot meet the new requirements and obligations without additional financial support. Their conclusion focused to the need to develop new concepts for funding to ensure future paediatric independent investigations, for example through specific grants from the EU Community or Member States.

5.1.2 Guidelines

In addition to legally binding legislation regulatory guidelines, concerning scientific and technical requirements for develop and register medicine for human use, were released by the regulatory authority (EMA, dedicate section of the site), after discussion with industry and experts. In the contest of clinical trial authorization and medicinal product registration system, the regulatory guideline cannot be substituted by any other guideline issued by scientific organizations and societies.

Two main regulatory EU non clinical documents considers the aim of non-clinical studies to support the development of medicinal products to be used in paediatric population:

1. Non-Clinical Safety Studies For The Conduct Of Human Clinical Trials For Pharmaceuticals (CPMP, 2009).
2. Non-clinical Testing in Juvenile Animals on Human Pharmaceuticals for Pediatric Indications (CHMP, 2008).

A Food and Drug Administration (FDA) guidance on non clinical issue of paediatric medicine is also available (FDA, 2006), recognizing the importance of animal data in predicting the potential drug toxicity, and those obtained in juvenile animal studies to provide information that might not be derived from standard adult toxicology studies or from safety data from adult humans.

The original "Non-Clinical Safety Studies For The Conduct Of Human Clinical Trials For Pharmaceuticals" guideline (CPMP, 2009) was approved in 1995, and revised on December 2009. Several new sections have been introduced, one of them was dedicated to clinical trials in paediatric populations: "when paediatric patients are included in clinical trials, safety data from previous adult human experience would usually represent the most relevant information and should generally be available before initiation of paediatric clinical trials. The appropriateness and extent of adult human data should be determined on a case-by-case basis. Extensive adult experience might not be available before paediatric exposures (e.g., for paediatric-specific indications). The conduct of any juvenile animal toxicity studies should be considered only when previous animal data and human safety data, including effects from other drugs of the same pharmacological class, are judged to be insufficient to support paediatric studies".

The CHMP guideline (CHMP, 2008) provides advice on investigation of findings that cannot be fully assessed in paediatric clinical trials, with reference to specific concerns, possible aggravation of expected findings and to establish safety factors. It highlights that drugs can have different safety profiles in adults compared to paediatrics and emphasizes that studies in juvenile animals should only be performed after careful consideration of available data, age of the intended paediatric population, duration of treatment and "cause for concern" identified. Additional data to develop a medicine for children can be necessary because

additional or different risk due to immature or growing organism can be possible and because children may be included earlier in drug development, before more extensive adult data available.

5.2 Difference between adults and children

We actually know that adverse events in children may not be always predicted from adult data, because a child is not a small adult and paediatric population has immature organs rapidly developing. The use of an approved adult drug in children only considering the pharmacokinetics (PK) may be problematic, because there are many relevant PK differences age-related. Amount of metabolism and kinetics detected in children (particularly neonates) can vary in significant way to those observed in adults, these differences can cause over or under exposure in paediatric population. The CHMP guideline (CHMP, 2008) provides the list of the major systems developing in age dependent manner and for which the functional differences existing between human neonates/infants and adults should be take into account. The guideline also note that the age ranges only apply to general development and it is not applicable to all endpoints related to that organ system. This should be taken into account in the design the paediatric drug development program and the individual studies (CHMP, 2008):

* Nervous system: Development up to adulthood
* Reproductive system: Development up to adulthood
* Pulmonary system: Development up to two years old
* Immune system: Development up to 12 years old
* Renal system: Development up to one year of age
* Skeletal system: Development up to adulthood
* Organs and/or systems involved in absorption and metabolism of drugs. Development of biotransformation enzymes up to adolescence

The sensitivity and the cross-species comparative postnatal development are among the major points to take into consideration using juvenile animals. There are in fact, evidence (Baldrick, 2004) that newborn or infant animals can be more (e.g. tetracycline, sodium salicylate, morphine, chloramphenicol) or less sensitive (eg metrazol, codeine, ethanol,) to drugs than adults. It is well known that potential differences in sensitivity of juvenile animals to drug toxicity can be due to the different stage of development that varies among species, strains, organ and in some case also in subcomponent of the same organs. A range of publications have summarized functional differences in early postnatal life among species used in toxicology testing. A number of these papers have arisen out of work performed by the Developmental and Reproductive Toxicology Technical Committee of the International Life Sciences Institute (ILSI) and Health and Environmental Sciences Institute (HESI). Organ systems examined included: central nervous, reproductive, pulmonary, renal, skeletal, cardiovascular, and immune systems (Baldrick, 2004). A series of articles reviewing the current knowledge on comparative postnatal function and physiologic development are discussed (Cappon et al., 2009) observing that in general the rat can be considered the appropriate specie, because most of the major organ systems maturation that occurs postnatally in humans also occurs during the postnatal period in rat. When the rat is not the relevant species mouse, minipig, dog and primate should be considered, comparative age categories are available

for rats, minipigs, dogs, non human primates and humans based on CNS and reproductive development (Gad, 2001; Baldrik, 2004; Beck et al., 2006). However the information on developmental stage across various species is not sufficient without knowledge of functional state at these age (Baldrick, 2004).

The comparison between animals and humans generally refers to the age categories described in the ICH guideline (ICH, 2000; CPMP, 2001):

• Preterm newborn infants.
• Term newborn infants (0 to 27 days).
• Infants and toddlers (28 days to 23 months).
• Children (2 to 11 years).
• Adolescents (12 to 16-18 years). (dependent on region)

The guideline recognizes that any categorization of the paediatric population is to some extent arbitrary, but provides a basis for thinking about non clinical and clinical study design in paediatric medicine development, and neonatal population is still an issue.

An overview of comparative development of main organ systems in man and different animal species, as a basis for species selection and protocol design was one of the topic of the workshop on juvenile animal testing organized by the EMA in July 2009, involving non-clinical assessors and experts of the Non-clinical Working Group of the PDCO and of the Safety Working Party (SWP) of the Committee for Human Medicinal Product. The presentations and the examples discussed during the workshop concerned brain, reproductive system, gastrointestinal system, metabolism, skeletal system, lungs, kidneys and immune system, pointing out that the maturation of different organs in various species is a key factor to be taken into account when designing juvenile animal studies in relation to early exposure of children to medicines (Silva-Lima et al., 2010).

5.3 Non clinical aspects

Non clinical safety assessment of new medicinal product intended to be administer in adult humans is essential to support clinical trials and eventual drug product registration. Once the company has identified and manufactured consistently a promising drug candidate the next step in development is to provide evidence to the regulatory authority that it is safe for the administration to humans. This evidence must be based on a well designed programme of appropriate non clinical and clinical studies, as those illustrated in Table 1. Non clinical drug development is a complex, regulatory-driven process designed primarily to assess the safety and viability of new molecular entities. Non clinical testing is conducted throughout all phases of drug development and, when well done, can maximize the chances of success in the clinical phases. Although the terms preclinical and nonclinical are used interchangeably it should take in mind that only studies necessary before the first administration in humans [First in Man (FIM) clinical trial], can be considered preclinical unlike other studies conducted during and in parallel to Phase II and III clinical trials (see Table 1). Considering their scientific objectives, non-clinical studies should be designed to provide information regarding the primary and secondary pharmacodynamic actions, safety pharmacology and toxicology of a compound. Regulatory requirements for safety and toxicity studies are more stringent than for pharmacology ones and they must be completed in compliance with Good Laboratory Practices (GLP) following the guidelines operating at the time.

Clinical Phase	Pre-clinical/Non clinical data (CPMP, 2009)
Phase I clinical trials conventionally examine the tolerability and the pharmacokinetics of new drugs; they are often conducted in healthy subjects, but may involve patients in studies with interventions that are known to be toxic. It should be noted that Phase I clinical trials now increasingly include persons with specific diseases persons for whom all conventional therapies have failed (e.g., terminal cancer or AIDS). Such studies may be designated as Phase I clinical trials where, in fact, they properly should be designated as mixed Phase I/II or pure Phase II clinical trials	• Pharmacodynamic • Safety pharmacology (CNS; CV; Respiratory) • Toxicology studies (Single dose toxicity, Repeated dose toxicity) • Initial genotoxicity • Initial reproductive toxicology • Local tolerance • Toxic\kinetic and Pharmacokinetic Studies
Phase II clinical trials primarily examine the short-term pharmacological toxicities and, to a lesser extent, the efficacy of new drugs; they are conducted in populations with specific diseases	• Completed battery of Genotoxicity • Completed battery Reproductive toxicity (male/female) • Extended repeated dose toxicity with toxicokinetic support • Extended pharmacokinetic studies
Phase III clinical trials primarily examine the pharmacological efficacy and, to a lesser extent, the short-term toxicities of new drugs. Phase III and IV clinical trials are designed to increase the survival or the quality of life of subjects suffering from a specific disease or condition	• Chronically used drugs: • Chronic toxicity (rodent; non-rodent) • Carcinogenicity • Supplemental studies (special safety concerns, as alerted)
Phase IV clinical trials, also known as post-marketing surveillance studies, primarily examine the long-term efficacy and toxicity of already-marketed drugs	

Table 1. The clinical phase and the supporting preclinical/non clinical data

5.3.1 Information available from adult human safety data

Safety evaluation programs of a new chemical product should normally include two relevant species rodent and non rodent, however for biotechnology-derived pharmaceuticals in certain justified cases one relevant species may be sufficient (e.g., when only one relevant species can be identified or where the biological activity of the biopharmaceutical is well understood). Upon completion of non clinical studies necessary to perform clinical trials and put a new drug on the market, the following information are available.

- Rodents and non rodents No Observed Effect Level (NOEL) or No Observed Adverse Effect Level (NOAEL) in mg/kg/day obtained after repeated dose administration in rats aged from 1 to 24 months (if necessary), and in dogs aged >5-6 months of age.
- Associated drug blood levels and exposition (Cmax and AUC).
- Pre-weaning exposure is only in utero or via the milk in pre- and postnatal reproduction toxicity studies in rats, since toxicokinetic data is not normally available in pre-weaning animals.

- Genotoxicity and carcinogenicity (if it is the case) potential.

In Europe these studies are performed taking into account the European Directive on the animal welfare. In November 2010, Directive 2010/63/EU (European Parliament and the Council of the European Union, 2010), this Directive revises Directive 86/609/EEC on the same subject (Council of the European Communities, 1986). Member States have until November 2012 to transpose the Directive into their respective national legislation and the new Directive will take full effect on 1 January 2013. Moreover to take into account the 3R principles (Reduce, Refinement and Replace) they are designed so that the maximum information is obtained from the smallest number of animals. While animal tests cannot predict all of the reactions a human, at the end of regulatory assessment of all these data available, there are sufficient knowledge of the intrinsic toxicological characteristics of a given substance and their target organs of toxicity.

Until the last few years the majority of studies on juvenile animals are essentially the repetition of those conducted in the adult, and performed mainly in the environmental regulatory setting, particularly to investigated neurotoxicity (Atchison et al., 1982; Benke & Murphy, 1975; Brodeur & Dubois, 1963; Cappon et al., 1997; Rice, 1988; Rigdon et al., 1989), or to support investigations certain drug classes used in a paediatric population, e.g., antibiotic, anti-emetic, and anti-asthma drugs (Baldrick, 2004).

Since the entry into force of the paediatric European and USA legislations, the interest of the regulators and pharmaceutical industry in non clinical juvenile toxicity studies has taken on characteristics of priority as also documented by recent publications on the subject (Baldrick, 2004; Cappon et al., 2009; Bailey & Mariën, 2009; Silva-Lima et al., 2010). To avoid production of unnecessary repetitive not interpretable data and to realize robust juvenile animal studies when they necessary, represent the main objectives requiring higher consideration than in the past.

Industry conducted recently a survey to establish whether any findings, other than changed sensitivity due to pharmaco/toxicokinetics or metabolism differences, have been seen in the juvenile animal studies currently performed after specific regulatory requirements, and whether the results could not have been predicted from the adult toxicology or found in routine adult animal studies. Ten pharmaceutical companies contribute the survey and shared their experiences with over 39 juvenile animal studies (29 in rats, nine in dogs and one single dermal study in minipigs). "Novel toxicity was only observed in four studies out of the 39 compiled, these comprised a single CNS drug in the rat, two anti-infective in the rat and one other anti-infective in the dog. In only one of these cases was it felt that the results observed were predictable from the pharmacology, and none of the four were predictable from the adult data. In all cases however, the novel toxicity was observed using routine toxicological techniques and not as a result of any sophisticated or complex design. In one case this was a combination of animal observations and histopathology and in the remaining three cases the toxicity was observed at histopathology" (Bailey & Mariën, 2009). Taking into consideration these data it might seem that we are not on the right track since juvenile animals are required by default rather than following a scientific rationale, but surely no definitive conclusions can be drawn based on so small database, and we can agree with the conclusion of Silva-Lima et al., 2010: "It is expected that with the increasing experience gained in this area by applicants and regulators, the criteria for requiring these studies will be further refined. An increased understanding of their ability to identify differences in activity between mature and immature systems, and of their predictive value for developing human organs will be gained. Research, follow-up discussions, and

experience sharing between all stakeholders will be continued and are encouraged in the EU regulatory framework". The increased database of toxicological studies in young/very young animals will contribute to better understand the level of predictability of juvenile animals for children. Currently there are examples of products, such as verapamil, phenobarbital and theophylline for which juvenile animal data help to predict age related toxicity in children (Baldrick, 2004).

In general, according both to CHMP (CHMP, 2008) and the CPMP guidelines (CPMP, 2009), the paediatric patients can be included in clinical trials when adequate pharmacokinetic, pharmacodynamic, clinical efficacy and safety data are available in human adults, this also implies, in most cases, the availability of a standard non-clinical data package. All available adult non clinical and clinical data must be reviewed and considered and their appropriateness and extent data should be determined on a case-by-case basis. These guidelines suggest that: results from repeated dose toxicity studies of appropriate duration (see also table 1 of CPMP, 2009), the core safety pharmacology package, the standard battery of genotoxicity tests, and relevant parts of the reproductive toxicity test program and also juvenile animal toxicity studies if necessary, should be available and reviewed before starting trials in paediatric population. Pharmaco- and toxico-kinetic assessment may be exceptionally useful to characterize juvenile toxicity studies since paediatric populations metabolize and respond to many substances in a very different manner than adults with mature. The data package should be justified, based on the characteristics of the clinical study and the intended paediatric population including age group(s), and the need for juvenile animal studies should be particularly taken into consideration in case of investigation of neonates (0-3 months) and when extensive adult experience might not be available before paediatric exposures (e.g., for paediatric-specific indications).

If previous animal work and clinical safety data are considered to be insufficient for a thorough safety assessment in the intended paediatric age group of interest, the juvenile studies should be run prior to the conduction of paediatric clinical trials. The need for pre-clinical juvenile toxicity testing may differ depending on the intended duration of exposure in the paediatric population. Consideration should also be given to whether pre-clinical and/or clinical studies have identified target organs vulnerable to toxicity, and whether these organ systems will be undergoing rapid development while patients are being exposed to a given drug. The children and adolescents could have higher recuperative capacities than adults, so it important to consider in the study design a period of recovery treatment-free assess the potential and timing of reversibility of any adverse effects seen. Assuming the need for juvenile study, the key elements described in the EU guideline (study design, age and duration, route of administration/doses, selection of species, pharmaco-kinetics/toxicokinetics) should be taken into account.

The analysis of published PIP decisions covering a period from August 2007 up to March 2009, revealed that, in most cases, the preferred species for juvenile animal studies was the rat; in a minority of cases, two species were required, the age animal used at the start of the study reflect the age of target paediatric population and generally, the principles stated in the guideline were followed (Silva-Lima et al., 2010). The same paper illustrates 4 case studies discussed at the EMA workshop related to a new molecular entity with a well known mode of action, an oncological new product intended to be administered to patients of all ages, a growth factor as a replacement therapy in premature newborns born after 28 weeks of gestation and a monoclonal antibody for a chronic non tumoral pathology emerging around 5 years of age.

5.3.2 Targeted approach

A targeted approach for juvenile animal study design may be used to specifically address identified toxicity concerns, and " primary factors to consider when designing a targeted juvenile animal study are: (1) ensure that the organ system of concern is undergoing similar developmental processes during the postnatal period as in the intended paediatric population; (2) define the age of exposure in the experimental species to ensure that the organ systems of concern are at the same stage of development in the animal species as in the intended human paediatric population; and (3) ensure that the appropriate endpoints are included to enable an in-depth investigation of the organ system of concern" (Cappon et al., 2009) The same authors illustrate two examples, based on real paediatric drug development programs and accepted by regulatory agencies to support those products, of targeted study, one for central nervous system and reproductive development and the other focused on liver and reproductive development. This approach support the chance to use specific case by case study design to perform suitable, interpretable and not repetitive non clinical studies in juvenile animals to support risk assessment for paediatric population.

5.3.3 Pre- and post-natal reproduction studies

The CHMP guideline the need for nonclinical testing in juvenile animals on human pharmaceuticals for paediatric indications (CHMP, 2008) supports the use of the modified pre- and postnatal development studies. It states in fact that before performing a juvenile animal toxicity study, it should be considered whether a developmental toxicity issue could be addressed in a modified pre- and postnatal development study in rats. Key factors that need to be examined include, but are not restricted to

•   the amount of the active substance and/or relevant metabolites excreted via the milk;
•   the resulting plasma exposure of the pups;
•   which organs under development that will be exposed during the pre-weaning period;
•   physical development and histopathology investigations.

The number of animals should be sufficient to draw scientifically sound conclusions, but a higher number of animals than necessary should be avoided. When a pre- and postnatal study is also being used to address a specific aspect of juvenile toxicity, such a study should be extended to include appropriate developmental endpoints: if specific developmental endpoints cannot be assessed within the context of pre- and postnatal studies, additional juvenile animal studies will be required. In the combined pre- and postnatal development toxicity study design (De Schaepdrijver & Bailey, 2009, Cappon et al., 2009) the maternal animal is dosed from implantation through post natal dose day 5. Dosing is then suspended for maternal animals and the offsprings are directly dosed until maturity (about 9 weeks). A clear advantage of this study is the reduced number of animals to be used, but it is not always suitable to investigated potentially additional end points related to target organs of concern, and excessive toxicity in the directly dosed pups can be observed (Cappon et., 2009, De Schaepdrijver et al., 2008, De Schaepdrijver & Bailey, 2009).

The guideline CHMP/SWP/169215/2005 is not rigid about the timing for performing juvenile animal studies, if these studies are considered necessary, they should preferably be available before the starting of clinical studies in paediatric populations, and pharmacokinetic data from humans and animals (including juvenile animals if available) should also be evaluated before the proposed paediatric clinical trial(s). More flexibility can

be considered for paediatric short-term investigations (PK or taste study) in which few doses are administered.

5.4 Conclusions

The needed and the efforts to develop and register medicine for paediatric population with the same level of scientific and clinical rigor as adult therapeutic products have advanced over the years through the actual legislation initiatives involving United States of America/FDA and EU Community and EMA. In the last five years many experience have been gained both by regulators and companies to assess the overall impact of non clinical juvenile studies on paediatric human pharmaceuticals risk assessment. The application of recent legislations have resulted in a significant increase in the number of juvenile animal studies request, in same case they were a simply repetition of the adult design in younger animals, but there are cases in which designs are being modified, and targeted designs were used, as we continue to gain knowledge from the submitted studies. Particularly important is to carefully consider the suitable age and the corresponding developmental stage of the children involved in the clinical trial in comparison to the development of the animals involved in the non clinical test if necessary. Children in the different interval of age considered by the ICH guideline (ICH, 2000; CPMP, 2001) can answer in different way to the same product, and neonatal population is still the main issue. As for standard adult non clinical study the benefit of harmonized design in juvenile studies should be recognized, harmonized study designs, enabling information-sharing across studies through standardized approaches to data collection and interpretation, are needed to optimize the information from these juvenile studies

At present there is general agreement that juvenile animal studies can be useful for safety determinations, especially when a problem is suspected, they are not prohibitively challenging to conduct, available data does not indicate that juvenile animal studies need to be conducted routinely to support clinical trials in paediatric patients, but might be needed under some circumstances and they should be designed on a case-by-case basis. Currently the data base of juvenile animals to investigate paediatric pharmaceutical product is still limited, however, and this conclusion might change as more studies are submitted and regulators and industry gain more experience with this issue.

6. Clinical: The need for adequate clinical trials methodologies

The benchmark for the assessment of the effect of any therapeutic intervention is the randomised clinical trial (RCT), its main assets being randomisation, to avoid bias in the allocation of subjects to treatments, blindness, to avoid bias in the evaluation of compared treatments, and the *a priori* choice of acceptable error margins, specifically type I and type II errors (Baiardi et al., 2011). However, the conduct of clinical trials in children causes several methodological, ethical and economic issues limiting paediatric research: small sample sizes, children exposure to the potential risks of a trial, and restricted paediatric medicines market are just the main examples.

Notwithstanding these multiple difficulties, understanding the effects of medicinal products in paediatric patients is an important goal that is shared by companies, regulatory authorities, health professionals and society as a whole (ICH, 2000; CPMP, 2001), and should be achieved without compromising the well-being of paediatric patients participating in clinical studies.

Traditional drug development approaches do not satisfy the requirements of research in the paediatric population. New approaches should be used to address the various practical, scientific and ethical issues that arise in paediatric research, as stated by a number of worldwide scientific and regulatory initiatives with particular reference, in Europe, to the Paediatric Regulation (European Parliament and the Council of the European Union, 2006). Methods for extrapolation of efficacy and safety data from adults to children, modelling and simulation, adoption of innovative study design and statistics have demonstrated to be the more suitable to produce real advancement in this field.

6.1 Status of the knowledge (What has been done)
6.1.1 Extrapolation

The difficulties in performing paediatric trials obliges physicians to extrapolate data from the adult to the paediatric population, the first approach usually employed to avoid useless studies in children, provided its limitations are properly defined.

Direct extrapolation can be made on the basis of previous clinical experience and scientific knowledge that allow to assume that the disease is the same in children and in adults, and that children have reached full maturity in those pharmacokinetic mechanisms involved in drug disposition and in the involved pharmacodynamic systems. In such cases, the relationship between the two populations is linear and allometric methods based on body weight or body surface area can be used to calculate the proportional dose in children (Bellanti & Della Pasqua, 2011).

To be able to use this type of approach, paediatric PK/PD data should be known and they should demonstrate a similar exposure/concentration curve as in the adult population. However, particularly in neonates and infants, the use of the allometric approach may fail to identify the appropriate dosing range (Bouzom & Walther, 2008; Johnson, 2005), thus reducing the access to a feasible extrapolation exercise.

Much more has to be done to facilitate the extrapolation approach also in younger children, and in particular to collect data on similar disease course and outcome, and similar primary endpoints for efficacy in adults and children. A decision-tree, similar to the one developed by the US Food and Drug Administration (Fig. 1), may be of help and is currently under evaluation by the EMA Paediatric Committee, that has also created a specific 'Extrapolation Group'.

6.1.2 Modelling and simulation

As extrapolation can have limited applicability in children, modelling and simulation (M&S) may play a pivotal role in reducing the needs of specific additional paediatric trials.

Clinical trial simulation (CTS) can be used to assess the impact of a range of design characteristics on the statistical power of a clinical trial, and thus to detect a treatment effect prior to exposing patients to an experimental drug (Bellanti & Della Pasqua, 2011). CTS investigates "what if" scenarios across a different range of conditions or design features (e.g. population size, stratification levels, dose range, sampling scheme, and even different endpoints) relying on the availability of accurate model parameters and corresponding distributions (Bellanti & Della Pasqua, 2011). The possibility to predict 'trial performance', and thus identify potential limitations in study and protocol design prior to its implementation, is one of the main advantages of such a virtual or statistical experiment (Bellanti & Della Pasqua, 2011; Manolis & Pons, 2009; Laer et al., 2009; Girard, 2005; Onar et al., 2009).
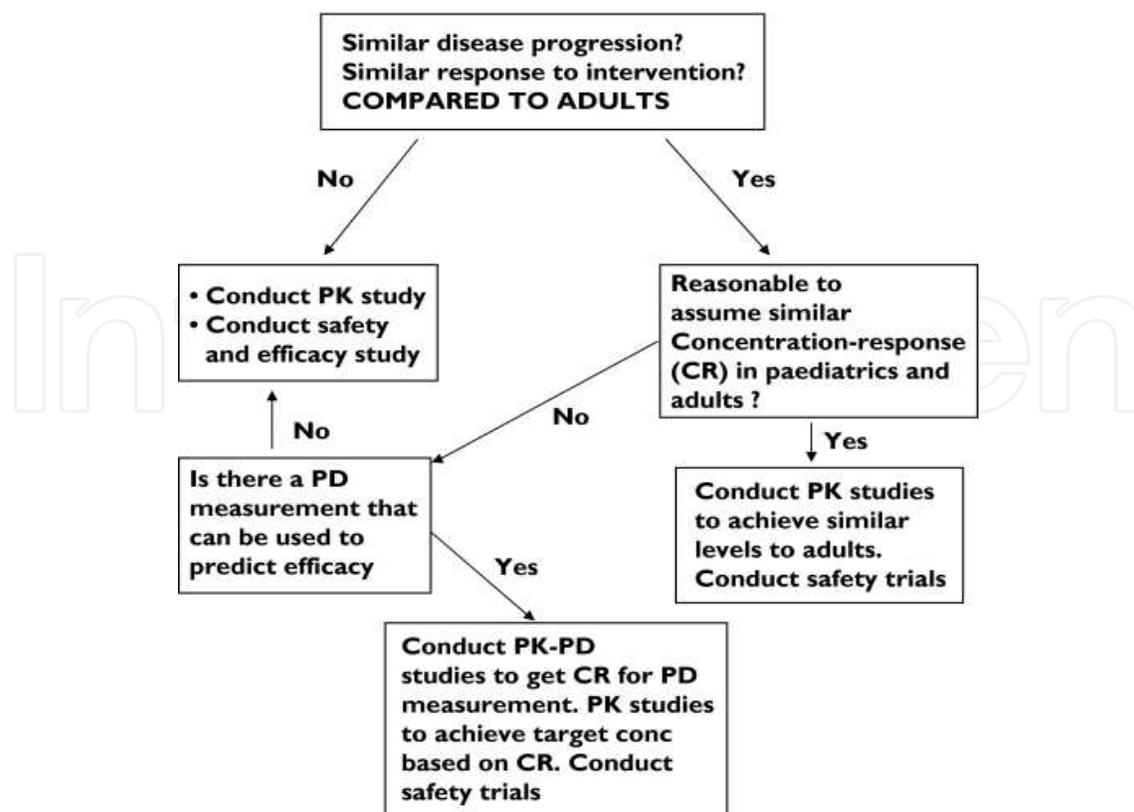
Fig. 1. Paediatric drug development decision tree for types of PK-PD studies required in children (Johnson, 2005)

CTS generally employs two types of models: first a drug–action (PKPD) model, which comprises pharmacokinetic and pharmacodynamic factors, and then a trial execution model, that simulates other important aspects of the trial (e.g. dropout, compliance and protocol deviations) (Bellanti & Della Pasqua, 2011; Santen et al., 2009).

6.1.2.1 PKPD model

PKPD models incorporate physiological differences between adults and children and between different age groups to evaluate variation in pharmacokinetics. This approach may allow conversion of the exploratory nature of first-in children studies into a confirmatory step (Johnson, 2005).

When applying bridging techniques, however, it is necessary to clearly understand the disease in question and therefore, both disease and disease progression models must be taken into account during the comparison of drug response and kinetics in adults and children (Manolis & Pons, 2009).

Disease models can also be applied to simulate treatment response; in fact, when disease models are combined with drug models, it is possible to explore the implications of different algorithms for dose adjustment (Manolis & Pons, 2009). It must be highlighted, however, that the application of sophisticated statistical methods, not achievable by standard linear regression techniques, are necessary to use disease models in the evaluation of drug–disease interactions and of the role of covariates in pharmacokinetics, pharmacodynamics and treatment outcome demands (Bellanti & Della Pasqua, 2011).

Bayesian statistical concepts are usually the basis on which these methods rely and they also include parameterisation based on hierarchical, non-linear mixed effects models that are

also known as population approaches, particularly suitable when information on individual subjects is limited (a common situation in pharmacokinetic and pharmacodynamic studies in children) as they use the population rather than the individual as the object of the investigation.

Population pharmacokinetic (pop PK) and population pharmacokinetic-pharmacodynamic (pop PKPD) models rely conceptually on pooled data across treatment cohorts or even across different studies (Anderson et al., 2006) and include the representation of three main components:

- a structural model that describes pharmacokinetics or pharmacodynamic characteristics;

- a statistical model describing between-subject variability; and

- an error model that accounts for the residual variability (Bellanti & Della Pasqua, 2011).

There are different advantages in using these types of approaches: it is possible to assess different clinical scenarios without exposing children to any risk, to explore drug, disease or covariate effects in a larger number of virtual patients compared to those enrolled in a real trial, and to assess the clinical relevance of covariates to drug exposure and to evaluate their effect on the treatment response (Anderson et al., 2006; Chatelut, 2008; Yim et al. 2005; Knibbe et al., 2002).

Moreover, the K-PD models, a specific group of nonlinear mixed effect approaches introduced into paediatric research, have been developed to describe exposure–effect relationships in the absence of drug concentration measurements (Manolis & Pons, 2009; Tod, 2008). These models are very useful if the rate-limiting step in drug disposition is the drug elimination from the biophase (Bellanti & Della Pasqua, 2011). On the other hand, data extrapolation across different scenarios (e.g. different doses, or populations) are not possible since no observations are available (Manolis & Pons, 2009).

6.1.2.2 Trial execution model

Trial execution models simulate some important aspects of the trial, such as dropout, compliance and protocol deviations (Gobburu & Lesko, 2009) and it is therefore possible to determine all possible outcomes under candidate trial designs, allowing such trial designs to be compared in a strictly quantitative manner.

6.1.3 Innovative designs

Many innovative designs have been described up to now, each design having intrinsic features that meet the requirements of the paediatric population even if there is no unique rule of thumb for choosing a specific approach (Baiardi et al., 2011).

6.1.3.1 Sequential design

This study design uses an a priori non fixed sample size and generally needs fewer patients compared to a fixed sample size design to reach a conclusion, thus guaranteeing some ethical advantages. It also ensures the possibility to stop the trial at any time during its course as soon as the scientific evidence of a superiority of one treatment against the other is proven (van der Lee et al., 2010). This approach has been developed in the 1960s, but not widely used in clinical trials: a review carried out by Goodman in 2009 showed that from 1963 to 2005, only 24 trials have been performed in the neonatal intensive care setting using the sequential design methodology, saving an average of 35% of the enrolled subjects when compared to a fixed sample size approach (Goodman, 2009). It is not usable to evaluate

survival but it could be useful to evaluate short treatments through surrogate endpoints (Baiardi et al., 2011).

6.1.3.2 Adaptive design

This approach allows modifications of the trial (e.g. sample size re-estimation, early stopping and adaptive randomization) to be made after its initiation and without invalidating the validity and integrity of the trial itself. Reports show that it has been used for dose-finding studies, for phase III trials and for phase IV trials in which the method allows the saving of up to a half of the subjects required by the traditional design (Chow & Chang, 2008). Adaptive designs are very attractive due to their flexibility and can be useful especially in early clinical development.

6.1.3.3 Bayesian design

The Bayesian design is the data dependent design *par excellence* (Schoenfeld et al., 2009).
Data from past studies are here used to form an *a priori* probability distribution for treatment effect, and then merged with the data of the current trial with the aim to provide an *a posteriori* distribution on which conclusions may be drawn (Baiardi et al., 2011).
This approach is particularly suitable for the paediatric population, since adult data can be used in designing the paediatric trial by taking advantage of past information for the sample size calculation or by directly including them into the study to generate the distribution (Goodman & Sladky, 2005).

6.1.3.4 Randomized withdrawal design

This type of design offers two main advantages to the paediatric population: first, patients have the opportunity to experience the potential benefits of the active treatment (Della Pasqua et al., 2007), and second, the individual receives the placebo for the minimum time possible. As a result, a better patient accrual may be achieved and moreover, testing the experimental drug against placebo on the responders increases the power of the comparison and requires a smaller sample size to achieve the same power of results (Baiardi et al., 2011).
This design has been recognised as the most appropriate for developing new treatments for juvenile idiopathic arthritis at the Paediatric Rheumatology Expert Meeting held in London in December 2009.

6.1.3.5 Randomized Placebo-Phase Design

The Randomized Placebo-Phase Design (RPPD) approach sets its innovation on the fact that the duration of the placebo trial is the shortest possible (Feldman et al., 2001). In fact, it is assumed that if the trial drug is active, the earlier it begins, the higher the probability to observe a response in short times, and therefore, in this type of study subjects receive placebo at different times. In more details, subjects are randomised to placebo for periods of different duration. At the end of those periods, all subjects receive the active treatment until it is possible to observe a response (Baiardi et al., 2011).
By guaranteeing the presence of a control group according to an intra-patient scheme, the blindness, and the randomisation, the RPPD trial can be classified as a RCT.

6.1.3.6 Three–stage clinical trial design

The methodological approach of the three-stage trial design combines the classical RCT and the randomised withdrawal trial with the aim to obtain the maximum level of information available from each subject.

During the first stage, subjects are randomised according to a traditional RCT. At the end of this first phase, patients responding to placebo or not responding to the trial treatment are then withdrawn from the study, while responders to the active treatment are assigned to the second phase of the trial, and non-responders to placebo are allocated to the third (Baiardi et al., 2011). Second phase subjects are then randomised again, while patients assigned to the third stage enter the following randomised withdrawal scheme: only those who respond to the drug in this phase continue the study and are again randomised to take either placebo or the active treatment (Honkanen et al., 2001).

Examples where the study may be potentially useful are in chronic conditions (for which it is expected to return to initial conditions when the active treatment is suspended), when the therapeutic efficacy in sub-populations has to be determined, in those cases in which efficacy in the general population has already been proved, or at the initial stages of the drug development when it is necessary to find dosages in small patients cohorts (Baiardi et al., 2011; Honkanen et al. 2001).

6.2 Research aim and results (What have we done)

Despite the potential of innovative research methods in collecting data on drug effects in children and/or developing clinical trials, it seems that their benefits as tools in pharmaceutical R&D has remained undervalued and sometimes ignored by key stakeholders (Cella et al., 2010; Abernethy & Burckart,2010). This attitude appears to contradict those ethical and scientific beliefs that emphasize the need for evaluation of the risk–benefit ratio in special populations, such as the paediatric one.

The Task-force in Europe for Drug Development for Young – TEDDY (Ceci et al., 2009) has performed analyses and surveys aimed at collecting data from published literature and registrative documents (i.e. EPARs [1]) to evaluate the status of paediatric clinical trials performed for drugs to be used in children (Baiardi et al, 2009), to explore the current status, limitations and perspectives of pharmacogenomic and pharmacogenetic paediatric clinical research (Krekels et al., 2009), and to at analysing the quality of the clinical trials for rare diseases.

Results showed that in the period 1995-2005, 60 drugs were licensed for use in children under the EMA centralised procedure with a total of 188 paediatric clinical trials included in their MA dossiers, mostly concerning diseases predominantly or exclusively affecting paediatric patients and serious or life-threatening diseases, occurring in both adults and paediatric patients, for which there are currently no or limited therapeutic options. PK studies are performed for almost 70% of drugs when they are intended for these diseases. Efficacy and safety studies are carried out for more than 90% in drugs intended for diseases affecting children only and for more than 75% in drugs for life-threatening diseases (Baiardi et al., 2009). Moreover, with reference to pharmacogenomic and pharmacogenetic research in the paediatric population, a rather equal distribution of activities across the different research categories throughout the world was found. More than 50% of the research activities are related to predisposition, i.e. exploratory studies aimed establishing the connection between a given genetic trait and the risk associated with a pathology or disease (Krekels et al., 2009).

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[1] The EPAR is the European public assessment report, a document provides a summary of the grounds for a EMA Committee opinion in favour of granting or refusing a marketing authorisation for a specific medicinal product.

Finally, the analysis of the clinical trials performed and published for 28 orphan medicinal products (OMP) approved by the EMA showed that even though all of these drugs can be used in children, paediatric studies were performed only in 17 out of the 28 medicines. The methodological quality of OMP dossiers is often criticised because of the several limitations identified in these dossiers: lack of controlled studies, of active comparator where available, of multicentre phase III trials with a suitable number of patients (particularly for diseases with a frequency from 5/100 000 to 5/10 000), insufficient exposure to the treatment, use of surrogate endpoints or weak proof of clinical benefit (Joppi et al., 2006). TEDDY research results, considering the number of clinical trials performed after the authorisation and the information still awaited for many of the drugs already authorised, confirm concerns raised on the quality of the OMP dossiers (unpublished material).

6.3 Ethical aspects in paediatric clinical trials

The main international reference for paediatric research is the ICH Topic E11 guideline 'Clinical Investigation of Medicinal Products in the Pediatric Population' (ICH, 2000) and was recognised by the European Union with the 'Note for guidance on clinical investigation of medicinal products in the paediatric population' (CPMP, 2001). The ICH guideline represents the methodological standard to perform scientifically correct and ethically sound paediatric clinical trials. In fact, on the basis of the assumptions that the paediatric population has the right to use medicines that have been appropriately evaluated and tested, and that it represents a vulnerable subgroup, the guideline introduces special measures to protect the rights of paediatric study participants and to shield them from undue risk.

The rights and well-being of children participating in clinical research in Europe have been for the first time assured by the provisions of the EU Directive 2001/20/EC (European Parliament and the Council of the European Union, 2001) that includes a specific article, Article 4, devoted to the protection of 'minors' and to the guarantee of their emotional, physiological and psychological specificities. This article establishes the condition for the start of a clinical trial involving minors, but does not include references to some relevant documents such as the Convention of Human Rights and Biomedicine (better known as Oviedo Convention) (Council of Europe, 1997) and its Additional protocol on Biomedical Research (Council of Europe, 2005), and ICH guideline (ICH, 2000; CPMP, 2001).

The entry into force of the Paediatric Regulation (European Parliament and the Council of the European Union, 2006a, 2006b), that is expected to increase the number of clinical trials carried out in the paediatric population, has highlighted the limits of the existing clinical trials legislation in particular with reference to children data protection, direct involvement into the 'consensus process' and respect of the children will during the entire clinical research (Altavilla et al, 2008).

In February 2008, the European Commission released updated recommendations on ethical aspects of clinical trials involving children (European Commission's Directorate-General for Health and Consumers, 2008) to tackle the weakness of the existing rules. This document provides a new regulatory context integrating principles contained in other various international ethical/legal source with the aim of ensuring the protection of subjects involved in biomedical research, while recognising the importance of benefits derived from research. The Recommendations also clarify the process of assessment of the benefit and risk balance, the processes of information and consent/assent according to age groups and level of minors' maturity, the process of ethical review of paediatric protocols, individual data protection and insurance issues. In particular, the Recommendations state that:

- the child should participate in the decision-making process together with the parents, according to his/her emerging maturity;
- information should be given by an experienced investigator, or his/her adequately trained delegate, to each parent/legal representative and to the child in language and wording appropriate to his/her age, psychological and intellectual maturity;
- minors should provide their assent. However, the minor's assent is not sufficient to allow participation in the research unless it is supplemented by the legal representative's informed consent;
- separate information sheets for adults and children, and separate consent and assent forms should be used. The child should be informed of the possibility to freely withdraw from the trial, at any time and for any reason, without any disadvantage or prejudice especially regarding medical care.

All of the above mentioned 'set of rules' valuable for protecting children during trials have represented a real advancement to assure a superior ethical context for children involved in a clinical trials. Nevertheless, some important surveys and enquires (Altavilla et al, 2008, 2011; European Forum for Good Clinical Practice (EFGCP) Ethics Working Party, 2010) demonstrate that the implementation of such provisions, and in particular of EU Directive 2001/20/EC (European Parliament and the Council of the European Union, 2001), suffered the lack of binding rules and EU guided coordination activities that led to great differences in children protection levels in different Member States. Such disparities are particularly relevant when related to consent/assent procedures, to the respect of the children's will, and to other fundamental rights (e.g., confidentiality and information). A variable lack of educational initiatives and debates involving local and national ethics committee members on the Paediatric Regulation and the European Ethical Recommendation (European Commission's Directorate-General for Health and Consumers, 2008) were also reported (Altavilla et al, 2011). This situation claims for new initiatives devoted to implement the existing rules and to identify better ways for the transposition of legislative provisions. To accomplish this and considering the increasing number of paediatric trials in Europe, all the main interested stakeholders (e.g., sponsors, investigators, ethics committees, regulatory bodies, patient associations) should be requested to take part in the efforts.

6.4 Future research

As stated by the Paediatric Regulation, since adults data could only partially be translated to children, drugs for children should be studied according to specific plans and methodologies; in addition, special attention should be devoted to assuring that paediatric studies are conducted under the highest methodological and ethical standards.

In a field where most clinical trials utilise a conservative design, the application of innovative methodologies offers a unique opportunity to develop medicinal products tailored to children. Power calculations can be improved by using clinical trial simulation, an approach that takes into account a multitude of factors.

7. Pharmacogenomics (pgx): The need of pgx tools in the light of the paediatric regulation

Pharmacogenomics is the investigation of variations in DNA and RNA characteristics in relation to drug response; a subset of PGx, pharmacogenetics studies the influence of variations in DNA sequence on drug response (Leeder, 2003). While the traditional

approach to diagnosis and treatment has been based around phenotypic definitions of disease and the identification of broad groups of patients with similar symptoms to be included in 'standardised clinical trials', the pharmacogenomics approach is based on the identification of specific genetic/genomics characteristics and aims at identifying "the right treatment for the right patient at the right time", the so called 'personalised medicine'.

Of particular interest is the fact that these '-omics' terms have been formulated to define approaches capable of identifying groups of biomarkers to be proposed for multiple purposes, such as a) to enable the detection of states of disease, b) to stratify patients based on biochemical profiles and to monitor disease progression and, in the specific field of medicines, and c) to orient the choice of therapy, identifying responders and predicting toxicity, paving the way to a customized therapy.

The increasing introduction of translational approaches in drug development using biomarkers and better defined cohorts, has the potential to increase the manageability, efficacy and safety of clinical trials while in the longer term possibly reducing their size, duration and cost.

7.1 Omics tools identification and methodological approaches
Common methodological approaches that can be applied to pharmacogenetics and pharmacogenomics studies are described below.

7.1.1 Case-control association study
The *case-control association study* is the study design generally used to assess pharmacogenetic effects. This approach examines the active treatment arm of a clinical trial and divides subjects into two groups: those with positive response and those with negative or no response. The groups are then genotyped for a particular candidate gene considered to be related to the treatment phenotype (Russo et al., 2011).

These types of studies are easy to perform, but they also present a number of potential biases or difficulties in interpretation. It is therefore necessary to ensure a good match between the genetic background of cases and controls to avoid biased sampling; techniques that can be employed to detect or eliminate the potential bias of population stratification are the match of cases and controls for ethnicity or the use of multiple unlinked markers (Pritchard & Rosenberg, 1999). Moreover, it is also indispensable to consider additional aspects such as sample size (Campbell et al., 1995), replication selection of candidate gene polymorphism (bioinformatic tools), observation bias (phenotyping and genotyping methods), linkage disequilibrium, allele or genotyped analysis, multivariate analysis, gene-gene and gene-environment interaction, and correction for multiple comparisons to guarantee the quality of the study and preventing false positive associations (Russo et al., 2011).

Efficient and powerful tools to identify inherited DNA sequence variations that contribute to phenotypic expression and variability are available to geneticists thanks to very high throughput DNA analysis technologies (e.g., *single nucleotide polymorphism [SNP] array*) and databases (HapMap project) harboring information about the genomic positions of DNA sequence variations. In fact, it is now possible to test a great amount of polymorphic markers for association with a particular phenotype in a single study, the *genome-wide association study* (GWAS).

GWASs are an important approach for revealing polymorphisms accounting for individual differences in drug efficacy and drug safety (Gurwitz & McLeod, 2009), as shown by

Crowley et al. and by the NHGRI GWAS catalog that summarized the results of 12 published pharmacogenomics GWASs (Crowley et al., 2009; National Human Genome Research Institute, n.d.) and showed that 6 of these GWASs evaluated the association of genetic variation with drug efficacy, five assessed adverse effects, and one examined a dose-response relationship (Russo et al., 2011). Despite their efficiency and potential for leading to useful clinical medicine and public health applications, however, genome-wide association studies have been used in only two drug clinical trials so far, each nonetheless providing relevant insights for future research (Russo et al., 2011, Maitland et al., 2007).

A new and promising field of research is *pharmacogenomics of miRNA* (Lagos-Quintana et al., 2001; Lau et al., 2001; Lee & Ambros, 2001), defined as the study of microRNAs and polymorphisms affecting miRNA function with the aim to predict drug behaviour and improve drug efficiency (Mishra et al., 2008; Mishra & Bertino, 2009). MiRNAs, small, single-stranded, 21-23 nucleotide-long, independent functional units of noncoding RNA, are drug targets that regulate expression of several important proteins in the cell and are differentially expressed in malignant versus normal cells, thus providing MiRNA pharmacogenomics with strong clinical implications (Mishra et al., 2007; Calin et al., 2002; Hon & Zhang, 2007; Iorio et al., 2005). MiR-polymorphisms, in fact, have the potential to be employed predictors of drug response and may lead to the development of more accurate methods of determining appropriate drug dosage based on a patient's genetic makeup, thus decreasing the likelihood of drug overdose (Russo et al., 2011; Mishra & Bertino, 2009).

7.1.2 Other tools

Other pharmacogenomic research areas are transcriptomics, metabonomics and proteomics. *Transcriptomics* is the study of gene transcripts, generally analyzed by cDNA expression microarrays which led to a number of exciting breakthroughs (Kieckle & Holland-Staley, 2003; McGregor, 2003). This approach has the advantage to include all genes of potential importance and therefore, provides the possibility to identify new therapeutic and diagnostic targets. On the other hand, its main disadvantage is to be a non-targeted genome-wide approach and therefore influenced by noise (i.e. expression signals of irrelevant genes) and increases the number of false positives (i.e. unimportant genes that are identified by chance) (Russo et al., 2011). Since acute lymphoblastic leukemia (ALL) is a 'liquid' tumour, relatively homogeneous and easy to isolate and characterize, it is ideal to assess global gene expression in cancer. Robust gene-expression profiling is a less labour-intensive and more automated alternative to the multiple methods that are currently used (e.g., immunochemistry, cytogenetics and molecular diagnostics), despite the possible limited availability of the appropriate source of sampling (e.g. blood, excreta, tissue), which is the major limitation of microarray studies (Armstrong et al., 2002; Golub et al., 1999; Ramaswamy & Golub, 2002; Moos et al., 2002; Ross et al., 2004).

Metabonomics or *metabolomics* is the study of metabolite profiling (Plumb et al., 2002; Reo, 2002). It has sampling limitations similar to transcriptomics, with one significant difference: metabonome represents an integrated response, in real time, to all endogenous plus all exogenous stimuli (e.g. drugs, chemical exposures, occupation, lifestyle, nutrition, age, gender) and it might offer the means to follow an individual patient's phenotype—as a function of age, nutrition, course of disease, or therapy (Russo et al., 2011). Metabolomics and "liver profile" test can therefore be considered analogous, except that metabolomics also includes measurement of metabolites and thus provides greater sensitivity. It is true that metabolite profiling can be performed only on easily available samples, nonetheless this

approach may be regarded as an extension of the present practice of clinical pharmacology (Nebert et al., 2003).

Proteomics is the study of all proteins encoded by the genome (Campbell & Ghazal, 2004) and has also been successful in certain areas of basic research. Although a recent study (Xing et al., 2004) estimated an average of 3.0 human proteins per gene, others have estimated that the true number of proteins per gene might be considerably higher (Russo et al., 2011). The limitation of proteomics—like transcriptomics and metabolomics—is represented by the types of source that must be sampled, e.g., blood, excreta or biopsy or tumour tissue in which relevant proteins exist.

7.2 The need to use pharmacogenomics tools to develop paediatric medicines

Recent data indicate that the general interest in the use of pharmacogenomics tools is increasing and in particular, it has been reported that today in some major companies up to 90% of molecules reaching the clinical development stage are associated to a biomarker strategy (Scarpa et al, in press).

The 35% of US approved drugs have pharmacogenetic information in their labels, and currently, 3 pharmacogenetic based drugs are licensed in the EU: herceptin (trastuzumab), glivec (imatinib mesilate) and erbitux (cetuximab). The marketing approved drug camptosar (irinotecan hydrochloride) is considered for re-labelling, based on post-approval research and the FDA decision to re-label the drug on the basis of the research's results.

Notwithstanding these considerable efforts and the claimed interest, the translation of 'omics' results into clinical practices is apparently growing at a very disappointing rate; in particular, there is for example no consolidated source of information on industry activities with enough detail to allow accurate estimates. This gap is probably due to proprietary and patent issues, which prevent public disclosure of information (Scarpa et al, in press).

In addition, it has been demonstrated that throughout the world more than 50% of the 'omics' research is related to predisposition, i.e. the investigation of the correlation between genetic traits and the probability or susceptibility for a given pathology or disease, and this type of exploratory studies provide no insights into the mechanisms of the disease or the drug action, neither it can be used to improve medical practice or support therapeutic solutions.

While many populations could take advantage from the introduction of 'omics' science aimed at developing personalised medicines, this approach could result of paramount importance particularly in 'small populations' for which the current trials approach, based on randomised controlled trials are not always applicable. Children represent the most significant example of such a case: in the paediatric field, in fact, few specific information are available because of the small number of patients and few resources invested to increase specific knowledge, and drugs are commonly used off-label or unlicensed and clinical trials result to be more difficult, longer and more expensive.

As explained in other sections of this chapter, there is a tangible need to base paediatric trials on new methodological approaches that take into account the peculiarities of this population, providing at the same time the highest scientific evidence from each enrollable subject and protecting as much as possible the patients exposed to the trial.

'Omics' approaches, by definition, aim at capturing the essence of the developmental processes that characterize maturation from birth through to adulthood, a particularly appealing methodology for the paediatric research context. However, as explained for

traditional research, it should be underlined that children should not be considered as small adults when they approach pharmacogenetic or pharmacogenomic studies. Many patterns of ontological development in the systems of the body illustrate how paediatric patients can differ from adults. Genotypes do not always correspond with expected phenotypes, making the exercise of deciding how to apply genomic research to paediatric medicine all the more complex (Scarpa et al, in press).

7.3 Pharmacogenomics tools in the paediatric populations: State of the art
Preliminary data on this issue have been provided within the context of the TEDDY Network of Excellence, an EU-funded project that dedicated a survey to the advancement of pharmacogenetic/pharmacogenomic application with the aim to explore the current status, limitations and perspectives of pharmacogenomic and pharmacogenetic clinical research in the paediatric population from an academic, regulatory and industrial perspective (Krekels et al., 2009). The main results reported by TEDDY showed that:

- innovative PGx research in the paediatric field is ongoing, but generally suffers from a non-standardized methodology, and a scarcity of recognized source of data (databanking tailored for children) and industrial interest and funds;
- few pharmaceutical companies declared ongoing pharmacogenomic- or pharmacogenetic-related research involving paediatric indications (Abbot, Altana Pharma, BD, Boherninger Ingelheim, Bristol-Myers Squibb, Ferring Pharmaceuticals, Genentech, GlaxoSmithKline, Isis Pharmaceuticals, Janssen-Cilag, Lilly, Merck, MGI Pharma Biologics, Novartis, Novo Nordisk, Roche, Sanofi Aventis, Servier, Taj Pharmaceuticals);
- the translation of pharmacogenetics and pharmacogenomics into the clinic is very slow. In addition, often pharmacogenetic and pharmacogenomic studies show contradictory results that reflect inconsistent research methods, small sample sizes, no replication studies, non-standardized outcome measures, or little consideration of potential covariates such as co-morbidity.

Pharmacogenetic studies in childhood conditions have been mainly developed in the following most common childhood conditions.

7.3.1 Attention-deficit/hyperactivity disorder
A multifactorial disorder characterized by physical hyperactivity and behavioural disinhibition, the attention-deficit/hyperactivity disorder (ADHD) usually appears during childhood or adolescence and often persists into adulthood. The usually prescribed psycho-stimulant is methylphenidate (MPH) that presents an estimated 70% response rate in ADHD affected children (Elia et al., 1991; Spencer et al., 1996).

According to pharmacogenetic studies, the inter-individual differences in stimulant-response may be related to genetic influences (Kirley et al., 2003; Langley et al., 2005; Gilbert et al., 2006; Winsberg & Comings, 1999; Roman et al., 2002; Purper-Ouakil, 2008; Kereszturi et al, 2008; da Silva et al., 2008) and the search for candidate genes associated with ADHD focused on the catecholamine system. Genes associated with increased risk for ADHD are the dopamine transporter (DAT1) (Purper-Ouakil, 2008), the dopamine receptors (DRD4 and DRD5) (Van Tol et al., 1992), serotonin transporter (5-HTT), and synaptosomal-associated protein (SNAP-25) (Husain et al., 2007; Faraone et al., 2005; McGough et al., 2006). Other genes of potential interest in pharmacogenetic studies include catehol-O-

methyltransferase (COMT) (Kereszturi et al, 2008), the adrenergic α2-receptor (ADRA2A and ADRA1A) (da Silva et al., 2008; Polankzyk et al., 2007; Elia et al., 2009) (Fig. 2). However, some pharmacogenetic studies show conflicting results. For example, in some of them individuals homozygous for the DAT1 10-repeat 480 bp-VNTR showed poorer outcome (Winsberg & Comings, 1999; Roman et al., 2002), whereas others report improved clinical outcome (Kirley et al., 2003) or no effect (Langley et al., 2005) on MPH response (Russo et al., 2011) (Fig. 2).

7.3.2 Growth hormone deficiency

GH deficiency (GHD) causing short stature is usually treated in children with GH replacement, carried out with fixed doses of human recombinant GH (hGH) adjusted for body weight or surface (Jorge et al., 2006).

Two of the most common isoforms of GHR in humans are generated by retention (full-length GHR, GHRfl) or exclusion of exon 3 (exon 3-deleted GHR, GHRd3) (Pantel et al., 2000). These isoforms present a widespread distribution in humans, with the frequency of each allele ranging from 68–75% for GHRfl and 25–32% for GHRd3 (Pantel et al., 2000; Dos Santos et al., 2004) (Fig. 2). It has been demonstrated that, among children with idiopathic short stature or who were born small for gestational age, patients with at least one GHRd3 allele presented 1.7 to 2 times more growth acceleration induced by hGH therapy than patients homozygous for the full-length isoform (Dos Santos et al., 2004). The study conducted by Jorge and colleagues demonstrated that patients carrying at least one GHRd3 allele had a significantly better growth velocity in the first year of hGH replacement and achieved a taller adult height when compared with patients homozygous for GHRfl alleles (Jorge et al., 2006) (Fig. 2).

7.3.3 Acute lymphoblastic leukemia

Cancer chemotherapy is the therapeutic class that could benefit more from PGt and PGx: anticancer agents, in fact, are often given at doses near to those that produce toxicity, show wide inter-patient variability in disposition and effects, and should therefore be administered at optimal doses for the best chance of cure (Russo et al., 2011).

In the USA, the leading cause of death by disease in children between 1 and 15 years of age is cancer and leukemia accounts for 33% of these deaths (Cheok & Evans, 2006). Twenty-five percent of all cancers in children is represented by Acute lymphoblastic leukemia (ALL). Treatment of ALL has undergone a significant progress, nonetheless long-term event-free survival rates are currently almost 80%, with 20% of patients who do not respond to standard therapy [Russo et al., 2011; Husain et al., 2007).

Polymorphisms in genes encoding enzymes that metabolize chemotherapeutic agents can modify treatment response. The thiopurine methyltransferase (TPMT) genetic polymorphisms and mercaptopurine toxicity are one of the best-studied examples in pharmacogenetics. Although 23 variant alleles have been identified to date (Ujiie et al., 2008), 3 variant alleles (TPMT*2 [Ala80Pro], TPMT*3A [Ala154Thr and Tyr240Cys] and TPMT*3C [Tyr240Cys]) account for >95% of low or intermediate TPMT enzyme activity: patients with TPMT deficiency are at very high risk of severe hematopoietic toxicity if treated with conventional doses of thiopurines (Cheok & Evans, 2006; Lennard et al., 1990; Yates et al., 1997). Patients who are heterozygous at the TPMT locus are at intermediate risk of dose-limiting toxicity and might require a modest dose reduction of approximately 35–

50%, whereas TPMT-deficient patients require a dose reduction of >90% (Cheok & Evans, 2006). Other important genes involved in ALL therapy are those codifying for the enzymes of the glutathione-S-transferase (GST) family. The polymorphisms of these genes have been associated with increased cancer incidence, therapy-related cancers and toxicity following chemotherapy (Cheok & Evans, 2006; Hayes et al., 2005). Polymorphisms of GSTM1, GSTP1, and GSTT1 exist in all populations. The GSTM1*0 (GSTM1 null) and GSTT1*0 (GSTT1 null) alleles represent deletions of GSTM1 and GSTT1 genes respectively and result in a loss of enzymatic activity (Rebbeck, 1997). The 1578 A > G transition in GSTP1 gives rise to the Ile105Val polymorphism, which confers reduced enzyme activity (Ye & Song, 2005); it is associated with high etoposide clearance in African-Americans treated with steroids (Kishi et al., 2004). Methotrexate (MTX) is also an important chemotherapeutic drug in the treatment of ALL. Methylenetetrahydrofolate reductase (MTHFR) is an essential enzyme in the folate/methotrexate metabolism pathway. About 10% of Caucasians show a genotypic variant of MTHFR (677 C >T; Ala222Val), which encodes a protein with about 30% of the wild-type activity (Frosst et al., 1995). This SNP has been linked to hepatotoxicity following methotrexate treatment (Ulrich et al., 2001). Another low-function variant of MTHFR results from the 1298 A >C (Glu429Ala) substitution; it has been reported to be protective for adult acute lymphocytic leukemia (Skibola et al., 1999; Wiemels et al., 2001) but not to altered effects of MTX in leukaemia (Krajinovic et al., 2004) (Fig. 2).

7.3.4 Asthma

The most common chronic disease among children, asthma affected in 2002 more than 30 million individuals in the USA reported having been diagnosed as having asthma, including 122 per 1000 children (Mattke et al., 2009).

The response to shortacting albuterol therapy in children with asthma is influenced by a common polymorphism in the coding region of ADRB2 gene (Fig. 2). Bleecker and colleagues have recently showed no pharmacogenetics affect of this genetic variant on therapeutic response when the patients were treated with inhaled corticosteroids plus longacting β2-agonists (Bleecker et al., 2007).

The other two modality of asthma treatment are corticosteroids and leukotriene modifiers, and polymorphisms of the genes (CRHR1, LTC4, ALOX5) involved in their modulation have been described (Fig. 2). In this example, the LTC4S -444 A>C promoter polymorphism has been associated to a reduced risk for asthma exacerbations when compared with individuals homozygous for reference allele (Sampson et al., 2000; Whelan et al., 2003; Husain et al., 2007); in other studies this observation was not consistent (Currie et al., 2003; Kedda et al., 2004) (Fig. 2).

8. Pharmacovigilance: The need for ad hoc measures

According to the World Health Organisation (WHO), pharmacovigilance is defined as "the science and activities relating to the detection, assessment, understanding and prevention of adverse effects or any other drug-related problem".

In adults pharmacovigilance predominantly refers to post-marketing surveillances.

The situation is different in children. Because of the large extend of unlicensed and off-label drug uses and the small population numbers intensive monitoring of adverse events has to be performed before and after marketing authorisation.

Childhood disease	Gene	PharmGKB ID	Genetic polymorphisms	[1]HGVS names	Polymorphism location and function	Biochemical and clinical effect(s)	PMID
Asthma	ADRB2	PA39	SNP (rs1042713)	NM_000024.4: c.46G>A	Coding region (Arg16Gly)	Arg-allele improves albuterol response	9399966; 10340917
			SNP (rs1042714)	NM_000024.4: c.79G>C	Coding region (Gln27Glu)	No association with response to albuterol	
	AC9	PA30	SNP (rs2230739)	NM_001116.2: c.2316A>G	Coding region (Ile772Met)	Met-allele improves albuterol response	15879435
	CRHR1	PA26874	SNP (rs242941)	NM_004382.3: c.122-1310C>A	Intron	Allele G improves lung function in response to inhaled corticosteroids	15128701; 19210659
	TBX21	PA36362	SNP (rs2240017)	NM_013351.1: c.99C>G	Coding region (His33Gln)	Gln-allele enhances the effects of inhaled corticosteroid on airway responsiveness	15604153
	LTC4S	PA235	SNP (rs730012)	NT_023133.12: g.24030224A>C	Promoter (-444 bp from start site)	Allele C has a reduced risk for asthma exacerbation	10992553; 14520724
	CYSLTR1	PA38453	SNP (rs320995)	NM_006639.2: c.927C>T	Coding region (Phe309Phe)	Allele C is associated with persistent asthma in males	19080797
	ALOX5	PA46	DIP (rs71921156)	–	5' near gene (-/GGGCGG)	Homozygous for mutant alleles (3,4,6 tandem repeats) reduces lung function responses to ABT-761 treatment	10369259; 12911785; 10369259
	GSDML	PA162390303	rs7216389	NM_001042471.1: c.236-1199G>A	Intron (locus controlling ORMDL3 expression)	T allele is associated with the risk of exacerbations of asthma	17611496; 18395550
ADHD	DRD4	PA27480	VNTR (2-11 repeats)	–	Coding region (48 bp sequence)	The shorter forms (2-4 rep alleles) have a blunted response to dopamine	1319557; 17979513; 19336242
	DAT1	PA311	VNTR (3, 9-10 repeats)	–	3'UTR (40 bp sequence)	Homozygosity of the 480-bp allele (10 rep) is associated with a poor response to MPH	10596245; 12172219; 18563707; 7717410
	5-HTT	PA312	DIP (rs12720056)	–	Promoter (-1212 to -1255)	The 44 bp deletion reduces transcription and protein levels	11425009; 18200432; 8632190
	SNAP-25	PA35980	SNP (rs3746544)	NM_003081.2: c.*239G>T NM_130811.1: c.*239G>T	3'UTR (Conserved element)	Allele T improves dose responses in comparison with the allele G	17979513; 15950004; 17023870
	COMT	PA117	SNP (rs4680)	NM_000754.2: c.472G>A	Coding region (Val158Met)	Val-allele or Val/Val genotype have a good MPH response	18214865
	ADRA2A	PA35	SNP (rs1800544)	NT_030059.12: g.31585029G>C	Promoter (-1291 bp from start site; maps to CpG island)	Allele G improves effects of MPH on inattentive symptoms after 1 month of treatment	17283289; 18200436
ALL	TPMT	PA356	SNP (rs1800462)	NM_000367.2: c.238G>C	Coding region (Ala80Pro)	TPMT*2, TPMT*3A and TPMT*3C alleles have low or intermediate TPMT enzyme activity with high risk of severe hematopoietic toxicity after thiopurines treatment	1973780; 9103127; 16491071
			SNP (rs1800460)	NM_000367.2: c.460G>A	Coding region (Ala154Thr)		
			SNP (rs1142345)	NM_000367.2: c.719A>G	Coding region (Tyr240Cys)		
	GSTT1	PA183	GSTT1*0	–	Large deletion	Loss of enzymatic activity	8198545; 9298582
	GSTM1	PA182	GSTM1*0	–	Large deletion	Loss of enzymatic activity	9298582; 9057653
	GSTP1	PA29028	SNP (rs1695)	NM_000852.3: c.313A>G	Coding region (Ile105Val)	Ile-allele confers high etoposide clearance in African-Americans treated with steroids	15862746; 12969965
	MTHFR	PA245	SNP (rs1801133)	NM_005957.3: c.665C>T	Coding region (Ala222Val)	Val-allele is a low-function variant (30% of the wild-type activity). It has a higher hepatotoxicity following MTX treatment	7647779; 10536004; 11418485; 11274424
			SNP (rs1801131)	NM_005957.3: c.1286A>C	Coding region (Glu429Ala)	Ala-allele is a lower-activity variant. It not alters MTX effects	
	GGH	PA432	SNP (rs11545078 9)	NM_003878.1: c.452C>T	Coding region (Thr151Ile)	Ile-allele is associated with 67% lower catalytic activity in the degradation of long-chain MTXPG, but not short-chain	16491071
GHD	GHR	PA28674	GHRfl (NM_000163)	–	Full lenght isoform	GHRd3 isoform had a better growth velocity in the first year of hGH replacement	10764769; 15208626; 16291702
			GHRd3 (AF210633)	–	Exon 3-deleted isofrom		

Legend: ADHD=attention-deficit/hyperactivity disorder; ALL, acute lymphoblastic leukemia; GHD= growth hormone deficiency, SNP,=ingle nucleotide polymorphism; DIP=deletion/insertion polymorphism; VNTR=variable number tandem repeat; MPH=methylphenidate; MTX=methotrexate; MTXPG=methotrexate polyglutamate; HGVS=Human Genetic Variation Society; PMID=PubMed Identifier

Fig. 2. Genetic polymorphisms in the most common childhood diseases (Russo et al. 2011)

Adverse drug reactions (ADRs) in children differ from those manifested in adults in terms of frequency, nature and severity due to their distinct pharmacokinetics and pharmacodynamics (Kearns et al., 2003).
There are also other issues to be considered with respect to the detection of adverse drug reactions in the paediatric population. Children can often not express their own drug therapy experience, which makes the determination of ADRs harder and takes more efforts and sensitivity towards the subject. The involvement of parents and carers therefore is even more important.

8.1 Sample size considerations

In the developed world, the paediatric population is small in general and relatively healthy when compared with for instance the elderly. Children and adolescents account for less than 25% of the population of 0-80years old only. Thus, the number of patients needing a

treatment is always going to be smaller hence, the power to detect adverse drug reactions is far more limited.

Furthermore, the paediatric population ranges from birth to the completion of the 18th year of age. With respect to the safety and efficacy of medicine, this cannot be seen as one homogenous population. Growth and development during these first years of life effect many physiological processes depending on age in different ways. This results in wanted and unwanted reactions to medicines that are not seen in adults and/or other paediatric sub-groups. To accommodate these differences the paediatric population has been sub-classified by the ICH into different age groups (Table 2).

< 37 weeks	Pre-term neonates
< 28 days	newborn and neonates
1months – 2years	infants
>2 - < 11years	children
>11	adolescents

Table 2. Paediatric age groups according to ICH and EMA guidelines

Developing medicines for children means that each age group needs to be studied separately. This poses another challenge, as certain numbers of participants are necessary in order to identify ADRs with statistical power. Many serious and severe ADRs are infrequent occurring in not more than 1 per 10,000 patients.

Therefore, in paediatric pharmacovigilance methods, which can systematically capture large populations, are essential.

8.2 Spontaneous reporting

Spontaneous ADR reporting is the most common method in pharmacovigilance. It has been introduced after the Thalidomide scandal in the late 1960s. Today spontaneous reporting is part of the legal duties of health care professionals in many countries around the world.

The system is well established and has many advantages but also disadvantages. Spontaneous ADR reporting is particularly useful for the detection of signals for new and unknown ADRs as it covers large populations, has low costs and is widely implemented.

However, it cannot be used to study incidence and prevalence as there is a significant under-reporting and unknown denominators, i.e. size of the population exposed and therefore at risk for ADRs. Also it does not allow for an early detection as by the time ADRs are reported the outcome of the ADR is already established, hence there is no possibility of any intervention for the individual patient.

Furthermore the data generated by the system are often biased because of both under-and over-reporting. The introduction of a drug to the market or promotion within the media may result in an increased awareness and thus increased reporting.

The highest value of spontaneous reporting is signal detection is. However, to detect a signal a minimum number of case report is needed. For the spontaneous ADR reporting this ranges between 3 and 9 cases (Edwards et al., 1990). How fast this number of cases can be collected depends on various factors such as the number of users, the frequency of the ADR and the reporting rate. This means that compared to adults, for the relatively small population of children in different age groups it will take much longer to receive the

appropriate number of cases to identify signals. Reports for children with rare disease, or relating to drugs not commonly used, result in even lower reporting rates and hence may never be identified as potential safety signal. Therefore increasing the awareness of ADR reporting in the paediatric population and the compilation of ADR reports on an international basis is crucial to retrieve relevant and important information on adverse drug reactions.

Despite all the limitations of spontaneous reporting, without the continuous reporting on suspicions of ADRs new hypothesis cannot be raised and consequently be tested by using other measures of pharmacovigilance. Therefore, spontaneous reporting will continue to be a crucial part in ADR reporting and efforts should be made to further improve it.

8.3 Active ADR surveillance

Stimulating clinicians and health care professionals regularly to report suspected ADRs and reminding them about the importance of reporting can significantly improve reporting rates. This may be a simple reminder sent on a weekly basis or the contribution of for instance pharmacists on the ward who will regularly ask for ADRs and document them accordingly. Furthermore tools can be made available to health care professionals which will make the reporting easier and also assure data collection is complete.

Various pilot-projects were set up in the past and have shown the effectiveness of active ADR surveillance (Carleton et al., 2009; Clarkson et al., 2004; Menniti-Ippolito et al., 2000). Unfortunately resource limitations usually have not allowed to implement these in general practice.

However, the results of these studies proof that if active surveillance is put in place the number of ADR reports significantly increases. Thus active ADR surveillance can help to overcome underreporting. Furthermore, active surveillance enables a more thorough data collection process and hence improved data quality. Establishing standardized methodologies can further improve and optimize the detection of ADRs.

Overall, active pharmacovigilance poses an important feature in the pharmacovigilance process which particularly in the paediatric population should be utilized more and replace the simple standard of spontaneous reporting.

8.4 Targeted ADR surveillance

Targeted pharmacovigilance focuses on monitoring the safety profiles of specific drug groups in relation to specific ADRs. Depending on the set up it can reach large populations under observation and at the same time provide denominator data to allow for incidence calculations. The AMSP study in Germany has proven that targeted pharmacovigilance in psychiatry is a valuable tool for the evaluation of ADRs as well as educating clinicians in the field of ADRs (Grohmann et al., 2004). Another study from the UK determined the feasibility of conducting a prospective targeted pharmacovigilance study to monitor adverse drug reactions associated with atypical antipsychotic therapy in children (Rani et al., 2009).

The projects showed that targeted pharmacovigilance can be very powerful but at the same time is resource intensive requiring a considerable amount of commitment from the participating clinicians including appropriate training. Nevertheless, the focused collection of practice based data is important. Methods accommodating all methodological issues still need to be developed using the continuously enhancing technological possibilities. The

payment of incentives or free access to all data collected during a surveillance could further enhance reporting.

The potential of this method has been realized by various stakeholders such as the EMA and the Royal College of Paediatric and Child Health which recommend in their guidelines on the conduct for paediatric pharmacovigilance the use of "targeted pharmacovigilance" to monitor drug safety in children (CHMP, 2007b; Royal College of Paediatrics & Child Health, 2004).

8.5 Computerized ADR surveillance

The use of computerized methods to improve the detection of adverse drug reactions in hospitalized patients is a promising approach in both paediatric and adult patients.

Laboratory parameters outside the normal range or the change of such within a certain period of time would allow to generate signals which can inform the clinician that an ADR has potentially developed or may develop in a particular patient. A simple example is the decrease of potassium in a patient receiving medication impacting on potassium levels such as loop diuretics or beta-mimetics.

Pilot studies have shown that such systems are feasible and the sensitivity is high. However, currently there is still a significant lack of specificity for those systems. The definition of rules is a particular challenge in the paediatric population as the normal ranges quickly change within different developmental stages (Haffner et al., 2005; Neubert et al., 2006).

Another promising approach which has also been implemented on paediatric wards are "trigger tools". Trigger tools use screening criteria to identify possible harm in a patient followed by an in-depth review of the patient chart for actual harm. The Canadian Association of Paediatric Health Centers Trigger Tool (CPTT) recently introduced the first validated comprehensive trigger tool to detect AEs in children hospitalized in acute care facilities (Matlow et al., 2011). Trigger tools do not solely report adverse drug reaction but also capture medication errors. With respect to medication safety in paediatric patients this is of importance; with respect to gaining effectively information on long-term safety outcomes there are clear limitations.

8.6 Education and increasing awareness of ADRs

The commitment and awareness of clinicians and other health care professionals involved with the paediatric patient is crucial for the detection and reporting of safety issues. This should be communicated early in the educational process and be part of the general training.

The involvement of paediatric pharmacologists is important to improve the understanding of general principles of how drugs are acting. However, to date there are only a few experts specifically trained in this field.

Within the recent activities around the paediatric drug development this need has been recognized. GRIP –Global Research in Paediatrics has been commissioned by the EU. One of its primary goals is the establishment of educational programmes in paediatric clinical pharmacology.

In addition to health care professionals knowledgeable in paediatric pharmacology and pharmacovigilance families and carers have to be actively involved and encouraged to report adverse reactions. Especially in the paediatric population as children cannot express theirselves, parents and carers play an important rule. They are often very closed to the

children and get the impression of how the child feels and whether changes in behaviour and/or well-being could be related to drug therapy. This information may not be gathered from the responsible health care professionals.

The new European regulation on pharmacovigilance supports this issues as it will allow that adverse drug reactions can be reported to the regulators and pharmaceutical companies by lay people such as patients, parents and carers.

8.7 Conclusions

In summary, pharmacovigilance is important in paediatric drug development. The need to further improve its methods has been realized by different stake holders including health care professionals and regulators. Various methods for ADR detection and reporting are available; to optimize ADR reporting in children a combination of methods needs to be used. Targeted pharmacovigilance is particularly to be promoted. It generates information on certain drugs or drug groups for short as well as long-term outcomes in large populations.

In addition, training of health care professionals and increasing the awareness towards paediatric drug safety is a key element to generate a sustainable drug safety culture in paediatrics.

9. Acknowledgment

10. References

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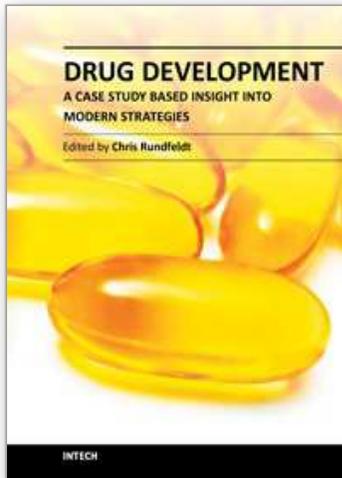
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