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Results of juvenile animal studies (JAS) and impact on anti-cancer medicine development and use in children

Project report

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1. Summary

Special scientific considerations apply to developing medicinal products for children because of their heterogeneity and potential for differences compared to adults. Several non-clinical and clinical EMA and ICH scientific guidelines address evaluating medicines in the paediatric population, including studies in juvenile animals (JAS), studies in specific populations such as neonates, and paediatric therapeutic areas such as for anti-cancer medicines.

This project was aimed to assess the impact and the utility of JAS on paediatric medicinal product development, and the example of anti-cancer medicines was chosen as they are expected to show toxicity that most likely affects developing tissues and organs, in both humans and animals.

A retrospective analysis was performed to identify cases where JAS for anti-cancer medicines were conducted as part of a development towards a paediatric use. Juvenile animal studies were searched in paediatric investigation plans (PIPs) for anti-cancer medicines that were agreed from mid-2007 (start of the Paediatric committee scientific evaluations) to December 2013. For 21 anti-cancer medicines (40% of anti-cancer medicine PIPs, 21/52), the development included at least 1 juvenile animal study (JAS) as a non-clinical measure in the agreed PIP. Results of JAS were available for 20 (out of these 21) anti-cancer medicines and were the basis for this report. In total 29 JAS were conducted for 201 anti-cancer medicines. The results of the JAS were compared to adult data (based on exposure, including the level and duration of exposure) and consequences for the paediatric development and use were assessed.

Besides non-specific effects on development landmarks (e.g. growth, eyes opening, age at puberty) or on reproductive organs the following was observed comparing adult and juvenile animal studies findings:

- new target organ toxicities for 8[2] products
- increased severity of toxicity for 8[3] products
- no major differences for 6 products

The results of this project can help to build up experience on the utility of the juvenile studies and contribute to shape the future requirements and assessments as well as to review how juvenile animal studies inform how to better evaluate anticancer medicines, protect and treat young children with cancer.

1 Detailed results and corresponding assessements for 3 medicinal products were removed from the report for reasons of confidentiality, however the total number of medicinal products assessed was mantained throughout the document.
2 Detailed results and corresponding assessement for 1 medicinal product beloging to this group were removed from the report for reasons of confidentiality.
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2. Introduction

Medicinal products were used for medical care and treatment of children off-label and even unlicensed in the absence of a systemic need for a specific paediatric development. In 2007, the implementation of the European paediatric regulation (EC) No 1901/2006 changed the approach of drug development and access for paediatric patients to medicines. The Paediatric Committee (PDCO) was created to scientifically evaluate medicines for a paediatric development through a Paediatric Investigation Plan (PIP) where there are unmet therapeutic needs in children.

The PIP is a research and development programme for ensuring that the necessary data are generated based on which a medicinal product may be assessed with the conclusion to recommend its use in a paediatric population and / or with the conclusion that it should not be used, for example because of concerns for safety or efficacy in a paediatric population. The agreed PIP therefore details which quality, non-clinical and clinical data are to be generated for supporting the targeted paediatric use (indication).

Special scientific considerations apply to developing medicinal products for children because of their heterogeneity and potential for differences compared to adults, for example, in respect of size, disease severity, growth and maturation of organs and functions, sensitivity and responsiveness to interventions. Several non-clinical and clinical EMA and ICH scientific guidelines address evaluating medicines in the paediatric population, including studies in juvenile animals (JAS), studies in specific populations such as neonates, and paediatric therapeutic areas such as for anti-cancer medicines (see References).

With the implementation of the paediatric regulation, the need for specific non-clinical studies including juvenile toxicity studies has to be discussed for each medicine by the PDCO, in order to anticipate adverse reactions possibly specific to children (that is, which do not occur in adults), to anticipate differences in pharmacodynamics and pharmacokinetics, to avoid studying children who could experience unacceptable adverse reactions as well as to inform investigating and treating paediatricians on how to use new medicines. This perspective of clinical utility of JAS is expressed in ICH E11 (2001), according to which the need for JAS should be based on developmental toxicology concerns, for the respective paediatric development program.

Similarly, according to the EMA guideline on the need for non-clinical testing in juvenile animals of pharmaceuticals for paediatric indications (2008), JAS should be performed on a case-by-case basis after consideration of available data, the age and duration of treatment of the intended paediatric population and pharmacodynamics in target organ(s)/tissue(s) with significant postnatal development. A key recommendation is that the presumed level of predictability of non-clinical studies in adult animals and of clinical trials in adult humans is to be taken into account for evaluating the need for JAS. Furthermore, it is stated that JAS may be warranted to further address a specific concern or to study reversibility or possible aggravation of the expected findings, as well as to establish safety factors, even if adverse reactions on developing organ(s) can be predicted from adult human or animal data.

In the ICH M3 (R2) guideline on non-clinical safety studies for the conduct of human clinical trials for pharmaceuticals (2009), the recommendation is that JAS should be considered only when previous animal and human safety data are judged insufficient. The same recommendation is made in the ICH S9 guideline on non-clinical evaluation for anticancer pharmaceuticals (2010). This guideline specifically applies to trials with children with an advanced cancer, and it is stated in the guideline that

studies in juvenile animals are not usually conducted in order to support inclusion of paediatric populations in studies for the treatment of their cancer.

In addition, animal testing should comply with the Directive 2010/63/EU on the protection of animals used for scientific purposes laying down the principles of the 3R rule (Replacement, Reduction and Refinement), according to which the use of young animals should be minimized.

After about 5 years of operation under the paediatric regulation, a number of JAS have been agreed to be conducted as part of a PIP, and consequently, JAS study result reports have now been submitted to the EMA. Therefore, this project was started to assess the impact and the utility of JAS on paediatric medicinal product development, and the example of anti-cancer medicines was chosen as they are expected to show toxicity that most likely affects developing tissues and organs, in both humans and animals.

For the paediatric therapeutic area of oncology, fewer medicine developments than in other paediatric therapeutic areas have been supported by scientific advices or regulatory assessments. A paediatric indication[4] has been established for only 9 of the 67 anti-cancer medicines that were centrally authorised from 1995 until the end of 2013. Paediatric clinical data have been submitted for very few other anti-cancer medicines (such as under Articles 45 or 46 of the paediatric regulation, not based on an agreed PIP). The paediatric regulation does not include an obligation to submit results of JAS unless part of an agreed PIP.

The objectives of the project were to identify the cases where JAS have been conducted since the implementation of the paediatric regulation, with a focus on the scientific grounds supporting their conduct and their design. A review of the results currently available is also made in order to establish the value of the information provided by the JAS, the consequences on the paediatric clinical development and the regulatory decisions taken after review by Scientific Committees at the EMA.

In contrast to other therapeutic areas, a first paediatric cancer trial has usually been restricted to children with limited life expectancy due to advanced malignant disease stage and lack of effective treatment, and such trials usually may not be expected to include very young children. In fact, paediatric oncology investigators often excluded children less than 1 or 2 years of age from the first and also from subsequent trials, likely to await the demonstration of safety and efficacy in older patients. However, the clinical development paradigm of anti-cancer medicines for children is changing: The goal is now to generate the necessary data that inform their most appropriate use in the paediatric population, based on the mechanism of action and the existing medical needs.

Due to the unacceptable burden of traditional cytotoxic treatments, unmet medical needs in paediatric oncology extend far beyond situations of advanced disease with limited life expectancy and span the different age ranges from infants to young adults. This also implies that successful developments are predicted to yield indications in paediatric populations with good chances for long-term survival. Consequently, the full paediatric anti-cancer medicine development and not only first paediatric trial(s) have to be considered with respect to the need for JAS in PIPs. Since novel targeted anti-cancer medicines typically target cell biology and developmental pathways with pleiotropic effects and often require chronic use, the considerations involved are inevitably more complex. The advent of seamless studies in early phases poses additional challenges in this respect.

This project report will help to build up experience on the utility of the juvenile studies and contribute to shape the future requirements and assessments as well as to review how juvenile animal studies inform how to better protect and treat young children in oncology as well as in other therapeutic areas.

[4] Products with paediatric indication were identified using the ATC code L01 and checking their paediatric use information, i.e. for an indication in section 4.1 of the Summary of product characteristics or as a paediatric posology in section 4.2.

3. Methods

A retrospective analysis was performed to identify cases where juvenile animal studies (JAS) for anti-cancer medicines were conducted as part of a development towards a paediatric use. The results of the JAS and consequences for the paediatric development and use were assessed.

3.1. Data collection

Juvenile animal studies were searched in paediatric investigation plans (PIPs) for anti-cancer medicines that were agreed from mid-2007 (start of the Paediatric committee scientific evaluations) to December 2013; other medicines, such as for supportive care, were excluded. For 21 anti-cancer medicines (40% of anti-cancer medicine PIPs, 21/52), the development included at least 1 juvenile animal study (JAS) as a non-clinical measure in the agreed PIP. Results of JAS were available for 20[5] out of these 21 anti-cancer medicines (no results available for veliparib) and are the basis for this report. In total 29 JAS were conducted for 20 anti-cancer medicines. There were cases of up to 3 JAS per active substance, where the studies were designed to be consecutive and to complement information, such as dose-range finding followed by definitive studies or studies separated for toxicokinetic and other endpoints.

Information and data on the concerned anti-cancer medicines were compiled from various sources:

- The PDCO Opinions on agreed PIP, with details of the non-clinical and clinical studies as well as the age subsets of patients waived to be studied or be include in trials. Most (80%, 41/52) of the developments as set out in the agreed PIPs are to include children less than 2 years of age. The treatment intention targeted in the respective developments was categorised by paediatric oncologists of the PDCO in reference to the EMA guideline on the evaluation of anticancer medicinal products in man (treatment administered with curative intent means to improve cure rate and survival or to relevantly decrease toxicity without loss of efficacy; treatment administered with the intent to achieve long-term disease control refers to those early lines of therapy for which reference therapies are established and next-line treatment options are likely to be meaningfully efficacious; palliative therapy mainly refers to last line settings where the prognosis for survival is poor and evidence-based reference therapies are difficult to identify). Accordingly, the targeted uses were curative (in 22 out of 52 PIPs; for 7 out of 20 products), long-term disease control (29/52; 13/20) or palliative (1/52; 0/20).
- The JAS study result reports submitted for example in support of a modification of an agreed PIP, for the verification of compliance with the agreed PIP at the time of adult marketing authorisation application. In addition, 4 reports were received from pharmaceutical companies upon invitation, outside of a procedure.
- The assessment reports of the CHMP and the PRAC concerning these JAS, as well as the summary of product characteristics of the concerned anti-cancer medicinal product.
- The EMA/PDCO summary reports documenting the scientific evaluation and conclusions.
- EMA Scientific advice letters that included preliminary assessments of these JAS study result.
- The investigator's brochures.

[5] Detailed results and corresponding assessements for 3 medicinal products were removed from the report for reasons of confidentiality; however the total number of medicinal products assessed was mantained throughout the document.

3.2. Assessment and comparison of juvenile with other animal data

The assessment was performed by a team of five experts (see 8. Acknowledgments) in non-clinical development who are working as assessors in their respective national agencies and who are members of the PDCO's Non-clinical Working Group (NcWG), one of them being also member of the EMA Safety Working Party (SWP). Each medicinal product was assigned to one of the assessors. Their assessments were discussed in meetings of the scientific team in order to reach a consensus. After discussion, the evaluation reports were modified by the assessors and reviewed by the scientific team leader. The reports were then circulated to the team for final comments to reflect the opinion of the team.

The objective was to compare, at corresponding exposure levels, the juvenile study results to the corresponding adult toxicity and then to the overall drug toxicity profile. A standard evaluation template was developed to document this assessment (see 10. Appendix).

Information that was collected and assessed included: the mechanism of action, the expected therapeutic dose/exposure, the targeted indication, the paediatric age subsets, the expected dosing regimen, a summary of the data from the juvenile animal studies, a summary of the data from the comparative adult animal study, all toxicities studies (details were provided only for those bringing relevant information for the conclusions), an overview of the PK profile, the toxicological profile of the product, a comparison of the adult and the juvenile animal data and the conclusions of the Working Group.

In most of the cases (17 out of 20) the animal species used in the study was the rat. The rat is broadly accepted as an appropriate and well-known model, for both practical and ethical reasons. The objectives of juvenile studies are to detect new unexpected safety risks or to assess if at equivalent exposure, the severity of the findings is increased compared to those observed in adults. Where the rat was an acceptable species to assess the toxicity profile of a drug, it is appropriate to use the rat to perform the juvenile studies. In rats, the development processes from neonates to adulthood can be studied over a brief period of time and are increasingly understood.

4. Results

4.1. Studies and data analysed

Overall, reports were available for 29 JAS with 20 anti-cancer medicines (products), and these were included in this analysis. For one product (imatinib), the JAS had been performed to support its use in a non-oncology indication (pulmonary hypertension) but the results are nevertheless included in this report. For another product (pixantrone), the JAS performed in the mouse was aimed at assessing sensitivity of juvenile animals to delayed cardiotoxicity, compared to adult mice.

The main motivations for a JAS included (but were not limited to):

- to specifically investigate toxicities seen in available non-clinical studies with concern for effects on growth and development. Available non-clinical studies included adult animal studies, peri-/ postnatal or foetal development studies, juvenile animal studies or safety pharmacology studies,
- to support the inclusion of patients of a lower paediatric age subset than considered supported so far by human and non-clinical data,
- to assess secondary pharmacology effects anticipated to interfere with normal development, and
- to assess effects of parent and metabolite(s) exposure on potentially related toxicities due to immaturity of clearance pathways.

In further 7 of the set of 52 anti-cancer medicinal products, on day 60 of the PIP procedure the PDCO questioned the lack of JAS in the proposed development strategy, but eventually no JAS were included in the final PIP opinion. This was based on supplementary data provided by the applicant and expert discussions on the reversibility of adverse effects observed in adult animal, on the possibility to safely monitor anticipated toxicities during the paediatric clinical trials, or on the lack of appropriate juvenile animal models. Non-clinical and paediatric clinical data with products of a similar mechanism of action were also taken into account. Finally, the scope of the paediatric clinical development may have been changed during the course of the PIP approval process (e.g. treatment of a paediatric tumour in older children only and no broad phase 1 clinical trial to be conducted in children covering all age ranges).

JAS were conducted, for 19/20 active substances, in a single species: the rat (16/20 products), the dog (1/20), the cynomolgus monkey (1/20) or the mouse (1/20) (Table 1). For one of the active substances (everolimus), two species have been used, i.e. a JAS was conducted in the rat, and a one-month toxicity study was performed in monkeys of about 1 year of age (studies performed before PIP application, for other indications).

For studies in rats, dosing started in very young rats (PND 7 to 10) in the JAS of 13 products. Of note, the targeted paediatric population of 11 of these 13 products included children less than 1 year of age.

After the dosing period, JAS of 17 products included a drug-free period between 2 weeks and up to 6 months to test reversibility.

Table 1: Active substances, paediatric use to be developed according to the agreed PIP and JAS study designs

Active substance (pharmacology)	**Paediatric use under development***	**Species/route of administration**	**Age of animals at treatment initiation - termination**	**Drug-free period**
Cabozantinib (Multi-target TKI including c-MET and VEGFR2)	From birth. Long-term disease control.	Rat / oral	DRF: PND 12 – 35 Pivotal studies: 1. Group a: PND 21 - 35 Group b: PND 21 - 70 2. Group a: PND 12 - 35 Group b: PND 12 - 70	 4 weeks 12 weeks 4 weeks
Cobimetinib (selective MEK1 TKI)	From 6 months. Long-term disease control.	Rat / oral	Pivotal: PND 10 - 38	
Dabrafenib	From 1 month. Long-term disease	Rat / oral	DRF: PND 7 –21,	

Active substance (pharmacology)	Paediatric use under development*	Species/route of administration	Age of animals at treatment initiation - termination	Drug-free period
(serine/threonine kinase inhibitor of BRAF and angiogenic TK (TIE2), SIK 1 and SIK 2	control.		PND 7 – 35, 7-35 Pivotal: PND 7 – 35	 6 weeks
Decitabine (A cytidine nucleoside analogue that acts via inhibition of DNA methyl transferases inducing DNA demethylation. This results in re-expression of silencing genes that inhibit cellular proliferation and induce cellular differentiation.	From 1 month. Curative.	Rat / iv-sc	Pivotal: Group a: PND 7 – 35 Group b: PND 11 – 35 (both intermittent dosing)	 5 weeks 5 weeks
Everolimus (a serine/ threonine kinase inhibitor of mTOR)	From birth. Long-term disease control.	Rat / oral Cynomolgus monkey / oral	DRF: PND 7 – 27 Pivotal: PND 7 – 70 1 year (28 days study)	13-26 weeks depending on subsets 2 weeks
Imatinib (multi-target TKI with specificity for the Bcr-Abl, PDGFR, and c-Kit tyrosine kinases)	From 6 months. Curative.	Rat / oral	DRF: PND 10 - 44 Pivotal: PND 10 - PND 70	 6-13 weeks depending on subset
Lenvatinib (split-kinase inhibitor of VEGFR1 (FLT1) and VEGFR2 (KDR))	From birth. Long-term disease control	Rat / oral	Pivotal: PND 7- 21, 21- 35 PND 21 – 77	 2 weeks 4 weeks
Midostaurin	From 3 months.	Rat / oral	DRF: PND 7 - 35	

Active substance (pharmacology)	Paediatric use under development*	Species/route of administration	Age of animals at treatment initiation - termination	Drug-free period
(multi-target tyrosine kinase inhibitor of several PKC isoforms, of the VEGFR and of the class III tyrosine protein kinases FLT3 and KIT)	Curative.		Pivotal: PND 7 - 70	6 weeks
Navitoclax (binding to multiple anti-apoptotic Bcl-2 family proteins including Bcl-XL, Bcl-2, Bcl-w and Bcl-B)	From 1 month. Long-term disease control.	Rat / oral	DRF: PND 7-21 Pivotal: PND 7 - 60	
Nilotinib (multi-target tyrosine kinase inhibitor of BCR-ABL, PDGF alpha and beta, c-kit, colony stimulating factor-1R (CSF-1R), discoidin domain receptor (DDR) and several of the ephrin receptor kinases)	From 1 year. Long-term disease control.	Rat / oral	DRF: PND 7 - 34 Pivotal: PND 7 - 70	5-8 weeks depending on subset
Ombrabulin inhibitor of tubulin polymerization and a vascular disrupting agent)	From birth. Long-term disease control.	Dog / 30 min iv infusions (impossible in young rats)	Pivotal: PND 21 - 182 PND 21 (single dose TK study)	13 weeks
Pazopanib (multi-target tyrosine kinase inhibitor of VEGFR-1, -2 and -3; PDGFR-α and β; c-KIT)	From 1 year. Long-term disease control.	Rat / oral	DRF: PND 9 – 21, PND 9 – 35, PND 21 – 35, PND 9 – 14 Pivotal: PND 21 – 62	4 weeks
Pixantrone (aza-anthracenedione	From 6 months. Curative.	Mouse / ip	Pivotal: PND 10 – 42	4-8 weeks

Active substance (pharmacology)	Paediatric use under development*	Species/route of administration	Age of animals at treatment initiation - termination	Drug-free period
with a molecular structure similar to other topoisomerase II inhibitors such as mitoxantrone and anthracyclines like doxorubicin)			(intermittent dosing)	depending on subset
Ponatinib (tyrosine kinase inhibitor (BCR-ABL))	From 1 year. Long-term disease control.	Rat / oral	Pivotal: PND 15 – 35	4 weeks
Regorafenib (multi-target tyrosine kinase inhibitor of angiogenic (VEGFR 2/3, TIE2), stromal (PDGFR-β, FGFR) and oncogenic (C-KIT, RET and BRAF) kinases)	From 6 months. Long-term disease control.	Rat / oral	Pivotal: PND 15 – 35	4 weeks
Sonidegib (Inhibitor of the Hedgehog signalling pathway)	From 4 months Long-term disease control.	Rat / oral	DRF: PND 14 - 34 Pivotal: PND 14 - 49	8 weeks
Trametinib (specific allosteric inhibitor of MEK1 and MEK2)	From 1 month. Long-term disease control.	Rat	DRF: PND 7 – 21, PND 7 - 35 Pivotal: PND 7 – 45	6 weeks

* This reflects the "Indication(s) to be targeted by the PIP" at the time the PIP was first agreed by the PDCO; for categorization, see section 2.1.

4.2. Results from JAS performed on 20 anti-cancer medicines

As part of this project, data were analysed by **product** with information related to the mechanism of action and the paediatric development. JAS study results were available for 20[6] products (referred to as cases in the following), including 14 tyrosine and/or serine-threonine kinases inhibitors, 1 Hedgehog signalling pathway inhibitor, 4 antineoplastic agents and 1 monoclonal antibody. Written and tabulated summaries of these JAS results are provided in sections 4.4. and 4.5. A matrix of findings by medicine is in section 7.3. .

When results of the JAS were compared to the results from repeat-dose toxicity studies of similar duration in adult animals, the major observations can be summarized as follows.

Besides non-specific effects on development landmarks (*inter alia,* growth, eyes opening, age at puberty) or on reproductive organs:

- new target organ toxicities were detected for 87 products. These included (but are not limited to) effects on brain, spinal cord and peripheral nerves (navitoclax: cerebellar hypoplasia and CNS related clinical signs, sonidegib: peripheral nerve and spinal cord degeneration), kidney (dabrafenib: tubular deposits, increased incidence of cortical cysts and tubular basophilia), heart (pazopanib: abnormal growth/maturation in kidney, lung, liver and heart), gastrointestinal (GI) tract (pazopanib: dilation of Brunner's gland ducts with associated glandular atrophy and dilation of the ampulla of Vater and/or pancreatic interlobular and extrapancreatic ducts with associated epithelial hyperplasia), lung-nasal cavity-eye (trametinib: increased alveolar macrophages, mineralization, inflammatory and degenerative changes in olfactory epithelium, corneal mineralization), (thymus-spleen (ombrabulin: lymphocyte necrosis). In addition, delayed learning/memory was seen in rats treated with everolimus.
- **Increased severity of toxicity** was observed for 8 products. This was exemplified by *mortality* occurring in juvenile animals at lower exposure or after a shorter duration of treatment than in adults (4 products; lenvatinib, ombrabulin, pazopanib and sonidegib). *Increased severity* of other non-fatal toxicities was also demonstrated, with several toxicities occurring at lower exposure or after a shorter duration of treatment (6 products[8]; lenvatinib, navitoclax, pazopanib, regorafenib and trametinib). Lenvatinib showed the most pronounced differences: mortalities occurred at exposure levels ~125 times lower in PND 7 rats, or ~12 times lower in PND 21 rats, than lethal exposure in adults, and eosinophilic exudates in the choroid plexus were observed following shorter treatment duration in pups than in adults.
- In the case of 4 products (cabozatinib, cobimetinib, midostaurin and nilotinib), mortality in juvenile animals occurred at lower doses or shorter treatment duration compared to adult data and a new target organ (lung) toxicity was observed with midostaurin (haemorrhages and mixed cell infiltration). For these products, however, the **apparent increased severity in toxicities** may have been associated with higher exposure levels occurring at the start of the juvenile studies when comparing toxicokinetics (TK) at the start of the JAS with adult data.
- Importantly, **no major differences** were detected in the toxicity profiles between juvenile and adult animals for 6 products[9] (decitabine, imatinib, pixantrone and ponatinib).

[6] Detailed results and corresponding assessements for 3 medicinal products were removed from the report for reasons of confidentiality; however the total number of medicinal products assessed was mantained throughout the document.

[7] One medicinal product not specified for reasons of confidentiality.

[8] One medicinal product not specified for reasons of confidentiality.

[9] Two medicinal products not specified for reasons of confidentiality.

- Appropriate toxicokinetics (TK) parameters were available for 15 products. In 11 cases, the **exposure levels** measured in juvenile animals before PND 21 were 2 to 13 times higher than in adult animals. When exposure levels were measured at PND 35, they were in most of the cases lower than in younger or adult animals. In some cases, these very high exposures in the youngest populations hampered data interpretation because of early mortality occurring at the highest doses due to excessively high exposure at the beginning of the study (see discussion in 5.3. JAS study designs).

In 2 cases (dabrafenib and trametinib) the pivotal studies used lower starting doses followed by escalated doses from PND 22 onwards in order to achieve a relatively stable exposure throughout the course of the study.

Study designs in some cases did not allow an accurate data interpretation because either the age of the animals at the start of the treatment did not fit with the age of the patient population (PND 21 to support studies in neonates: ombrabulin), or because the duration of the study was too short (ponatinib). In several cases, dose adaptation might have allowed a more stable exposure throughout the study. In one case, the dosing regimen was different between juvenile and adult studies; however, it should be kept in mind that for example monthly dosing in adult animals would not make sense in a short term JAS.

4.3. Consequences for product development and information

JAS results had the following impact on the further paediatric development:

- **Requests for additional information** were made in 4 cases[10] (dabrafenib, navitoclax and pixantrone), which were a new JAS and a comparison of the results with adult data.
- **Inclusion of youngest paediatric age subsets** in clinical trials for 7 products[11] (dabrafenib, lenvatinib, navitoclax, ombrabulin and pixantrone) was mandated to await results of additional JAS.
- **Waivers of further paediatric trials** in children less than 2 years were granted because of safety concerns for 3 products (dabrafenib, lenvatinib and pazopanib). In another case, the company proposed a waiver below 1 year of age because of CNS toxicity (navitoclax). For dabrafenib, a waiver below 1 year of age was granted because of renal toxicity.
- Recommendations on the **design of clinical trials** were made for 4 products[12] (lenvatinib, regorafenib and trametinib); these were a staggered approach to include patients in the trials, specific monitoring and recommendations on the dosing.
- JAS results have been submitted to the CHMP for 12 products. The results of JAS were assessed by the CHMP and, for most products that were granted a marketing authorization, **reflected in the SmPC,** even when the authorised indication was limited to the adult population.
- **Recommendation against the use in children** less than 2 years of age was made in two cases (lenvatinib and pazopanib).
- In two cases (lenvatinib and everolimus), the results of the JAS lead to **specific monitoring** in the pharmacovigilance plan.

[10] One medicinal product not specified for reasons of confidentiality.

[11] Two medicinal products not specified for reasons of confidentiality.

[12] One medicinal product not specified for reasons of confidentiality.

Two products were authorised in children; results of JAS did not bring new information for imatinib. In the second case (everolimus), it was indicated in the SmPC (until long-term paediatric data became available) that the potential for risks identified in the JAS to a long-term use in children is unknown.

4.4. Written summaries of results and appraisal of JAS

For each of the following summaries, the case assessment report is available from the EMA Paediatric Medicines Office. Major findings and consequences of JAS are tabulated in section 4.5. A summary overview is in Table 3.

4.4.1. Cabozantinib

Cabozantinib is a multi-target tyrosine kinase inhibitor of c-Met and VEGFR2. A PIP was agreed for the treatment of paediatric patients from birth onwards with refractory malignant solid tumours that are associated with MET, VEGFR2 and RET pathway activation as a result of mutation, overexpression, or amplification (Fleuren et al. 2013) and for the treatment of advanced or metastatic medullary thyroid cancer.

Outcome of the evaluation[13]: At the same dose levels, exposure in juvenile animals aged PND 21 and older was of the same order of magnitude compared to the adult animals. In younger pups exposure levels were 2 to 4 times higher (data collected during the dose range finding study).

Two definitive juvenile studies were performed starting either at PND 21 (0.3, 1, or 2 mg/kg) or at PND 12 (1, or 2 mg/kg). Similar target organs and/or toxicities were observed in both the juvenile studies and the adult studies.

When cabozantinib was dosed orally at 2 mg/kg/day to juvenile rat cohorts (dose intervals: PND 12-35 or 12-70) treatment related unscheduled deaths were observed.

In the previously conducted definitive juvenile toxicity study, cabozantinib dosed orally at 2 mg/kg/day to slightly older juvenile rat cohorts (dose intervals: PND 21-35 or 21-70) resulted in no mortalities, but in otherwise similar test article-related toxicity as observed in the PND 12 cohorts.

No test article related mortalities were observed in the 1 mg/kg/day cohorts in either study.

These results suggest that the juvenile rat from the PND 12 cohorts (correlating to a < 2-year paediatric population) may be more sensitive to cabozantinib-related toxicity than are the juvenile rat dosed from PND 21 (correlating to a > 2-year paediatric population). Higher exposure levels may also have contributed to apparent increased sensitivity.

Outcome of review by CHMP/PDCO: The results of the juvenile study were reviewed by the CHMP and reflected in the SmPC (section 5.3) mentioning:

"Juvenile rats (comparable to a >2-year old pediatric population) administered cabozantinib showed increased WBC parameters, decreased haematopoiesis, pubescent/immature female reproductive system (without delayed vaginal opening), tooth abnormalities, reduced bone mineral content and density, liver pigmentation and lymph node lymphoid hyperplasia. Findings in uterus/ovaries and decreased haematopoiesis appeared to be transient, while effects on bone parameters and liver pigmentation were sustained. Juvenile rats (correlating to a <2 year pediatric population) showed similar treatment-related findings, but appeared to be more sensitive to cabozantinib-related toxicity at comparable dose levels".

[13] Outcome of the evaluation= outcome of the evaluation for this project

Following review of the second juvenile study PDCO endorsed the statement that based on the dose-related and generally manageable toxicity observed at the 1 mg/kg/day dose level with the PND 12 cohorts, it would appear that paediatric populations <2-years of age may be able to receive cabozantinib without risk of significant toxicity; however, a lower starting dose than in paediatric populations > 2-years of age would be required.

Lessons learnt:

These data showed an increased exposure levels and an apparent increased sensitivity to cabozantinib in the youngest rat (PND < 12) populations which emphases the importance of the adequacy of JAS study designs to support the paediatric population.

4.4.2. Cobimetinib

Cobimetinib is a tyrosine kinase inhibitor of MEK. A PIP was agreed for the treatment of paediatric patients from 6 months onwards with solid malignant tumours.

Outcome of the evaluation: At the same dose levels, exposure in juvenile animals (PND 10) was increased compared to the animals on PND 38 or adult animals. The difference in exposure was of the order of 2- to 11-fold and 3-to 4-fold, respectively.

At the same dose levels, exposure in PND 38 animals was decreased compared to the adult animals. The difference in exposure was of the order of 2-fold.

Mortalities in juvenile rats were observed at a lower dose than in adult animals, probably due to the higher exposure at the beginning of the study. On the other hand, in juvenile rats, cobimetinib administration resulted in similar toxicological findings as seen in the pivotal toxicity studies in adult animals.

Outcome of the review by CHMP/PDCO: The PDCO reviewed the results and no action was taken. The results of the juvenile study were reviewed by the CHMP and reflected in the SmPC (section 5.3) mentioning that cobimetinib systemic exposures were higher on post-natal day 10 than on post-natal day 38, and that in juvenile rats, cobimetinib administration resulted in similar changes as seen in the pivotal toxicity studies in adult animals, although mortality occurred at a lower dose than in adult animals.

Lessons learnt: As an increased exposure was observed in juvenile animals at the start of dosing, a dose adjustment approach with a progressive increase according to exposure would have been more suitable for the pivotal study.

4.4.3. Dabrafenib

Dabrafenib is a serine/threonine kinase inhibitor of BRAF and angiogenic TK (TIE2), SIK 1 and SIK 2. A PIP was initially agreed for the treatment of adolescent patients from 12 years of age with melanoma containing BRAF V600 activating mutations and for the treatment of paediatric patients from 1 month onwards with solid malignant tumours containing BRAF V600 activating mutations.

Outcome of the evaluation: At the same dose levels, exposure in juvenile animals (PND 7, PND 22) was 2-4 times higher compared to PND 35 and adult animals. Dose levels were escalated on PND 22 in an effort to maintain consistent exposures for the remaining duration of the dosing. Novel target organs which are kidney and bone were observed in juvenile animals. Severe and irreversible kidney toxicity (lesions containing dabrafenib and a metabolite) observed at all doses in rats treated from PND

7 or PND 22 was most likely associated with kidney function maturation. In addition, a 2- to 4-day earlier vaginal opening was observed.

Outcome of the review by CHMP/PDCO: The CHMP reviewed the results of the JAS and the conclusions were reflected in the SmPC. It was mentioned that the studies in juvenile animals have shown effects of dabrafenib which had not been observed in adult animals and that the safety and efficacy of dabrafenib have not yet been established in children (Section 4.2). The toxicities, that is, effects on growth (shorter long bone length), renal toxicity (tubular deposits, increased incidence of cortical cysts and tubular basophilia and reversible increases in urea and/or creatinine concentrations), testicular toxicity (degeneration and tubular dilation) and earlier vaginal opening, were listed (Section 5.3). Paediatric effects are classified as important potential risks.

After review of the results by the PDCO, an additional juvenile study was planned in the PIP to further evaluate the renal toxicity and to reconsider the inclusion of paediatric patients less than 1 year of age in clinical trials, which had initially been deferred until it would be supported by the results of the additional juvenile animal toxicity study. Subsequently, a waiver of studies in children less than 1 year of age was granted as dabrafenib is likely unsafe in patients of this age, however uncertainties remain regarding the use from this age onwards.

Lessons learnt: Novel target organs were observed, i.e. kidney and bone. This observation cannot be explained by unexpectedly high plasma levels of dabrafenib, especially as the kidney lesions were observed at the low dose of the juvenile study, at and below clinically relevant exposure levels. Nephrogenesis is complete at birth in human but not in rat up to PND 14. The complete maturation occurs up to 6 weeks after birth in rats and up to 6 months to 1 year in children.

4.4.4. Decitabine

Decitabine is a cytidine nucleoside analogue inducing DNA demethylation by inhibition of DNA methyl transferases. Decitabine induces re-expression of silencing genes that inhibit cellular proliferation and induce cellular differentiation. A PIP was agreed for the treatment of paediatric patients from 1 month onwards with AML who have high-risk cytogenetics, or are refractory to, or have a relapse after first-line treatment.

Outcome of the evaluation: Similar target organs and/or toxicities were observed in both the juvenile and the adult animal studies. Although the effects on the male reproductive organs could have been predicted from the adult animal studies and the pharmacology of the product, this juvenile study was informative with respect to the reversibility of the changes that are induced when exposing animals before the sexual maturation (functional capacity of the testes had not been severely compromised but rather caused a delay in testis and sperm maturation and in the age of attainment of sexual maturity).

Limited toxicokinetics data in adult rats were available. However, based on toxicokinetics data at different ages from the juvenile rat study and from a single dose TK study in juvenile rats, it appears that exposure does not differ significantly as a function of age.

Outcome of the review by CHMP/PDCO: The results of the juvenile study were reviewed by the CHMP and reflected in the SmPC (section 5.3) mentioning that neurobehavioural development and reproductive capacity were unaffected when neonatal/juvenile rats were treated at dose levels inducing myelosuppression.

Lessons learnt: Although the effects on the male reproductive organs could have been predicted from the adult animal studies and the pharmacology of the product, the JAS was informative with respect to the reversibility of the changes to the male reproductive organs when exposing animals before the

sexual maturation. The design of the JAS had very well anticipated to this, by ending administration at the age of PND 35 and including a recovery period until PND 70.

Interestingly as well, because of the need to apply intermittent administrations due to the toxicity of the product and in order to mimic clinical dosing regimens, the study included two cohorts, ensuring that each age-window had been covered in one of both cohorts.

4.4.5. Everolimus

Everolimus is a serine/threonine kinase inhibitor of mTOR. A PIP was agreed for the treatment of SEGA (subependymal giant cell astrocytoma) in paediatric patients from birth with tuberous sclerosis complex.

Outcome of the evaluation: At the same dose levels, exposure in juvenile animals (PND 7) was increased compared to the adult animals. The difference in exposure was of the order of 3- to 5-fold in males and 7- to 11-fold in females.

Apparent increased severity of toxicity was observed in juvenile animals, as evidenced by lenticular toxicity occurring at lower exposure levels. However, this effect appears to be rat-specific.

In addition, novel toxicities or target organs were observed in juvenile animals. These were delays in attainment of developmental landmarks (eye opening, sexual maturation) in males and females and increased latency time in learning and memory in the water maze test in males.

Outcome of the review by CHMP/PDCO: The CHMP reviewed the results of the study. In order to evaluate the developmental delay observed in the juvenile rats, which is classified as an important potential risk, routine pharmacovigilance activities include a detailed review in the PSUR and additional activities such as a targeted follow-up of all serious spontaneous reports, serious post-marketing surveillance study reports, and serious reports from other programs where data are being handled as solicited and all clinical trial serious adverse events reports, using a targeted event questionnaire / checklist. The risks identified in the JAS which were developmental delays and lens findings were reflected in the SmPC (Votubia, sections 4.2 and 5.3):

"In juvenile rat toxicity studies, systemic toxicity included decreased body weight gain, food consumption, and delayed attainment of some developmental landmarks, with full or partial recovery after cessation of dosing. With the possible exception of the rat-specific lens finding (where young animals appeared to be more susceptible), it appears that there is no significant difference in the sensitivity of juvenile animals to the adverse reactions of everolimus as compared to adult animals. Toxicity study with juvenile monkeys did not show any relevant toxicity."

"The potential for growth/developmental delays with long-term treatment in SEGA patients is unknown".

Lessons learnt: The results of the JAS impacted the pharmacovigilance plan.

4.4.6. Imatinib

Imatinib is tyrosine kinase inhibitor, with specificity for the Bcr-Abl, PDGFR, and c-Kit tyrosine kinases. A PIP was agreed for the treatment of paediatric patients with newly diagnosed Philadelphia chromosome (BCR-ABL translocation)-positive acute lymphoblastic leukaemia integrated with chemotherapy after induction therapy. A second PIP was agreed for the development of imatinib in pulmonary arterial hypertension (PAH) in paediatric patients from 6 months onwards for which a JAS was requested.

Outcome of the evaluation: At the same dose levels, exposure in juvenile animals decreased from PND 10 to PND 64 (2- to 4-fold), then started to increase throughout adulthood (comparison with adult exposure data collected on day 28 ofthe 26-week adult toxicity study). No new target organ was identified in the JAS with respect to the known target organs in adult rats, but at higher doses the treatment affected growth (effects on: body weight, sexual maturation, crown to rump length and slightly lower values for the length and/or width of the femur and tibia noted at 60 mg/kg/day at the end of the treatment period. Femur and tibia effects were still apparent at the end of recovery; there were no adverse effects on bone mineral density or histomorphometry of the tibia).

Outcome of review by CHMP/PDCO: The results of the juvenile study were reviewed by the CHMP and reflected in the SmPC (section 5.3): "*No new target organs were identified in the rat juvenile development toxicology study (day 10 to 70 postpartum) with respect to the known target organs in adult rats. In the juvenile toxicology study, effects upon growth, delay in vaginal opening and preputial separation were observed at approximately 0.3 to 2 times the average paediatric exposure at the highest recommended dose of 340 mg/m^2. In addition, mortality was observed in juvenile animals (around weaning phase) at approximately 2 times the average paediatric exposure at the highest recommended dose of 340 mg/m^2*".

Lessons learnt: At exposure levels close to expected paediatric exposure, treatment effects in juvenile rats have induced growth and sexual maturation delays.

4.4.7. Lenvatinib

Lenvatinib is a split-kinase inhibitor of VEGFR1 (FLT1) and VEGFR2 (KDR). A PIP was agreed for the treatment of children from birth with 131I-refractory follicular or papillary thyroid carcinoma and for the treatment of children from birth with a first relapse of osteosarcoma.

Outcome of the evaluation: At the same dose levels, exposure in very young animals (PND 7) was the same as in adults but exposure in young animals (PND 21) was decreased compared to the very young and adult animals due to higher metabolic rate around weaning age. The difference in exposure was of the order of 2-fold.

Similar target organs and/or toxicities were observed in both the juvenile and the adult animal studies.

Apparent increased severity of toxicity was observed in juvenile animals, as evidenced by mortality occurring at lower exposure levels. The difference in sensitivity was of the order of 125-fold and 12-fold in term of dose and exposure (AUC) for rats treated from PND 7 or PND 21 respectively.

Apparent increased severity of toxicity was observed in juvenile animals, as evidenced by toxicity occurring following shorter treatment duration (eosinophilic exudate in the blood vessel of choroids plexus in the brain).

Outcome of the review by CHMP/PDCO: After review of the DRF JAS results by the PDCO, a waiver was also agreed for children younger than 2 years of age with osteosarcoma since mortality was observed at doses significantly lower than in adults when pups were dosed from PND 7 and the initiation of the clinical trials in children below 12 years of age was deferred and should be considered after the assessment of the results of the definitive study. Furthermore, in order to monitor and ensure a safe exposure in younger children from 2 years onwards, measures were added such as the monitoring of skeletal bone age or the monitoring of risks associated with cardiovascular and renal system in the paediatric clinical trial. Additionally, after review of the definitive JAS results by the PDCO, further new measures were added for the monitoring of skeletal growth and the risks associated with renal system and with the gastrointestinal tract.

The results were reviewed by the CHMP and a recommendation against the use in children below 2 years of age was made (sections 4.2 of the SmPC). Furthermore, the effects seen in the JAS were listed in the SmPC (section 5.3) mentioning mortality for dosing initiated at PND 7 and at PND 21 that were respectively 125- or 12-fold lower compared with the exposure at which mortality was observed in adult rats, as well as growth retardation, secondary delay of physical development, and lesions attributable to pharmacologic effects (incisors, femur [epiphyseal growth plate], kidneys, adrenals, and duodenum).

Lessons learnt: Mortality was the dose-limiting toxicity in juvenile rats and the results clearly suggest an increasing sensitivity to toxicity with decreasing age. Though mortality was attributed to complications related to primary duodenal lesions, additional toxicities to immature target organs may also have contributed.

There are remaining uncertainties with respect to the exact aetiology and hence the extrapolation to children of the increased toxicity risk. It is therefore unknown until what corresponding age in children this increased sensitivity may persist, and caution is needed in the absence of clinical data.

4.4.8. Midostaurin

Midostaurin is a multi-target tyrosine kinase inhibitor of several PKC isoforms, of the VEGFR and of the class III tyrosine protein kinases FLT3 and KIT. A PIP was agreed for treatment of paediatric patients from 3 months onwards with FLT3 mutated AML, newly diagnosed or in first relapse.

Outcome of the evaluation: At the same dose levels, exposure in juvenile animals was increased by a factor of 5 to 8 in the younger pups (PND 7) compared to PND 70 rats and animals from the adult animal studies.

Apparent increased severity of toxicity in younger pups (PND 7), which is mortality, was observed in the dose range finding study following 1 or 2 administrations at doses causing no such effects in adult animals even after chronic administration. This is most likely due to higher exposure levels in very young pups.

A novel target organ, which is lung, was observed in juvenile animals. On the other hand, adverse effects on female or male reproductive organs, as seen in repeated dose toxicity and fertility studies, were not observed in the juvenile study.

Outcome of the review by CHMP/PDCO: The results of the juvenile study have not yet been reviewed by these scientific committees.

Lessons learnt: Toxicokinetics could have been obtained from the DRF study. As excessive toxicity, probably linked to high exposure, was observed in the young rats (mortality), a dose-adjustment approach would have been more suitable for the pivotal study (e.g., using a lower starting dose and then escalate).

The lung toxicity is not readily explained, although a link to VEGFR mediated pulmonary hypertension and thromboembolic events is known. Effects on reproductive organs were observed in adult animals at high exposure levels not achieved during the JAS, which may explain why similar effects have not been observed in juvenile animals.

4.4.9. Navitoclax

Navitoclax binds to multiple anti-apoptotic Bcl-2 family proteins including Bcl-XL, Bcl-2, Bcl-w, and Bcl-B. A PIP was agreed for the treatment of ALL, NHL in children from 1 month onwards.

Outcome of the evaluation: At the same dose levels, exposure in juvenile animals at PND 7 was higher compared to PND 60 and adult animals. The difference in exposure was of the order of 2.5- to 3-fold.

Apparent increased severity of toxicity was observed in juvenile animals, as evidenced by toxicity occurring at lower exposure levels.

Novel toxicities or target organs were observed in juvenile animals. While adult rat studies did not reveal any CNS effects, juvenile rats had lower brain weights, associated with cerebellar hypoplasia at exposure levels in the estimated human efficacious range. At higher exposures, CNS related clinical signs were also observed, in addition to necrosis of the basal ganglia, primarily affecting the globus pallidus.

Outcome of the review by CHMP/PDCO: In order to characterize the brain toxicity seen in juvenile animals, the PDCO requested the applicant to provide additional data (comparison between juvenile and adult rats regarding brain concentration, penetration, mode of transport across the blood-brain-barrier, Bcl-2 protein expression in developing brain) before the initiation of the paediatric clinical trials. No paediatric trial was started so far.

Lessons learnt: The relevance of the novel toxicities observed in JAS to humans cannot be excluded in view of the involvement of Bcl-2 protein in brain development and the clinically relevant exposure levels at which the brain effects occurred in the juvenile rat. Additional data should be produced to further characterize the juvenile brain toxicity. No brain exposure data were provided for juvenile rats. Considering that navitoclax is a P-gp substrate (Vogler et al. 2011), its brain concentration may be expected to be low in adults (as observed in adult rats), but may be assumed to be higher in juvenile rats below 1 month of age and in children below 6 months of age because of P-gp immaturity.

Of note, a modification of the agreed PIP was requested to change the age cut-off of the waiver from less than 1 month to less than 1 year of age due to safety concern (brain toxicity). The PDCO however concluded that additional data were needed to agree on this proposal and implemented requirements for additional non-clinical data to be provided before the first paediatric trial may be initiated.

4.4.10. Nilotinib

Nilotinib is a multi-target tyrosine kinase inhibitor of BCR-ABL, PDGF alpha and beta, c-kit, colony stimulating factor-1R (CSF-1R), discoidin domain receptor (DDR) and several of the ephrin receptor kinases, but not inhibiting the majority of other protein kinases such as Src. A PIP was agreed for the treatment of paediatric patients with Ph+ CML in children from 1 year onwards.

Outcome of the evaluation: At the same dose levels, exposure in very young animals (PND 7) was increased compared to the young (PND 70) and adult animals. The difference in exposure was of the order of 2- to 13-fold when compared to young animals and of the order of 2- to 10-fold when compared to adult animals.

Similar target organs and toxicities were observed in both the juvenile and the adult animal studies.

Apparent increased severity of toxicity that is, mortality, was observed in the DRF juvenile rat study. As no exposure levels were available from the DRF study, it can be assumed (based on high exposure levels at PND 7 in the pivotal juvenile study) that the exposure levels in the DRF where mortality and moribundity occurred were much higher than the exposure measured in the pivotal juvenile and adult studies. No necropsy findings were considered related to the administration of nilotinib. The cause of death could not be determined for those animals found dead during the DRF study.

Outcome of the review by CHMP/PDCO: The results of the juvenile study were reviewed by the CHMP and reflected in the SmPC (section 5.3) mentioning that a reduction in body weight in both genders and delayed preputial separation in males were observed and that the juvenile animals did not exert increased sensitivity to nilotinib relative to adults and that the toxicity profile in juvenile rats was comparable to that observed in adult rats. JAS results were available before a paediatric trial was expected to start.

Lessons learnt: The availability of TK data from the DRF study could have been useful for the interpretation of the observed mortalities in this study.

4.4.11. Ombrabulin

Ombrabulin is an inhibitor of tubulin polymerization and a vascular disrupting agent. A PIP was agreed for the treatment of paediatric patients from birth with metastatic or locally advanced rhabdomyosarcoma soft tissue sarcoma and for the treatment of paediatric patients from birth with relapsed/progressive non- rhabdomyosarcoma soft tissue sarcoma.

Outcome of the evaluation: Apparent increased severity of toxicity was observed in juvenile animals (dogs), as evidenced by toxicity (mortality, myocardial necrosis/fibrosis, gallbladder necrosis, degenerative lesions in testes and epididymides) occurring at lower dose levels (and probably at lower exposure levels).

In addition, mortality was observed in juvenile animals following a single ombrabulin administration at doses not causing such an effect in adult animals, even following repeated cyclic administration.

Novel toxicities, such as lymphocyte necrosis in the thymus and the spleen, were also observed in juvenile animals.

Limited toxicokinetics data were available. Furthermore, the dosing frequency in juvenile studies (weekly infusions) was different from the adult studies (one infusion every three weeks) which made the comparison difficult.

Outcome of the review by CHMP/PDCO: Results of the juvenile study were reviewed by the PDCO. Toxicities observed (deaths) were deemed related to the pharmacology of the drug, which acts on tumours but also on normal tissues, which is also the reason why the safety margins are narrow. The design of the second JAS, a 6-months repeat-dose toxicokinetic study to evaluate the deaths observed in the first study, was transformed into a single-dose administration as the PK data collected during the first JAS were not deemed reliable due to a bioanalytical issue. Results of the second study are awaited, and required to be supportive, before the initiation of paediatric clinical trials. No paediatric trial was started so far.

Lessons learnt: The dosing regimen in JAS should have been aligned with the regimen in the corresponding adult study. To note: The development of ombrabulin was discontinued due to unfavourable results in three adult indications (sarcoma, ovarian and lung cancer).

4.4.12. Pazopanib

Pazopanib is a multi-target tyrosine kinase inhibitor of VEGFR-1, -2 and -3; PDGFR-α and β; c-KIT. A PIP was agreed for the treatment of rhabdomyosarcoma (RMS), non-rhabdomyosarcoma soft tissue sarcoma (NRSTS) or Ewing sarcoma family of tumours (ESFT) in children from 1 year onwards.

Outcome of the evaluation: At the same dose levels, exposure in juvenile animals at the highest doses of 100 or 300 mg/kg was slightly increased compared to the adult animals.

Apparent increased severity of toxicity was observed in juvenile animals treated from PND 9 compared to PND 21, as evidenced by mortality occurring at lower exposure levels and following shorter treatment duration. Increased severity of toxicity to teeth was also observed. The difference in sensitivity was of the order of 2-fold for mortality and of the order of 5-fold for the toxicity of the teeth.

In addition, toxicity to the adrenals and bone was observed in juvenile animals following shorter treatment duration compared to older animals.

Furthermore, different novel toxicities or target organs were observed in juvenile animals. These were in rats dosed from PND 21 dilation of Brunner's gland ducts with associated glandular atrophy and dilation of the ampulla of Vater; pancreatic interlobular and extrapancreatic ducts with associated epithelial hyperplasia (findings in Brunner's gland not reversible); in rats dosed from PND 9 abnormal organ growth/maturation in kidney, lung, liver and heart.

Outcome of the review by CHMP/PDCO: The results were reviewed by the CHMP and a recommendation against the use in children below 2 years of age was made (sections 4.2 and 4.4 of the SmPC). Furthermore, the effects seen in the JAS were listed in the SmPC (section 5.3) mentioning mortalities and abnormal growth/maturation in kidney, lung, liver and heart observed in very young rats (PND 9 to 14) at exposures below clinical anticipated exposures and a similar toxicological profile as the adults in young rats (PND 21 to 62). Furthermore, increased risk for bone and teeth effects in human paediatric patients is indicated.

The results were reviewed by the PDCO and a waiver in children less than 2 years was granted for lack of safety (their inclusion had initially been requested for the second paediatric trial). The results also suggested inter-patient variability for PK parameters (especially in younger patients) and enabled to provide considerations for the calculation of dosing parameters.

Lessons learnt: The JAS revealed safety concerns relevant for youngest children and provided grounds to exclude this specific age subset from drug development.

4.4.13. Pixantrone

Pixantrone is an antineoplastic agent, next generation anti-tumour aza-anthracenedione with a molecular structure related to other topoisomerase II inhibitors such as mitoxantrone and anthracyclines like doxorubicin. A PIP was agreed for the treatment of non-Hodgkin lymphoma in paediatric patients from 6 months onwards.

Outcome of the evaluation: Similar target organs and toxicities were observed in both the juvenile and adult mouse studies.

While no precise comparison is feasible because of the lack of exposure data in adult mouse studies and the different dosing regimen, it can at least be concluded that cardiotoxicity is not significantly higher in juvenile animals.

Outcome of the review by CHMP/PDCO: The PDCO considered the juvenile study and the sudden death evaluation should be awaited before inclusion of children less than 12 years of age in the first trial. The PDCO also considered that more evidence from adults should be sought to understand the potential of the medicine, in particular with respect to improved cardiac safety compared to anthracyclines and considered that the initiation of the paediatric clinical studies should await a positive benefit/risk assessment and a trend to reduce cardiotoxicity.

Lessons learnt: The dosing regimen in JAS should have been aligned with the regimen in the corresponding adult study.

4.4.14. Ponatinib

Ponatinib is a tyrosine kinase inhibitor of BCR-ABL, as well as mutated forms of the protein that cause resistance, including the T315I "gatekeeper" mutant that causes resistance to TKI inhibitors such imatinib, nilotinib and dasatinib. A PIP was agreed for the treatment of paediatric patients from 1 year onwards with chronic (CP), accelerated (AP) or blast phase (BP) CML, whose CML is resistant or are intolerant to prior tyrosine kinase inhibitor (TKI) therapy as well as for the treatment of paediatric patients from 1 year onwards with Ph+ ALL, whose ALL is resistant or who are intolerant to prior TKI therapy.

Outcome of the review by CHMP/PDCO: Results of the JAS have been reviewed by the CHMP and reflected in the SmPC (section 5.3) mentioning that, mortality related to inflammatory effects was observed in animals treated with 3 mg/kg/day, and reductions in body weight gain were observed at doses of 0.75, 1.5 and 3 mg/kg/day during the pre-weaning and early post-weaning treatment phases and that ponatinib did not adversely affect important developmental parameters in the juvenile toxicity study.

Outcome of the evaluation: At the same dose levels, exposure in juvenile PND 15 animals was higher compared to the adult animals. The difference in exposure was of the order of 2- to 3-fold, when comparing to adult animals of a separate study and 8- to 9-fold when comparing to PND 35 animals from the juvenile study. Exposure in PND 35 animals was decreased compared to adult animals (3- to 4-fold). The decrease in exposure between PND 15 and PND 35 is likely due to maturation of metabolism over this time period. Dose adaptation was not included during the study to account for the change in exposure and doses in the juvenile animal study were limited by mortality.

Bone lesions are seen in general toxicity studies in adult rats , but not in juvenile rats, but it may well be that these effects were not pronounced because of the lower exposure levels in juvenile animals.

Kidney findings observed in adult rats were considered to be exacerbation of normal age-related nephropathy, which has been well described in rats, and are thus not considered relevant to human risk assessment, and would not be expected in the juvenile animals.

No new toxicities were observed in juvenile animals.

Lessons learnt: Doses were limited by tolerability (mortality) in preweaning animals, likely due to the increased exposure in pre-weaning rats, relative to the and decrease in exposure post-weaning. Effects on bone seen in adults were not observed in juvenile animals, which might be due to a higher exposure in adult animals. Effects on kidney seen in adults were not observed in juvenile animals, which might be due to normal age-related nephropathy.

The juvenile rats appeared to be more sensitive to ponatinib toxicity than adult rats. This increased sensitivity may have been due to high plasma drug concentrations observed in preweaning rats.

The lack of dose adaptation during the juvenile study makes it difficult to evaluate the relative sensitivity of juvenile rats to effects of the compound previously observed in general toxicity studies, particularly bone findings.

4.4.15. Regorafenib

Regorafenib is a multi-target tyrosine kinase inhibitor of angiogenic (VEGFR 2/3, TIE2), stromal (PDGFR-β, FGFR) and oncogenic (C-KIT, RET and BRAF) kinases. A PIP was agreed for the treatment of paediatric patients from 6 months onwards with a solid malignant tumour integrated with anti-cancer therapy.

Outcome of the evaluation: At the same dose levels, exposure in juvenile animals (PND 15) was the same order of magnitude than in adult animals. Similar target organs and/or toxicities were observed in both the juvenile and the adult animal studies. However, as toxicities occurred at lower exposure, the data point out to increased sensitivity of juvenile animals. Interestingly, however, the juvenile toxicity study showed that delayed development of sexual organs and impaired growth of teeth and bone was only partially or not reversible, respectively. Moreover, these effects were observed at clinically relevant exposure levels.

Outcome of review by CHMP/PDCO: The results of the juvenile study were reviewed by the CHMP and reflected in the SmPC (section 5.3) to clarify that alterations of teeth and bones and adverse effects in the reproductive system were more pronounced in young and growing animals as well as in juvenile rats and indicate a potential risk for children and adolescents.

Lessons learnt: The juvenile toxicity study showed, apart from the known toxicity in adults, that paediatric patients may also be more susceptible to adverse effects in bone, teeth and development of sexual organs. It should be noted that the major metabolites in rats are only present in trace amounts in humans. As the metabolites could be (partially) responsible for the adverse effects observed, relevance for humans is uncertain.

4.4.16. Sonidegib

Sonidegib is a Hedgehog signalling pathway inhibitor via a Smoothened (SMO) antagonism. A PIP was agreed for the treatment of paediatric patients from 4 months onwards with hedgehog pathway-activated medulloblastoma.

Outcome of the evaluation: In the juvenile toxicity study, similar toxicities have been seen as in adult animals, with the exception of a non-reversible degeneration of the sciatic nerve and spinal cord observed in juvenile rats, only. Target organs in juvenile rats also included male reproductive tissues and hair follicles, which were not reported in the adult rat studies but were similar to effects in adult dogs described previously. In addition, delayed physical sexual development (vaginal opening and preputial separation) was observed.

At the same dose levels, exposure in juvenile animals (PND 14) was similar to adult animals.

Apparent increased severity of toxicities, that is mortality, was observed in juvenile animals, as evidenced by mortalities occurring at lower exposure levels. The cause of death was not determined. The difference in sensitivity was of the order of 4- to 6-fold.

Outcome of the review by CHMP/PDCO: The JAS results were reviewed by CHMP and the nerve findings were listed as a new toxicity in the EPAR, however the results were not summarised in the SmPC.

Lessons learnt: In juvenile rats, the sciatic nerve and spinal cord findings may have been due to the early bone growth plate closure resulting in growth cessation causing compression on the still growing nerves and spinal cord.

The findings on the male reproductive tract, hair follicles and sexual development - although not observed in the adult rat study - were in line with the pharmacological mechanism of action on developmental pathways and were not unexpected given that these target organs had already been identified in dog studies. This underpins the importance of all available data (including pharmacodynamics) for the interpretation of juvenile animal findings.

4.4.17. Trametinib

Trametinib is a multi-target tyrosine kinase inhibitor of MEK1 and MEK2. A PIP was agreed for the treatment of paediatric patients from 1 month onwards with solid malignant tumours (excluding melanomas) with known or expected RAS, RAF, or MEK pathway activation and for the treatment of adolescent patients with melanoma containing BRAF V600 activating mutations.

Outcome of the evaluation: At the same dose levels, exposure in pre-weaning (PND 13) juvenile animals was increased compared to the adult animals. The difference in exposure was of the order of 5- to 12-fold (comparison could be made for one dose only). Exposure levels in post-weaning animals were in the same range as adult animals at the same dose level. This was taken into account in the design of the pivotal juvenile toxicity study since doses were escalated on PND 22 to maintain constant exposure levels.

Apparent decreased severity of toxicities, that are mortality and skin toxicity, was observed in juvenile animals, as evidenced by toxicities occurring at higher exposure levels (or not occurring) than in adults.

Apparent increased severity of toxicities, that are bone and kidney, was observed in juvenile animals, as evidenced by more severe toxicities occurring at similar or lower exposure levels. The difference in sensitivity was of the order of 2.1- to 3.5-fold for bone toxicity and at least 3.5-fold for kidney toxicity.

Apparent increased severity of toxicities was observed in juvenile animals, as evidenced by toxicities (bones and mineralization of soft tissues) occurring following shorter treatment duration.

Novel toxicities or target organs (heart, lung, eye, Harderian gland, nasal cavity/sinuses) were observed in juvenile animals, as well as delayed sexual maturity in females.

Outcome of the review by CHMP/PDCO: In order to characterize the potential effects on sexual maturation seen in juvenile animals from the DRF study, the PDCO suggested the applicant to conduct the subsequent pivotal juvenile rat toxicity study (PND 10 to PND 45) to cover the relevant periods of sexual maturation.

Considering the high exposure levels observed in the youngest rats and that the NOAEL was not identified in the juvenile rats, the PDCO recommended to adopt an age staggered approach for the inclusion of paediatric patients in clinical trials with particular attention to the starting dose and to include parameters to monitor adverse effects on the bone.

Lessons learnt: The increased exposure in pre-weaning rats was taken into account to optimise the design of the pivotal study. Doses were escalated in the pivotal study to keep a constant exposure level.

4.5. Major findings and consequences of JAS on development and / or use in children

Table 2: Findings in juvenile compared to adult animals and regulatory actions taken after review by Scientific Committees

Active substance	**Difference in exposure at similar dose**	**Novel toxicities/ target organs**	**Difference in severity of toxicities**	**Data missing/not clear to conclude / uncertainties**	**Comments / regulatory and/or development impact of JAS results**
Cabozantinib	At the same dose levels, exposure in JA > PND 21 was comparable to adult animals. In younger pups exposure was higher than in older rats.		In pups < PND 21, mortality occurred at lower exposure than in older rats		The results of the juvenile studies were reviewed by the CHMP and reflected in the SmPC. JAS was ongoing when PIP application was reviewed. Second but not first trial to be open from birth onwards.
Cobimetinib	3- to 4- fold increase on PND 10 2-fold decrease on PND 38 compared to exposure in adult animals		Mortalities at lower doses compared to adult animals	Variability in exposures pre-/post-weaning	The results of the juvenile study were reviewed by the CHMP and reflected in the SmPC. Mortalities were observed at lower doses in juvenile animals but the available data do not allow exposure comparison. The PDCO identified growth and neurodevelopmental long-term concerns. At the time of the start of paediatric trials, JAS results were already available.
Dabrafenib	2- to 4-fold increase in PND 7 and PND 22 animals compared to PND 35 or adult animals	Kidney, Bone			PDCO: An additional juvenile study was planned in the PIP to further evaluate the renal toxicity and the inclusion of paediatric patients less than 1 year of age

Active substance	Difference in exposure at similar dose	Novel toxicities / target organs	Difference in severity of toxicities	Data missing / not clear to conclude / uncertainties	Comments / regulatory and / or development impact of JAS results
					in clinical trials was initially deferred. Subsequently, studies in children less than 1 year of age were waived for safety reasons. The results of the juvenile study were reviewed by the CHMP and reflected in the SmPC. Risk management plan: paediatric effects specified as important potential risks
Decitabine				Lack of TK data in comparative adult study	The results of the juvenile study were reviewed by the CHMP and reflected in the SmPC.
Everolimus	Increase in younger rats (PND 7): 3- to 5-fold increase in males; 7- to 11-fold increase in females	Delays in development landmarks (eye opening, sexual maturation) Increased latency for learning and memory in males	Lenticular toxicity at lower levels		The results of the juvenile study were reviewed by the CHMP and reflected in the SmPC. PV: developmental delay considered as important potential risk included in routine PV activities.
Imatinib		No new target organs, but effects on growth, delay in vaginal opening			The results of the juvenile study were reviewed by the CHMP and reflected in the SmPC.

Active substance	Difference in exposure at similar dose	Novel toxicities / target organs	Difference in severity of toxicities	Data missing / not clear to conclude / uncertainties	Comments / regulatory and / or development impact of JAS results
		and preputial separation around paediatric clinical exposure, mortality at approx. 2 times the paediatric clinical exposure.			
Lenvatinib	Exposure in very young animals (PND 7) was the same as in adults but exposure in young animals (PND 21) was decreased compared to the very young and adult animals. The difference in exposure was of the order of 2-fold.		Mortality at lower exposure (125- and 12-fold in rats from PND 7 and 21) Toxicity (eosinophilic exudate in blood vessel of choroid plexus) after shorter treatment duration		PDCO: Waiver below 2years of age Required additional monitoring during clinical studies The results of the juvenile study were reviewed by the CHMP and reflected in the SmPC: Recommendation against use in children less than 2 years of age Results of the juvenile studies reported in the SmPC Risk management plan: Bone and teeth abnormalities in the paediatric population considered important potential risk

Active substance	Difference in exposure at similar dose	Novel toxicities/ target organs	Difference in severity of toxicities	Data missing/not clear to conclude / uncertainties	Comments / regulatory and/or development impact of JAS results
Midostaurin	Exposure 5- to 8-fold higher in PND 7 pups compared to PND 70 animals and adult animals	Lung	Acute mortality in young pups (PND 7) at doses not causing such effect in adult animals. No effects on reproductive organs although seen in adult repeated dose and fertility studies.	Mortality probably attributable to very high exposure.	Not yet reviewed by CHMP.
Navitoclax	Exposure 2.5- to 3-fold higher in very young rats (PND 7) compared to PND 60 or adult rats	Lower brain weight, cerebellar hypoplasia - Necrosis of basal ganglia, CNS-related clinical signs	Toxicity at lower exposure and/or following shorter exposure		PDCO: A modification of the agreed PIP was requested by the applicant to change the age cut-off of the waiver from less than 1 month to less than 1 year of age due to safety concern (brain toxicity). The PDCO however concluded that additional data / information was needed to agree this request. JAS results were available before a paediatric trial was expected to start. No paediatric trial was started so far.
Nilotinib	2- to 13- and 2- to 10-fold increase in very young rats (PND 7) compared to young (PND 70) and adult		Mortality in young pups (PND 7) at doses not causing such effect in adult animals.	Mortality occurred in DRF and can be assumed to have been associated with high exposure	The results of the juvenile study were reviewed by the CHMP and reflected in the SmPC. JAS results were available before a paediatric trial was expected to start.

Active substance	Difference in exposure at similar dose	Novel toxicities / target organs	Difference in severity of toxicities	Data missing / not clear to conclude / uncertainties	Comments / regulatory and / or development impact of JAS results
	animals, respectively				
Ombrabulin		Lymphocyte necrosis	Mortality following shorter treatment duration Heart histopathologic lesions at lower exposure	Lack of reliable TK data in juveniles Different dosing regimen	PDCO required waiting for results of ongoing JAS before starting a paediatric trial. No paediatric trial was started so far. Development reported to have been discontinued due to lack of efficacy in adult indications.
Pazopanib	Slightly higher exposure in juvenile rats compared to adults at high doses	Dilatation of Brunner's gland ducts Decreased secretory content of prostate Abnormal growth / maturation of kidney, lung, liver and heart	Mortality and teeth toxicity at lower exposure (2- and 5-fold, respectively) and after shorter treatment duration Adrenals and bone toxicity after shorter treatment duration		PDCO: Inclusion of children below 2 years of age had initially be requested for the second paediatric trial, but was subsequently waived. CHMP: Recommendation against use in children less than 2 years The results of the juvenile study were reflected in the SmPC
Pixantrone			While no precise comparison is feasible because of the lack of exposure data in adult mouse studies and the different dosing regimen, it can at least be	Lack of TK data in comparative adult study Different dosing regimen	The PDCO considered that more evidence from adults should be sought to understand the potential of the medicine, in particular, with respect to cardiac safety expected to be better than with mitoxantrone or doxorubicin.

Active substance	Difference in exposure at similar dose	Novel toxicities/ target organs	Difference in severity of toxicities	Data missing/not clear to conclude / uncertainties	Comments / regulatory and/or development impact of JAS results
			concluded that cardiotoxicity is not significantly higher in juvenile animals.		The PDCO considered that the initiation of the paediatric clinical studies should await a positive benefit/risk assessment and a trend to reduce cardiotoxicity. For inclusion of children less than 12 years of age in the first trial, the juvenile study and the sudden death evaluation was to be awaited.
Ponatinib	Exposure (in terms of AUC) was significantly higher in PND 15 animals compared to PND 35 animals. The difference was of a factor 8-9. Compared to the adult exposure (in terms of AUC) at the same dose exposure was 2-3 times higher in PND 15 animals and ~3.5 times lower in PND 35 animals.		No effects in juveniles on bone and kidneys although seen in adult repeated-dose studies.	Lack of dose adaptation pre-/ post-weaning Juvenile study duration too short	CHMP: The results of the juvenile study were reflected in the SmPC including the fact that ponatinib did not adversely affect important developmental parameters in the juvenile toxicity study.
Regorafenib			Toxicity more pronounced in young and growing animals.		CHMP: The results of the juvenile study were reviewed by the CHMP and reflected in the SmPC (section 5.3) to clarify that alterations of teeth and bones and adverse

Active substance	Difference in exposure at similar dose	Novel toxicities / target organs	Difference in severity of toxicities	Data missing / not clear to conclude / uncertainties	Comments / regulatory and / or development impact of JAS results
					effects in the reproductive system were more pronounced in young and growing animals as well as in juvenile rats and indicate a potential risk for children and adolescents.
Sonidegib		Non-reversible nerve fibre degeneration (sciatic nerves and thoracic spinal cord)	Mortality at lower exposure (4- to 6-fold in rats from PND 14)		The JAS results were reviewed by CHMP and the nerve findings were listed as a new toxicity in the EPAR, however the results were not summarised in the SmPC.
Trametinib	5- to 12-fold increase in pre-weaning rats (PND 13)	Eye, lung, heart, nasal cavity, Harderian gland	Decreased (mortality and skin) at higher exposure or not occurring Increased (bone and kidney) at lower exposure and shorter treatment duration		PDCO: Considering the high exposure levels observed in the youngest rats and that the NOAEL was not identified in the juvenile rats, the PDCO recommended to adopt an age staggered approach for the inclusion of paediatric patients in clinical trials with particular attention to the starting dose and to include parameters to monitor adverse effects on the bone.

5. Discussion

This report is on juvenile animal studies (JAS) that were performed for the development of anti-cancer medicines as potential treatments for children with a malignant disease. Clinical research and development with such patients is evolving and is summarised in the next section to support the discussion of JAS.

5.1. Evolving drug development in paediatric oncology and haematology

Since about 1970, systematic approaches to medical care of children with a cancer have included a range of therapies such as anti-neoplastic medicines, surgery and radiation therapy as well as diagnostic and supportive measures, compiled into treatment protocols (care guidance) issued by paediatric oncology groups. One of the protocols' objectives is to provide recommendations that are comprehensive and that can be applied to most concerned patients, including newly-diagnosed patients of all ages.

The incidence of cancer is notably high during the first years of life compared to older paediatric age groups (Figure 1). Cancers occurring in the first (0-4) years of life are acute myeloid leukaemia (AML), MLL-rearranged infant acute lymphoblastic leukaemia (ALL), non-medulloblastoma brain tumours (e.g., anaplastic ependymoma, astrocytoma, brain stem tumours), neuroblastoma, retinoblastoma, hepatoblastoma, Shh-aberrant medulloblastoma and extracranial germ cell tumours.

Subsequent to treatment improvements, there is now long-term survival (5 year or longer) for more than 75% of youngest patients (0-4 years) with cancer (Smith et al. 2010) (Figure 2). For cancers such as non-medulloblastoma brain tumours, the outcome improvement is likely due to surgery and radiation therapy, because little progress was made in chemotherapies (Smith et al. 2010). Effective and safe anti-cancer medicines are still sought for all age paediatric ranges.

For treating children with anti-cancer medicines during their first years of life, most treatment protocols include empirical rules for adapting doses, which however have not been validated (Pizzo and Poplack 2011, table 15.5, p 451). Particularly for infants with cancer, data are scarce according to a comprehensive review (van den Berg, van den Anker, and Beijnen 2011), and it was reported that rules did not match the ontogeny of metabolism or elimination pathways.

Experience with cytotoxic anti-cancer medicines in children

Clinical trials are conducted in children with cancer by academic cooperative groups (Adamson 2015), using multi-agent chemotherapies that subsequently revolutionised the standard(s) of care. On the side of safety, a recurring observation was that children were able to tolerate substantially higher dose-intensity than adults, presumably due to more robust organ function during the acute treatment phase (e.g., better cardiovascular, hepatic and renal functional reserves). It is assumed that this partly accounted for the dramatic successes of the treatment paradigms introduced largely empirically. Moreover, upon completion of successful oncologic therapy many paediatric cancer patients, with some notable exceptions (e.g., CNS tumour patients), demonstrate remarkable recovery from acute toxicities and impairments as evidenced by improvement of activities of daily life, catch-up growth and psychomotor development including return to regular school.

With improved survival, concerns about late effects of traditional cytotoxic medicines have come to the forefront of research efforts in the academic groups (Pritchard-Jones et al. 2013). Secondary malignancies have since long been linked to exposure to certain classes of cytotoxic agents. The emerging data indicate that survival achieved by traditional combination chemotherapy protocols

comes at the cost of latent organ damage resulting in progressive impact on health and quality of life over time.

Clinical trials and treatment protocols continue to co-exist. Only a proportion of paediatric cancer patients is treated in clinical trials, because trial participation is voluntary, trials sites are fewer than treatment centres and trial eligibility is restricted. In contrast to treatment protocols, children younger than one year of age were historically excluded from most paediatric oncology trials. This may be expected for first or early trials (Figure 3), which mostly include patients after a number of failed treatments are and who are thus no longer in the youngest age. However, this was also found for later phase trials, into which often newly-diagnosed patients were recruited (Figure 3.

Evaluation of novel anti-cancer medicines

Early paediatric trials with anti-cancer medicines are motivated by the intention to provide a potential anti-tumour treatment option, at a likely active dose (e.g. see EMA Addendum on paediatric oncology). In fact, some of the novel and targeted medicines may be expected to have such anti-tumour activity that large and sustained treatment effects can occur in early trials. An example is the first paediatric crizotinib trial, with complete and durable responses in 7 out of 9 paediatric patients with an anaplastic large-cell lymphoma, refractory to therapy and for whom there was no known curative treatment (Mossé et al. 2013). This trial, like other early trials in patients with an advanced leukaemia or lymphoma, included the option to proceed to a potentially curative treatment (such as high-dose chemotherapy and haematopoietic stem cell transplantation). In addition, more early paediatric trials emerge where inclusion criteria permit recruitment of children for whom no curative treatment is known, rather than of only those for whom no active treatment is known.

Some early trials include extensions (about 6 months to 2 years), when it is expected that patients may receive the experimental medicine for a prolonged period of time to explore therapeutic effects (phase 2), at times in combination. Recruiting children with cancer, 31 trials were registered in EudraCT as only phase 1 and 29 as phase 1 / 2 trials in the last five years (since 2011). Seamless approaches (from early phase to therapeutic exploratory or confirmatory) are employed in adults (Manji et al. 2013) and are also a model for trials with children. The success potential has been highlighted by FDA oncologists (Prowell, Theoret, and Pazdur 2016), appraising an example of an early trial with over one thousand adult patients.

However, such evolving approaches present the same issue as conventional first cancer trials, in that the safety data are very limited and cannot be extrapolated to subsequently recruited patients with better prognosis, as also highlighted by paediatric oncology trialists. From their perspective, it appears, a gap remains between a "phase 1" population and the paediatric populations in subsequent trials. They seem to regret the lack of data to inform on developmental toxicities: "Unfortunately, the extent of overlap between normal developmental networks and cancers' [oncogene] addictions is not necessarily obvious before testing in large numbers of children" (Gore, DeGregori, and Porter 2013).

The molecular and cellular pathways that are altered in tumour cells and so drive tumorigenesis ("addiction", "dependency") very often have an indispensable physiological function(s) during normal development, which may be affected by novel classes of targeted non-cytotoxic anti-cancer medicines; therefore, this provide a strong biological rationale to hypothesize that adverse reactions of targeted agents may be more severe in young children who have not completed growth and organ development and maturation. Examples: homeobox gene overexpression in leukaemia and normal haematopoiesis; hedgehog signalling in medulloblastoma, rhabdomyosarcoma and normal bone development; anaplastic lymphoma kinase activation in lymphomas and in normal neuronal and visceral muscle development; adapted from (Gore, DeGregori, and Porter 2013) as well as IGF-1R/PI3K/AKT in several cancers and in physiologic cardiac hypertrophy and mature B cell persistence; RET kinase in several

cancers and in the development and function of parafollicular cells (Dy and Adjei 2013); EWSR1 in Ewing sarcoma and in normal cell survival in the central nervous system and in the regulation of genomic integrity (Cantile et al. 2013). The pathways for intended on-target effects are moreover remarkably conserved through evolution (e.g., Aiello and Stanger 2016; Scotting, Walker, and Perilongo 2005), suggesting that animal studies may also be useful with respect to toxicities of targeted medicines in humans.

However, against the background that clinical efficacy more than non-clinical safety questions may have driven development in paediatric oncology, it is remarkable that for a brain tumour with unfavourable outcome, a study in young mice has been conducted with the first anti-cancer medicine directed against a tumour-specific target (Kimura, Ng, and Curran 2008). This is another sign of the change in the field and of the interest to improve development also from a safety point of view.

Taken together, no scientific strategy was found for generating safety data to inform on targeted anti-cancer medicines in youngest cancer patients, in spite of the cancer incidence, unmet needs, the expected survival and the need for specific data. There is moreover a gap between paediatric populations included in trials and those expected to benefit from the treatments, as early trials in children with an advanced cancer do not inform a use in a broader population. Toxicities observed in adults can be expected also in children when treated with anti-cancer medicines; however, absence of certain toxicities in adults is not predictive of their absence in children; such safety cannot be extrapolated. In contrast to the majority of cancers in adults, overall survival is high in most paediatric cancers, last-line situations may be reversed, and quality of survival is a research focus.

For historic reasons explained above, the experience with the assessment of anti-cancer medicines for children in the regulatory setting is scarce. By the end of 2013, only 9 out of 67 centrally authorised anti-cancer medicines had a paediatric indication[14], and few paediatric data sets have been submitted for regulatory assessment. In contrast to the requirements to submit results of paediatric clinical trials for assessment and / or to make them publicly available, there is no obligation to submit results of JAS, unless these are part of an agreed PIP.

5.2. Juvenile animal data

A group of non-clinical experts from EU national agencies and members of the PDCO's Non-clinical Working Group (NcWG) assessed the results of juvenile animal toxicity studies (JAS) of 20 anti-cancer medicinal products for which PIPs had been agreed by the PDCO. The results of JAS were compared to the results of toxicity studies of similar duration performed in adult animals based on exposure data, when available.

The results of the JAS are presented in section 4.2. by type of finding and in section 4.3. by their impact on product development, use and information.

Importantly, **no major differences** were detected in the toxicity profile between juvenile and adult animals for 6 products. For another 4 products, it is likely that increased toxicity was only apparent, because exposures at the start of the study were higher in juvenile compared to adult animals. However, in 10 other products, **new toxicities or more severe toxicities** were observed in juvenile animals.

The most worrying findings were seen with navitoclax, dabrafenib and lenvatinib, as illustrated in the following:

[14] Products with paediatric indication were identified using the ATC code L01 and checking their paediatric use information, i.e. for an indication in section 4.1 of the Summary of product characteristics or as a paediatric posology in section 4.2.

A juvenile toxicity study was performed with **navitoclax**, an inhibitor of Bcl-2, Bcl-xL, and Bcl-w antiapoptotic proteins, because it was known that the Bcl-2 family of proteins is involved in brain development including particularly Bcl-xL in the cerebellum (Vogel 2002; Lindsten et al. 2000; Lindsten and Thompson 2006). While adult rat studies did not reveal any CNS effects, juvenile rats had lower brain weights, associated with cerebellar hypoplasia at exposure levels in the estimated human efficacious range. At higher exposures, CNS related clinical signs were also observed, in addition to necrosis of the basal ganglia, primarily affecting the globus pallidus. As navitoclax does not seem to cross the blood-brain barrier it was thought that the effect occurred at the time of blood brain barrier immaturity. The PDCO considered that additional data should be provided to ascertain this assumption. As little is known about Bcl-2 family proteins expression in children, specifically in children's brain tissue the exact clinical relevance of these findings is unknown. The Bcl-2 family of proteins are widespread during embryonic development. Proliferating neuroepithelial cells of ventricular zones as well as the postmitotic cells of the cortical plate, cerebellum, hippocampus and spinal cord express Bcl-2 family proteins. Postnatally, Bcl-2 family proteins are principally retained in the granule cells of the cerebellum and dentate gyrus of the hippocampus. Thereafter, Bcl-2 family proteins expression in the CNS declines with aging (Merry et al. 1994).

While no renal toxicity was detected in adult rats given **dabrafenib**, juvenile toxicity studies revealed renal tubular deposits containing dabrafenib and/or its metabolites, increased incidence of cortical cysts and tubular basophilia and reversible increases in urea and/or creatinine concentrations, even when the treatment started on PND 21. This observation cannot be explained by unexpectedly high plasma levels of dabrafenib, especially as the kidney lesions were observed at the low dose of the juvenile study. Kidney toxicity was observed in adult patients, but the severity of the effect was considered lower than with vemurafenib, another BRAF inhibitor, and was not indicative of a relationship with these severe effects seen in the juvenile animals (Jhaveri KD, Sakhiya V, and Fishbane S 2015). Nephrogenesis is complete at birth in human but not in rat up to PND 14, whereas kidney maturation occurs up to 6 weeks after birth in rats and up to 6 months to 1-2 year in children, with GFR increasing rapidly in the first two weeks of life. Afterwards, GFR corrected for body surface area (BSA) increases more slowly to reach adult levels between 1 to 2 years of age. Tubular maturation is completed between 1 and 2 years of age (see guideline on the investigation of medicinal products in the term and preterm neonate). It was considered that the renal toxicity seen in juvenile animals was linked to kidney function immaturity. Nevertheless, metabolic clearance immaturity may also have played a role as after administration of a single oral dose of ^{14}C-dabrafenib the majority of the dose was excreted via the faeces in rat (90 to 93%). Faecal excretion was predominant in all species, but urinary excretion was greater in humans than in rats and dogs. The safety concern was considered high enough so that a PIP waiver was granted for children younger than 1 year of age and the results of the JAS were reflected in the SmPC with a safety warning.

A JAS was performed on **lenvatinib** to assess potential increased toxicity in juvenile animals. Similar target organs and/or toxicities were observed in both the juvenile and the adult animal studies, but an apparent increased severity of toxicity was observed in juvenile animals, as evidenced by mortality occurring at lower exposure levels. The difference in sensitivity between PND 7 and PND 21 was of the order of 125-fold and 12-fold in terms of dose and exposure (AUC), respectively. The cause of death was not determined for the youngest population (PND 7), but was attributed to gastro-intestinal lesions complicated by an inflammatory reaction to infection when treatment started in PND 21 rats. Increased severity of additional target organ toxicities was evidenced, and a waiver in children younger than 2 years of age (end maturation of the gastrointestinal tract) was granted and the results of the JAS were reflected in the SmPC together with a warning.

The examples show that severe toxicities were detected in juvenile animals which may have a significant safety impact in the population of youngest children, either related to pharmacology/toxicity

affecting organ/systems undergoing maturation (lenvatinib, navitoclax) or related to immaturity of clearance pathways (dabrafenib). The case of dabrafenib was unexpected.

Generally, immature metabolism is known to lead to higher exposure and this can be modelled, but it is more difficult to predict target organ toxicity linked to immaturity of clearance pathways inducing higher exposure to toxic parents and / or metabolites. Understanding the ontogeny of renal and metabolic clearance pathways may help interpreting study results.

5.3. JAS study designs

These JAS bring general information that may be applicable to other therapeutic fields. For example, they show that reversibility of the findings and that toxicokinetic (TK) data are important to estimate the clinical relevance of the JAS. However, conclusions may have been hampered by inappropriate study designs:

In many cases exposure levels were higher in the youngest rats and then decreased up to PND 35, to increase slightly, up to adult levels, thereafter. In some cases, excessive toxicity due to very high exposure levels at the beginning of the study led to termination of the high dose group. Dose adaptation to achieve rather stable exposure was applied in only two cases. A short-term DRF study including TK may help determine the dosing regimen.

In one case, the study duration was shorter than in older animals to properly assess and compare age-related effects on development. In addition, different species were used and TK data were incomplete. Interpretation of results may also have been difficult because the dosing regimen was different in adults and juvenile animals in one case (ombrabulin).

The dosing regimen or route of administration of the JAS should be aligned with that of the adults and should relate to the anticipated clinical dosing regimen as much as possible. Similar endpoints pertaining to the juvenile evaluation should be included in adult studies, to permit proper comparison.

In two cases, the JAS were undertaken with the intention to support treatment of neonates, but started only on PND 21 so that no effects in younger rats were investigated.

5.4. Clinical development and regulatory impact

Following the assessment of the non-clinical data in view of the indication pursued with the paediatric development (PIP), JAS were mandated to be completed for 7 anti-cancer medicinal products (and for 6 other anti-cancer medicines happened to be completed; details in section 7.3.) before the anti-cancer medicine could be administered in a trial to the youngest patients, in most cases those younger than 1 to 2 years of age, depending on the cause for concern were. The completion of these studies was to be awaited so that it could be checked if their results supported the administration to youngest children and if any precautions were to be considered, such as specific dosing clinical rules or adverse events monitoring. However, older children could already receive the medicine in the trial, based on the existing data (see also Figure 4).

As demonstrated, results of JAS studies led to PIPs modifications (studies in younger children were waived), improved the safety monitoring and were reported in the SmPCs and/or EPAR, in some cases accompanied with warnings for safety reasons.

Waiving studies in youngest patients can narrow the paediatric population included in clinical trials to those for whom exposure to the experimental treatment is likely more safe; however, once the medicine is available, it may be used in a broader paediatric population, also outside the authorised indication, in and outside of clinical trials.

Better understanding and dissemination of these non-clinical data should in principle allow a safer treatment of younger patients, who are still lacking adequately developed medicines for the treatment of their cancer.

6. Conclusions

As described above, some first studies in children with a cancer may lead to long-term treatment, particularly if seamless clinical designs are applied, and long-term survival may occur. In addition, the incidence of cancer in paediatric patients aged less than 2-3 years is comparable to older paediatric populations. Across the PIPs for 20 anti-cancer medicinal products that were reviewed, there were 14 where the protocols of the first paediatric trial were to permit inclusion of children less than 1 or 2 years of age, and it was considered important to determine as much as possible, if unacceptable toxicities may occur because of too severe or not-readily-detectable side effects.

Contrary to conventional cytotoxic agents, which may be considered toxic by design and induce usually predictable haematologic, intestinal, testicular and cardiac toxicities, targeted therapies were originally expected to show safer toxicity profiles. Experience collected so far with these therapies, both in humans (e.g., Le Tourneau et al. 2010) and in animals, point to very different toxicity profiles attributable to off-target or secondary pharmacology, sometimes unexpected and severe effects, particularly when interfering with organ/systems undergoing maturation. Juvenile toxicity studies were conducted proactively by companies or deemed warranted by the PDCO, often because the youngest age of patients to be included in trials was lowered during evaluation of the proposed PIP.

This review of juvenile animal studies (JAS) was conducted in the therapeutic area of oncology, which had been chosen as a model area as it was hypothesised that anti-cancer medicines will likely reveal toxicities and affect maturation and development, and would therefore allow appraisal of the usefulness of JAS. The juvenile animal toxicity studies with anti-cancer medicines under paediatric development have brought new information in a substantial portion of the cases, including previously unknown toxicities (new target organ toxicities, 8 cases) and including cases with toxicities that likely would severely harm youngest patients (8 cases). Conversely, some juvenile studies were found to support the administration of an anti-cancer medicine starting at the adult maximum tolerated dose in a trial involving the youngest patient populations (6 cases). It is also important that some findings in adults and / or juvenile animals were already shown to be clinically relevant, such as early growth plate closure induced by hedgehog inhibitors, leading to select the paediatric patients.

The results of JAS evaluated in this report showed that these studies can be informative to support the paediatric development of medicinal products in oncology, either because they bring to the fore new toxicities or enhanced toxicities when compared to adult studies, or because they showed that the safety profile was comparable in juvenile and adult animals which suggest that adult safety data collected may well be extrapolated to the younger populations.

Considering the high number of oncology drugs currently under development and the fortunately relatively low number of paediatric compared to adult cancer patients, discussions are ongoing between companies, academicians, parent representatives and regulators to understand if prioritization for efficacy of these drugs could be achieved (Pearson et al. 2016). Juvenile studies should not delay paediatric trials, but serve to improve the provisions to safeguard against intolerable harms and to monitor for age-specific risks in trials of new anti-cancer medicines including the youngest paediatric patients (Figure 4, p 45). The main objective of most juvenile studies is to well inform conducting clinical research rather than to prevent the use of the medicine. In addition, it is important to make this information well available for all potential sponsors/investigators involved in paediatric clinical trials and to paediatricians considering to use these medicines off-label.

In summary, results of JAS studies led to PIPs modifications (studies in younger children were waived), improved the safety monitoring and were reported in the SmPCs and/or EPAR, in some cases accompanied with warnings for safety reasons.

Based on the experience documented in this project, JAS may provide valuable information for a given anti-cancer medicine leading to protection of the youngest population from potentially harmful effects or to appropriate monitoring during the clinical studies and clinical use.

On this basis, it is recommended to consider the need for juvenile animal studies whenever a scientific rationale exists, when there are specific concerns about the safety profile for the targeted paediatric population, and animal models are expected to generate data relevant to support clinical paediatric development. It is also recommended to use tools such as predictive PK/PD modelling for improving paediatric trials particularly with young patients and these should become an integral part of paediatric medicine development.

The non-clinical and clinical studies conducted to support a paediatric development (reflected in the PIP) are planned in such a way that, taken together, the results can support the most appropriate use of the anti-cancer medicine in all relevant paediatric age ranges. In PIPs, approaches to include youngest patients in trials should be tailored to the case, avoiding unnecessarily staggering -and thereby delaying- inclusion by age.

In the future, the role of non-clinical studies as part of strategies for accelerating paediatric oncology development including in youngest children could be further discussed and optimised as part of PIPs.

7. Annexes

7.1. Glossary

Term	Explanation
Adverse effects	Include in animals for example alteration of: morphology, functional capacity, growth, development or life span.
Agreed PIP	A PIP that had been agreed by the PDCO issuing an Opinion and EMA issuing a Decision on the application for agreement of a PIP.
Applicant	The pharmaceutical company or person applying for the agreement of a Paediatric Investigation Plan or requesting the granting of a Product-Specific Waiver.
Children	Used synonymously with paediatric population (from birth to the day before the 18th birthday)
Decision	The legal act issued by the European Medicines Agency, which puts into effect the Opinion of the Paediatric Committee.
Deferral	The possibility to request marketing authorisation for the use of the medicine in adults, before completing one or more of the studies /measures included in a PIP. The Paediatric Committee may grant a deferral to avoid a delay in the availability of the medicine for adults.
Marketing Authorisation	When a Marketing Authorisation is granted, the pharmaceutical company may start selling the medicine in the relevant country (in the whole European Union, if the procedure was a centralised one).
No Observed Effect Level	The level of exposure in an animal at which no (biologically or statistically) significant increases in frequency or severity of any effect were found between the exposed population compared to its appropriate control
No Observable Adverse Effect Level	The level of exposure in an animal at which no (biologically or statistically) significant increases in the frequency or severity of any adverse effects were found in the exposed population compared to its appropriate control.
Opinion	The result of the evaluation by the Paediatric Committee of the European Medicines Agency. The opinion may grant a product-specific waiver, or agree a PIP.
Paediatric investigation plan (PIP)	Set of studies and measures, usually including clinical studies in children, to evaluate the benefits and the risks of the use of a medicine in children, for a given disease or condition. A PIP may include “partial” waivers (for example, for younger children) and/or a deferral (see below).
Pharmaceutical form	The physical aspect of the medicine (the form in which it is presented), for example: a tablet, capsule, powder, solution for injection, etc. A medicine can have more than one pharmaceutical form.
Route of administration	How a medicine is given to the patient. For example: for oral use, for intramuscular use, for intravenous use, etc. The same medicine, or the same pharmaceutical form, may be given through more than one route of administration.

Term	Explanation
Waiver	An exemption from conducting studies in children, for a given disease or condition. This can be granted for all children (product-specific waiver), or in specific subsets (partial waiver): for example, in boys or in children below a given age.

7.2. Abbreviations

Acronym	Explanation
ALL	Acute lymphoblastic leukaemia
AML	Acute myeloid leukaemia
BCR-ABL	Breakpoint cluster region-Abelson tyrosine kinase, an abnormal protein that is found in some types of leukaemia
BW	Body weight
CHMP	Committee for Medicinal Products for Human Use
CSF-1R	Colony Stimulating Factor-1R
CTD	Common Technical Document
DDR	Discoidin Domain Receptor
DRF	Dose Range Finding study
EMA	European Medicines Agency
ES	Ewing Sarcoma
FLT3	Fms-Like Tyrosine kinase-3
HGG	High-grade glioma
ICH	International Conference on Harmonisation for Technical Requirements for Registration of Pharmaceuticals for Human Use
IGF-1R	Insulin-like Growth Factor-1 Receptor
JAS	Juvenile animal study / studies
LOAEL	Lowest Observed Adverse Effects Level
LOEL	Lowest Observable Effect
MEK	Mitogen Activated Protein Kinase
NHL	Non-Hodgkin lymphoma
NOAEL	No Observable Adverse Effect Level
NOEL	No Observed Effect Level
NRSTS	Non-Rhabdomyosarcoma Soft Tissue Sarcoma
PDCO	Paediatric Committee

Acronym	Explanation
PDGF	Platelet-Derived Growth Factor
PIP	Paediatric Investigation Plan
PKC	Protein Kinase C
PND	Post Natal Day
PV	Pharmacovigilance
RMS	Rhabdomyosarcoma
VEGF	Vascular Endothelial Growth Factor

7.3. Table JAS results

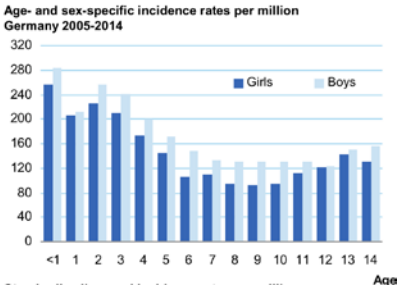
Table 3: Results of JAS with 20[15] anti-cancer medicines and consequences (as of 30 July 2015)

Medicine / **Findings**	**Cabozantinib**	**Cobimetinib**	**Dabrafenib**	**Decitabine**	**Everolimus**	**Imatinib**	**Lenvatinib**	**Midostaurin**	**Navitoclax**	**Nilotinib**	**Ombrabulin**	**Pazopanib**	**Pixantrone**	**Ponatinib**	**Regorafenib**	**Sonidegib**	**Trametinib**	**Total number**
New target toxicity			x		x				x		x	x				x	x	**8**
Increased mortality at same or lower exposure or shorter duration							x				x	x				x		**4**
Increased severity at same or lower exposure							x		x			x			x		x	**6**
No major differences				x		x							x	x				**6**
Additional JAS or information needed			x						x				x					**4**
All paediatric trials waived based on JAS																		**0**
Youngest waived based on JAS			x				x					x						**3**
JAS available before youngest studied (m = mandated)	x	x	m	x	x		m		m		m	x	m			x		**13**
Monitoring adapted							x								x		x	**4**
Dosing adapted																	x	**2**
Long-term follow-up concern based on JAS			x	x												x		**3**
Recommendation against use in subset							x					x						**2**
Results in SmPC	x	x	x	x	x	x	x			x		x		x	x		x	**12**
Discontinued									-		x							**1**

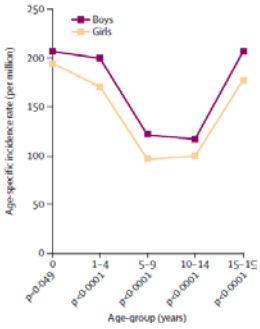
[15] Detailed results and corresponding assessements for 3 medicinal products were removed from the report for reasons of confidentiality, however the total number of medicinal products assessed was mantained throughout the document.

7.4. Figures

Figure 1: Incidence of cancer in the paediatric population



German Childhood Cancer Registry, Annual Report 2015 (p 17)



European data on cancer incidence in children and adolescents (Steliarova-Foucher et al. 2004)

SEER Incidence Rates by Age at Diagnosis
All Sites, 2009-2013

Age	Both Sexes, Rate per 100,000
<1	23.6
1-4	21.9
5-9	12.7
10-14	13.8
15-19	22.2
20-24	35.7

U.S. National Cancer Institute (NCI) Surveillance, Epidemiology, and End Results (SEER) Program (analysis of all cancers, May 2016)

The above figures were corrected neither for neuroblastoma stage 4S / MS, nor for acute myeloid leukaemia in Down syndrome, because these represent no more than 20% of all youngest patients.

Figure 2: Survival of children with cancer

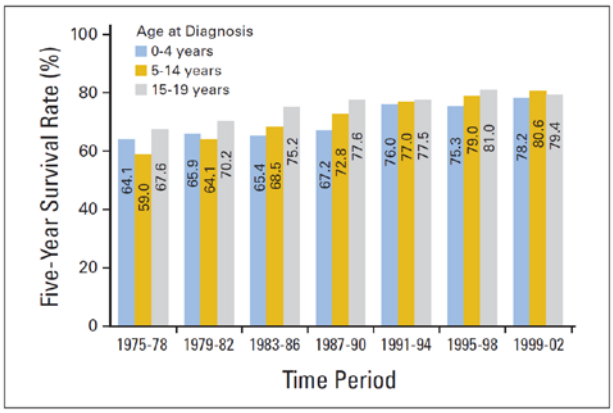


Fig 4. Five-year survival rates for all cancers combined in children by age group and period of diagnosis from 1975 to 2002, with follow-up of vital status through 2006, according to data from the Surveillance, Epidemiology, and End Results 9 (SEER 9) Registries.

Paediatric cancer outcome improvement (Smith et al. 2010)

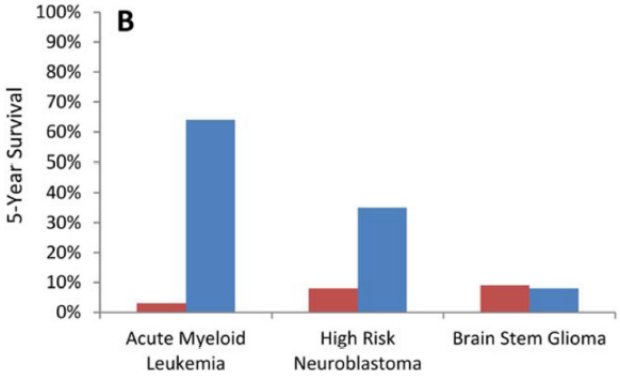
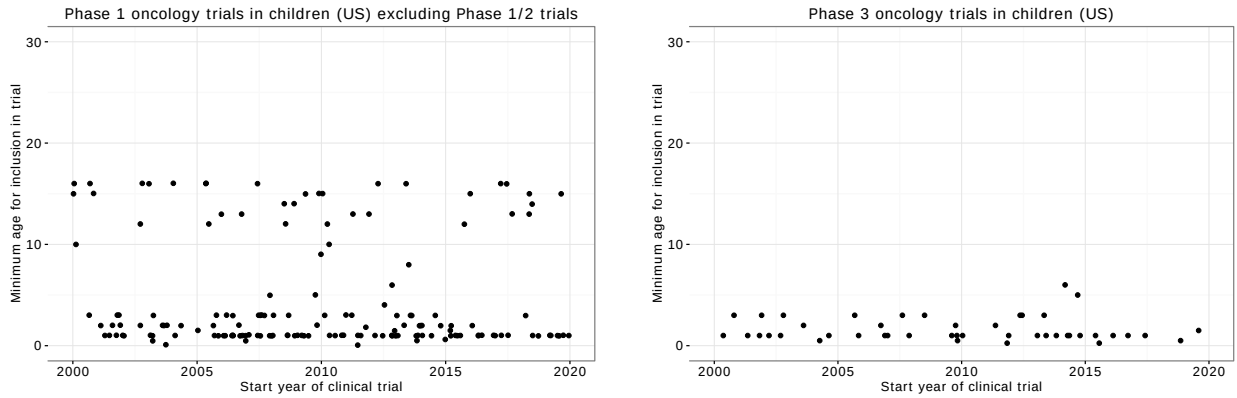


FIGURE 1.
Approximate 5-Year Survival for Children Treated in 1960 (red bars) Versus Children Treated in the Year 2000 (blue bars). (A) Over the past 50 plus years, significant improvements in outcome have been made in a range of childhood cancers; (B) whereas, for certain cancers, despite intensification of treatment, progress has been more limited or has not been realized.

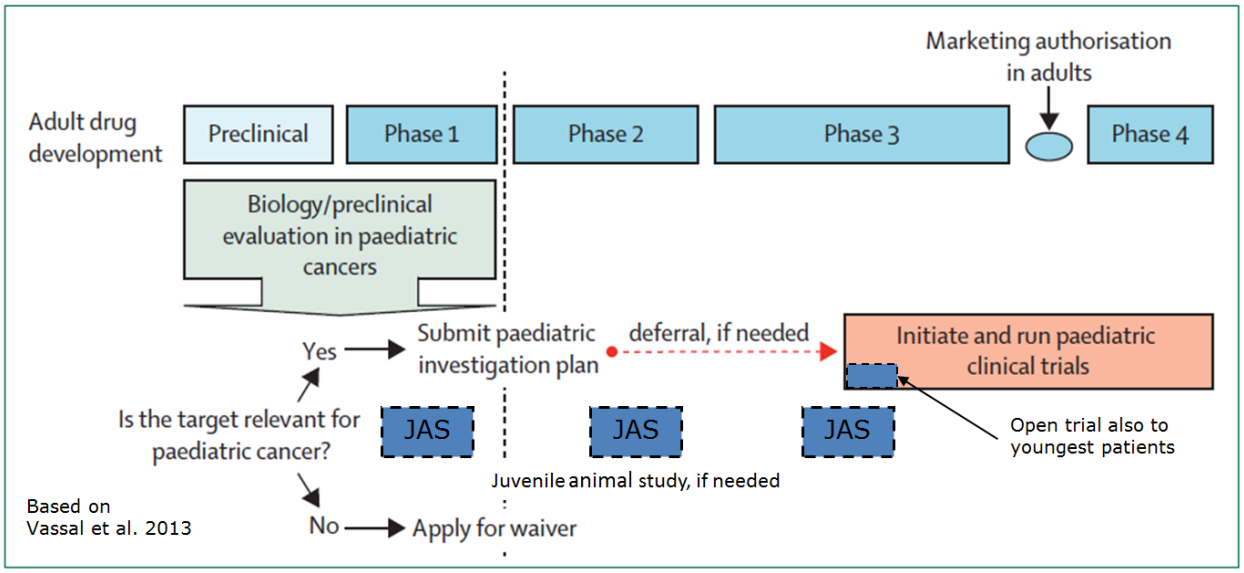
Outcome improvement by malignant disease (Adamson 2015)

Figure 3: Paediatric age groups in cancer trials



Minimum age of phase 1 (left) and 3 (right) paediatric oncology interventional clinical trials (analysis using information in ClinicalTrials.Gov, 2016)

Figure 4: Options for timing of juvenile animal studies (JAS), if needed



The figure shows options for timing of juvenile animal studies (JAS), if needed to support paediatric trials and use (see 6. Conclusions), superimposed on a proposal for the novel paediatric drug-development pathway (Vassal et al. 2013). In cases where a JAS is considered needed, one of the options is to open recruitment into a paediatric clinical trial to youngest patients as soon as these results are available (and support such recruitment). The choice of the appropriate option is the result of a scientific assessment.

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9. References

Pharmaceutical legislation and guidelines

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Guideline on the need for non-clinical testing in juvenile animals of pharmaceuticals for paediatric indications (EMEA/CHMP/SWP/169215/2005)

Guideline on the role of pharmacokinetics in the development of medicinal products in the paediatric population (EMEA/CHMP/EWP/147013/2004/Corr)

Addendum on Paediatric Oncology (EMEA/CPMP/EWP/569/02) to the guideline on the evaluation of anticancer medicinal products in man (EMA/ CHMP/205/95/Rev. 4)

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ICH M3 (R2) guideline on non-clinical safety studies for the conduct of human clinical trials for pharmaceuticals (EMA/CPMP/ICH/286/1995)

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10.

10. Appendix

10.1. Assessment template

Active substance

Mechanism of action:

Expected therapeutic dose/exposure:

Expected dosing regimen:

Juvenile study justification:

Targeted indication:

Paediatric age subsets:

Summary of the data from juvenile animal studies

Title				
Age at initiation				
Dosing regimen				
Endpoints [16]	mortality, clinical signs, body weights, food consumption (only in Phase 2 animals), hematology, blood chemistry, physical and functional development, behavioral observations, toxicokinetics, bone measurement, and macroscopic and microscopic pathology.			
Doses (mg/kg)	**NOEL/NOAEL /LOAEL**	**Toxicities**[17]	**Cmax (ng/mL or □g/mL)**	**AUC (ng.h/mL or □g.h/mL)**
Dose 1				
Dose 2				
Dose 3				

<Duplicate this table in order to provide a summary of the preceding dose-range finding study, only if this brings additional relevant information.>

[16] Select endpoints as indicated in the study report

[17] Dose-limiting toxicities should be reflected in bold

Summary of the data from comparative adult animal study

<Choose adult study comparable to juvenile animal study. The adult study should be chosen based on the following criteria: same species and closest treatment duration. If there is a specific reason to compare to a different species, a short explanation should be provided.

PK data (half-life and metabolism) should be provided.>

Title [18]				
Age at initiation				
Dosing regimen				
Endpoints[19]	mortality, clinical signs, body weights, food consumption (only in Phase 2 animals), hematology, blood chemistry, physical and functional development, behavioral observations, toxicokinetics, bone measurement, and macroscopic and microscopic pathology.			
Doses (mg/kg)	**NOEL/NOAEL /LOAEL**	**Toxicities**[20]	**Cmax (ng/mL or μg/mL)**	**AUC (ng.h/mL or μg.h/mL)**
Dose 1				
Dose 2				
Dose 3				

[18] If no study report available, please indicate briefly where the information comes from (e.g. IB + date, CHMP assessment + date etc.)

[19] Select endpoints as indicated in the study report

[20] Dose-limiting toxicities should be reflected in bold

All toxicity studies

The following studies were reviewed and provided relevant information for the conclusions:

<Fill in the following table with available adult animal studies results (including results in other species) that provided additional information.>

Title	NOEL / NOAEL / LOAEL	Additional findings

The following studies were reviewed but did not provide relevant information for the conclusions:

<Fill in the following table with available adult animal studies results (including results in other species) that did not provide useful information.>

Title

Overview of PK profile

$t_{1/2}$

Absorption:

Distribution:

Metabolism:

Excretion:

Toxicological profile of the medicinal product

In adult animal studies, the targeted organs were <…>.

In juvenile animal studies, the targeted organs were <…>.

Comparison of the adult and juvenile animal data

<Indicate if enough information was available in adult studies and juvenile studies to make comparison and conclusion (indicate if same specific endpoints)>

Toxicological profile of the medicinal product:

Pharmacokinetics comparison:

<Compare PK profile observed in juvenile study to adult data>

NOAEL juvenile <species> = ……. corresponding to an exposure of …….

Toxicities observed at a higher dose were:

NOAEL adult <species> = …….. corresponding to an exposure of …….

Toxicities observed at a higher dose were:

<Indicate if effects observed in juvenile studies were reversible>.

Conclusion

Title of the non-clinical juvenile study <specify species, route, age at treatment initiation, exposure duration, recovery period>

<Tick the most appropriate conclusion(s). More than one option is possible, e.g. similar toxicities + increased sensitivity.>

	At the same dose levels, exposure in juvenile animals was <increased/decreased> compared to the adult animals. **The difference in exposure was of the order of …-fold.**
	Similar target organs and/or toxicities were observed in both the juvenile and the adult animal studies.
	Apparent <increased/decreased> severity of toxicity <(that is, mortality)> was observed in juvenile animals, as evidenced by toxicity occurring at <lower/higher> exposure levels.
	Apparent <increased/decreased> severity of toxicity <(that is, mortality)> was observed in juvenile animals, as evidenced by toxicity occurring following <longer/shorter> treatment duration.
	Novel toxicities or target organs were observed in juvenile animals. These were ……..
	The toxicities observed in juvenile animals occurred at <lower/higher> exposure levels compared to adult animals. The difference in sensitivity was of the order of …-fold.
	Other:
	Data were missing/ not clear enough to conclude.