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Review

Paediatric Strategy Forum for medicinal product development of epigenetic modifiers for children ACCELERATE in collaboration with the European Medicines Agency with participation of the Food and Drug Administration

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Abstract The fifth multistakeholder Paediatric Strategy Forum focussed on epigenetic modifier therapies for children and adolescents with cancer. As most mutations in paediatric malignancies influence chromatin-associated proteins or transcription and paediatric cancers are driven by developmental gene expression programs, targeting epigenetic mechanisms is predicted to be a very important therapeutic approach in paediatric cancer. The Research to Accelerate Cures and Equity (RACE) for Children Act FDARA amendments to section 505B of the FD&C Act was implemented in August 2020, and as there are many epigenetic targets on the FDA Paediatric Molecular Targets List, clinical evaluation of epigenetic modifiers in paediatric cancers should be considered early in drug development. Companies are also required to submit to the EMA paediatric investigation plans aiming to ensure that the necessary data to support the authorisation of a medicine for children in EU are of high quality and ethically researched.

The specific aims of the forum were i) to identify epigenetic targets or mechanisms of action associated with epigenetic modification relevant to paediatric cancers and ii) to define the landscape for paediatric drug development of epigenetic modifier therapies. DNA methyltransferase inhibitors/hypomethylating agents and histone deacetylase inhibitors were largely excluded from discussion as the aim was to discuss those targets for which therapeutic agents are currently in early paediatric and adult development.

Epigenetics is an evolving field and could be highly relevant to many paediatric cancers; the biology is multifaceted and new targets are frequently emerging. Targeting epigenetic mechanisms in paediatric malignancy has in most circumstances yet to reach or extend beyond clinical proof of concept, as many targets do not yet have available investigational drugs developed. Eight classes of medicinal products were discussed and prioritised based on the existing level of science to support early evaluation in children: inhibitors of menin, DOT1L, EZH2, EED, BET, PRMT5 and LSD1 and a retinoic acid receptor alpha agonist. Menin inhibitors should be moved rapidly into paediatric development, in view of their biological rationale, strong preclinical activity and ability to fulfil an unmet clinical need. A combination approach is critical for successful utilisation of any epigenetic modifiers (e.g. EZH2 and EED) and exploration of the optimum combination(s) should be supported by preclinical research and, where possible, molecular biomarker validation in advance of clinical translation. A follow-up multistakeholder meeting focussing on BET inhibitors will be held to define how to prioritise the multiple compounds in clinical development that could be evaluated in children with cancer.

As epigenetic modifiers are relatively early in development in paediatrics, there is a clear opportunity to shape the landscape of therapies targeting the epigenome in order that efficient

and optimum plans for their evaluation in children and adolescents are developed in a timely manner.

1. Introduction

The fifth multistakeholder Paediatric Strategy Forum was organised by ACCELERATE [1] in collaboration with the European Medicines Agency (EMA) with participation of the Food and Drug Administration (FDA) and focussed on epigenetic modifiers in children and adolescents.

Previous multistakeholder Paediatric Strategy Forums [2–4] have been successful in evaluating preclinical and clinical research and providing an opportunity for constructive interactions between relevant stakeholders (patient advocates, clinicians, academic experts, biotechnology/pharmaceutical companies, and regulators) on topics relatively late in development. The aim of the forums is to share information and advance learning, in a precompetitive setting, which may direct subsequent clinical investigation strategies and inform regulatory decisions on the development of medicines for children with cancer [5]. This forum was held early in the stage of development of epigenetic modifying drugs as a class with the expectation that emerging science would aid in understanding the landscape of development. The goal of this meeting was to discuss the ongoing development of epigenetic modifying drugs and provide recommendations to support their introduction into the care of children with malignancies.

DNA methylation and covalent histone modifications are precisely and dynamically controlled by various epigenetic modifying proteins and are critical aspects of transcriptional regulation. Dysregulation of these enzymes may result in cancer [6]. Epigenetic changes are particularly important in paediatric cancers, as most alterations in paediatric malignancies influence chromatin-associated protein in the background of a typically simple genomic landscape [7,8]. The goal of epigenetic therapies is to disrupt the equilibrium created by the initial alteration, restore the epigenetic balance, and revert malignant cells to a more normal condition. In recent years, epigenetic modifiers have become the focus of many cancer clinical trials in adults. Currently, several drugs have been approved for clinical use in oncological practice and many more are on the horizon. The full implementation of the Research to Accelerate Cures and Equity (RACE) for Children Act and the FDARA amendments to section 505B of the FD&C Act in the United States of America (USA) in August 2020 requires that certain drugs and biological products be assessed early in paediatric cancers if the drug or biological product is directed at a molecular target determined to be substantially relevant to the growth or progression of a paediatric cancer [9]. The ongoing evaluation by the EU on both the paediatric and orphan regulations will potentially further enhance the regulatory environment in Europe.

The specific aims of the forum were i) to identify epigenetic targets or mechanisms of action relevant to paediatric cancer and ii) to define the landscape for paediatric drug development of epigenetic modifying drugs and iii) identify opportunities to further enable their development in paediatric cancers. DNA methyltransferase inhibitors/hypomethylating agents and histone deacetylase (HDAC) inhibitors were largely excluded from discussion as the aim was to discuss the targets which were earlier in development.

The Paediatric Strategy Forum was held over two days in Philadelphia, Pennsylvania (USA), in January 2020. A comprehensive overview of the scientific rationale for epigenetic modifiers in paediatric leukaemia, central nervous system tumours and solid tumours was presented and potential epigenetic therapeutic targets were identified. This was followed by a description of pharmacological and clinical information on seventeen compounds being developed as epigenetic modifiers and provided a basis for overall conclusions and recommendations.

The forum was advertised, and expressions of interest were sought from the pharmaceutical/biotechnology industry (if they wished to present data on relevant medicinal products, a condition for their participation), academic experts, international regulatory authorities and patient advocates.

Seventy-two participants from both North America and Europe including academic experts, patient advocates (from Children's Cancer Cause, Coalition Against Childhood Cancer, KickCancer, Solving Kids' Cancer and the Andrew McDonough B + Foundation), EMA (including the Paediatric Committee [PDCO]) and FDA regulators and representatives from 11 biopharmaceutical companies discussed the scientific rationale for the epigenome to be considered a valid therapeutic target, with evidence explored across classes of paediatric malignancies.

2. Epigenetic modifiers in paediatric malignancies

Paediatric cancers in general harbour few mutations in the coding genome, suggesting that oncogenesis is at least partly led by epigenetic mechanisms. As most mutations that do occur in paediatric malignancies

influence chromatin-associated protein or transcription factors, and paediatric cancers are driven by developmental gene expression programs typically in the background of a low mutational burden, targeting epigenetic mechanisms, chromatin-based control of gene expression is potentially a very important therapeutic approach in paediatrics. Oncogenic mutations in genes controlling epigenetic processes lead to dependence on specific epigenetic pathways, and fusion oncoproteins are frequently critically involved in epigenetic events. One objective of epigenetic modifiers is to "reprogram" cancer cells and facilitate their normal differentiation. In addition, combination regimens that include an epigenetic modifier may make cells more responsive to immunotherapies, prevent resistance to chemotherapy and prevent lineage shifts. Epigenetic therapy is thought to have several modes of anticancer action: i) increasing expression of tumour suppressor genes and thereby causing tumour cell death, ii) increasing differentiation and thus reducing stemness and proliferation and iii) enhancing the immune response (e.g. through enhanced expression of immune related molecules such as MHC class I on tumour cells, by inducing expression of tumour antigens that the immune system can recognize, or by a more direct effect on immune cells).

2.1. Scientific rationale for epigenetic modifying therapies in paediatric leukaemia

Paediatric leukaemia frequently results from fusion oncoproteins that involve transcription factors or chromatin regulators (e.g. MLL-rearrangements, ETV6-RUNX1, RUNXI1-RUNX1T1, PML-RARα) or other abnormalities of transcription factors (e.g. PAX5, NOTCH1, IKZF1) leading to an aberrant transcriptional program and differentiation state. Moreover, the success of therapies targeting PML-RARα in acute promyelocytic leukaemia [10] and leukaemia with IDH1/2 mutations in adults by inducing differentiation [11] is a testament to the efficacy of therapeutically modifying the epigenome. Homoeotic genes (including Hox/Meis) are highly expressed in normal haematopoietic stem cells and subtypes of leukaemia and transform haematopoietic cells. Homoeotic gene expression is controlled by histone methylation [12]. *MLL1* (*KMT2A*) is located at 11q23 and is consistently rearranged in infant acute leukaemia (more than 70%) [13,14] and in subtypes of acute myeloid leukaemia (AML) [15]. MLL-rearranged acute lymphoblastic leukaemia (ALL) has a particularly poor prognosis [13]. Leukaemic transformation is highly dependent on the menin-MLL interaction as MLL1 maintains Hox gene expression during development and is necessary for appropriate haematopoietic development [16–18]. MLL fusions perturb normal chromatin regulatory complexes. The binding of menin to MLL1 leads to upregulation of Hox gene transcription and leukaemia in MLL-rearranged AML and ALL [16–24]. There are a number of therapeutic opportunities for targeting MLL-fusion-driven leukaemia by targeting enzymes that are important for its activity (e.g. DOT1L and CDK9) and through protein-protein interactions (e.g. MLL-menin, BRD4-acetyl lysine and WDR5). Inactivation of DOT1L leads to decreased MLL-AF9 target gene expression and inhibits leukaemia development [25–29]. A phase I trial of a DOT1L inhibitor in patients with MLL-rearranged leukaemia documented minimal toxicity and inhibition of 60–70% of *HOX*/*MEIS* expression, but also illustrated that responses, when observed, may take time to occur and suggested that meaningful antitumour activity would require deep and consistent DOT1L inhibition and use in combination with other drugs [30,31]. Treatment of MLL-r cells with a novel menin-MLL inhibitor, VTP-50469, leads to loss of menin and DOT1L binding on chromatin, producing substantial gene expression/protein changes and dramatic responses in MLL-ALL PDX models [34]. NPM1-mutant leukaemia, a subtype in about 8% of childhood AML16, is also sensitive to the menin-MLL inhibitor, VTP-50469. Similar to MLL-r, treatment with VTP-50469 causes loss of menin-chromatin binding and suppression of the leukaemic gene transcription program which leads to decreased MLL-associated gene expression. Dramatic activity in preclinical models of AML (MLL-rearranged and NPM1 mutant) have been observed [32,33,34].

2.2. Scientific rationale for epigenetic modifiers in paediatric central nervous system tumours

Evidence has clearly shown that there is an association between epigenetic events and the development of several paediatric brain tumours [35]. It is postulated that the driver event is often a genetic abnormality leading to major epigenetic disorders that preclude early embryonic cells from undergoing normal differentiation, and therefore maintain some stemness, which make cells very prone to proliferation and malignancy. Specifically, it has been demonstrated that some embryonal tumours including medulloblastomas [36], embryonal tumour with multilayered rosettes [35], atypical teratoid rhabdoid tumours (ATRT) [35], paediatric high-grade glioma and posterior fossa group A ependymomas [36] are driven by a prenatal oncogenic event [37]. Furthermore, removal of the driver mutation, the lysine-to-methionine amino acid substitution on histone 3 variants (K27MH3) in diffuse intrinsic pontine glioma (DIPG), directly promotes differentiation, suggesting the effect of a differentiation blockade can be reversed [38].

In particular, DIPG and glioblastomas in children and young adults are developmental defects and involve H3K27M and histone 3.3 G34R/V mutations (H3.3 G34R/VM). There is a topographical and age association between different histone mutations and tumour anatomical location, with cortical tumours often having G34 mutations while midline tumours mostly have K27M mutations [39–44]. H3 K27M mutations are specific for midline high grade gliomas and lead to drastic decrease in the levels of H3K27 dimethylation and tri-methylation. Notably, abnormalities in H3 trimethylation are also observed in posterior fossa group A ependymomas, where overexpression of EZH inhibitor protein (EZHIP) mimics the effects of K27M [45]. They can also be a consequence of mutations in the SWI/SNF complex in ATRT and of KDM6A loss, an H3K27me3 demethylase, in subgroups of medulloblastomas. Thus, abnormal methylation of H3K27 is a general mechanism involved in brain tumour oncogenesis, either through mutation in H3.3/H3.1 genes, overexpression of EZHIP, or through pathogenic imbalance of histone methyl transferases and histone demethylases in other brain tumour entities.

These central nervous system tumours with histone mutation are examples of stalled development through defective spread of major chromatin marks – inhibition of methyltransferase activity. K27-mutated histones inhibit methyltransferases and promote loss of methylation and H3 K27M is essential for tumour maintenance [46,47].

H3 K27M cells are more vulnerable to DNA demethylation triggering viral mimicry. High-grade gliomas defined by H3 K27M exhibit global loss of H3 K27 trimethylation and reciprocal gain of H3 K27 acetylation, respectively, shaping repressive and active chromatin landscapes. H3 K27ac is enriched at repeat elements, resulting in their increased expression, which in turn can be further amplified by DNA demethylation and HDAC inhibitors providing a potential therapeutic vulnerability and unique opportunities for successful monotherapy [48].

There are additional somatic mutations specific to tumour locations, for example, gain-of-function mutations in *ACVR1* occur in tumours of the pons mainly in conjunction with H3.1 K27M, whereas *FGFR1* mutations/fusions can occur in thalamic tumours associated with H3.3 K27M [42] and *BRAFV600E* with *H3K27M* mutations [49]. In addition, in glioblastoma, the presence of *H3F3A* mutations frequently occur concurrently with *TP53* and with *ATRX* (α-thalassaemia/mental retardation syndrome X-linked), and mutations/genetic alterations; the latter are strongly associated with alternative lengthening of telomeres and specific gene expression profiles [39]. These additional somatic mutations substantially expand potential therapeutic strategies and offer opportunities for rational combination therapies which are critically needed, for example, enhancer of zeste homologue 2 (EZH2) and ACVR1 inhibitors or EZH2 with PDGFRA or FGFR inhibitors.

2.3. Scientific rationale for epigenetic modifiers in paediatric non–central nervous system solid tumours

Rhabdoid tumours are the paradigm of epigenetically driven cancers, as *SMARCB1* loss occurs in more than in 90% of these tumours [50–52]. Other rare paediatric *SMARCB1*-deficient cancers include undifferentiated chordomas [53], renal medullary carcinomas [54], epithelioid sarcomas [55], fibromyxoid chondrosarcoma and myoepithelial carcinomas. Fusion-driven sarcomas (e.g. Ewing's sarcoma) and synovial sarcomas also act through SWItch/Sucrose Non-Fermentable (SWI/SNF) dysfunction [56]. Malignant peripheral nerve sheath tumour (MPNST) is a rare and very aggressive cancer which is driven by epigenetic events and in 90% there is inactivation of SUZ12 or EED [57]. Genetic alterations in epigenetic modifiers are obviously the key oncogenic drivers in a few paediatric extracranial solid tumours, such as rhabdoid tumour (inactivating mutations or deletions in SMARCB1 and SMARCA4), synovial sarcomas (translocation disrupting the BAF complex) and MPNSTs [7] (deleterious genetic variants in the polycomb repressive complex 2 [PRC2] complex); genetic alterations are also recurrently reported in lower proportion in other tumour types, where they are thought to play oncogenic roles as "second actors", such as neuroblastoma (deletions of CHD5, mutations in the BAF complex genes) [58,59], osteosarcoma (mutations in ATRX and PRC2 genes) [60], Ewing's sarcoma (mutations in EZH2) [6] and Wilms' tumour (alterations in BCOR, CHD4, ARID1A) [61]. SMARCB1-deficient cancers are good candidates for EZH2 inhibition [62], but in most instances, EZH2 inhibition is not sufficient and combinations are required. EZH2 inhibition may also be effective in *ATRX*-mutated and *MYCN*-amplified neuroblastoma [63]. BET inhibitors need to be further evaluated in *MYCN*-driven [64–66], as well as in BRD3- and BRD4-fusion positive midline NUT carcinomas, and synergy with other drugs including PI3K inhibitors in neuroblastoma and JAK2 inhibitors needs to be investigated further [67,68].

3. Epigenetics and immunotherapy

Many epigenetic modifiers (DNMTs, HDAC, lysine-specific demethylase 1 [LSD1], EZH2, SETDB1, G9a and CDK9 inhibitors) induce viral mimicry in preclinical studies [69]. Antitumour effects include upregulation of endogenous retroviruses, activation of the viral defence response and induction of cell death; this in turn increases an adaptive immune response [70,71]. Viral mimicry comprises DNA demethylation leading to reactivation of repeats (endogenous retroviruses,

Key conclusions of the Paediatric Strategy Forum

- As most mutations in paediatric malignancies influence chromatin-associated protein or transcription factors and paediatric cancers are driven by developmental gene expression programs, targeting epigenetic mechanisms, chromatin-based control of gene expression, is predicted to be a very important therapeutic approach in paediatrics, which has in most circumstances yet to reach or extend beyond clinical proof of concept, as many targets do not yet have drugs available.
- Epigenetics is an evolving field, and new targets continue to emerge.
- As there are many epigenetic targets on the RACE for Children Act FDARA amendments to section 505B of the FD&C Act Paediatric Molecular Target List, paediatric development of epigenetic modifying drugs should be considered early.
- Preclinical studies are critically needed to support early clinical evaluation of epigenetic modifiers in paediatrics.
- A combination approach is critical for the majority of epigenetic modifiers, either with other epigenetic modifiers or molecularly targeted agents.
- Eight classes of medicinal products were discussed and prioritised, based on the level of science to support early evaluation in children: inhibitors of menin, DOT1L, EZH2, EED, BET, PRMT5 and LSD1 and retinoic acid receptor alpha agonists.
- Menin inhibitors should be moved rapidly into paediatric development, in view of their biological rationale, strong preclinical activity and ability to fulfil an unmet clinical need.
- A follow-up multistakeholder meeting focussing on BET inhibitors will be held to define how to prioritise the multiple compounds in clinical development that may be evaluated in children and to prioritise subsets of childhood cancer for initial testing.
- The importance of combinations of EZH2 inhibitors with other products, for example EED inhibitors, was clearly evident and it was proposed that early phase studies of EZH2 inhibitors have a short monotherapy component leading on to a combination phase.
- Other potential combinations include DOT1 and menin-MLL inhibitors.
- New exciting approaches to target fusion proteins are by "degraders" through proteolysis targeting chimeras (PROTAC) and CRISPR-Cas9.
- An LSD1 inhibitor has demonstrated significant preclinical activity in cell lines and mouse xenograft model of Ewing's sarcoma and the results of early phase study are awaited with interest.
- It is envisioned that the discussions in the Forum will inform academics and companies in their future plans and help define the landscape for paediatric drug development of epigenetic modifying compounds.

dsRNA formation, pattern recognition activation (MDA5/MAVS/IRF7), interferon response, which decreases cancer cell "fitness" and increases adaptive immune response (CD8 T cell–dependent). The best strategy to exploit viral mimicry in paediatric malignancy needs to be determined. INFORM2 NivEnt is combining HDAC inhibition to augment the response to checkpoint inhibition aiming to create an immunogenic tumour microenvironment through viral mimicry [72].

In addition to their potential antitumour effect, epigenetic modifier therapies may also influence the immune response. For instance, it has recently been shown that MHC class I presentation is dependent on PRC2-mediated silencing; therefore, EZH2 inhibition may also act by increasing MHC class I presentation and enhancing the T-cell antitumour response [73]; EZH2 inhibition could thereby act on the immune escape of tumour cells to immunotherapy [74].

4. Future directions including targeting fusion proteins

A challenge for the future is to directly target abnormal transcription factors in childhood cancers, a class of proteins that are often critical disease drivers but are notoriously difficult to target with small molecules. In Ewing's sarcoma, 80–90% of tumours have the EWS/FLI1 fusion and 5–10%, the EWS/ERG fusion, and there are few other recurrent mutations [75]. Accordingly, EWS/FLI1 is a clear dependency in EWS/FLI1-positive cell lines [76] and has been shown to recruit chromatin regulators such as the BAF complex to control enhancer activation states. In the case of very poor prognosis, group 3 medulloblastoma amplifications of both c-Myc and the homoeobox transcription factor OTX2 have been described. Furthermore, OTX2 is overexpressed in most group-3 medulloblastomas and is present at most active enhancers in these tumours, suggesting that OTX2 itself or its downstream targets may represent opportunities for therapeutic development [77].

Numerous other paediatric solid tumours also harbour transcription factor fusions in an otherwise simple genomic background, such as alveolar rhabdomyosarcoma (PAX-FOXO1) and desmoplastic small round cell tumour (EWS-WT1). An approach to target fusion proteins is by "degraders" through natural "glue-like" molecules. For example, studies have shown that the mechanism by which immunomodulatory drugs, such as thalidomide, lenalidomide and pomalidomide, exert antitumour effects in multiple myeloma is by binding to the CRL4(CRBN)E3 ubiquitin ligase and redirecting its substrate specificity to bind and degrade IKZF1 and IKZF3, essential transcription factors in multiple myeloma [78,79]. Another degrader approach is through the development of engineered molecules known as proteolysis-targeting chimeras (PROTACs) [80–82]. PROTACs are heterobifunctional molecules comprising two distinct chemical moieties: a small molecule that can bind to the target protein of interest, linked to a second small molecule that binds to an E3

ligase, such as cereblon. Degraders act by bringing the target protein (e.g. EWS-FLI1) and an E3 ligase complex in close proximity, resulting in ubiquitination of the target protein followed by proteasome-mediated degradation. The first PROTACs targeting androgen and oestrogen receptors have just entered clinical trials. This approach has substantial potential as a therapeutic strategy for fusion-driven paediatric cancers which hitherto have been very challenging to target.

5. Relevance of the FDA Molecular target list for epigenetic modifiers and paediatric investigation plans

There are many epigenetic targets on the FDA Paediatric Molecular Targets List, including ACVR1, BRD3-NUTM1, BRD4-NUTM1, CDK12, DDX3X, DOT1L, ETS gene fusion, EWSR1-FLI1, EZH2, H3 G34R/V, Hi3 K27M, IDH1 and IHD2, menin, MLL, MYC, NFkappaB, NSD3-NUTM1, NTRK, PAX-FOXO1, SYT-SSX, TERT, ZNF532-NUTM1, BCOR, MAGE-A3, WT1 and YAP1.

In accordance with EMA Paediatric Regulation (EC) No 1901/2006, all applications for marketing authorisation for new medicines must have either a product-specific waiver or a paediatric investigation plan (PIP) agreed, which could also be deferred for studies planned to be commenced or completed after the marketing authorisation in adults. A PIP is a development plan aimed at ensuring that the necessary data to support the authorisation of a medicine for children are of high quality and ethically researched. In terms of clinical measures, a PIP must include studies generating data on early development (dose finding and efficacy signal seeking) as well as studies generating pivotal evidence. There are currently (January 2020) only 6 published PIPs agreed for medicines known to have an epigenetic mechanism of action: decitabine, azacitidine, guadecitabine, enasidenib, ivosidenib and molibresib. None of these PIPs have yet been completed. Epigenetic modifiers as a class are at a much earlier stage of development even for adults and hence requirement for a PIP/paediatric study plan has not been triggered yet for most of the compounds.

6. New medicinal products

Eight classes of medicinal products were discussed at the forum: menin, DOT1L, EZH2, EED, BET, protein arginine methyltransferase 5 (PRMT5), LSD1 inhibitors and RARα agonist (Table 1).

Table 1
Medicinal products discussed at the Paediatric Strategy Forum.

Class of medicinal product	Product	Target	Company
Menin inhibitor	SNDX-5613	Menin	Syndax Pharmaceuticals
DOT1L inhibitors	EPZ-5676 (pinometostat)	DOT1L	Epizyme[a]
EZH2 inhibitors	EPZ-6438 (tazemetostat)	EZH2	Epizyme[a]
	PF-06821497	EZH2	Pfizer-
	CPI-1205	EZH2	Constellation Pharmaceuticals
	CP0209	EZH2	Constellation Pharmaceuticals
EED inhibitors	MAK683	EDD	Novartis
BET inhibitors	GSK525762 (Molibresib/)	BET	GlaxoSmithKline
	CC-90010	BET	Celgene
	CC-95775	BET	Celgene
	BMS-986158	BET	Bristol Myers Squibb
	BI 894999	BET	Boehringer Ingelheim International GmbH
	CPI 0610	BET	Constellation Pharmaceuticals
	AZD5153	BET	AstraZeneca
LSD1	SP-2577 (Seclidemstat)	LSD1	Salarius
PRMT5 inhibitors	PF-06939999	PRMT5	Pfizer
	GSK3326595	PRMT5	GlaxoSmithKline
Retinoic acid receptor alpha agonist	SY-1425	Retinoic acid receptor alpha	Syros

[a] Publicly available data reviewed by independent presenter.

7. Discussion

The epigenetic landscape is an evolving field and is highly relevant to many paediatric cancers as most alterations in paediatric tumours influence chromatin-associated proteins or transcription factors and paediatric cancers are strongly driven by developmental gene expression [6,7]. Furthermore, paediatric malignancies otherwise tend to have relatively simple genomes with few mutations in readily druggable targets such as kinases. Targeting epigenetic mechanisms is thus predicted to be a very important therapeutic approach in paediatric malignancies, but it has yet to achieve its full potential. Many targets do not yet even have inhibitory drugs available. There is a clear opportunity to exploit the cellular vulnerability in select cell populations, potentially restricted to specific windows of development because of the epigenetic/microenvironmental states. The challenge is to rationally screen for vulnerabilities of specific oncogenic cellular states. The development of epigenetic modifying drugs in paediatric malignancies must be driven by scientific evidence to provide the best therapeutic approaches for children with cancer. Genome-wide characterisation of chromatin states in cancer, in addition to genome-wide functional dependency screening with technologies such as CRISPR-Cas9 [83], can uncover cellular

dependencies on transcription factors and their target genes that may be exploited for future cancer therapeutic approaches.

DNA methyltransferase inhibitors/hypomethylating agents and HDAC inhibitors were not discussed in this forum as they have been evaluated in paediatrics. Recurrent mutations affecting IDH1/2, TET2 or DNMT3a, in central nervous system tumours and leukaemia, cause DNA methylation, resulting in aberrantly hypomethylated or hypermethylated genomes. The use of inhibitors of DNA methyl transferases has proved to be efficient in some myeloid leukaemias with a hypermethylated genome. Beyond adult leukaemias with mutations in DNA methylating enzymes, hypermethylated tumours were considered to be potential good candidates for hypomethylating agents, such as DNMT inhibitors. In children, for example, posterior fossa ependymomas with cpG island methylator phenotype ("CIMP+") [84] and SHH-ATRT [85] have hypermethylated chromatin, suggesting a potential for hypomethylating agents. However, the use of such medicinal products in conventional doses and as single agents in adult solid tumours has not met expectations and has remained disappointing. Similarly, inhibition of HDAC activity prompts tumour cells to enter apoptosis; therefore, the utility of HDAC inhibitors for the treatment of cancer has been investigated and several HDAC inhibitors have been developed. Haematological malignancies seem to be particularly sensitive, and vorinostat, for instance, has been approved for the treatment of cutaneous lymphomas. More recent classes of HDAC inhibitors such as panobinostat have also shown promising preclinical results in paediatric cancers such as DIPG [86].

It was agreed by participants that as i) the biological rationale for the menin-MLL interaction driving the malignant transformation of MLL-rearranged or NPM1 acute leukaemias is very strong, ii) there is substantial documented pre-clinical activity of menin inhibitors and iii) there is a substantial unmet clinical need as the prognosis of leukaemias with *MLL1* rearrangement is poor with no approved therapies specifically targeting MLL, the paediatric development of menin inhibitors should be accelerated [33,34]. Early phase trials of menin inhibitors should commence in paediatrics now, in parallel to, and incorporating data from, ongoing adult studies.

DOT1L mediates histone H3 K79 methylation in *MLL*-rearranged leukaemia and is an important target. Although trials to date of DOT1L inhibitors in paediatrics have not produced substantial responses and the need for a continuous infusion regimen has been logistically challenging [31], the participants of the forum believed further evaluation of DOT1L inhibitors was warranted. The maximum tolerated dose was not achieved in previous early phase trials and further dose escalation may result in an improved pharmacodynamic effect or new molecules with better pharmacokinetic properties might demonstrate improved efficacy. Furthermore, these agents should potentially be combined with menin-MLL inhibitors.

Activating enhancers of EZH2 mutations or aberrations of the SWI/SNF complex (e.g. mutations or deletions of the subunits SMARCB1 or SMARCA4) can lead to aberrant histone methylation, oncogenic transformation, and a proliferative dependency on EZH2 activity. PRC2 is an epigenetic regulator primarily responsible for trimethylation of histone H3 on lysine 27 (i.e. H3 K27me3). It is made up of 3 subunits: EED, EZH2 and SUZ12. EZH2 catalyses the monomethylation, dimethylation and trimethylation of H3 K27. PRC2 is the only human protein methyltransferase that can methylate H3K27, and H3K27 is thought to be the primary substrate for PRC2. Abnormal trimethylation of H3K27 is tumourigenic in a broad spectrum of human cancers; however, aggressive tumours appear less sensitive to histone methyltransferase inhibition (at least from EZH2 inhibition) than slower growing tumours [87–91]. Patient selection by *EZH2*-activating mutations identifies more responsive tumours, although in follicular lymphoma; a subset of patients without activating mutations was also reported to respond. Unlike HDAC and BRD proteins, which have more global effects, EZH2 typically regulates lineage-specific transcription programs critical for cell identity. The EZH2 inhibitor tazemetostat recently received FDA-approval for epithelioid sarcoma [55] and has had clinical activity in other BAF (SWI/SNF-A) mutant tumours such as malignant rhabdoid tumour [52]. Based on data from a number of research groups, there is also preclinical evidence to support the exploration of EZH2 inhibitor therapy in neuroblastoma.[58,59]

EED has a dual role of binding H3 K27me3: PRC2 recruitment and allosteric activation. There is a potential and interesting role for combining EZH2 and EED inhibitors, although toxicity may be increased. Importantly, in K27M-mutant gliomas or EZHIP-overexpressing posterior fossa group A ependymomas where H3K27me3 levels are already very low, further decrease in these levels through increased EZH [38,92] and/or EED inhibition may result in tumour-specific cell death, while normal cells with high H3K27me3 levels would be relatively spared. It was proposed that early combination clinical trials of EZH2 inhibitors with EED inhibitors are designed and executed, with short monotherapy phases of the EZH2 and EDD inhibitors leading to evaluation of the combination.

BET inhibitors [64,65] have at least three areas of interest in paediatric malignancies in i) NUT midline tumours [93], ii) *MYCN*-amplified malignancies [94,95] and iii) fusion-driven malignancies. There is however controversy relating to their role in *MYCN*-amplified malignancies, as it is uncertain if the drug concentrations necessary to achieve a biological effect *in vivo* can

be reached in clinical practice due to toxicity, notably thrombocytopaenia [66,96] and there is limited evidence for tumour-regressing activity. Furthermore, the pan-BET inhibitors have been challenging to administer in adults and monotherapy has resulted in modest anti-tumour activity and class toxicity effects such as bone marrow and gastrointestinal tract toxicity, transaminitis and fatigue. The focus should be on BET inhibitors with broader therapeutic index, which will make it possible to use them with various combination partners and on those BET inhibitors with blood-brain barrier penetrance allowing the targeting of paediatric central nervous system malignancies. The situation is further complicated by the presence of at least seven pan-BET inhibitors in clinical development. There is interest in second-generation BET inhibitors that, in contrast to dual-bromodomain BET inhibitors that bind with similar affinities to the first (BD1) and second (BD2) bromodomains, show selective inhibition of the BD2 bromodomain [97]. The more limited impact of BD2-selective inhibition on global transcription patterns may lead to an enhanced therapeutic window for selected cancers. The relevant paediatric population is not large enough to accommodate pivotal clinical trials. It was therefore proposed to hold a multistakeholder follow-up meeting focussing on BET inhibitors to define how to prioritise investigation of BET inhibitors in children and evaluate their specific roles.

PRMT5 is another relevant potential target [98–101]. Early preclinical and human genetic data suggest that PRMT5 inhibitors may have a role in the treatment of a variety of tumour types including gliomas and lymphomas in children. First-generation PRMT5 inhibitors are currently undergoing clinical testing. However, it needs to be determined if these early generation compounds have the appropriate antitumour, safety and pharmacodynamic properties (e.g. ability to cross the blood-brain barrier) for development in paediatric cancers. More adult clinical and preclinical data in paediatrics are needed to support testing and development of PRMT5 inhibitors in children.

LSD1 is an epigenetic eraser and demethylates monomethylated and dimethylated H3K4 (activating mark) and H3K9 (repressive mark) [102,103]. EWS/FLI (11; 22) is the most common fusion in Ewing's sarcoma resulting in the repression of vital tumour suppressor genes by the activity of LSD1. LSD1 is overexpressed in 60% of Ewing's tumours [104,105] and is correlated with poor overall survival [106]. LSD1 possesses not only FAD-dependent enzymatic histone demethylase activity through its amine oxidase domain but also interacts with a myriad of proteins and protein complexes [103]. Studies suggest that catalytic inhibition of LSD1 is insufficient to impact tumour cell viability and that disruption of LSD1 protein-protein interactions may be required in certain tumour types, such as Ewing's sarcoma [107]. Seclidemstat is a small molecule inhibitor of

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(continued on next page)

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both LSD1's catalytic and protein-rotein interaction capacity and has significant preclinical activity in cell lines and mouse xenograft models of Ewing's sarcoma [108]. The results of the ongoing early phase study (NCT03600649) which is also open to recruitment for adolescents aged 12 years and older are awaited with interest.

SY-1425 (tamibarotene), a potent and selective RARα agonist has been developed to overcome selectivity and pharmacokinetic liabilities associated with all-trans retinoic acids [109]. A subset of non-APL AML patients (30%) have an alteration in the regulatory region of the genome that drives increased RARA expression and are RARA-positive by a novel blood-based biomarker test that predicts sensitivity to SY-1425 [109,110]. SY-1425 is being developed in combination with azacitidine in RARA-positive adult AML. Early results demonstrated high complete response rates and a rapid onset of clinical responses in RARA-positive newly diagnosed unfit AML [111]. The high clinical unmet need, strong preclinical rationale and favourable safety profile in adults supports development in RARA-positive paediatric AML, where a proportion of paediatric AML is RARA-positive. SY-1425 has antiproliferative, proapoptotic and on-target prodifferentiation effects in RARA-positive paediatric AML *in vitro* and marked antileukaemia activity in *vivo* [111]. In view of the rarity of the population, it was proposed to approach paediatric development through collaboration with the PedAL/EUPAL [112].

A combination approach is important for most epigenetic modifiers, either with other epigenetic modifiers (e.g. EZH2 and EED) or molecular targeted agents (ACVR1, PDGFRA or FGFR inhibitors) or immunotherapies, and exploration of the optimum combination should be underpinned by extensive preclinical research in advance of clinical translation. Early phase clinical studies should be designed with this perspective in mind and have a short monotherapy component leading on to a combination phase in the same trial.

Preclinical studies are needed to support early clinical evaluation of epigenetic modifiers in paediatrics, and this theme was consistent for all medicinal products discussed. The extent and depth of the preclinical studies depend on the strength of the underlying biological hypothesis. For example, if the target is a well-defined oncogenic driver, then less preclinical evidence is required. As a combination approach is likely to be required to achieve meaningful antitumour activity for the vast majority of epigenetic modifiers, it is important that preclinical studies investigate the utility of epigenetic modifiers with other drugs. There remains a shortage of facilities for these preclinical investigations; hopefully, the international preclinical testing initiatives (Innovative Therapies for Children with Cancer Paediatric Preclinical Proof-of-concept Platform [ITCC-P4] [113], National Cancer Institute's Paediatric Preclinical Testing Consortium [PPTC] [114] and the Foundation for the National Institutes of Health [FNIH]) [115]will fulfil these requirements.

It is anticipated that this Paediatric Strategy Forum, which was held early in the paediatric development of epigenetic modifiers in children, when most companies had not yet clarified their plans for paediatric development and before reaching agreement with regulatory authorities on these plans, will be beneficial. It is envisioned that the discussions in the forum will inform academics regarding the design of clinical investigation strategies and companies in their future plans and help to define the landscape for paediatric drug development of epigenetic modifiers.

8. Conclusion

The epigenetic landscape is an evolving field and is highly relevant to many paediatric cancers; the biology is innovative and new targets are frequently emerging. Targeting epigenetic mechanisms is potentially a very important therapeutic approach in paediatric malignancy, one that in most circumstances has yet to reach or extend beyond proof of concept. One reason for this is that many targets do not yet have drugs available. As there are many epigenetic targets on the FDA's Relevant Paediatric Molecular Target List, the new regulatory landscape should benefit children with malignancy. A combination approach is important for many epigenetic modifiers and exploration of the optimum combination will need to be supported by extensive preclinical research. Menin inhibitors should be moved rapidly into paediatric development, in light of their biological rationale, strong preclinical activity and ability to fulfil an unmet clinical need. A follow-up multistakeholder meeting focussing on BET inhibitors will be held to define how to prioritise the multiple compounds that might be evaluated in children and to determine which molecular subtypes to prioritise for testing. As epigenetic targeted drugs are early in development, there is a major opportunity to define the landscape of epigenetic modifiers to develop efficient and optimal plans for their evaluation in children and adolescents.

Author contribution

Study concepts - ADJP, GV, KN, DK, GR, KS and FB. Manuscript preparation - ADJP, KS, FB, GV, DK, GR, LVM, MS and NB. Study design, data acquisition, quality control of data analysis and algorithms, data analysis and interpretation, manuscript editing and manuscript review - All authors.

Conflicts of interest statement

MLM is an employee of Syndax Pharmaceuticals Inc. PB is a scientific advisor for Novartis, Syndax and Servier. DDC and MR are consultants for Loxo Oncology and receive research support from Advanced Cell Diagnostics. PCA is an employee of Sanofi. SB is an employee of Day One Therapeutics Inc. ADD is an employee of Salarius Pharma. BG is an employee of Novartis. MH is an employee of AstraZeneca. PH is an employee of Boston Pharmaceuticals. IJ is an employee of Pfizer. MJK is an employee of Syros Pharmaceuticals. MK is an employee of Bristol Myers Squibb. ZN is an employee of Celgene. AS is an employee of Constellation Pharma. TT is an employee of Boehringer Ingelheim. ADJP has participated in advisory boards for Novartis, Takeda, Merck, Lilly and Celgene. All remaining authors have declared no conflicts of interest.

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