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Invited Review: Dysregulation of chromatin remodellers in paediatric brain tumours – SMARCB1 and beyond

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Dysregulation of chromatin remodellers in paediatric brain tumours – SMARCB1 and beyond

Mutations in chromatin remodelling genes occur in approximately 25% of all human tumours (Kadoch *et al. Nat Genet* 45: 592–601, 2013). The spectrum of alterations is broad and comprises single nucleotide variants, insertion/deletions and more complex structural variations. The single most often affected remodelling complex is the SWI/SNF complex (SWItch/sucrose non-fermentable). In the field of paediatric neuro-oncology, the spectrum of affected genes implicated in epigenetic remodelling is narrower with *SMARCB1* and *SMARCA4* being the most frequent. The low mutation frequencies in many of the SWI/SNF mutant entities underline the fact that perturbed chromatin remodelling is the most salient factor in tumourigenesis and could thus be a potential therapeutic opportunity. Here, I review the genetic basis of aberrant chromatin remodelling in paediatric brain tumours and discuss their impact on the epigenome in the respective entities, mainly medulloblastomas and rhabdoid tumours.

Keywords: AT/RT, Medulloblastoma, SMARCA4, SMARCB1, SWI/SNF

Chromatin and its components

Chromatin is a dynamic structure that organizes the genome in eukaryotic cells and thus influences cell type-specific gene expression. The term – originally coined by the cell biologist Walther Flemming in the 19th century and back then describing various staining patterns of the nucleus as observed under the light microscope – relates to the DNA and the attached histone proteins (as well as their modifications) [1]. Specifically, it comprises the four core histones, H2A, H2B, H3 and H4, which are wrapped into a DNA segment of 147 bp length [2]. The histone H1 links nucleosomes and is not directly involved in the compaction of DNA but is important to facilitate higher order chromatin structure [3]. The spatial organization of this complex as well as its covalent modifications (for example, by the demethylation or methylation of specific histone residues) influence transcriptional programmes and consequently have a direct effect on the physiological differentiation of cells [4].

Histone modifications as targets in tumourigenesis

The N-terminal histone tail, which protrudes out of the nucleosome, is an important regulatory structure and can be subject to several post-translational modifications. These include, for instance, trimethylation of the

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amino acid lysine in position 27 of histone 3 which exerts a repressive influence on gene expression or its acetylation which usually enhances gene expression. Other amino acids as part of the histone H3 tail, which can be modified either by methylation or by demethylation, are the lysines in position 4 or 27 (H3K4 and H3K27).

Further post-transcriptional modifications which influence the histone code are small protein modifications such as ubiquitin and SUMO (small ubiquitin modifiers). Ubiquitin commonly acts as a signal for proteomic degradation of the marked proteins. The SUMO protein is able to recruit further proteins which carry SUMO-interacting domains (SIM) and can thus act as a scaffold to assemble large protein complexes. It has recently been found that the addition of these residues in turn may influence the presence of other histone marks such as H3K9me3 [5].

The methylation or demethylation of the aforementioned amino acids is mediated by a set of enzymes which in turn can become the target of oncogenic mutations. The KDM family of demethylases catalyses the demethylation of lysine residues and is frequently mutated in non-small cell lung and urothelial cancers [6]. In particular, KDM6A is prototypically affected in acute myeloid leukaemia, where the loss of this protein leads to a higher degree of chemotherapy resistance [7]. While chromatin-modifying enzymes influence gene transcription by directly targeting amino acids of various histones, chromatin remodellers affect the steric conformation of the histone code and are thus less well defined than the focal effects of chromatin modifiers.

Chromatin remodellers in paediatric brain tumours – families and functions

Broadly, chromatin remodelling complexes can be classified into four different families according to the phylogenetic similarities in their ATPase domains [8]: imitation switch (ISWI), chromodomain helicase DNA-binding (CHD), switch/sucrose non-fermentable (SWI/SNF) and INO80 [9]. These protein families differ by their protein composition but also by their functional effects on chromatin configuration. The ISWI and CHD complexes are pivotal for the maturation of nucleosomes [10] (that is, the proper assembly and configuration of nucleosomes after DNA replication processes) and for correctly spacing these to enable transcription. The SWI/SNF complexes open up the chromatin by introducing irregular spaces between nucleosomes and by ejecting nucleosomes. This mechanism exposes DNA segments to transcription factors. INO80 is implicated mostly in the exchange of histones in nucleosomes without, however, changing the spacing of the nucleosomes [11,12].

Among these families of chromatin modifiers, mutations in the SWI/SNF complex play a dominant role in human tumourigenesis. In a meta-analysis of 44 whole genome/exome sequencing studies, Kadoch *et al.* found mutations of SWI/SNF subunits in 19.6% of all cancer types analysed [13]. Among them, *ARID1A* was the most frequently mutated gene (mainly in endometrioid and clear cell ovarian cancer). Overall, *SMARCA4*, *ARID1A*, *ARID1B*, *ARID2* and *PBRM1* were mutated significantly above the background mutation rate.

In paediatric oncology, the distribution is remarkably different and narrower. Recently published pan-cancer analyses [14,15] have found *SMARCB1* and *SMARCA4* as the only significantly mutated genes of this type, which were affected by single nucleotide variants (SNVs) in paediatric tumours. In these studies, 38.2% of all atypical teratoid rhabdoid tumor (ATRT) samples, 6.67% of all Burkitt lymphomas and 4.76 % of all wingless-related integration site (WNT) medulloblastomas displayed *SMARCB1* point mutations. *SMARCA4* was found to be mutated in 46.67% of all Burkitt lymphomas, 19.05% of all WNT- and 10% of all Group 3 medulloblastomas. No other SWI/SNF complex members were among the significantly mutated genes.

Although these studies are comprehensive in that they comprise samples of most cancer entities, they are particularly enriched in paediatric brain tumours. An independent analysis of several published whole-exome studies from paediatric tumours, which I performed in this review, not only confirms the importance of *SMARCA4* and *SMARCB1* but also highlights the importance of *ARID1A/B* in paediatric entities (Figure 1; Table 1). ARID mutations occur recurrently in neuroblastomas, rhabdomyosarcomas and a small fraction of Wilms tumours [16-19]. In neuroblastoma, up to 10% of all samples harbour mutations in either *ARID1A/B* or *SMARCA4* and many of these tumours belong clinically to the high-risk group. Based on these numbers, it is hard to determine which subunits of the SWI/SNF complex are most often affected in paediatric oncology, but it is safe to state that *SMARCB1* and

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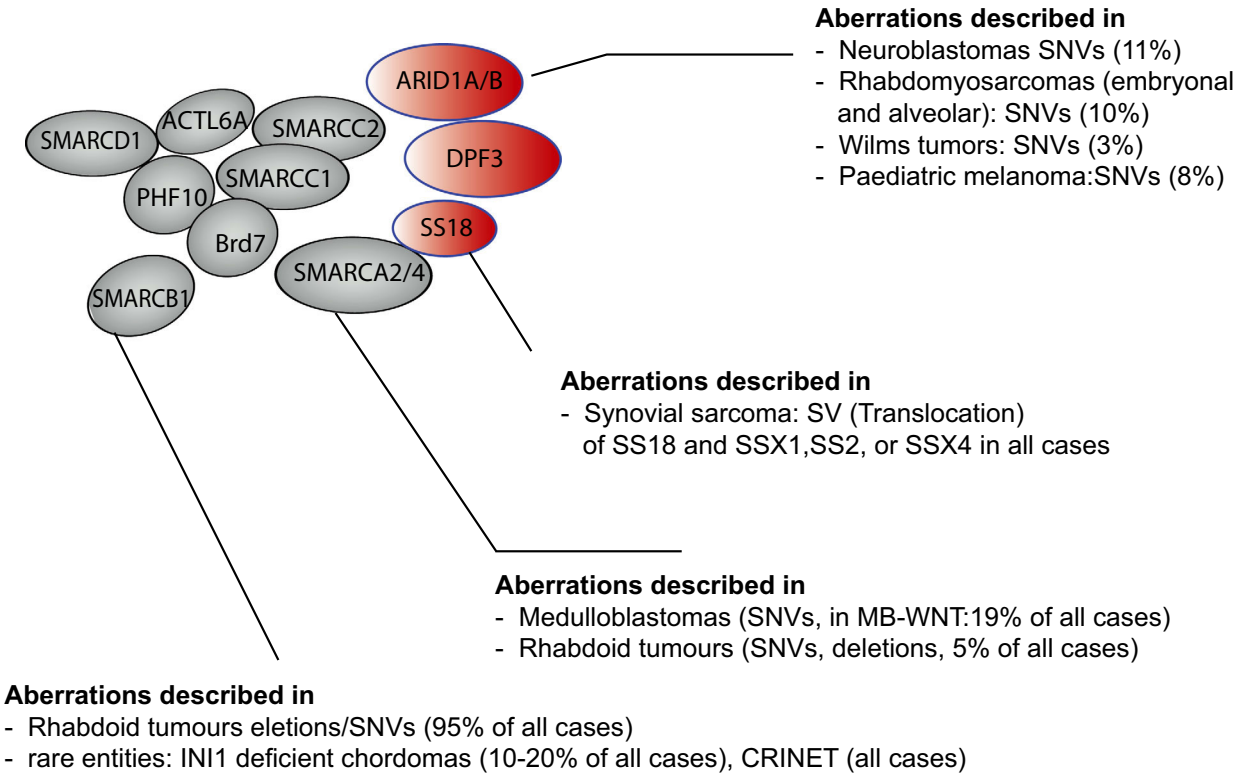

Figure 1. An overview on SWItch/sucrose non-fermentable aberrant paediatric entities. CRINET, cribriform neuroepithelial tumours.

SMARCA4 aberrations occur more often in the tumours of infancy and childhood than in adult entities.

In the setting of paediatric tumours in general or paediatric brain tumours specifically, mutations of SWI/SNF members thus seem to be the prevailing theme. A notable exception is *CHD5*. The CHD proteins (comprising CHD1–9) have been implicated in the neuroblastoma – the 1p36 locus, which is often lost in high-risk neuroblastomas, encompasses the *CHD5* locus. Point mutations of this protein have been described in breast and ovarian [33,34] cancers but to the best of my knowledge have not been reported in paediatric malignancies.

In neuroblastoma, both the deletion of *CHD5*, as part of the complex CHD, as well as a lower expression of CHD5 have independently been identified as markers of a bad prognosis [35]. Unlike the case of SWI/SNF subunit inactivation (which is mostly homozygous), most *CHD5* aberrations in neuroblastoma are heterozygous, and homozygous inactivation might be achieved by epigenetic silencing as mutations or structural variants of the second allele are seldom found. Although CHD5 has been ascribed tumour suppressive functions in glioma cell lines [36], a *bona fide* role as a tumour suppressor in paediatric brain tumours remains to be established.

Similarly, the links of INO80 aberrations to cancer in general and paediatric neuro-oncology specifically are sparse. While it is known that this complex is required for oncogenic transcription in, for example, non-small cell lung cancer and colon cancer [37,38], so far no recurrent oncogenic mutations have been identified. Similarly, although germline mutations of SWI complex members result in more or less well-characterized (neuro)developmental disorders (such as Coffin-Siris syndrome, which is characterized by mutations in *ARID1A*, *ARID1B*, *SMARCA4*, *SMARCB1*, *SMARCE1* or the developmental transcription factor *SOX1* [39] or Rett syndrome-like phenotypes in which *SMARCA1* is mutated [40]), links between brain tumourigenesis and the ISWI complex are largely missing.

Thus, the subunits of the SWI/SNF complex – or BAF complex ('BRG1- or HBRM-associated factors') are most often affected by genetic alterations in paediatric brain tumours.

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Table 1. An overview on SWI/SNF aberrations in paediatric entities

Group of entities	*Entity*	*Frequency of SWI/SNF gene alterations*	*References*
Leukaemia and lymphomas	Early T-cell precursor ALL	None	Zhang *et al.* [20]
	AMKL	One *SMARCA2* mutation in 14 cases	Gruber *et al.* [21]
	Hypodiploid acute leukaemia	None	Holmfeld *et al.* [22]
	Follicular lymphoma	None detected	Ozawa *et al.* [23]
	Burkitt lymphoma	2/4 cases *SMARCA4* mutations	Richter *et al.* [24].
Brain tumours	Pilocytic astrocytomas	None	Jones *et al.* [11]
	High-grade gliomas	None	Sturm *et al.* [11]
	Ependymomas	None	Mack *et al.* [11]
	ATRT	*SMARCB1* aberrations in all samples subjected to WES	Johann *et al.* [25]
Kidney tumours	Wilms tumour	*ARID1A* copy number changes and SNVs in low frequency (2/117), two *ARID1A* germline variants	Gadd *et al.* [11]
	Adrenocortical carcinomas	None	Zheng *et al.* [11]
	Clear cell sarcoma of the kidney	None	Roy *et al.* [26,27]
Neuroblastoma	Neuroblastoma	*ARID1A/B* alterations in 8/71 tumours	Sausen *et al.* [16]
		ARID1B alterations in 5/72 samples (6.9%)	Lee *et al.* [28]
Sarcoma	Rhabdomyosarcoma (embryonal and alveolar)	6/60 samples with *ARID1A* mutations (four frameshift/nonsense mutations), four in eRMS, two in aRMS	Seki *et al.* [17]
		4/20 samples with *ARID1A* or *ARID1B* mutations, two samples with *SMARCB1* mutations, one with a *SMARCA4* mutation	Kohsaka *et al.* [18]
	Osteosarcoma	None detected (WES of 31 tumours)	Bousquet *et al.* [29], Kovac *et al.* [30]
	Synovial sarcoma	Translocations involving *SS18* and *SSX1*, *SSX2* or *SSX4* in all cases	Banito *et al.* [31]
	Ewing's sarcoma	None	Crompton *et al.* [11]
Others	Paediatric melanoma	2/23 samples harboured mutations in *ARID1A* and *ARID1B*	Lu *et al.* [32]

SWI/SNF, SWItch/sucrose non-fermentable.

Three of a kind? Structure and functions of three BAF complexes

The SWI/SNF complex – initially identified 20 years ago in yeast [41,42] – is a highly conserved protein complex with pleiotropic, mainly activating, functions in the epigenome of mammalian cells. It helps to keep the balance between stem cell properties and the differentiation of cells. An important structural basis for this function is the fact that components of the BAF complex can be exchanged during cellular differentiation. For example, as neuronal progenitors mature into neurons, they lose the ACTL6A/BAF53A and PHF10/BAF45A components of the npBAF complex which are replaced by ACTL6B/BAF53B and DPF1/BAF45B or DPF3/BAF45C. Thus, turning the neuronal progenitor BAF complex to a neurone-specific BAF complex (see Figure 2**a**) [43]. This conversion, or subunit switching, seems to be an important characteristic in acquiring cell type-specific functions. Beyond these tissue-specific BAF complexes (cBAF), recent studies have highlighted a further structurally different variant of the SWI/SNF complex, which differs profoundly in its protein composition. Specifically, aside from the cBAF complex and the pBAF complex (polybromo BAF complex), the non-canonical SWI/SNF complex (see Figure 1) has been identified [44,45].

The modular assembly of these complexes has recently been uncovered within the frame of a large-scale mass spectrometric study [44]. In the three BAF types, the SMARCC and SMARCD subunits represent the common backbone. By addition of SMARCB and SMARCE, the so called BAF core is formed. The addition of GLTSCR1 then paves the way for further differentiation into the ncBAF complexes, while the addition of SMARCB1 and SMARCE1 builds up the BAF core. This core BAF complex is then further branched into the cBAF complex (by the addition of ARID1, 'ARID-dependent branching') or the pBAF complex (by the addition of ARID2). The addition of the ATPase module (in

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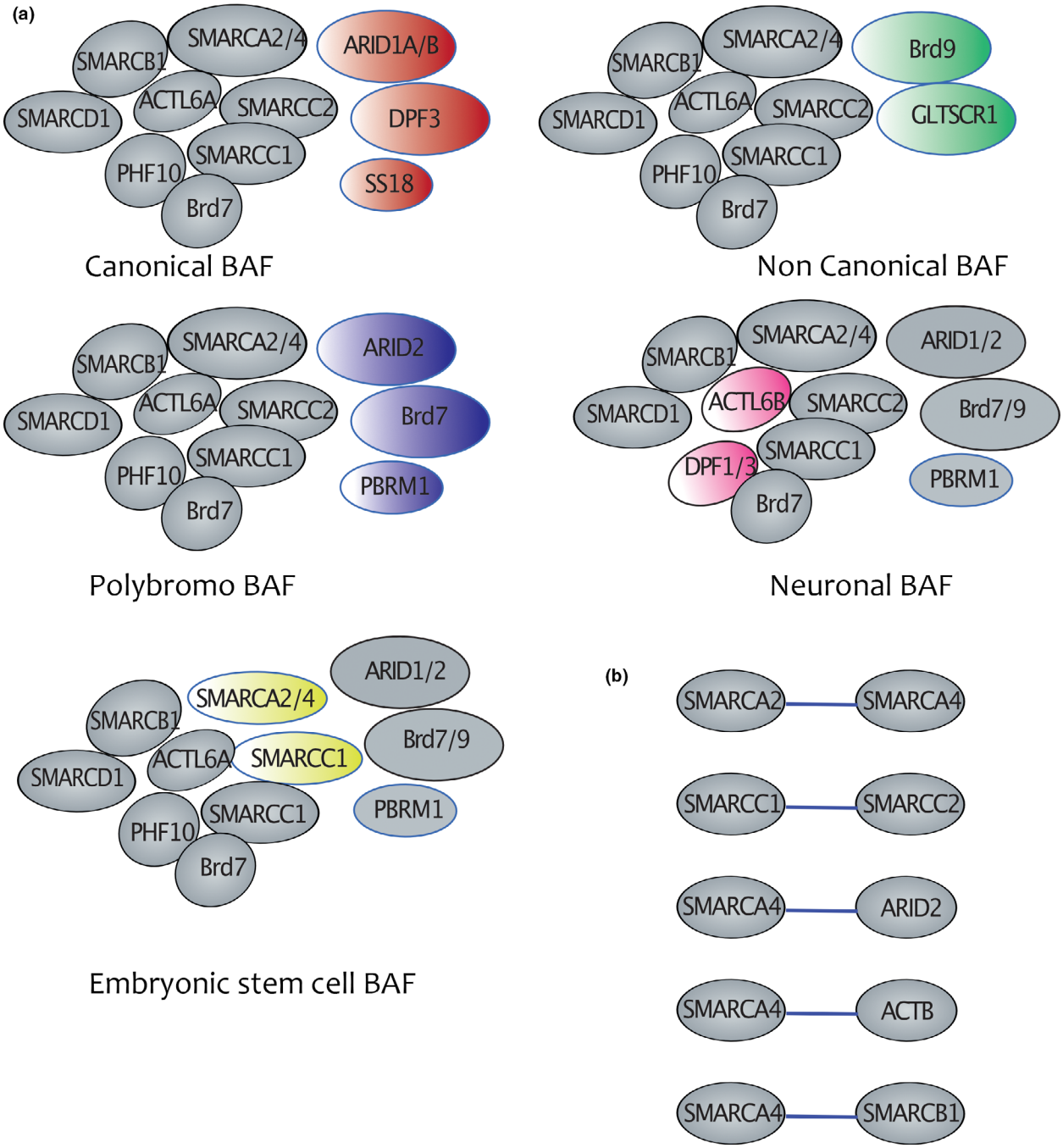

Figure 2. The PRC2 complex and an overview on its non-canonical functions in rhabdoid tumours. (**a**) Different variants of the BAF complex. (**b**) Previously described dependencies of BAF subunits.

all three types of complexes) finalizes the assembly process.

Although different cellular functions of these complexes have not been fully elucidated, the different composition of the complexes may suggest that a loss of classic SWI/SNF function (such as for instance in rhabdoid tumours) may be compensated by an increase in, for example, ncBAF functioning – which would then constitute a putative therapeutic vulnerability. Due to the fact that no mutational or structural aberrations in the ncBAF-specific

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proteins (such as, for example, GLTSCR1 and Brd9) have been uncovered so far, the suitability of these as potential drug targets is thus an important, potentially clinically exploitable, consequence [45,46].

Along this line, it is noteworthy that the ncBAF complex lacks ARID1, ARID2 and SMARCB1 subunits and may hint towards the use of Brd9 inhibition in both ARID1-altered entities and SMARCB1-altered entities. The identification of dependencies between SWI/SNF complex members is an active field of investigation. Recently, Schick *et al.* have comprehensively mapped which proteins become vital for cell survival after, for example, SMARCA4 knock-down. This knowledge may in future be used to target SMARCA4-deficient tumours [47] (see also Figure 2**b**).

Functional insights into how the different BAF complex members exert distinct functional roles are sparse. For the pBAF complex, an interesting contribution came from Wei *et al.* who found that targeting Brd9 in pancreatic beta cells was able to enhance the interaction between the vitamin D receptor and the pBAF complex, thus increasing beta cell survival and ameliorating hypoglycaemia in animal models [48].

Among other specific roles that have been ascribed to the pBAF complex (or its core unit Brd7) are the regulation of neural crest development in association with subunits of the CHD complex [49,50] as well as the maintenance of embryonal stem cell transcription [51].

Interestingly, the pBAF complex also seems to play a role in increasing the vulnerability of cancer cells to T cell-mediated killing, which may offer the prospect to sensitize tumour cells to immunotherapeutic approaches by using Brd7 inhibitors [52].

It can be assumed that many more of these molecular switches, which result in an increased recruitment of specific BAF subtypes, exist. This may potentially be relevant in the oncological setting.

In the context of cancer, the BAF complex has mainly been recognized as an important tumour suppressor. Jagani *et al.* showed that the inactivation of SMARCB1, being a key component of the BAF complex, leads to an overactivation of potentially oncogenic sonic hedgehog (SHH) signalling [53]. This experimental finding is backed by studies in primary tumours, which found an overexpression of SHH components in a subgroup of ATRT [25]. Not only MYC-dependent signalling but also the expression of MYC itself are also repressed by an intact SWI/SNF complex [54,55].

Earlier studies have pointed out that the transition from the G1 to S phase is impaired by direct binding of the SWI/SNF complex [56] and can thus induce G1 arrest [57], this goes along with a downregulation of E2F targets (such as E2F1, CDC6, cyclin A and cyclin D1) [58,59]. This function of the SWI/SNF complex is critically dependent on an intact RB protein [59].

Beyond these functions at the level of oncogenic signalling, the BAF complex is pivotal in maintaining genome integrity [60]. This may happen via different routes. For example, Kapoor *et al.* pointed out that Mec1 kinase (a yeast orthologue of the human *ATM* gene) is positively regulated by the SWI/SNF complex [61]. Moreover, the SWI/SNF complex induces and maintains high levels of γH2A.X, which in turn provides binding sites for the checkpoint factor MDC1 [62].

The importance of the SWI/SNF complex for genome integrity is somewhat counteracted by the observation that many cancers, which display SWI/SNF subunit mutations, do not display a high degree of chromosomal instability.

While the interplay of the BAF subcomplexes is subject to further functional studies, the 'bottom-up' approach of genomic analysis has helped to identify paediatric brain tumours which harbour mutations in SWI/SNF complex members.

SMARCA4 mutations in paediatric brain tumours – the genomic basis

In the field of paediatric brain tumours, alterations in *SMARCA4* have been described in medulloblastoma and rhabdoid tumours, with the cardinal difference being that the alterations in rhabdoid tumours are commonly homozygous, whereas in medulloblastomas only one allele is affected.

A number of studies [63-65] found *SMARCA4* aberrations in medulloblastomas at varying frequencies between 4% and 11.5% respectively. Notably, most of these tumours belonged to the molecular Group 3 and a minority to the WNT subgroup. It is noteworthy that these mutations in medulloblastoma co-occur with other SNVs, which are typical for the respective MB subgroups such as *CTNNB1* in WNT and gains/amplifications of *MYC* in MB-Group 3 [65]. Mutations in *SMARCA4* are relatively depleted in the SHH subgroup of medulloblastomas [66]. This can potentially be

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explained by a recently described dependency of SHH signalling on wild-type SMARCA4 [67] – it can thus be assumed that this oncogenic pathway can only be maintained in the presence of an intact SMARCA4.

The heterozygous mutations in medulloblastoma are thus in contrast to those in rhabdoid tumours where they are presumed to be the sole oncogenic events. For medulloblastomas, this raises the hitherto unanswered question as to whether these mutations are really the initiating event in tumourigenesis or rather bystanders which promote tumour formation at a later stage.

The protein structure of SMARCA4 comprises five functional domains: the QLQ domain (amino acids 171–206) and the HSA domain (amino acids 460–532) at the N-terminal end of the protein, as well as two helicase-binding domains (the SNF2 domain or helicase ATP-binding domain, amino acids 830–995 and the C-terminal helicase domain, amino acids 11480–1310), the Brd domain. The bromodomain of SMARCA4 is localized C-terminally. It has been found to be dispensable for tumour cell proliferation and its pharmacological inhibition did not repress the growth of rhabdoid tumour cells *in vitro* [68].

The mutations detected in medulloblastomas were (almost) exclusively found in the DEAD-like helicase domain and the helicase C-terminal domain. Unlike in rhabdoid tumours where loss of *SMARCA4* (either by a somatic SNV or by cytogenetic alterations) is usually homozygous, the mutations found in medulloblastomas were mostly heterozygous. In rhabdoid tumours, *SMARCA4* alterations are seldom found – they account for approximately 5% of all rhabdoid tumours and are thus exceedingly rare [69]. The complete loss of the protein likely has different functional consequences than the point mutations in medulloblastoma, in which SMARCA4 is still expressed although functionally compromised [69,70].

Mechanistic consequences of *SMARCA4* mutations and their consequence on the epigenome

The functional role of SMARCA4 is complemented by SMARCA2 – both proteins are 75% identical and are expressed in different cell types and stages of differentiation [71]. As a consequence of this functional similarity, SMARCA2 becomes vital for tumour cell survival in SMARCA4 mutant cell lines [72]. The functional consequences of the various SMARCA4 mutations affecting the different protein domains have only recently been investigated. Mutations in the DNA-binding domain of SMARCA4, as would be expected, inhibit its binding to nucleosomes and thus increase the amount of mobile SMARCA4 in a cell, in contrast to mutations in the ATPase-binding domain that prevent the release of SMARCA4 from bound chromatin by decreasing ATP hydrolysis [73].

Despite these opposing mechanistic effects, the broad epigenetic consequences of introducing heterozygous mutants of the ATPase-binding domain and the DNA-binding domain [73] on chromatin accessibility seem to be similar. Consistent with the role of the SWI/SNF complex as an 'opener' of the chromatin, there were more genomic sites losing accessibility than gaining in the ATAC-seq analysis of cells with *SMARCA4* mutations.

A prominent epigenetic pattern, which was present in all heterozygous mutants, was the loss of H3K27Ac while the H3K4me3 mark was still present at the same sites, thus suggesting a transformation of these sites from active to poised enhancers [73]. It is worthwhile noting that the so called super-enhancers – clustered enhancers with abundant H3K27Ac signal – were equally affected by this loss of H3K27Ac signal. This is in contrast to the effect of SMARCB1 loss on the epigenome, which was found to alter the distribution of classical enhancers but to leave super-enhancers relatively unaltered [74] (Figure 3). Whether this differential effect on super-enhancers when comparing *SMARCB1* and *SMARCA4* mutant cells also results in a differential sensitivity to bromodomain inhibition (which mainly affects super-enhancer-driven genes) has not been comprehensively studied so far.

Similar to SMARCB1, the interplay of SMARCA4 and the PRC1 and PRC2 complexes has been actively investigated. Mutations in the ATP-binding domain of SMARCA4 have been shown to increase genome-wide binding of PRC1 by Stanton *et al.* [75]. The increased deposition of PRC1 components (such as Ring1b, which was specifically investigated in this work) was mostly due to a loss of direct binding between the BAF complex and the PRC1 complex. Furthermore, it was demonstrated that H3K4me3 and other epigenetic marks, which characterize active promoters, were strongly associated with sites that gained PRC1 signals thus suggesting that, in particular, promoter regions

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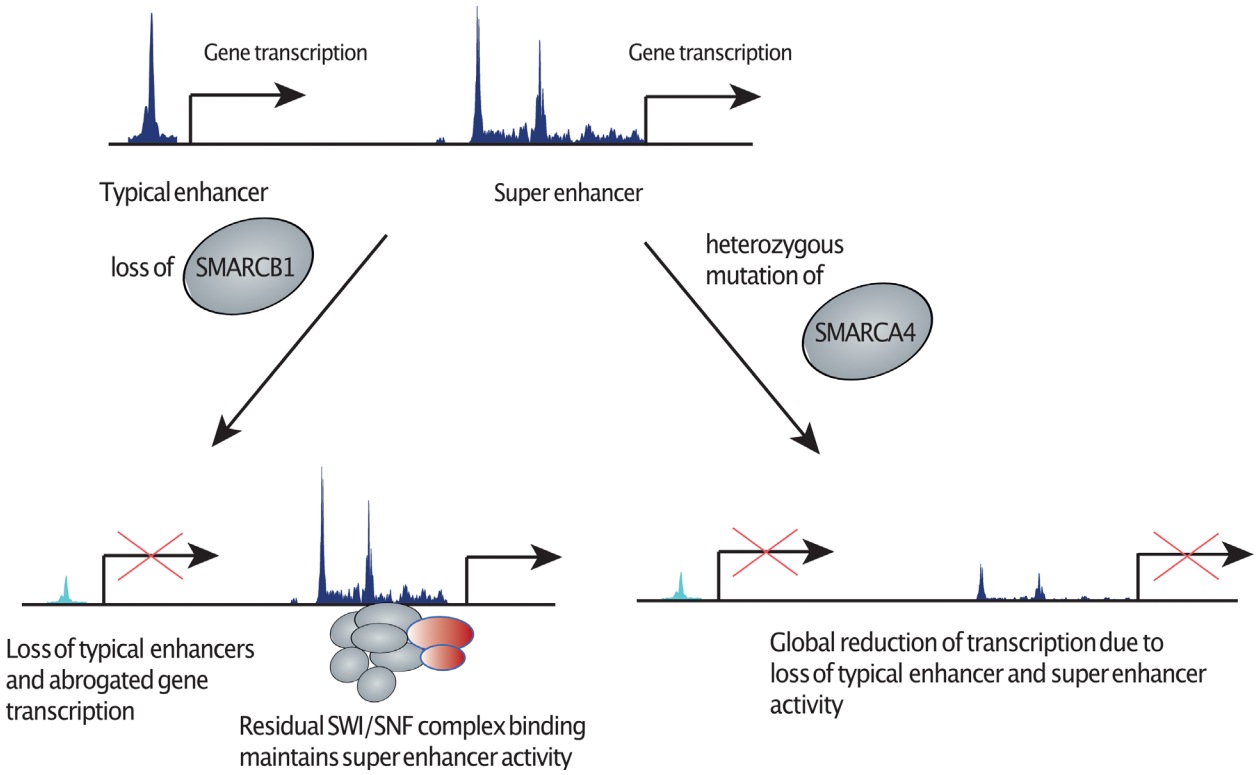

Figure 3. Diverging effects of SMARCB1 loss and SMARCA4 mutations on enhancers and super-enhancers. SWI/SNF, SWItch/sucrose non-fermentable.

were affected by this increase of PRC1. As PRC1 binding prompts the recruitment of PRC2 complex factors, the study also found consecutive increases in H3K27me3, the repressive mark which is deposited by the PRC2 complex. Although a genome-wide increase in H3K27me3 has been suggested in *SMARCB1*-deleted or mutant entities as well, the deletion of *ARID1A*, another component of the SWI/SNF complex, did not result in an increase of neither H3K27me3 nor Ring1b.

Putative drug targets in *SMARCA4* mutant entities

Only few preclinical studies have been conducted specifically assessing the vulnerabilities of *SMARCA4* mutant paediatric brain tumours. However, recent discoveries from various non-CNS entities, with bi- or monoallelic alterations of BRG1, may fuel drug treatment studies in medulloblastoma and rhabdoid tumours as well. For example, the interplay of the PRC2 complex and the SWI/SNF complex has propelled research on the use of EZH2 inhibitors in various SWI/SNF compromised entities, among them SMARCA4-deficient lung cancers, thoracic sarcomas and small cell carcinoma of the ovary hypercalcaemic type (SCCOHT). A seminal study by Januario *et al.* [76] found that the expression level of SMARCA2 – the ATPase subunit which is a paralogue of SMARCA4 – is predictive of the response to EZH2 inhibition. Notably, the cell line panel, which was investigated in this work, did not contain paediatric brain tumours but nonetheless these results may be generalizable to, for example, medulloblastomas or ATRT. The proof of concept for a specific vulnerability of SMARCA2- and SMARCA4-deficient cancer cells towards EZH2 inhibition was presented by Chan-Penebre *et al.* showing a differential sensitivity towards the EZH2 inhibitor tazemetostat in SCCOHT [77].

Beyond targeting EZH2, other approaches have aimed to exploit the dependency of SMARCA4-deleted cancers on SMARCA2. The synthesis of orally available SMARCA2/4 inhibitors has paved the way for preclinical studies, which have underlined the dependency of SMARCA4-deficient cancers (such as oesophageal

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carcinoma [78]) on SMARCA2. It remains to be seen, if these promising preclinical experiments can be translated into clinical success.

Outside the epigenetic field, other putative targets which have been highlighted in SMARCA4-deficient entities are RTKi. In SCCOHT, a CRISPR-Cas9 screening approach detected PDGFR signalling as a putative vulnerability and the use of Ponatinib to inhibit this axis proved to be efficacious preclinically [79]. How far these vulnerabilities represent Achilles heels in all SMARCA4-deficient entities and could thus be expanded to paediatric brain tumours remain to be determined.

SMARCB1 alterations in paediatric brain tumours

Rhabdoid tumours represent the prototypic example of SMARCB1 compromised entities – the first description of SMARCB1 as genetic target in rhabdoid tumours dates back to the 1990s. After the initial description of rhabdoid tumours as a series of case reports in the 1980s [80-82], Versteege *et al.* found deletions of *SMARCB1* in established rhabdoid tumour cell lines [83]. Since then, several studies have confirmed this gene as the most important genetic hit in rhabdoid tumours, which are otherwise genomically very stable [84,85]. While all ATRT share a loss of INI-1 (the protein encoded by the *SMARCB1* gene), the genetic basis of this loss differs between tumours. Rhabdoid tumours of the periphery (eMRT) more often than CNS rhabdoid tumours display homozygous losses affecting *SMARCB1*, which also cover several kBP beyond this gene. [86]. In contrast, rhabdoid tumours of the central nervous system have a more complex deletion pattern. For example, the recently identified major ATRT subgroups, ATRT-TYR, ATRT-SHH and ATRT-MYC, [25,87] differ in their inaxctivation pattern of *SMARCB1* (see Table 2).

The functional consequences of these findings are still largely unknown but codeletions in genes other than *SMARCB1* may well contribute to the divergent transcriptomic profiles found in the three ATRT subgroups.

The spectrum of *SMARCB1*-deleted entities in paediatric neuro-oncology goes beyond rhabdoid tumours and also comprises a set of rare entities, specifically CRINETs (cribriform neuroepithelial tumours) and SMARCB1-deleted chordomas. Antonelli *et al.* found an absence of INI-1 staining in four out of eight paediatric chordomas [88]. As was later shown, these INI-1-negative chordomas indeed form a separate molecular subgroup when compared to ATRT and INI-1 intact chordomas. The INI-1-negative chordomas differ from classic chordomas also by the absence of further cytogenetic aberrations such as chromosome 9 or chromosome 10 deletions, which are commonly observed in classic chordomas [89]. In line with the evidence from other INI1 deleted tumour entities, INI-1-deficient chordomas seem to have a particularly bad prognosis [90]. The immunohistochemical absence of INI-1 thus prompts the question if these tumours are susceptible to the same therapeutic approaches as ATRT such as, for example, EZH2 inhibition, which have been studied widely in MRT. A clinical trial using the EZH2 inhibitor tazemetostat [91] has shown two partial responses in children with INI-1-deficient chordomas.

In contrast to INI-1-deficient chordomas, CRINETs are indeed highly similar to ATRT, in particular to subgroup ATRT-TYR, [92] and it is conceivable that this entity represents a histological variant of this ATRT subgroup with an excellent survival.

Beyond these entities, *SMARCB1* mutations are rarely found in paediatric neuro-oncology and evidence is circumstantial. Recently, El-Ayadi *et al.* have described a case of a medulloblastoma with concurrent *IDH1* and a (heterozygous) *SMARCB1* mutation [93]. However, unlike SMARCA4 and Arid1a/Arid1b, the protein does not seem to be a protagonist in the pathogenesis of these tumours.

Table 2. Overview on the type of genetic inactivation of *SMARCB1* in various entities

Entity (and subgroup)	*Prevailing type of SMARCB1 inactivation*	*Localization*
ATRT (TYR)	Whole chromosome loss of chr.22 in one allele, point mutations in the other allele	CNS
ATRT (SHH)	Mostly point mutations	CNS
ATRT (MYC)	Homozygous 'broad' loss	CNS
INI-1-def chordoma	Point mutations on one allele, broad losses on the other allele	CNS
CRINET	Point mutations	CNS

CRINET, cribriform neuroepithelial tumours.

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The epigenetic consequences of *SMARCB1* alterations

Unlike SMARCA4, SMARCB1 is not a part of the ATPase subunit of the SWI/SNF complex and the functional consequences of its deletion may thus differ from the aforementioned SMARCA4 aberrations. As mentioned previously in the context of SMARCA4, a well-studied mechanistic consequence of SWI/SNF complex perturbations is the activation of the PRC2 complex. Initial evidence towards an antagonism between the PRC2 complex and the SWI/SNF complex came from studies in *Drosophila melanogaster*. PcG proteins – the analogues of the PRC2 complex in fly – were found to block SWI/SNF-induced nucleosome mobilization [94,95]. The human analogue, the PRC2 complex, is composed of four subunits [96,97]: SUZ12, RBBP4/7, AEBP2 and EZH2 – with the latter two being the methyltransferases that are responsible for placing methyl groups at the lysine residue 27 of Histone 3, thus inducing H3K27me3.

With the advent of inhibitors of the EZH2 methyltransferase activity, the interplay between the SWI/SNF complex and the PRC2 proteins once more came into focus. McCabe *et al.* found that the EZH2 inhibitor GSK126 could be used to abrogate mutation-induced hyper-trimethylation of H3K27 in EZH2 mutant lymphoma [98]. Subsequently, Knutson *et al.* showed a regression of rhabdoid tumour flank xenografts when being treated with the EZH2 inhibitor EPZ-6438 [99]. Along a similar line, it was described that EZH2 inhibition was able to abrogate self-renewal and sensitize to radiation [10].

These *in vitro* and *in vivo* data support the use of EZH2 inhibitors in preclinical studies. However, more recent findings from both functional and descriptive studies, which characterize epigenetic features of SMARCB1-deficient tumours, suggest that the role of EZH2 may be more complex. Kim *et al.* confirmed that SWI/SNF mutant cell lines were in general more susceptible to EZH2 inhibition than SWI/SNF intact cell lines and virtually all cell lines were sensitive to EZH2 knock-down [11]. However, the introduction of a non-catalytically active, mutant EZH2 in these cell lines, which were unable to restore H3K27me3, rescued the effect of EZH2 knock-down in cell lines insensitive to pharmacological EZH2i. This speaks for a role of the non-catalytic subunit of EZH2 in tumourigenesis and indicates that pharmacological decrease of H3K27me3 may not be the only mechanism by which EZH2 exerts pro-tumourigenic influence. Along a similar line, it has been found previously that the reduction of H3K27me3 does not correlate with the tumour response when treating rhabdoid tumour xenografts with EZH2 inhibitors [12].

Adding to these mechanistic and preclinical insights, immunohistochemical and ChIP sequencing studies have shed a new light on EZH2 as a drug target. Hasselblatt *et al.* [13] and Kakkar *et al.* [14] found that about 50% of all ATRTs do not display increased but rather diminished H3K27me3 signals. In a large epigenetic study examining the presence of the ENCODE marks in ATRT primary samples, Erkek *et al.* [15] did not observe a global increase of the H3K27me3 mark when comparing ATRT to other embryonal brain tumours. However, specifically, neuronal differentiation genes were found to be affected by increased H3K27me3 deposition. Notably, it was found that in many sites which bound EZH2, no or only a very faint signal of H3K27me3 was detected. At these genes, abundant SMARCA4 binding was present, thus suggesting that residual SWI/SNF complex binding may play a role in maintaining the expression of, for example, cell cycle genes despite the overexpression of EZH2. This may suggest that EZH2 inhibition as the sole treatment in the clinical setting could not be enough to abrogate all oncogenic effects present in the epigenome of rhabdoid tumours and that the inhibition of SMARCA4 may represent a rationale to tackle this disease. Further support for this approach came from a histopathological study, showing that SMARCA2 staining is often absent in malignant rhabdoid tumours [16]. Thus, SMARCB1-deficient tumours may be critically depending on the SMARCA4 (the paralogue of SMARCA2) and SMARCA4 inhibition as a concept to exploit synthetic lethality could be a concept of preclinical studies.

In addition, the putative dependency on SMARCA4 in the absence of SMARCA2 may hold the promise of testing substances or substance combinations, which have been used in the context of SMARCA2/4 mutant entities such as CDK inhibitors (which have been tested successfully in SCCOHT). An additional interesting concept which so far has not been fully exploited when treating rhabdoid tumours in the clinic is the use of EZH2 as a sensitizer for other therapeutics. In

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SMARCA4 mutant lung cancer, the combination of EZH2 inhibition and topoisomerase inhibition has shown efficacy both *in vitro* and *in vivo*. This dependency is likely mediated by a SWI/SNF-induced repression of EGFR. It can be speculated that SMARCB1-deleted entities may show the same vulnerability [17].

With regard to the direct influence of SMARCB1 on the epigenome, Nakayama *et al.* [18] demonstrated that SMARCB1 is important for mediating the stability of SWI/SNF chromatin binding without, however, influencing the stability of the complex itself. Beyond that SMARCB1, re-expression in MRT cell lines contributed to a widespread gain of the activating marks H3K27Ac and H3k4me1, whereas the levels of H3K4me1 did not change significantly. These findings have been paralleled by a study from Wang *et al.* [74] confirming the importance of SMARCB1 for sharpening the enhancer landscape. Adding to that, they showed that super-enhancer activity is maintained by residual SWI/SNF complex members in the SMARCB1-deficient setting, thus retaining the expression of genes which form cell identity. However, the landscape of typical enhancers is drastically changed by the deletion of SMARCB1 thus affecting mainly tumour suppressor genes which are downregulated.

On the contrary to these reports which dwell on the activating function of SMARCB1, there is also growing evidence on its repressive role in specific cell types or developmental stages. Langer *et al.* [19] recently studied the role of INI-1 in human embryonic stem cells (hESC). Performing ATAC-seq, they found that SMARCB1 represses the accessibility of key pluripotency genes, thus contributing to differentiation. In line with its roles as an important protagonist in neurogenesis, differentiation of hESC into neuronal cells was not possible after deletion of SMARCB1. These findings substantiate further publications that have shown the indispensability of the SWI/SNF complex as a whole for self-renewability and neuronal differentiation [73,110,111].

Going beyond the canonical BAF complex, recent literature has underlined the dependency of SMARCB1-deficient cells on the non-canonical BAF complex. In the absence of SMARCB1, Brd9 becomes critical in stabilizing the remaining SWI/SNF complex [46] and displays a distinct binding pattern which differs from the canonical BAF complex. Remarkably Michel *et al.* found a tight colocalization of Brd9 and SMARCA4 in the absence of SMARCB1 underlining that Brd9 plays a pivotal role in stabilizing the residual SWI/SNF complex.

Targeting Brd9 is possible and *in vivo* active inhibitors are available [112]. It has been attempted *in vitro* in rhabdoid tumours [113] and *in vivo* in other entities [114].

In summary, the impact of SMARCB1 (or its absence) on the epigenome remains complex. While recent years have seen a surge in publications studying the epigenetic landscape of these tumours, a clearly amenable epigenetic target remains yet to be identified. The paralogue dependency on SMARCA2, which has been described in SMARCA4-deficient entities, may be a concept that also applies to SMARCB1-deficient entities, given the emerging role of the residual SWI/SNF complex. EZH2 – arguably the best studied epigenetic target in rhabdoid tumours – continues to be in the focus of further investigations. However, both the limited effect of monotherapies with EZH2 inhibitor in the clinical setting and the mechanistic data which suggest non-canonical functions of this enzyme may demand sophisticated combinations with other epigenetic therapies or immunotherapeutic approaches.

ARID1A/ARID1B and *ARID2* mutations

While *ARID1A* and *ARID1B* mutations are very common among solid neoplasms in adulthood (for example, in oesophageal carcinomas, lung cancers reviewed in [115]), their occurrence in paediatric neuro-oncology seems to be limited to medulloblastomas. Northcott *et al.* [66] and Jones *et al.* [64] found mutation frequencies of 2% and 8%, respectively, in both cases tightly associated with the WNT subgroup. The vast majority (10/11 mutations) are stop-gain mutations and possibly contribute to expressing a truncated protein in the cell.

As described previously, ARID1A and ARID1B are close paralogues and in analogy to the paralogues SMARCA2 and SMARCA4, inactivating ARID1A mutations render ARID1B essential. While this dependency so far cannot be exploited clinically, AURKA inhibition seems to be synthetically lethal with ARID1A mutations at least in colorectal cancer cells [116] and thus merits further investigation.

ARID2 is present only in the pBAF complex and not the cBAF complex. Mutations have been described in

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non-small cell lung cancer and hepatocellular carcinoma but not in paediatric brain tumours [117,118].

Although there are no substances available that interfere directly with ARID expression or chromatin binding, a number of surrogate targets have been described in the context of ARID mutant tumours – such as the kinase ATR, which was found to be essential for cell survival in ARID mutant entities [119]. Thus, although ARID mutations are rare in paediatric neuro-oncology, the findings from other entities may hold the promise of being transferred to ARID mutant medulloblastomas as well.

Summary and perspective

Mutations of the SWI/SNF complex subunits and the consecutive changes in epigenetic and transcriptional programmes are important genetic contributors to tumourigenesis. In a remarkably high proportion of tumours, it is clear that these aberrations represent the initiating event for the formation of cancer cells. However, translating this knowledge into therapeutic possibilities so far has been difficult, owing to the fact that the majority of these mutations constitute loss of function events which impact widely on gene transcription. The strategies which have been pursued to tackle these diseases have so far aimed at limiting or reversing the epigenetic consequences which result from SWI/SNF perturbation. This path is further strengthened by recent investigations which have revealed functional dependencies between members of the SWI/SNF complex [47]. With the advent of the first available SMARCA4 inhibitors, the concept of paralogue dependency and the idea of targeting the residual SWI/SNF complex may become more prominent in the clinic as well. Beyond epigenetic therapies, further drug targets have recently emerged. Despite the fact that rhabdoid tumours display low mutation frequencies, two recent studies found a substantial degree of immune cell infiltration and a response of ATRT to checkpoint blockade [86,120]. Thus, novel and extratumoural therapeutic opportunities exist and it is worthwhile studying them together with classical chemotherapy or epigenetic treatments as a future prospect.

Conflict of interest

The author does not declare any conflict of interest.

References

1 Olins DE, Olins AL. Chromatin history: our view from the bridge. *Nat Rev Mol Cell Biol* 2003; **4**: 809–14
2 Biran A, Meshorer E. Concise review: chromatin and genome organization in reprogramming. *Stem Cells* 2012; **30**: 1793–9
3 Hergeth SP, Schneider R. The H1 linker histones: multifunctional proteins beyond the nucleosomal core particle. *EMBO Rep* 2015; **16**: 1439–53
4 Jambhekar A, Dhall A, Shi Y. Roles and regulation of histone methylation in animal development. *Nat Rev Mol Cell Biol* 2019; **20**: 625–41
5 Ninova M, Godneeva B, Chen YA, Luo Y, Prakash SJ, Jankovics F, et al. The SUMO ligase Su(var)2–10 controls hetero- and euchromatic gene expression via establishing H3K9 trimethylation and negative feedback regulation. *Mol Cell* 2019; **77**: 571–85.
6 Li J, Tao X, Shen J, Liu L, Zhao Q, Ma Y, et al. The molecular landscape of histone lysine methyltransferases and demethylases in non-small cell lung cancer. *Int J Med Sci* 2019; **16**: 922–30
7 Stief SM, Hanneforth AL, Weser S, Mattes R, Carlet M, Liu WH, et al. Loss of KDM6A confers drug resistance in acute myeloid leukemia. *Leukemia* 2020; **34**: 50–62
8 Clapier CR, Iwasa J, Cairns BR, Peterson CL. Mechanisms of action and regulation of ATP-dependent chromatin-remodelling complexes. *Nat Rev Mol Cell Biol* 2017; **18**: 407–22
9 Narlikar GJ, Sundaramoorthy R, Owen-Hughes T. Mechanisms and functions of ATP-dependent chromatin-remodeling enzymes. *Cell* 2013; **154**: 490–503
10 Aydin OZ, Vermeulen W, Lans H. ISWI chromatin remodeling complexes in the DNA damage response. *Cell Cycle* 2014; **13**: 3016–25
11 Zhou CY, Johnson SL, Lee LJ, Longhurst AD, Beckwith SL, Johnson MJ, et al. The yeast INO80 complex operates as a tunable DNA length-sensitive switch to regulate nucleosome sliding. *Mol Cell* 2018; **69**: 677–88
12 Eustermann S, Schall K, Kostrewa D, Lakomek K, Strauss M, Moldt M, et al. Structural basis for ATP-dependent chromatin remodelling by the INO80 complex. *Nature* 2018; **556**: 386–90
13 Kadoch C, Hargreaves DC, Hodges C, Elias L, Ho L, Ranish J, et al. Proteomic and bioinformatic analysis of mammalian SWI/SNF complexes identifies extensive roles in human malignancy. *Nat Genet* 2013; **45**: 592–601
14 Grobner SN, Worst BC, Weischenfeldt J, Buchhalter I, Kleinheinz K, Rudneva VA, et al. The landscape of genomic alterations across childhood cancers. *Nature* 2018; **555**: 321–7
15 Ma X, Liu Y, Liu Y, Alexandrov LB, Edmonson MN, Gawad C, et al. Pan-cancer genome and transcriptome analyses of 1,699 paediatric leukaemias and solid tumours. *Nature* 2018; **555**: 371–6

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16 Sausen M, Leary RJ, Jones S, Wu J, Reynolds CP, Liu X, et al. Integrated genomic analyses identify ARID1A and ARID1B alterations in the childhood cancer neuroblastoma. *Nat Genet* 2013; **45**: 12–17
17 Seki M, Nishimura R, Yoshida K, Shimamura T, Shiraishi Y, Sato Y, et al. Integrated genetic and epigenetic analysis defines novel molecular subgroups in rhabdomyosarcoma. *Nat Commun* 2015; **6**: 7557
18 Kohsaka S, Shukla N, Ameur N, Ito T, Ng CK, Wang L, et al. A recurrent neomorphic mutation in MYOD1 defines a clinically aggressive subset of embryonal rhabdomyosarcoma associated with PI3K-AKT pathway mutations. *Nat Genet* 2014; **46**: 595–600
19 Gadd S, Huff V, Walz AL, Ooms A, Armstrong AE, Gerhard DS, et al. A Children's Oncology Group and TARGET initiative exploring the genetic landscape of Wilms tumor. *Nat Genet* 2017; **49**: 1487–94
20 Zhang J, Ding L, Holmfeldt L, Wu G, Heatley SL, Payne-Turner D, et al. The genetic basis of early T-cell precursor acute lymphoblastic leukaemia. *Nature* 2012; **481**: 157–63
21 Gruber TA, Larson Gedman A, Zhang J, Koss CS, Marada S, Ta HQ, et al. An Inv(16)(p13.3q24.3)-encoded CBFA2T3-GLIS2 fusion protein defines an aggressive subtype of pediatric acute megakaryoblastic leukemia. *Cancer Cell* 2012; **22**: 683–97
22 Holmfeldt L, Wei L, Diaz-Flores E, Walsh M, Zhang J, Ding L, et al. The genomic landscape of hypodiploid acute lymphoblastic leukemia. *Nat Genet* 2013; **45**: 242–52
23 Ozawa MG, Bhaduri A, Chisholm KM, Baker SA, Ma L, Zehnder JL, et al. A study of the mutational landscape of pediatric-type follicular lymphoma and pediatric nodal marginal zone lymphoma. *Mod Pathol* 2016; **29**: 1212–20
24 Richter J, Schlesner M, Hoffmann S, Kreuz M, Leich E, Burkhardt B, et al. Recurrent mutation of the ID3 gene in Burkitt lymphoma identified by integrated genome, exome and transcriptome sequencing. *Nat Genet* 2012; **44**: 1316–20
25 Johann PD, Erkek S, Zapatka M, Kerl K, Buchhalter I, Hovestadt V, et al. Atypical teratoid/rhabdoid tumors are comprised of three epigenetic subgroups with distinct enhancer landscapes. *Cancer Cell* 2016; **29**: 379–93
26 Ueno-Yokohata H, Okita H, Nakasato K, Akimoto S, Hata J, Koshinaga T, et al. Consistent in-frame internal tandem duplications of BCOR characterize clear cell sarcoma of the kidney. *Nat Genet* 2015; **47**: 861–3
27 Roy A, Kumar V, Zorman B, Fang E, Haines KM, Doddapaneni H, et al. Recurrent internal tandem duplications of BCOR in clear cell sarcoma of the kidney. *Nat Commun* 2015; **6**: 8891
28 Lee SH, Kim JS, Zheng S, Huse JT, Bae JS, Lee JW, et al. ARID1B alterations identify aggressive tumors in neuroblastoma. *Oncotarget* 2017; **8**: 45943–50
29 Bousquet M, Noirot C, Accadbled F, Sales de Gauzy J, Castex MP, Brousset P, et al. Whole-exome sequencing in osteosarcoma reveals important heterogeneity of genetic alterations. *Ann Oncol* 2016; **27**: 738–44
30 Kovac M, Blattmann C, Ribi S, Smida J, Mueller NS, Engert F, et al. Exome sequencing of osteosarcoma reveals mutation signatures reminiscent of BRCA deficiency. *Nat Commun* 2015; **6**: 8940
31 Banito A, Li X, Laporte AN, Roe JS, Sanchez-Vega F, Huang CH, et al. The SS18-SSX oncoprotein Hijacks KDM2B-PRC1.1 to drive synovial sarcoma. *Cancer Cell* 2018; **33**: 527–41.e8
32 Lu C, Zhang J, Nagahawatte P, Easton J, Lee S, Liu Z, et al. The genomic landscape of childhood and adolescent melanoma. *J Invest Dermatol* 2015; **135**: 816–23
33 Gorringe KL, Choong DY, Williams LH, Ramakrishna M, Sridhar A, Qiu W, et al. Mutation and methylation analysis of the chromodomain-helicase-DNA binding 5 gene in ovarian cancer. *Neoplasia* 2008; **10**: 1253–8
34 Wong RR, Chan LK, Tsang TP, Lee CW, Cheung TH, Yim SF, et al. CHD5 downregulation associated with poor prognosis in epithelial ovarian cancer. *Gynecol Obstet Invest* 2011; **72**: 203–7
35 Kolla V, Zhuang T, Higashi M, Naraparaju K, Brodeur GM. Role of CHD5 in human cancers: 10 years later. *Cancer Res* 2014; **74**: 652–8
36 Bagchi A, Papazoglu C, Wu Y, Capurso D, Brodt M, Francis D, et al. CHD5 is a tumor suppressor at human 1p36. *Cell* 2007; **128**: 459–75
37 Zhang S, Zhou B, Wang L, Li P, Bennett BD, Snyder R, et al. INO80 is required for oncogenic transcription and tumor growth in non-small cell lung cancer. *Oncogene* 2017; **36**: 1430–9
38 Lee SA, Lee HS, Hur SK, Kang SW, Oh GT, Lee D, et al. INO80 haploinsufficiency inhibits colon cancer tumorigenesis via replication stress-induced apoptosis. *Oncotarget* 2017; **8**: 115041–53
39 Karaca E, Harel T, Pehlivan D, Jhangiani SN, Gambin T, Coban Akdemir Z, et al. Genes that affect brain structure and function identified by rare variant analyses of Mendelian neurologic disease. *Neuron* 2015; **88**: 499–513
40 Lopes F, Barbosa M, Ameur A, Soares G, de Sa J, Dias AI, et al. Identification of novel genetic causes of Rett syndrome-like phenotypes. *J Med Genet* 2016; **53**: 190–9
41 Workman JL, Kingston RE. Alteration of nucleosome structure as a mechanism of transcriptional regulation. *Annu Rev Biochem* 1998; **67**: 545–79
42 Luger K, Mader AW, Richmond RK, Sargent DF, Richmond TJ. Crystal structure of the nucleosome core particle at 2.8 A resolution. *Nature* 1997; **389**: 251–60
43 Lessard J, Wu JI, Ranish JA, Wan M, Winslow MM, Staahl BT, et al. An essential switch in subunit composition of a chromatin remodeling complex during neural development. *Neuron* 2007; **55**: 201–15

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44 Michel BC, D'Avino AR, Cassel SH, Mashtalir N, McKenzie ZM, McBride MJ, et al. A non-canonical SWI/SNF complex is a synthetic lethal target in cancers driven by BAF complex perturbation. *Nat Cell Biol* 2018; **20**: 1410–20
45 Mashtalir N, D'Avino AR, Michel BC, Luo J, Pan J, Otto JE, et al. Modular organization and assembly of SWI/SNF family chromatin remodeling complexes. *Cell* 2018; **175**: 1272–88.e20
46 Wang X, Wang S, Troisi EC, Howard TP, Haswell JR, Wolf BK, et al. BRD9 defines a SWI/SNF sub-complex and constitutes a specific vulnerability in malignant rhabdoid tumors. *Nat Commun* 2019; **10**: 1881
47 Schick S, Rendeiro AF, Runggatscher K, Ringler A, Boidol B, Hinkel M, et al. Systematic characterization of BAF mutations provides insights into intracomplex synthetic lethalities in human cancers. *Nat Genet* 2019; **51**: 1399–410
48 Wei Z, Yoshihara E, He N, Hah N, Fan W, Pinto AFM, Vitamin D, et al. Switches BAF complexes to protect beta cells. *Cell* 2018; **173**: 1135–49.e15
49 Bajpai R, Chen DA, Rada-Iglesias A, Zhang J, Xiong Y, Helms J, et al. CHD7 cooperates with PBAF to control multipotent neural crest formation. *Nature* 2010; **463**: 958–62
50 Chandler RL, Magnuson T. The SWI/SNF BAF-A complex is essential for neural crest development. *Dev Biol* 2016; **411**: 15–24
51 Kaeser MD, Aslanian A, Dong MQ, Yates JR 3rd, Emerson BM. BRD7, a novel PBAF-specific SWI/SNF subunit, is required for target gene activation and repression in embryonic stem cells. *J Biol Chem* 2008; **283**: 32254–63
52 Pan D, Kobayashi A, Jiang P, Ferrari de Andrade L, Tay RE, Luoma AM, et al. A major chromatin regulator determines resistance of tumor cells to T cell-mediated killing. *Science* 2018; **359**: 770–5
53 Jagani Z, Mora-Blanco EL, Sansam CG, McKenna ES, Wilson B, Chen D, et al. Loss of the tumor suppressor Snf5 leads to aberrant activation of the Hedgehog-Gli pathway. *Nat Med* 2010; **16**: 1429–33
54 Weissmiller AM, Wang J, Lorey SL, Howard GC, Martinez E, Liu Q, et al. Inhibition of MYC by the SMARCB1 tumor suppressor. *Nat Commun* 2019; **10**: 2014
55 Stojanova A, Tu WB, Ponzielli R, Kotlyar M, Chan PK, Boutros PC, et al. MYC interaction with the tumor suppressive SWI/SNF complex member INI1 regulates transcription and cellular transformation. *Cell Cycle* 2016; **15**: 1693–705
56 Strober BE, Dunaief JL, Goff SP. Functional interactions between the hBRM/hBRG1 transcriptional activators and the pRB family of proteins. *Mol Cell Biol* 1996; **16**: 1576–83
57 Trouche D, Le Chalony C, Muchardt C, Yaniv M, Kouzarides T. RB and hbrm cooperate to repress the activation functions of E2F1. *Proc Natl Acad Sci U S A* 1997; **94**: 11268–73
58 Zhang ZK, Davies KP, Allen J, Zhu L, Pestell RG, Zagzag D, et al. Cell cycle arrest and repression of cyclin D1 transcription by INI1/hSNF5. *Mol Cell Biol* 2002; **22**: 5975–88
59 Versteege I, Medjkane S, Rouillard D, Delattre O. A key role of the hSNF5/INI1 tumour suppressor in the control of the G1-S transition of the cell cycle. *Oncogene* 2002; **21**: 6403–12
60 Ribeiro-Silva C, Vermeulen W, Lans H. SWI/SNF: Complex complexes in genome stability and cancer. *DNA Repair* 2019; **77**: 87–95
61 Kapoor P, Bao Y, Xiao J, Luo J, Shen J, Persinger J, et al. Regulation of Mec1 kinase activity by the SWI/SNF chromatin remodeling complex. *Genes Dev* 2015; **29**: 591–602
62 Xu Y, Sun Y, Jiang X, Ayrapetov MK, Moskwa P, Yang S, et al. The p400 ATPase regulates nucleosome stability and chromatin ubiquitination during DNA repair. *J Cell Biol* 2010; **191**: 31–43
63 Pugh TJ, Weeraratne SD, Archer TC, Pomeranz Krummel DA, Auclair D, Bochicchio J, et al. Medulloblastoma exome sequencing uncovers subtype-specific somatic mutations. *Nature* 2012; **488**: 106–10
64 Jones DT, Jager N, Kool M, Zichner T, Hutter B, Sultan M, et al. Dissecting the genomic complexity underlying medulloblastoma. *Nature* 2012; **488**: 100–5
65 Robinson G, Parker M, Kranenburg TA, Lu C, Chen X, Ding L, et al. Novel mutations target distinct subgroups of medulloblastoma. *Nature* 2012; **488**: 43–8
66 Northcott PA, Buchhalter I, Morrissy AS, Hovestadt V, Weischenfeldt J, Ehrenberger T, et al. The whole-genome landscape of medulloblastoma subtypes. *Nature* 2017; **547**: 311–7
67 Shi X, Wang Q, Gu J, Xuan Z, Wu JI. SMARCA4/Brg1 coordinates genetic and epigenetic networks underlying Shh-type medulloblastoma development. *Oncogene* 2016; **35**: 5746–58
68 Papillon JPN, Nakajima K, Adair CD, Hempel J, Jouk AO, Karki RG, et al. Discovery of orally active inhibitors of brahma homolog (BRM)/SMARCA2 ATPase activity for the treatment of brahma related gene 1 (BRG1)/SMARCA4-mutant cancers. *J Med Chem* 2018; **61**: 10155–72
69 Hasselblatt M, Gesk S, Oyen F, Rossi S, Viscardi E, Giangaspero F, et al. Nonsense mutation and inactivation of SMARCA4 (BRG1) in an atypical teratoid/rhabdoid tumor showing retained SMARCB1 (INI1) expression. *Am J Surg Pathol* 2011; **35**: 933–5
70 Hasselblatt M, Nagel I, Oyen F, Bartelheim K, Russell RB, Schuller U, et al. SMARCA4-mutated atypical teratoid/rhabdoid tumors are associated with inherited germline alterations and poor prognosis. *Acta Neuropathol* 2014; **128**: 453–6

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71 Reisman DN, Sciarrotta J, Bouldin TW, Weissman BE, Funkhouser WK. The expression of the SWI/SNF ATPase subunits BRG1 and BRM in normal human tissues. *Appl Immunohistochem Mol Morphol* 2005; **13**: 66–74
72 Wilson BG, Helming KC, Wang X, Kim Y, Vazquez F, Jagani Z, et al. Residual complexes containing SMARCA2 (BRM) underlie the oncogenic drive of SMARCA4 (BRG1) mutation. *Mol Cell Biol* 2014; **34**: 1136–44
73 Hodges HC, Stanton BZ, Cermakova K, Chang CY, Miller EL, Kirkland JG, et al. Dominant-negative SMARCA4 mutants alter the accessibility landscape of tissue-unrestricted enhancers. *Nat Struct Mol Biol* 2018; **25**: 61–72
74 Wang X, Lee RS, Alver BH, Haswell JR, Wang S, Mieczkowski J, et al. SMARCB1-mediated SWI/SNF complex function is essential for enhancer regulation. *Nat Genet* 2017; **49**: 289–95
75 Stanton BZ, Hodges C, Calarco JP, Braun SM, Ku WL, Kadoch C, et al. Smarca4 ATPase mutations disrupt direct eviction of PRC1 from chromatin. *Nat Genet* 2017; **49**: 282–8
76 Januario T, Ye X, Bainer R, Alicke B, Smith T, Haley B, et al. PRC2-mediated repression of SMARCA2 predicts EZH2 inhibitor activity in SWI/SNF mutant tumors. *Proc Natl Acad Sci U S A* 2017; **114**: 12249–54
77 Chan-Penebre E, Armstrong K, Drew A, Grassian AR, Feldman I, Knutson SK, et al. Selective killing of SMARCA2- and SMARCA4-deficient small cell carcinoma of the ovary, hypercalcemic type cells by inhibition of EZH2. in vitro and in vivo preclinical models. *Mol Cancer Ther* 2017; **16**: 850–60.
78 Ehrenhofer-Wolfer K, Puchner T, Schwarz C, Rippka J, Blaha-Ostermann S, Strobl U, et al. SMARCA2-deficiency confers sensitivity to targeted inhibition of SMARCA4 in esophageal squamous cell carcinoma cell lines. *Sci Rep* 2019; **9**: 11661
79 Lang JD, Hendricks WPD, Orlando KA, Yin H, Kiefer J, Ramos P, et al. Ponatinib shows potent antitumor activity in small cell carcinoma of the ovary hypercalcemic type (SCCOHT) through multikinase inhibition. *Clin Cancer Res* 2018; **24**: 1932–43
80 Biggs PJ, Garen PD, Powers JM, Garvin AJ. Malignant rhabdoid tumor of the central nervous system. *Hum Pathol* 1987; **18**: 332–7
81 Bonnin JM, Rubinstein LJ, Palmer NF, Beckwith JB. The association of embryonal tumors originating in the kidney and in the brain. A report of seven cases. *Cancer* 1984; **54**: 2137–46
82 Ho PS, Lee WH, Chen CY, Chou TY, Wang YC, Sun MJ, et al. Primary malignant rhabdoid tumor of the brain: CT characteristics. *J Comput Assist Tomogr* 1990; **14**: 461–3
83 Versteege I, Sevenet N, Lange J, Rousseau-Merck MF, Ambros P, Handgretinger R, et al. Truncating mutations of hSNF5/INI1 in aggressive paediatric cancer. *Nature* 1998; **394**: 203–6
84 Lee RS, Stewart C, Carter SL, Ambrogio L, Cibulskis K, Sougnez C, et al. A remarkably simple genome underlies highly malignant pediatric rhabdoid cancers. *J Clin Invest* 2012; **122**: 2983–8
85 Kieran MW, Roberts CW, Chi SN, Ligon KL, Rich BE, Macconaill LE, et al. Absence of oncogenic canonical pathway mutations in aggressive pediatric rhabdoid tumors. *Pediatr Blood Cancer* 2012; **59**: 1155–7
86 Chun HE, Johann PD, Milne K, Zapatka M, Buellesbach A, Ishaque N, et al. Identification and analyses of extra-cranial and cranial rhabdoid tumor molecular subgroups reveal tumors with cytotoxic T cell infiltration. *Cell Rep* 2019; **29**: 2338–54.e7
87 Torchia J, Golbourn B, Feng S, Ho KC, Sin-Chan P, Vasiljevic A, et al. Integrated (epi)-genomic analyses identify subgroup-specific therapeutic targets in CNS rhabdoid tumors. *Cancer Cell* 2016; **30**: 891–908
88 Antonelli M, Raso A, Mascelli S, Gessi M, Nozza P, Coli A, et al. SMARCB1/INI1 involvement in pediatric chordoma: a mutational and immunohistochemical analysis. *Am J Surg Pathol* 2017; **41**: 56–61
89 Hasselblatt M, Thomas C, Hovestadt V, Schrimpf D, Johann P, Bens S, et al. Poorly differentiated chordoma with SMARCB1/INI1 loss: a distinct molecular entity with dismal prognosis. *Acta Neuropathol* 2016; **132**: 149–51
90 Beccaria K, Tauziede-Espariat A, Monnien F, Adle-Biassette H, Masliah-Planchon J, Pierron G, et al. Pediatric chordomas: results of a multicentric study of 40 children and proposal for a histopathological prognostic grading system and new therapeutic strategies. *J Neuropathol Exp Neurol* 2018; **77**: 207–15
91 Meng T, Jin J, Jiang C, Huang R, Yin H, Song D, et al. Molecular targeted therapy in the treatment of chordoma: a systematic review. *Front Oncol* 2019; **9**: 30
92 Hasselblatt M, Oyen F, Gesk S, Kordes U, Wrede B, Bergmann M, et al. Cribriform neuroepithelial tumor (CRINET): a nonrhabdoid ventricular tumor with INI1 loss and relatively favorable prognosis. *J Neuropathol Exp Neurol* 2009; **68**: 1249–55
93 El-Ayadi M, Egervari K, Merkler D, McKee TA, Gumy-Pause F, Stichel D, et al. Concurrent IDH1 and SMARCB1 mutations in pediatric medulloblastoma: a case report. *Front Neurol* 2018; **9**: 398
94 Kennison JA, Tamkun JW. Dosage-dependent modifiers of polycomb and antennapedia mutations in Drosophila. *Proc Natl Acad Sci U S A* 1988; **85**: 8136–40
95 Tamkun JW, Deuring R, Scott MP, Kissinger M, Pattatucci AM, Kaufman TC, et al. brahma: a regulator of Drosophila homeotic genes structurally related to the yeast transcriptional activator SNF2/SWI2. *Cell* 1992; **68**: 561–72
96 Kim KH, Roberts CW. Targeting EZH2 in cancer. *Nat Med* 2016; **22**: 128–34

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97 Pasini D, Di Croce L. Emerging roles for polycomb proteins in cancer. *Curr Opin Genet Dev* 2016; **36**: 50–8
98 McCabe MT, Ott HM, Ganji G, Korenchuk S, Thompson C, Van Aller GS, et al. EZH2 inhibition as a therapeutic strategy for lymphoma with EZH2-activating mutations. *Nature* 2012; **492**: 108–12
99 Knutson SK, Warholic NM, Wigle TJ, Klaus CR, Allain CJ, Raimondi A, et al. Durable tumor regression in genetically altered malignant rhabdoid tumors by inhibition of methyltransferase EZH2. *Proc Natl Acad Sci U S A* 2013; **110**: 7922–7
100 Alimova I, Birks DK, Harris PS, Knipstein JA, Venkataraman S, Marquez VE, et al. Inhibition of EZH2 suppresses self-renewal and induces radiation sensitivity in atypical rhabdoid teratoid tumor cells. *Neuro Oncol* 2013; **15**: 149–60
101 Kim KH, Kim W, Howard TP, Vazquez F, Tsherniak A, Wu JN, et al. SWI/SNF-mutant cancers depend on catalytic and non-catalytic activity of EZH2. *Nat Med* 2015; **21**: 1491–6
102 Kurmasheva RT, Sammons M, Favours E, Wu J, Kurmashev D, Cosmopoulos K, et al. Initial testing (stage 1) of tazemetostat (EPZ-6438), a novel EZH2 inhibitor, by the Pediatric Preclinical Testing Program. *Pediatr Blood Cancer* 2017; **64**: e26218
103 Hasselblatt M, Johann PD, Kool M, Fruhwald MC. Reduced histone H3 K27 trimethylation is encountered in about 50% of atypical teratoid/rhabdoid tumors (AT/RT) but is not associated with molecular subgroup status and outcome. *Acta Neuropathol* 2017; **134**: 817–8
104 Kakkar A, Biswas A, Goyal N, Suri V, Sharma MC, Gupta D, et al. The expression of cyclin D1, VEGF, EZH2, and H3K27me3 in atypical teratoid/rhabdoid tumors of the CNS: a possible role in targeted therapy. *Appl Immunohistochem Mol Morphol* 2016; **24**: 729–37
105 Erkek S, Johann PD, Finetti MA, Drosos Y, Chou HC, Zapatka M, et al. Comprehensive analysis of chromatin states in atypical teratoid/rhabdoid tumor identifies diverging roles for SWI/SNF and polycomb in gene regulation. *Cancer Cell* 2019; **35**: 95–110.e8
106 Rao Q, Xia QY, Wang ZY, Li L, Shen Q, Shi SS, et al. Frequent co-inactivation of the SWI/SNF subunits SMARCB1, SMARCA2 and PBRM1 in malignant rhabdoid tumours. *Histopathology* 2015; **67**: 121–9
107 Fillmore CM, Xu C, Desai PT, Berry JM, Rowbotham SP, Lin YJ, et al. Author correction: EZH2 inhibition sensitizes BRG1 and EGFR mutant lung tumours to TopoII inhibitors. *Nature* 2018; **563**: E27
108 Nakayama RT, Pulice JL, Valencia AM, McBride MJ, McKenzie ZM, Gillespie MA, et al. SMARCB1 is required for widespread BAF complex-mediated activation of enhancers and bivalent promoters. *Nat Genet* 2017; **49**: 1613–23
109 Langer LF, Ward JM, Archer TK. Tumor suppressor SMARCB1 suppresses super-enhancers to govern hESC lineage determination. *Elife* 2019; **8**. https://doi.org/10.7554/eLife.45672
110 Ho L, Ronan JL, Wu J, Staahl BT, Chen L, Kuo A, et al. An embryonic stem cell chromatin remodeling complex, esBAF, is essential for embryonic stem cell self-renewal and pluripotency. *Proc Natl Acad Sci U S A* 2009; **106**: 5181–6
111 Rada-Iglesias A, Bajpai R, Swigut T, Brugmann SA, Flynn RA, Wysocka J. A unique chromatin signature uncovers early developmental enhancers in humans. *Nature* 2011; **470**: 279–83
112 Martin LJ, Koegl M, Bader G, Cockcroft XL, Fedorov O, Fiegen D, et al. Structure-based design of an in vivo active selective BRD9 inhibitor. *J Med Chem* 2016; **59**: 4462–75
113 Kramer KF, Moreno N, Fruhwald MC, Kerl K. BRD9 inhibition, alone or in combination with cytostatic compounds as a therapeutic approach in rhabdoid tumors. *Int J Mol Sci* 2017; **18**: 1537
114 Bevill SM, Olivares-Quintero JF, Sciaky N, Golitz BT, Singh D, Beltran AS, et al. GSK2801, a BAZ2/BRD9 bromodomain inhibitor, synergizes with BET inhibitors to induce apoptosis in triple-negative breast cancer. *Mol Cancer Res* 2019; **17**: 1503–18
115 Wu JN, Roberts CW. ARID1A mutations in cancer: another epigenetic tumor suppressor? *Cancer Discov* 2013; **3**: 35–43
116 Wu C, Lyu J, Yang EJ, Liu Y, Zhang B, Shim JS. Targeting AURKA-CDC25C axis to induce synthetic lethality in ARID1A-deficient colorectal cancer cells. *Nat Commun* 2018; **9**: 3212
117 Manceau G, Letouze E, Guichard C, Didelot A, Cazes A, Corte H, et al. Recurrent inactivating mutations of ARID2 in non-small cell lung carcinoma. *Int J Cancer* 2013; **132**: 2217–21
118 Zhao H, Wang J, Han Y, Huang Z, Ying J, Bi X, et al. ARID2: a new tumor suppressor gene in hepatocellular carcinoma. *Oncotarget* 2011; **2**: 886–91
119 Williamson CT, Miller R, Pemberton HN, Jones SE, Campbell J, Konde A, et al. ATR inhibitors as a synthetic lethal therapy for tumours deficient in ARID1A. *Nat Commun* 2016; **7**: 13837
120 Leruste A, Tosello J, Ramos RN, Tauziede-Espariat A, Brohard S, Han ZY, et al. Clonally expanded T cells reveal immunogenicity of rhabdoid tumors. *Cancer Cell* 2019; **36**: 597–612.e8

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