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Translational Aspects of Epithelioid Sarcoma – Current Consensus

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Abstract

Epithelioid sarcoma (EpS) is an ultra-rare malignant soft-tissue cancer mostly affecting adolescents and young adults. EpS often exhibits an unfavorable clinical course with fatal outcome in ~50% of cases despite aggressive multimodal therapies combining surgery, chemotherapy, and irradiation. EpS is traditionally classified in a more common, less aggressive distal (classic) type, and a rarer aggressive proximal type. Both subtypes are characterized by a loss of nuclear INI1 expression, most often following homozygous deletion of its encoding gene *SMARCB1* – a core subunit of the SWI/SNF chromatin remodeling complex. In 2020, the EZH2 inhibitor tazemetostat was the first targeted therapy approved for EpS, raising new hopes. Still, the vast majority of patients did not benefit from this drug or relapsed rapidly. Further, other recent therapeutic modalities, including immunotherapy, are only effective in a fraction of patients. Thus, novel strategies, specifically targeted to EpS, are urgently needed. To accelerate translational research on EpS and eventually boost the discovery and development of new diagnostic tools and therapeutic options, a vibrant translational research community has formed in past years and held two international EpS digital expert meetings in 2021 and 2023. This review summarizes our current understanding of EpS from the translational research perspective and points to innovative research directions to address the most pressing questions in the field, as defined by expert consensus and patient advocacy groups.

Keywords

Epithelioid sarcoma; SMARCB1; INI-1; Tazemetostat

INTRODUCTION

Epithelioid sarcoma (EpS) was first described by Franz Enzinger in 1970 as a sarcoma simulating a granuloma or a carcinoma(1) (Fig. 1). Its characteristic nodular appearance, epithelioid morphology with frequent necrosis, and involvement of fascial and tendon structures were the leading causes for misdiagnosis(2). These initial reports mostly referred to distal anatomic sites, representing the classic variant of EpS. Furthermore, due to frequent challenges in diagnosis, patients may experience long delays of up to 36 months until effective treatments can be administered, thus further lowering eventual treatment success(3,4). Almost three decades later (1997), Guillou *et al.* described a 'proximal' type clinical variant of EpS, with an even more aggressive course and worse prognosis than the distal type, which correlated in most but not all cases with a distinct morphology composed of large epithelioid cytology, marked atypia with frequent rhabdoid features and mostly lacking granuloma-like pattern(5). Modena *et al.* was the first group (2005) to define the EpS pathogenesis by identifying SMARCB1/INI1 inactivation in a cohort of proximal but not distal (classic-type) EpS cases using fluorescence-in-situ-hybridization (FISH) and array-based comparative genomic hybridization (CGH) methodology(6). Subsequent molecular and genomic studies have shown that biallelic inactivation of the *SMARCB1* gene (SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily b, member 1, aka *BAF47, INI1,* or *SNF5*) resulting in SMARCB1/INI1 deficiency drives the pathogenesis of 95% EpS, regardless of their clinical presentation and histotype(7,8). Moreover, extended chromosome 22q copy number loss in genes flanking the *SMARCB1* locus (22q11.23) occurred in one-third of EpS, however, recurrent co-occurring genetic events were rare in EpS(8). The first targeted therapy for EpS, tazemetostat, an EZH2 inhibitor was FDA-approved in 2020(9). Nevertheless, tazemetostat is insufficient to fully cure patients and curative therapy, other than surgery and sometimes radiation therapy, remain elusive.

1. Epidemiology and demographics

EpS is an ultra-rare soft tissue sarcoma (prevalence of <2 per 100,000), with a crude incidence rate (IR) ranging from 0.03/100,000 to 0.05/100,000. According to the SEER database of almost 1,000 cases, EpS mostly affects the adult population, with a peak in the fifth decade of life (mean age at diagnosis: 46 years)(10). The IR increases with age (higher in patients over 35 years), while is very low (0.01) in the childhood population. Patients were predominantly white (80%), male (55%), and fell within the three middle age categories (cumulatively 83%). In this dataset, younger age (pediatric group) was significantly associated with better outcomes by univariate analysis. Moreover, when only including EpS defined by loss of SMARCB1 expression, there was no significant age difference between the two clinical subsets, with a mean age at diagnosis being 35-years old for both distal-type and proximal-type groups (range: 14–71 years) (7). No relevant gender

predominance has been observed (M:F ratio: 1.6), nor a significant impact on survival was found for gender and race. (10,11). Overall, the prognosis of patients with EpS is poor, with a 5-year relative survival of 50% (5,8,12).

2. Pathology and molecular pathology

Pathology:

EpS represents a SMARCB1/INI1-deficient mesenchymal neoplasm exhibiting a predominant epithelioid cytomorphology and epithelial immunophenotype(11). As stated above, the two clinical types show distinct histology in most cases, with the distal-type having a pseudo-granulomatous growth, with a mixture of relatively bland spindle and epithelioid cells, while the proximal-type is composed of solid sheets or nests of large epithelioid cells with densely eosinophilic cytoplasm and rhabdoid phenotype(11). Occasional cases composed of predominantly large cells are seen in distal locations. Immunophenotypically, both types show expression of epithelial membrane antigen (EMA), low- and high- molecular weight cytokeratins, and consistent loss of SMARCB1 (INI1) nuclear expression (Fig. 2). Unlike most carcinomas, EpS show positivity for CD34 in >50% of cases. Depending on the clone applied, ERG staining can be seen in half of the cases, but mostly in the distal type, which can cause confusion with vascular lesions(13).

Molecular pathology:

SMARCB1 biallelic inactivation drives the pathogenesis of virtually all EpS. *SMARCB1* encodes a subunit of two of the three SWI/SNF chromatin-remodeling complexes (canonical BAF (cBAF), polybromo-associated BAF (PBAF), but not non-canonical BAF (ncBAF)), which regulates gene transcription through nucleosomes modifications(14). Most EpS harbor homozygous deletions, while in a smaller subset only heterozygous *SMARCB1* loss-of-function (LOF) alterations are detected(7,8,15,16). In the latter group, the second *SMARCB1* alteration remains undefined, as there is limited evidence to support epigenetic silencing through either hypermethylation or miRNA deregulation(16–18). The types of LOF abnormalities are variable, with large arm-level deletions on chr22q predominating, and less frequent focal intragenic deletions or mutations (nonsense, frameshift)(7,8,15). *SMARCB1* alterations are mostly somatic in EpS, with a rare case with presumed constitutive heterozygous alteration reported(7). By next generation sequencing (NGS), EpS typically display diploid profiles and do not exhibit microsatellite instability (https://www.cbioportal.org/), but harbor a higher mutation rate compared to malignant rhabdoid tumors (MRT)(16), which also are defined by SMARCB1 deficiency(19). *CDKN2A/B* deletions are the only recurrent copy number alterations (CNA), apart from *SMARCB1,* reported in up to one-third of cases, but the genomic profiles include many CNA across the genome, mostly non-recurrent(7,8,16).

Molecular Diagnosis:

In most cases molecular testing is not required, as loss of SMARCB1 expression by immunohistochemistry is sufficient to support the diagnosis of EpS in the appropriate clinicopathologic context (anatomic location, cytokeratin positivity, epithelioid/rhabdoid phenotype). Although occasional studies had suggested that retained SMARCB1 expression

is seen in small subsets of EpS (21% of proximal and 6% of distal types)(20), most experts are in agreement defining SMARCB1 deficiency as an essential diagnostic criterion(11). Moreover, alleged alternative inactivation of other SWI/SNF subunits have been proposed in rare cases of EpS retaining SMARCB1 expression(20), however, no studies have confirmed this hypothesis(8). Molecular testing may be used in challenging differential diagnosis with other SMARCB1-deficient tumors, in particular with MRT of soft-tissues or the kidney or atypical teratoid/rhabdoid tumor (ATRT) of brain in pediatric patients, as MRT show no CNA nor mutations apart from the *SMARCB1* locus(21). The wide spectrum of *SMARCB1* alterations impacts on the sensitivity of various methodologies applied in the molecular diagnosis. Thus, FISH and CGH mostly detect large deletions, while multiplex ligation-dependent probe amplification is sensitive in detecting small exonic deletions(7,15,17). Other methods such as DNA-based targeted NGS and whole-exome sequencing (WES) are optimal for intragenic deletions or mutations(8) (Fig. 3).

3. Comparative molecular alterations with other SMARC-deficient neoplasms

Parallels with other SMARCB1 deficient neoplasms:

SMARCB1 deficiency is seen in a number of other soft tissue and bone tumors, most of which also display epithelioid or rhabdoid morphology (Table 1; Fig. 4). However, the incidence and type of *SMARCB1* LOF alterations vary depending on the histotype. For instance, loss of SMARCB1/INI1 protein expression is seen in 50–70% of epithelioid malignant peripheral nerve sheath tumors (MPNSTs)(22), 50% of epithelioid schwannomas(8,23), 30% of soft tissue myoepithelial tumors(8), the majority of poorly differentiated chordomas,(8) and almost all MRTs(21) and SMARCB1-deficient sinonasal carcinoma(24). Overall, similar to EpS, where *SMARCB1* homozygous and heterozygous deletions were reported in 80–100% of cases, *SMARCB1* deletions also predominate in poorly differentiated chordomas (75–89%)(8). In MRTs, *SMARCB1* point mutations/intragenic deletions ranged from 55–60% in somatic cases and 71% in germline cases(21,25). Conversely, for epithelioid MPNSTs and epithelioid schwannomas, a slight majority of cases showed only monoallelic *SMARCB1* point mutations/intragenic deletions (58% and 60% respectively).(8,26) Finally, 60% (3 of 5) soft tissue myoepithelial tumors lacking *EWSR1* gene rearrangements showed homozygous *SMARCB1* deletions.(7)

Studies on recurrent genetic alterations (other than *SMARCB1*) among various SMARCB1-deficient mesenchymal tumors are largely limited to case reports or small case series(8). Overall, molecular alterations cooccurring with *SMARCB1* appear to be rare among SMARCB1-deficient mesenchymal tumors(8). *SMARCB1* alterations were the sole recurrent genomic alterations in a whole exome sequencing study of 35 MRTs.(21) An array-based CGH study demonstrated chr22q loss (comprising the *SMARCB1* locus) in a case of poorly differentiated chordoma without other chromosomal gains or losses(27). Similar recurrent losses of chr22q or heterozygous *SMARCB1* deletion were noted in a small series of extra-axial chordomas, while transformation to a poorly differentiated chordoma resulted in homozygous deletion of SMARCB1(28). A similar progression was reported in a single case of SMARCB1-deficient conventional chordoma which transformed

into a poorly differentiated chordoma with whole-genome-doubling(29). WES of a case of soft tissue myoepithelial tumor showed an *RB1* frameshift deletion(30). Recurrent *CDKN2A* deletions were reported in 31% of epithelioid MPNSTs and 20% of epithelioid schwannomas in a panel-based NGS study(26).

Parallels with SMARCA4 neoplasms:

SMARCA4 encodes one of the ATPase subunits of the BAF chromatin-remodeling complex and, similar to SMARCB1, plays a tumor suppressor role, as recurrent inactivation mutations are increasingly detected in a variety of human neoplasia often displaying an undifferentiated rhabdoid phenotype(31). A rare subset of tumors that are typically associated with biallelic *SMARCB1* inactivation have been reported to show instead *SMARCA4* deficiency, including 2% of MRT(32) and exceptionally rare cases of EpS(20).

Among *SMARCA4*-deficient undifferentiated neoplasms, two groups have emerged based on their simple versus complex karyotypes. In the first group of genomically stable tumors are included ATRT and MRT, small cell carcinoma of the ovary, hypercalcemic type (SCCOHT)(33,34) and *SMARCA4*-deficient uterine sarcoma(35,36), while in the second group defined by a complex karyotype are mostly undifferentiated/ dedifferentiated carcinomas that have arisen from a precursor lesion with intact SWI/SNF function. Some examples from the latter category include dedifferentiated uterine endometrioid adenocarcinoma(37,38), undifferentiated/dedifferentiated urothelial carcinoma(39),and undifferentiated/rhabdoid carcinomas of the gastrointestinal tract(40). In some cases, however, the presence of a precursor lesion is not demonstrable. One such example are the so-called thoracic *SMARCA4*-deficient undifferentiated tumors, which have been the source of much debate as they occur in younger patients, often limited epithelial marker expression and lacking a precursor carcinoma component in most cases. These tumors show diffuse sheets of variably discohesive epithelioid to rhabdoid cells, with frequent reactivity to CD34, SALL4, and/or SOX2, thus overlapping with MRTs(41,42). Their transcriptomic/ immunohistochemical profiles are distinct from EpS, in that developmental genes are enriched and SMARCA2 expression is often co-deficient(41,43,44). However, detailed genomic studies have shown significant overlap with lung carcinomas in most cases and thus are currently regarded by most experts as an undifferentiated/dedifferentiated form of lung carcinoma(41,44).

4. Translational genomics

Two independent groups representing co-authors on this review have published genomic landscape studies: one study using WES and deep RNA-sequencing (RNA-seq) showed retained dysfunctional *SMARCB1* expression in 12 of 16 distal, pediatric/young adult-associated EpS biopsies and two of two distal EpS cell lines (as well as elevated *GLI3, FYN*, and *CXCL12* expression for distal EpS)(45), whereas the parallel report combining targeted DNA-based sequencing and FISH showed *SMARCB1* genetic alterations in all but three distal and one proximal out of 44 EpS tumor samples, despite loss of nuclear SMARCB1 expression by immunohistochemistry in all cases(8). Moreover, four additional proximal EpS showed only heterozygous *SMARCB1* alterations using these methods(8).

Both studies represent relatively small patient population samplings, and merit additional expanded studies.

RNA-sequencing (RNA-seq).

As mentioned previously(16,43), despite a rather small number of cases (n=11) relative to the 7000+ RNA-seq expression profiles(46), EpS formed distinct group from all other tumors, including MRT or other SWI/SNF-deficient tumors, in UMAP projection of expression profiles. In that series, EpS was split in two slightly different homogenous groups, which do not seem to be related to the anatomic site of the tumor nor the specific histology or clinical aspect, one being closer to MRTs than the other. Further studies are needed to investigate the significance of this transcriptional dichotomy. In one case authors were able to identify a gene fusion involving *SMARCB1* and *DNAI3*, resulting in *SMARCB1* truncation at the end of exon 3. Despite loss of SMARCB1 by IHC, identification by RNA-seq of *SMARCB1* alterations remains challenging due to: i) the mRNA decay process that degrades RNA carrying a truncating mutation, and ii) the cells from the tumor microenvironment still express wildtype SMARCB1, thus loss of SMARCB1 expression (or of the other SWI/SNF genes known to be involved in EpS: SMARCA4, SMARCC1 or SMARCC2) is therefore barely seen. By RNA-seq, we could identify a *SMARCB1* truncating mutation in a single case of EpS, while loss of expression was noted in only four cases. No mutation nor loss of expression of SMARCA4, SMARCC1 or SMARCC2 was seen.

DNA-methylation.

Array-based DNA-methylation profiling and classification has been established as a powerful new diagnostic tool for brain tumors(47) and had transformative impact in reclassification of known brain tumor entities and led to the ongoing discovery of more and more distinct entities. Recently, a similar DNA-methylation based classifier was brought *en route* for sarcomas(48) and is currently being validated in several subsets of sarcomas(49–51). A recent landmark paper demonstrated the power of multi-omics molecular profiling of sarcomas in adolescent and young adult patients in which 2 EpS patients were included(52). Interestingly, of the two cases confirmed by centralized expert pathology review, one could not be assigned to DNA-methylation based classification due to ‘assay failure or no analysis’, while the other was assigned to the methylation class ‘unclassified’(52). This result is in stark contrast to the other sarcoma entities (>10) included in this study, highlighting the difficulties seen especially for EpS. The technical conundrum may be related on one hand to the relatively high stroma content and inflammatory background seen in most EpS compared to other sarcomas, which is likely responsible for the challenges faced with DNA-methylation based classification of EpS without a stringent enrichment for tumor-bearing tissue (unpublished observations T.G.P.G. and S.P-V.). A recent methylome profiling study focusing only on *SMARCB1*-deficient neoplasms, showed that all classic and most proximal EpS cases tested formed distinct clusters from MRT and ATRT(53). Only 2 cases of proximal-type EpS clustered together with the MRT group, however, it remains unclear if this finding also translates into similar clinical outcomes.

Proteomics.

Advances in proteomic technologies have translated into an in-depth characterisation of protein and post-translational modification levels in tumour specimens at high resolution(54,55). To date proteomic analyses in EpS have been undertaken in tissue samples using gel electrophoresis coupled to mass spectrometry and in the VA-ES-BJ cell line (RRID:CVCL 1785) by mass spectrometry and protein arrays. These studies have led to several biological findings including the demonstration that the actin depolymerization and capping protein CAPZB is overexpressed in EpS patient specimens(56) and that silencing of this protein leads to a reduction in VA-ES-BJ cell growth and migration(57). Furthermore, the use of antibody arrays to assess the activation status of receptor tyrosine kinases in VA-ES-BJ line highlighted the potential of combination therapies involving EGFR and c-Met inhibition(58) or mTOR and c-Met inhibition(59) to overcome EpS cell proliferation. These studies illustrate the power of proteomics to identify candidate targets for drug development. Future studies incorporating deep proteomic profiling of EpS patient specimens and integration with parallel genomic studies are likely to reveal new opportunities for prognostication and therapy selection(60).

5. Biology of the SWI/SNF complex in normal and cancer cells

SWI/SNF complexes utilize the power of adenosine triphosphate (ATP) hydrolysis to remodel nucleosome-DNA interactions, thereby facilitating DNA accessibility and activating the transcription of lineage-specific genes required for cell differentiation(61). Mammalian SWI/SNF (mSWI/SNF) complexes are heterogeneous, multi-subunit protein complexes that are composed of subunits encoded by 29 genes. Based on their characteristic subunit compositions, mSWI/SNF complexes exist in three distinct forms: cBAF, PBAF, and ncBAF (also known as GBAF, GLTSCR1/1L-containing BAF)(62,63) (Fig. 5). The specific subunits in each form are ARID1A/B and DPF2 (cBAF); PBRM1, ARID2, and BRD7 (PBAF); and GLTSCR1/GLTCR1L and BRD9 (ncBAF). ncBAF is also characterized by the absence of SMARCB1, SMARCE1, DPF1/2/3, and ARID1/2; ncBAF uniquely localizes to CTCF sites and promoters, and ncBAF exhibits distinct gene regulation compared to the other two forms of mSWI/SNF complexes(64). Because tumor cells depend on ncBAF for proliferative maintenance in cancers driven by core cBAF subunit perturbations (e.g., EpS and MRT), the ncBAF-specific subunits GLTSCR1/GLTCR1L and BRD9 are potential targets for synthetic lethality(64).

Exome sequencing studies have revealed that the genes encoding the mSWI/SNF complex subunits are collectively mutated in >20% of cancers(65), as well as several developmental disorders (Table 1). Notably, some subunits frequently undergo mutation in specific cancer types; the existence of these driver mutations indicates that aberrant mSWI/SNF complexes lacking specific subunit functions can cause oncogenic transformation in specific cellular lineages(65,66). *SMARCB1*, encoding the INI1/BAF47/hSNF(65) protein, is a core subunit of both the cBAF and PBAF assembly forms of mSWI/SNF complexes. The loss of immunohistochemically-detectable expression of SMARCB1 occurs in ~98% of soft tissue/kidney MRT and brain ATRT cases, >90% of EpS cases, and other bone and soft tissue tumors with epithelioid or rhabdoid morphology(15,67–70). In the context of MRT, the loss

of SMARCB1 lowers the affinities of mSWI/SNF complexes for chromatin, significantly reducing the occupancy of aberrant mSWI/SNF complexes on enhancers and bivalent gene promoters; these changes lead to the repression of key genes involved in cell differentiation and tumor suppression(14). Although the loss of SMARCB1 is suspected to play a key role in EpS pathogenesis, preliminary studies suggest that SMARCB1 rescue may not completely inhibit progression in models using stably transfected cell lines(14). Whereas 98% of MRT cases exhibit biallelic inactivation of SMARCB1, as well as a stable genome with a low mutational burden(21), genome-wide studies of EpS have revealed the loss of SMARCB1 protein through several alternative mechanisms involving genomic complexity and high mutation rates(8,16,45). These findings suggest that, in addition to the loss of SMARCB1, other signaling pathway mutations may contribute to EpS pathogenesis.

6. Early clinical trials and targeted therapy

As recently reviewed elsewhere(71,72), conventional therapeutic options for patients with EpS provide limited benefit, with only 15–27% of overall responses in first-line therapy, and a median duration of response (mDOR) of 3–6 months. Although this has been improved by tazemetostat, an EZH2 inhibitor (EZH2i) (25% responses in first-line therapy with a mDOR of 9.5 months)(73), the vast majority of tumors remain resistant to this therapy, thereby supporting the early enrollment of patients in clinical trials that evaluate innovative complementary therapeutic approaches.

Immune therapies:

Although SMARCB1-deficient tumors show a low tumor mutational burden (TMB), EpS tend to highly express PD-L1 and have extensive cytotoxic T cell infiltration(74,75), which are predictive factors of response to checkpoint inhibitor therapy. In line with this observation, tumor responses to agents targeting the PD1/PD-L1 axis have been reported in several SMARCB1-deficient sarcomas, including at least 5 EpS(76), notably as part of early phase clinical trials (partial response on pembrolizumab(77) or in the form of case reports(74,78,79). Most recently, results from the AcSé Pembro trial, which evaluated pembrolizumab in ultra-rare sarcoma, reported one complete response and three prolonged stable disease, out of six EpS patients(80). Noteworthy, three (out of 12) patients with SMARCA4-deficient sarcomas or MRT also presented prolonged partial responses, overall suggesting increased sensitivity of SWI/SNF-defective sarcomas to anti-PD-1 antibodies. Intriguingly, despite presenting a near terminal disease and prior EZH2i treatment, a patient had an unexpected complete response which was prolonged over 11 months on nivolumab and ipilimumab therapy (81). Whether previous EZH2i exposure plays a role in this deserves further exploration. Several preclinical results and clinical observations suggest that EZH2 inhibitors have immunomodulatory properties that could synergize with immune checkpoint blockers targeting the PD1/PD-L1 axis (82–84). This is currently being evaluated in some academic clinical trials (NCT04705818, NCT05407441). Ongoing clinical trials that evaluate targeted therapies or immune therapies for EpS are currently summarized in Table 2. Further studies are required to determine the role and sequencing of targeted therapy (e.g. EZH2i and pazopanib).

Beyond immune checkpoint inhibitors (ICI), cell therapy may also bring benefit in EpS. A case report showed promising outcomes in a patient with advanced EpS who received autologous immune enhancement therapy based on activated and expanded NK cells and T cells(85). Although NY-ESO-1 and MAGE-A4 antigens have been frequently detected in various soft-tissue sarcomas(86,87) and clinical trials with engineered T cell receptors (CAR-T cells) are currently underway in synovial sarcoma, CAR-T therapy has not yet been investigated in EpS.

Oncolytic viruses (OVs):

OVs are viruses that have been genetically engineered to selectively replicate and kill cancer cells(88). In addition to their capacity of directly killing the infected tumor cell, OVs have also the capacity to manipulate the tumor immune microenvironment and trigger, in many cases, a tumor specific immune response(89). In fact, within the tumor OVs exert their anti-tumor activity in many different ways: (1) They infect and replicate in infected cells triggering an immunological cell death(90), allowing also tumor antigens release in the microenvironment; (2). Due to their own nature and their capability to interact with many pathogen recognition receptors (PRRs), OVs can activate resident dendritic cells to pick up tumor antigens and migrate to the near lymph nodes to present these tumor-antigens to naïve T cells(90); (3) In the tumor microenvironment OVs create a local inflammation that results in enhanced T cells recruitment often converting cold tumor into hot ones, for this specific reason OVs have been very often associated with immune checkpoints inhibitors (ICIs)(91); (4) OVs can be genetically or ‘chemically’ engineered to deliver tumor antigens to dendritic cells (DCs) to generate a specific T cell response(92); (5) OVs can be genetically engineered to produce immune-active molecules such as cytokines to boost particular arms of the immune system(93) or to produce ICIs(94) to further boost and shape the anti-tumor immune response(94). Interestingly, tumor response was recently reported in a patient with EpS enrolled in a Phase 1 study evaluating nivolumab in combination with RP3, a genetically modified herpes simplex type 1 virus (HSV-1) that expresses exogenous genes (anti-CTLA-4 antibody, CD40 ligand and h4-1BBL), designed to directly destroy tumors and generate an anti-tumor immune response when injected in tumor lesions (NCT04735978). Although this isolated case report does not allow distinguishing whether the clinical benefit derives from RP3 and/or nivolumab, this suggests that OVs represent an interesting and innovative strategy. Further, OV can be easily decorated with tumor specific antigens or neo-antigens to direct and concentrate the immune response towards the tumor(95,96), a feature can be easily and inexpensively adapted to many tumor types (NCT05492682). To this end, we have adapted our antigen discovery pipeline to EpS and discovered few new tumor-specific antigens that can be used to decorate oncolytic viruses to treat EpS in the future in a tumor-specific or even personalized way.

Targeted therapies:

The registration of tazemetostat in January 2020 has been a breakthrough in the treatment of EpS, but primary and acquired resistance still represent a major challenge. Activity was originally observed in the dose-escalation Phase 1 trial, where 5/13 patients with SMARCB1-negative tumors showed clinical benefit (stable disease or response), including two patients with EpS whose disease stabilized on treatment for more than 20

months(97). This prompted the design of a Phase 2 trial, which confirmed the activity of tazemetostat in EpS, with a 15% overall response rate (9/62 patients)(73). Importantly, responses were more frequent in first-line patients (25% ORR), and were durable (median duration of 9.5 months). As with any targeted therapy, patients eventually relapsed and acquired resistance to EZH2i which represents a major challenge. Several mechanisms of rezistance to EZH2i have recently been described, including the non-catalytic activity of EZH2(98), the induction of autophagy (99), the loss of NSD1-dependent H3K36me2, which is required to activate SWI/SNF target genes(100), and more recently mutations from the RB1/E2F axis that uncouple EZH2-dependent differentiation and cell cycle control (bioRxiv.2023.02.06.527192). Importantly, the former mechanism was recently described in patients, with a primary resistance sample harbored *CDKN2A/2B* inactivating mutations, and two acquired resistance samples showed a missense mutation of $EZH2^{Y666N}$, and a bi-allellic loss of function mutation in *RB1,* respectively. Similarly, primary or acquired resistance tumors showed increased gene expression of S/G2/M-phase associated Gene Ontology pathways, suggesting the decoupling of proliferation and PRC2-regulated differentiation. Some of these resistance mechanisms may be addressed by inhibiting EED, the scaffold subunit of the polycomb repressive complex 2 (PRC2): the EED inhibitor MEK683 recently showed a 15% overall response rate (ORR) in a series of 14 EpS patients (of whom 80% received at least one prior therapy), with 35% of patients being treated for > 1 year(101). Further, small molecules that inhibit both EZH1 and EZH2, such as valemetostat, CPI-1205, or HH2853, may also be additional benefit. At ASCO 2023, a very promising response rate of 28% (10/32 patients, including one complete response lasting more than 270 days), was reported in the phase 1 trial evaluating HH2853 in pre-treated EpS patients (NCT04390737). Disease control rate was 78%. Although this EZH1/2 inhibitor displayed a higher rate of gastro-intestinal and hematological toxicities than tazemetostat, this supports that dual EZH1/2 inhibitors may be more potent than first-generation EZH2 inhibitors. Beyond targeting PRC2, other therapies that exploit intra-complex synthetic lethality, such BRD9 degradation (NCT05355753, NCT04965753), may also bring benefit in EpS(64).

7. Translational research and disease models

In EpS, most of the existing models represent the proximal type, reflecting its biological aggressiveness compared to the distal type, with inherent challenges in generalizing these findings to all EpS. However, the characteristics of the original tumors remain unknown in some models. An extensive number of EpS cell lines (2D or 3D) has been established through years and recently characterized(102,103) highlighting the differences between ES subtypes as well as age of patients(45) of which most were summarized in a prior review in 2015(104).

Since then, several new cell lines, PDX, organoid derived models (PDO: patient derived organoids; ODX: organoid derived xenografts) have been published that have already proven invaluable to perform functional experiments within conditions closer to the physiological tumor environment, summarized in Table 3. However, relatively few pediatric cell lines or xenograft models exist for investigation. One proximal-type PDX model (ES-1) with a corresponding 2D cell line was recently characterized and confirmed to fully recapitulate

the clinical tumor of origin and was exploited to comparatively assess the effectiveness of the EZH2 inhibitor EPZ-011989, doxorubicin-ifosfamide combination and gemcitabine, showing similar anti-cancer activity of these agents(99). Yet, immune-competent models are still lacking which hampers the generation and testing of new hypotheses on the immune infiltrates in EpS and the effect of different approaches, such as chemotherapeutics, targeted therapies and immune therapy, to boost an immune response.

8. Current state-of-the-art for translational research on EpS including the tumor microenvironment and extracellular vesicles

The tumor microenvironment (TME), which includes immune cells, stromal cells, vasculature, and extracellular matrix (ECM), plays a diverse and complex role in tumor progression(105). Until recently the characterization of the immune microenvironment of EpS has been limited to case reports. Kodet *et al.* identified lymphocyte clusters ($CD20^+$) and macrophages ($CD68^+$) in a portion of pediatric EpS samples(106). Gong et al. showed the presence of tumor infiltrating lymphocytes ($CD3^+/CD4^+/CD8^+$) and macrophages ($CD68^+/CD163^+$) in an adult patient who responded to camrelizumab(78). A recent genomic study performed on a large cohort of SMARCB1-deficient neoplasms (18 EpS, 40 MRT, 49 ATRT) focused on immune cell deconvolution from RNA sequencing datasets(53). Their results showed a predominance of M2 macrophages and CD8+ lymphocytes in the TME of these tumors, further supporting EpS being an immunogenic neoplasm that may benefit from inmmune checkpoint inhibitors. With increasing evidence that the immune composition is associated with treatment response and patient outcomes in other sarcoma subtypes, studying the immune activity in EpS may have implications for clinical decision-making(107). The ECM remain largely unstudied in EpS(108), however Rasmussen et al. identified gene modules associated with ECM and cell adhesion to be differentially expressed between proximal and distal EpS at the transcriptomic level(45). More recently, extracellular vesicles have been shown to mediate interactions between tumor cells and TME, promoting tumor-specific processes(109). Using an EpS cell line model, Aoki and colleagues found that sarcoma cells promote invasion and metastasis by releasing CD147 as microvesicles, which stimulates the production of matrix metalloproteinases (MMPs) by fibroblasts in the TME(110). However, the full diagnostic and prognostic potential of extracellular vesicles in EpS is yet to be explored.

9. Translational research on EpS – patients' perspective

From the perspective of patient organizations, there are three main areas for action.

Research priorities:

Defining and better understanding the biology of EpS in terms of the two types distal versus proximal, or other molecular classification, is needed. New research and treatment approaches also must be explored, most notably immunotherapies such as ICI and oncolytic viruses, precision oncology, and nano-based therapies. In addition to these approaches, the role of the cell-of-origin, microbiome and the tumor microenvironment needs to be explored.

Finally, developing biomarkers that would allow early detection of primary tumors or metastasis, as well as understanding mechanisms of EpS tumor cell spreading are required.

Research organization:

From a patient's perspective, international cooperation between researchers and clinicians is key to success. It is of outmost importance to establish a centralized biobank making tissue samples physically and digitally available to the translational research community as a basis for development of new cell lines, mouse models, and organoids. The connection will be strengthened through translational and interdisciplinary expert meetings. Another opportunity for intensified collaboration is the use of existing international research/clinical platforms.

Contribution of patient organizations:

Patient organizations provide the link between the stakeholders: researchers, clinicians, and patients. They provide awareness and education about the disease and ensure that it is detected in time and that delayed or incorrect diagnoses are avoided. They are committed to ensure that patients receive the best possible treatment and assist researchers in collecting biospecimens. Providing a specialized website with information about the disease for patients, physicians, and researchers is a central component of their work.

10. Open questions and future directions for translational research on EpS

Opportunities for expanded biological and drug development studies in EpS very much depend upon patient-clinician-research laboratory collaborations to build a single-site or federated tumor bank for expanded genomic landscape and proteomic studies, as well as cell line and patient-derived xenograft model development. For the latter, distal and/or pediatric models are especially few and critical for new development. Although tazemetostat has shown efficacy in EpS, its exact mechanism of action in patients is still only partially understood. In order to move forward, we collectively think that elucidating resistance mechanisms using sequential patient biopsies, studying cross-resistance mechanisms with next-generation EZH1/2 inhibitors, as well as deciphering potential immunomodulatory effects of EZH2i in patients, will be crucial in designing future rationale combinatorial approaches, notably with immune therapies, to hopefully eventually cure patients. Once the latter are understood, other drugs might one day be combined for synergy, tumor regression, and eventually potential cures for patients' benefit.

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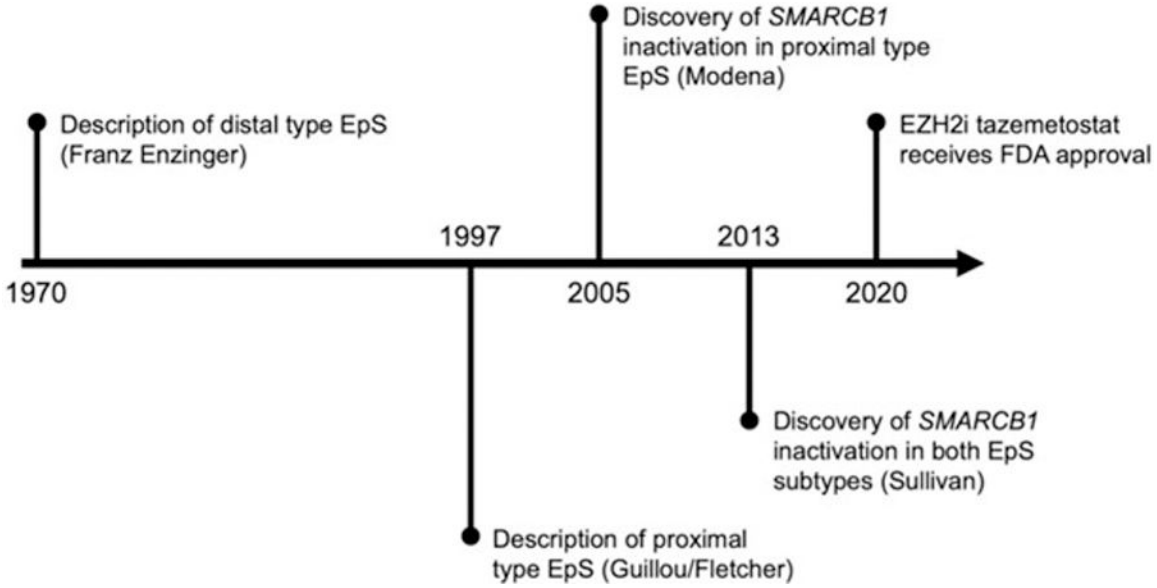

Fig. 1 |.
Timeline of EpS indicating major events/discoveries

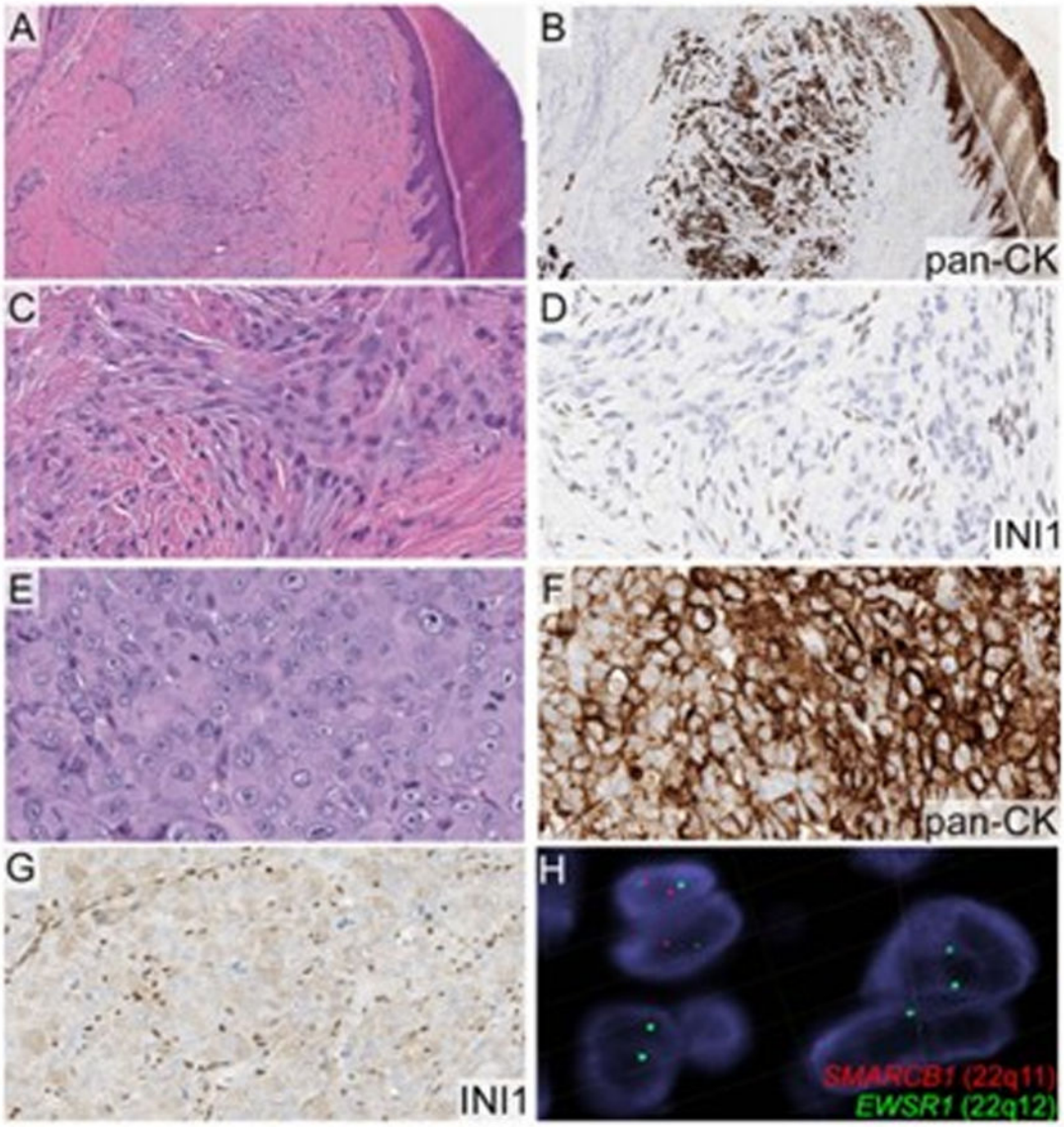

Fig. 2 |.

Prototypical pathologic, immunohistochemical and molecular features. A–D: classic/distal-type. E–G: proximal-type. H: FISH analysis showing *SMARCB1* (red probe) homozygous deletion using *EWSR1* as the reference 22q12 control probe (green).

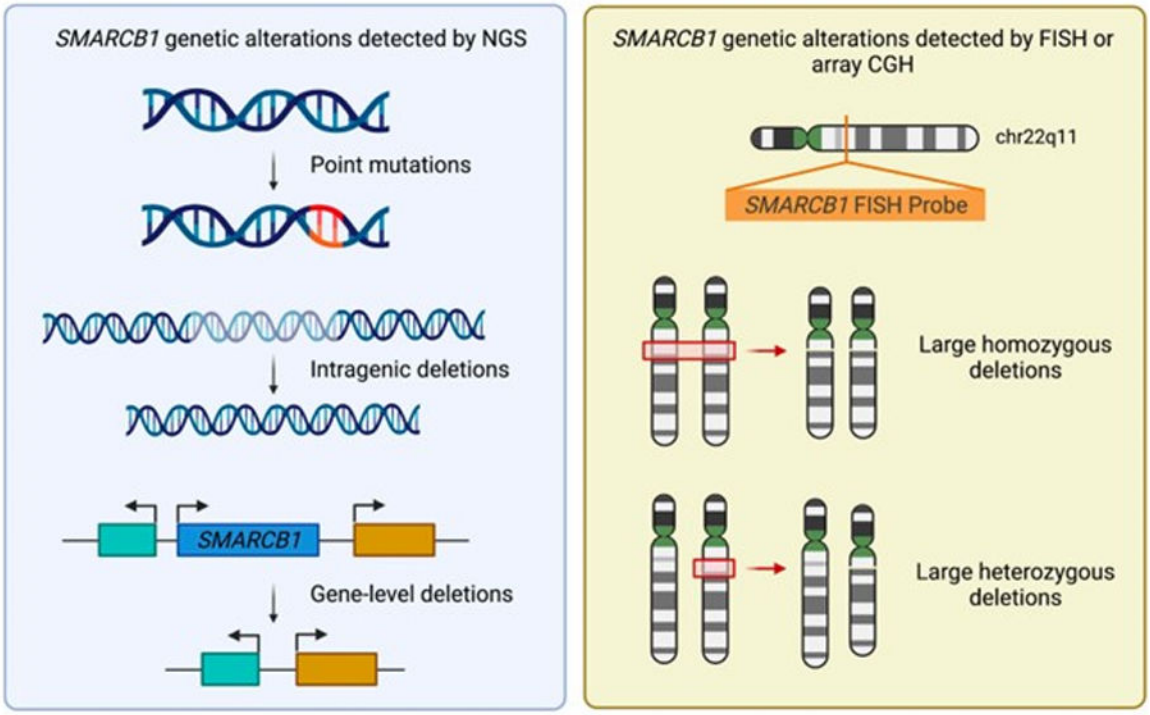

Fig. 3 |.
SMARCB1 genetic alteration types and molecular detection methods

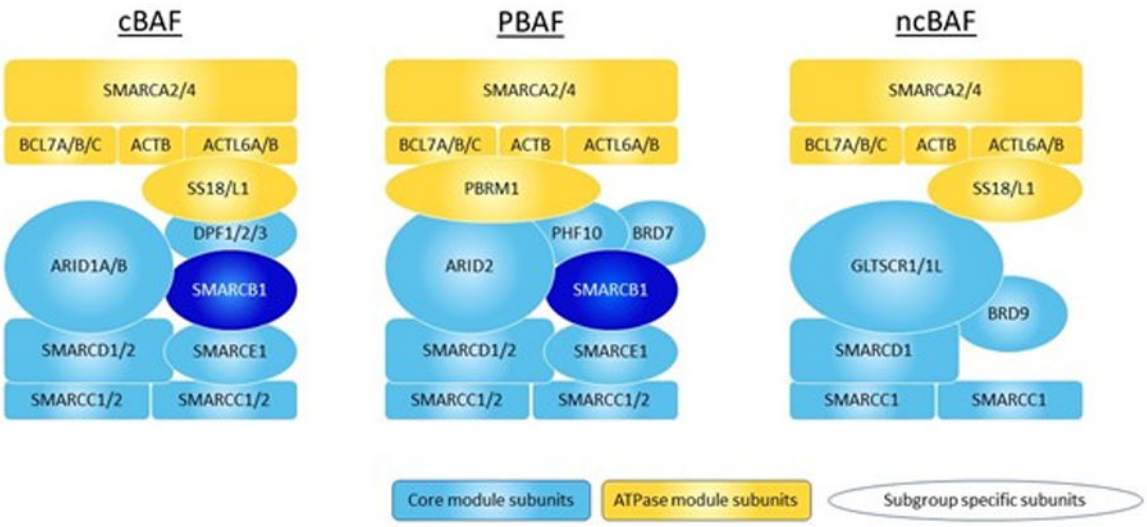

Fig. 4 |.
Scheme of SWI/SNF complexes

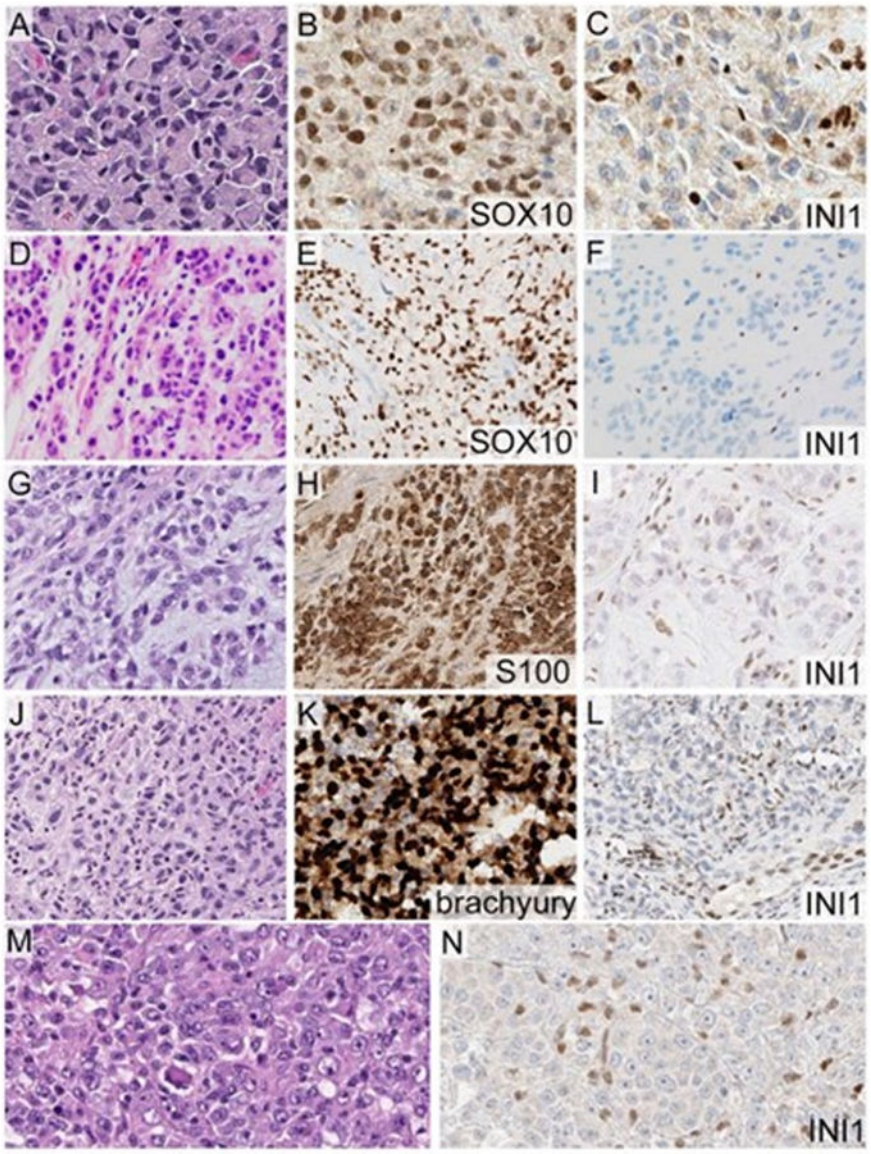

Fig. 5 |.

Comparative histologic appearance of EpS and related neoplasms. A–C: Epithelioid MPNST. D-F: Epithelioid schwannoma. G–I: Soft tissue myoepithelial tumor. J–L: Poorly differentiated chordoma. M–N: MRT.

Table 1 |

List of SWI/SNF complex members and known alterations in cancer

	Subunit		mBAF subgroup			Representative cancer types with SWI/SNF subunit alteration	References
	Gene	Protein	cBAF	PBAF	ncBAF		
Core module	SMARCB1	BAF47	○	○		Malignant rhabdoid tumor Epithelioid sarcoma Schwannomatosis	(7,15,19,111–113)
	SMARCC1/2	BAF155/BAF170	○	○	C1		
	SMARCE1	BAF57	○	○		Multiple spinal meningingioma	(114,115)
	SMARCD1/2	BAF60A/60B	○	○	D1		
	ARID1A/B	BAF250A/B	○			Ovarian clear cell carcinoma Endometrial clear cell carcinoma Bladder cancer Neuroblastoma	(116–120)
	ARID2	BAF200		○		Non-small cell lung cancer Hepatocellular carcinoma	(121,122)
	GLTSCR1/1L	GLTSCR1/1L			○		
	DPF1/2/3	BAF45B/D/C	○				
	PHF10	BAF45A		○			
	BRD7	BRD7		○			
	BRD9	BRD9			○		
ATPase module	SMARCA2/4	BRM/BRG1	○	○	○	Small cell cancer of the ovary, hypercalcemic type SMARCA4-deficient thoracic sarcoma	(33,34,43,123)
	BCL7A/B/C	BCL7A/B/C	○	○	○		
	ACTB	ACTB	○	○	○		
	ACTL6A/B	BAF53A/B	○	○	○		
	SS18/L1	SS18/L1	○		○	Synovial sarcoma (SS18-SSX fusion)	(124)
	PBRM1	BAF180		○		Clear cell renal carcinoma, cholangiocarcinoma	(125)

Table 2 |

Summary of ongoing trials including EpS patients

NCT identifier	Phase	Treatment	Study population	Setting
NCT05407441	I/II	Tazemetostat (EZH2i) Nivolumab (aPD-1) Ipilimumab (aCTLA-4)	AT/RT SMARCB1-deficient primary CNS malignant tumors, SMARCA4-deficient primary CNS malignant tumors, MRTs, rhabdoid tumor of the kidney (RTK), EpS, chordoma	Metastatic / Advanced
NCT04416568	II	Nivolumab (aPD-1) Ipilimumab (aCTLA-4)	MRT, RTK, EpS, chordoma (poorly differentiated or de-differentiated), AT/RT, other SMARCB1-negative malignant tumors (with PI approval)	Metastatic / Advanced
NCT04204941	III	Tazemetostat (EZH2i) + doxorubicin vs Doxorubicin + placebo	Advanced soft tissue sarcoma Advanced EpS	Metastatic / Advanced
NCT05286801	II	Atezolizumab (aPD-L1) Tiragolumab (aTIGIT)	SMARCB1- or SMARCA4-deficient tumors	Metastatic / Advanced
NCT04390737	I/II	HH2853	Solid tumors and lymphoma	Metastatic / Advanced
NCT05415098	I	APG-5918 (EEDi)	Nasopharyngeal Carcinoma, castration resistant prostate cancer, gastric cancer, ovarian clear cell carcinoma, mesothelioma, sarcoma, non-Hodgkin lymphoma B Cell Lymphoma, EpS	Metastatic / Advanced
NCT03069378	II	Talimogene Laherparepvec (T-VEC) Pembrolizumab (aPD-1)	Sarcoma, EpS, cutaneous angiosarcoma	Metastatic / Advanced
NCT05142631	II	Frucidinib (aVEGFR)	desmoplastic small round cell tumor, epithelioid hemangioendothelioma, solitary fibroma or second-line and posterior line treatment of angiosarcoma.	First-line metastatic / advanced
NCT05355753	I / II	CFT8634 (BRD9 degrader)	SMARCB1-null tumors	Metastatic / advanced
NCT04965753	I	FHD-609 (BRD9 degrader)	Synovial sarcoma, *SMARCB1*-null tumors	Metastatic / advanced
NCT04705818 (CAIRE)	II	Tazemetostat (EZH2i) Durvalumab (aPD-L1)	Pancreatic adenocarcinoma, colorectal cancer solid tumors with tertiary lymphoid structures, soft tissue sarcoma	Metastatic / advanced

Table 3 |
Summary of published EpS PDX, organoids and cell lines since 2015

(for prior models before 2015 see reference(104))

PDX	Cell line	PDO/ODX	Subtype	Age	Sex	primary site	Metastatic	Cell line/PDX source	Year	INI1 status	Additional mutations	Investigator/ Reference
STSP1	STSP1		proximal	unknown	F	groin	yes (lymphatic)	primary	2022	unknown	NF1	Wang et al. (126)
ES-1-PDX	ES-1		distal	28	M	forearm		primary	2019	lost		Stacchiotti et al.(99)
J000078604			proximal	22	F	Chest wall muscle		primary	2019	lost	BCR, CDKN2A	Berlow et al. (127).
unknown									2018			Lu et al. (128)
COA-171			unknown (only meeting abstract available)						2022			Hutchins et al.(129)
		OICI-EPS-0530	distal	22	M	Perineum	no		2022	lost		Wakamatsu et al.(130)
		OICI-EPS-0486	distal	50	M	Prox thigh	yes (s.c.)		2022	lost		Wakamatsu et al.(130)

PDO, patient derived organoids; ODX, organoid derived xenografts; PDX, patient derived xenografts