



## Original Research

# Comprehensive molecular profiling of sarcomas in adolescent and young adult patients: Results of the EORTC SPECTA-AYA international proof-of-concept study



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## KEYWORDS

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Personalised medicine

**Abstract Background:** Adolescent and young adult (AYA) patients with cancer are poorly recruited to molecularly targeted trials and have not witnessed the advances in cancer treatment and survival seen in other age groups. We report here a pan-European proof-of-concept study to identify actionable alterations in some of the worst prognosis AYA cancers: bone and soft tissue sarcomas.

**Design:** Patients aged 12–29 years with newly diagnosed or recurrent, intermediate or high-grade bone and soft tissue sarcomas were recruited from six European countries. Pathological diagnoses were centrally reviewed. Formalin-fixed tissues were analysed by whole exome sequencing, methylation profiling and RNA sequencing and were discussed in a multidisciplinary, international molecular tumour board.

**Results:** Of 71 patients recruited, 48 (median 20 years, range 12–28) met eligibility criteria. Central pathological review confirmed, modified and re-classified the diagnosis in 41, 3, and 4 cases, respectively. Median turnaround time to discussion at molecular tumour board was 8.4 weeks. whole exome sequencing (n = 48), methylation profiling (n = 44, 85%) and RNA sequencing (n = 24, 50%) led to therapeutic recommendations for 81% patients, including 4 with germ line alterations. The most common were for agents targeted towards tyrosine kinases (n = 20 recommendations), DNA repair (n = 18) and the PI3K/mTOR/AKT pathway (n = 15). Recommendations were generally based on weak evidence such as activity in a different tumour type (n = 68, 61%), reflecting the dearth of relevant molecular clinical trial data in the same tumour type.

**Conclusions:** We demonstrate here that comprehensive molecular profiling of AYA patients' samples is feasible and deliverable in a European programme.

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## 1. Introduction

Survival for many poor prognosis adolescent and young adult (AYA) cancers has been static for decades. AYA representation in initiatives like the International Cancer Genome Consortium [1] is scanty. Poor AYA trial recruitment hinders learning from affiliated molecular programmes [2], and despite a multiplicity of genomic profiling programmes across Europe [3–8], few explicitly target AYA patients. Therefore, in 2019, we opened a European programme targeting AYA patients with high-risk cancers: the European Organisation for Research and Treatment of Cancer (EORTC) Screening Cancer Patients for Efficient Clinical Trial Access (SPECTA)-AYA study [9], with multi-layered molecular diagnostics to maximise clinically useful data per sample, and discussion at an international molecular tumour board (MTB). We report here the results of the first completed pilot cohort, which recruited high-risk sarcomas.

## 2. Methods

### 2.1. Study protocol and patient enrolment

Patients aged 12–29 years with newly diagnosed or recurrent intermediate or high-grade bone and soft tissue sarcoma were recruited. For newly diagnosed patients, the diagnostic biopsy and/or definitive resection specimen were analysed. For recurrent disease, tissue was from the latest recurrence. Clinico-pathological data were collected according to the EORTC-SPECTA protocol (NCT02834884) [8], open in 17 European countries.

### 2.2. Sample workflow and molecular analysis

Formalin-fixed, paraffin-embedded (FFPE) tumour and whole blood were transported to the Integrated Bio-Bank of Luxembourg. Sections were stained with haematoxylin and eosin and assessed for tumour cell

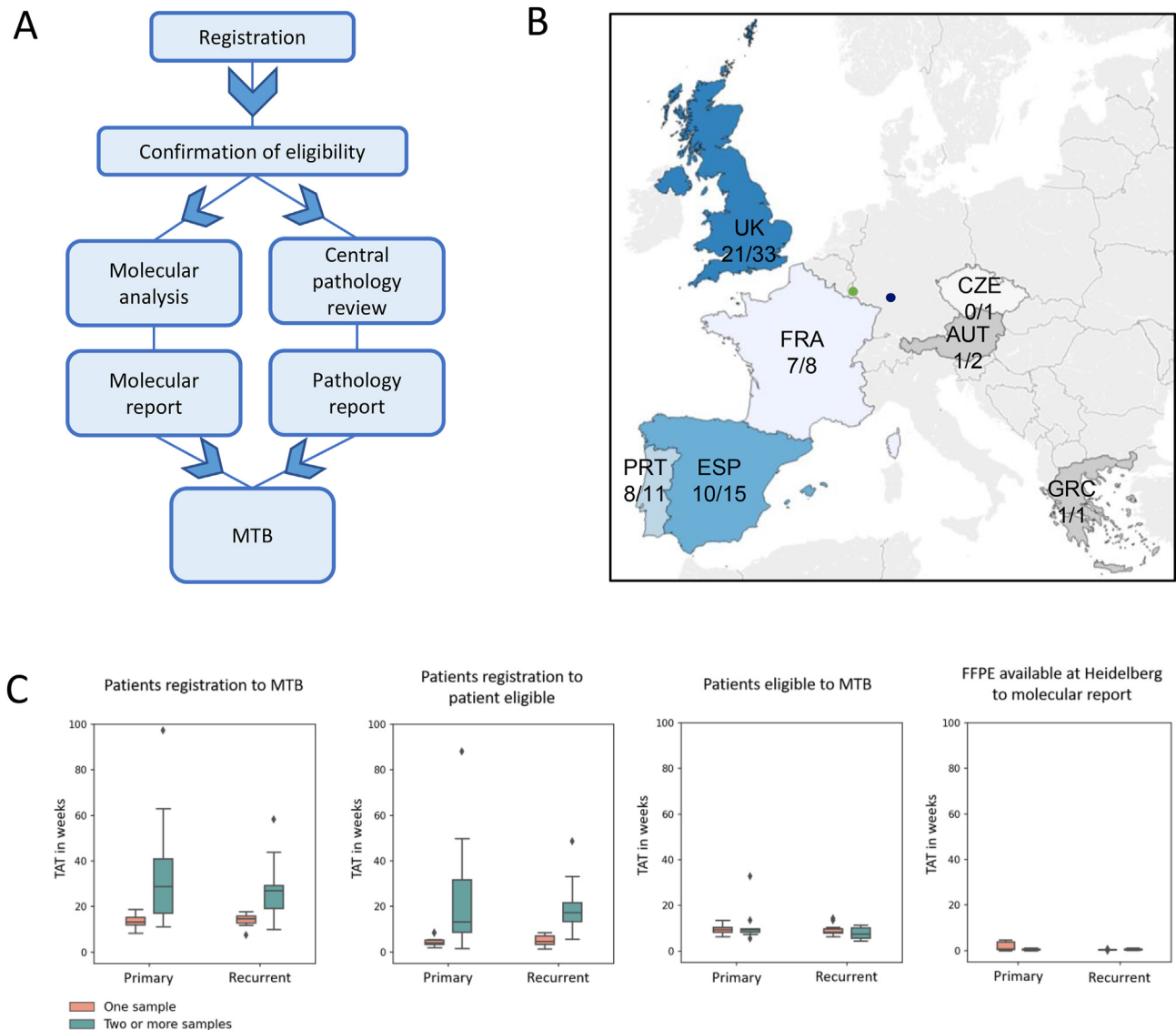


Fig. 1. Patient recruitment and sample handling. (a) Sample flow diagram. Confirmation of eligibility was performed by IBBL. Once eligibility was confirmed, central pathology review, including standard molecular testing, and comprehensive, multi-omic molecular analysis proceeded in parallel and results were discussed at a monthly, multidisciplinary molecular tumour board, integrating clinical patient characteristics, to produce a definitive, molecularly informed MTB diagnosis. (b) Participating countries. Number of sequenced (numerator) and enrolled (denominator) patients from each country. Green dot: IBBL, Luxembourg. Blue dot: DKFZ, Heidelberg (c) Left panel: Overall Turnaround time (TAT) in weeks from patient registration to molecular tumour board (MTB). Boxplots show TAT for patients profiled during primary therapy and at recurrence. TAT is shown separately for cases where the initial sample passed quality metrics (orange) and where multiple samples were required (green). Three components of the overall TAT are shown in the panels on the right. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.) IBBL, Integrated BioBank of Luxembourg.

content. DNA and RNA were extracted using the QIAamp DNA FFPE tissue kit (Qiagen, ref. 56404) and miRNeasy FFPE Kit (Qiagen, ref. 217504) and transported to the German Cancer Research Centre (DKFZ), Heidelberg. Eligibility required 10% minimum tumour cell content and successful nucleic acid extraction (Fig. 1a). For ineligible samples, additional paraffin blocks were requested. Proportions of patients failing quality control between different histotypes were compared using Chi-square and Fisher exact tests.

Haematoxylin and eosin and additional submitted diagnostic slides were scanned at 40 $\times$  magnification onto the Agoko platform along with local pathology reports, including any local molecular testing results ([www.agoko.be](http://www.agoko.be)) and reviewed by two sarcoma pathologists (PS and EW). Minimum immunohistochemical stain sets were recommended for specific histologies (Supplementary Table 1). Additional stains and/or fluorescent *in situ* hybridisations (FISH) were performed to confirm diagnosis where tissue permitted.

Table 1  
Clinical characteristics of the patient population.

	N	%		
Male	27	56%		
Female	21	44%		
<u>Diagnosis</u>	<u>Referring site diagnosis</u>		<u>Central pathology review</u>	
Ewing sarcoma	16	29%	15	31%
Osteosarcoma	8	16%	10	21%
Embryonal rhabdomyosarcoma	4	8%	5	10%
Alveolar rhabdomyosarcoma	2	4%	2	4%
Pleomorphic rhabdomyosarcoma	1	2%	0	0%
Spindle cell rhabdomyosarcoma	0	0%	1	2%
Rhabdomyosarcoma	1	2%	0	0%
Synovial sarcoma	4	8%	4	8%
BCOR sarcoma	0	0%	3	6%
Epithelioid sarcoma	2	4%	2	4%
Desmoplastic small round cell tumour	1	2%	1	2%
Angiosarcoma	1	2%	1	2%
Clear cell sarcoma	1	2%	1	2%
Undifferentiated round cell sarcoma	2	4%	1	2%
Undifferentiated high-grade round and spindle cell tumour, possibly a sarcoma	0	0%	1	2%
Teratoma	0	0%	1	2%
Dedifferentiated liposarcoma	1	2%	0	0%
Spindle cell sarcoma	1	2%	0	0%
Sarcoma NOS	2	4%	0	0%
Sarcoma NOS/PNET	1	2%	0	0%
<u>Treatment status at study entry</u>				
Treatment naive	21	44%		
Post neo-adjuvant treatment	4	8%		
Recurrent disease				
Local recurrence	4	8%		
Distant metastasis	19	40%		
<u>Prior treatment</u>				
Surgery	41	85%		
Radiotherapy	24	50%		
Chemotherapy	46	96%		
<u>Follow-up status at data cutoff</u>	<u>N</u>	<u>%</u>		
Alive	32	66%		
Dead	14	29%		
Lost to follow-up	2	4%		

To avoid delays in releasing molecular results, a preliminary MTB was undertaken prior to definitive histological review for some cases.

Whole exome sequencing (WES, matched tumour and blood), tumour RNA sequencing and DNA methylation profiling (Infinium MethylationEPIC Kit, Illumina) were performed at DKFZ as previously described [5]. DNA and RNA quality were assessed by Agilent DNA and RNA integrity numbers and DV200 index (RNA). Cases were classified by methylation profile according to a calibrated prediction score (sarcoma classifier version 12.2), using  $\geq 0.9$  as a diagnostic threshold [10]. A spectrum of molecular biomarkers were considered (derivation described in the study by Horak et al. [5], Groschel et al. [11] and Hubschmann et al. [12]), and single base substitution mutational signatures [13] and homologous recombination repair deficiency phenotype (HRD) scores were derived as described previously [5,14,15]. Germ line variants in a panel of cancer predisposition genes (described in the

study by Horak et al. [5]) were evaluated according to American College of Medical Genetics and Genomics criteria. Data are deposited on the European Genome-Phenome Archive (EGAS00001005840).

Monthly MTBs included EORTC representatives, sarcoma oncologists, central review pathologists, DKFZ molecular leads and referring clinicians, producing clinically validated reports containing combined pathological/molecular final diagnoses, actionable molecular alterations and treatment recommendations, based on a schema as previously described (Supplementary Table 2 and [5]).

### 3. Results

#### 3.1. Patient characteristics

Between April 2019 and July 2020, 71 patients were recruited (Fig. 1b). Samples were inadequate for 23 patients (<10% tumour cell content or inadequate size,

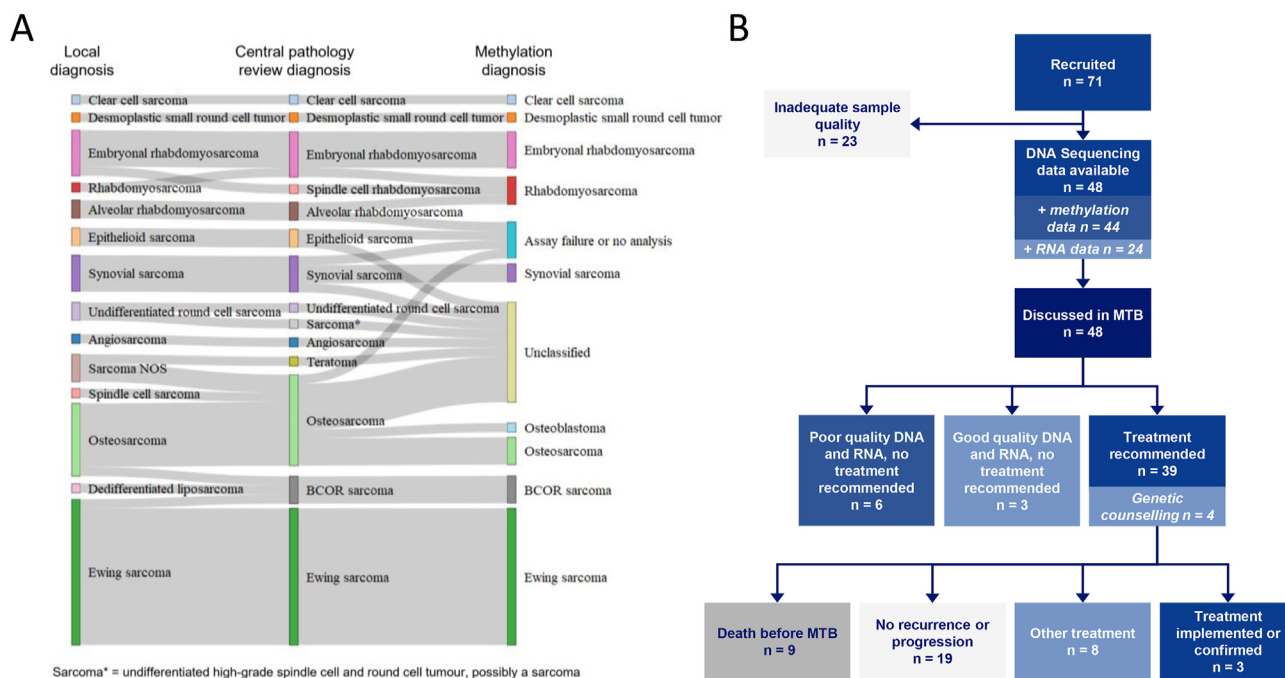


Fig. 2. Sample assignment, patient recruitment and treatment recommendations. (a) Sankey plot summary of central pathology review. Final agreed central pathology review diagnosis is shown in the middle column. The diagnosis submitted by local pathologists is on the left. The outcome of the sarcoma methylation classifier is on the right for all cases eligible for methylation analysis. (b) Consort diagram describing patient recruitment, eligibility and outcomes.

$n = 12$ ) or poor quality nucleic acid ( $n = 11$ ): 25 newly diagnosed and 23 recurrent sarcomas were evaluable. Non-eligible patients had Ewing sarcoma ( $n = 8$ ), osteosarcoma ( $n = 5$ ), sarcoma not otherwise specified (NOS) ( $n = 6$ ) alveolar rhabdomyosarcoma ( $n = 1$ ) and other histotypes ( $n = 3$ ). No central review was performed for excluded patients. There were no significant differences in the proportions of eligible and ineligible patients between bone and soft tissue sarcomas or between Ewing sarcomas, osteosarcomas and other histotypes.

Baseline characteristics are shown in Table 1. At database lock (17th February 2021), median follow-up 11.6 months (range 2.6–19.6), 14 patients had died. Male:female ratio was 1.3:1. Median age was 19 years (range 6–28 years) at diagnosis and 20 years (range 12–28 years) at registration (Supplementary Fig. 1). Recurrent samples were taken at median 32 weeks (range 2–242 weeks) after prior treatment. Most patients had received chemotherapy ( $n = 46$ ) and surgery ( $n = 41$ ); 24 patients had received radiotherapy.

### 3.2. Turnaround time from patient registration to MTB discussion

All times reported are median (range). Global reporting time from registration to MTB was 16 weeks (7.6, 97.2, Fig. 1c). Time to confirm eligibility was 4.4 weeks (1.4, 8.6) if the first sample was adequate and 17.2

weeks (1.6, 88) if inadequate. Lengthy delays were due to the intervals between diagnostic and definitive resections, and sourcing of alternative samples. The interval between arrival at Integrated BioBank of Luxembourg and confirmation of eligibility was 0.6 weeks (0.2, 4.6) and from eligibility to MTB discussion was 8.4 weeks. Central pathological review was 9 weeks (5, 50) for all samples, including those where additional diagnostic tests were required. DKFZ molecular analysis from sample arrival to completion was 6.9 weeks (2.9, 17.4).

### 3.3. Central pathology review and comparison with methylation classification and molecular analysis

Additional immunostaining was required for 35 patients and FISH confirmed two BCOR fusions. Review pathologists agreed with site diagnoses in 41 cases (85%) and disagreed in 7 cases (Fig. 2a). Three cases (sarcoma NOS,  $n = 2$  and spindle cell sarcoma NOS,  $n = 1$ ) were re-classified as osteosarcomas. Three cases recruited with Ewing sarcoma, osteosarcoma and liposarcoma were re-classified as sarcomas with BCOR alterations, and one sarcoma NOS was re-classified as undifferentiated neuroectodermal-type tumour arising from a teratoma.

In parallel, 46 cases were analysed using the methylation classifier [10]. Two cases, estimated on bioinformatic analysis to have <10% tumour content, were

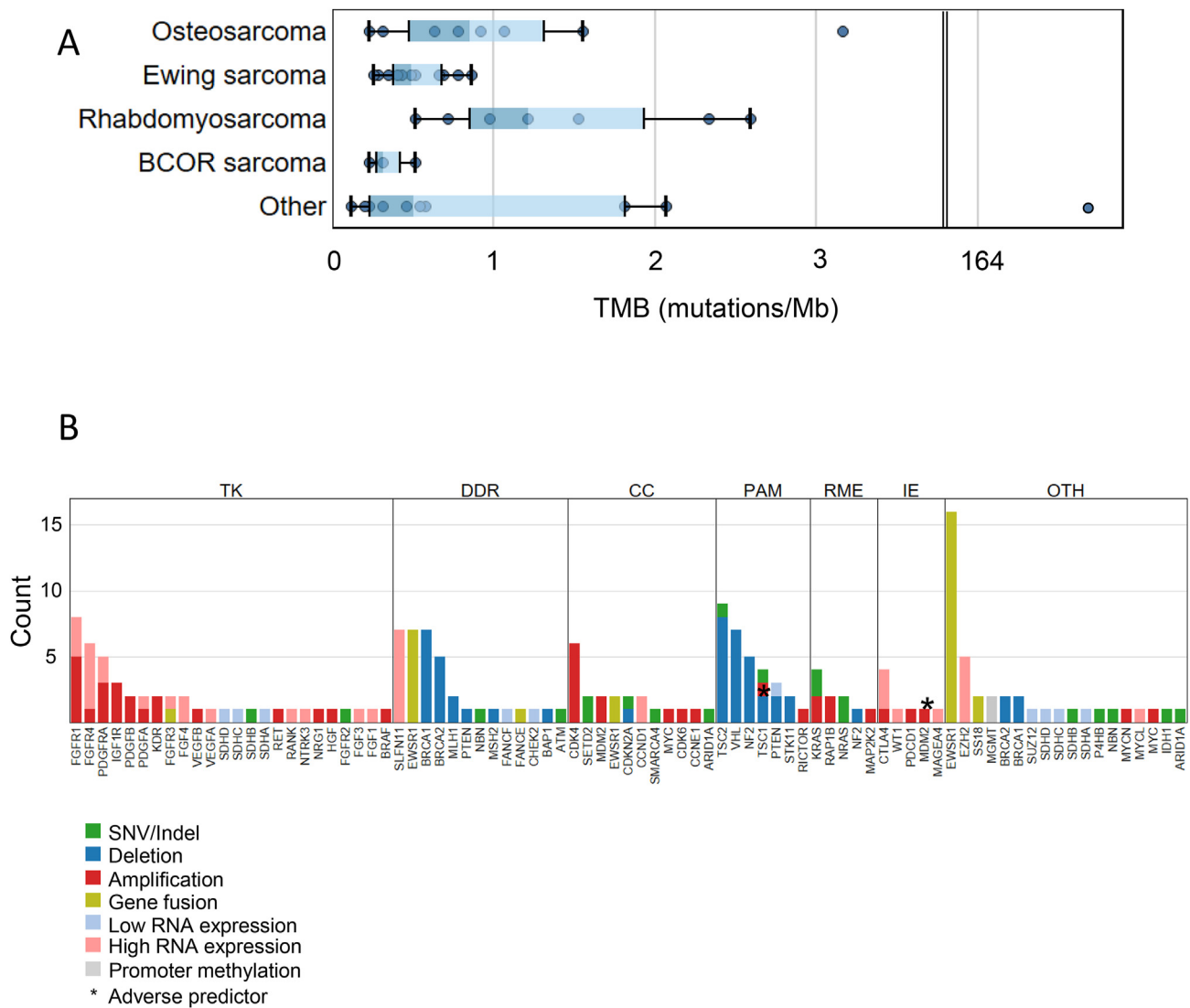


Fig. 3. Molecular results of the cohort. (a) Tumour mutational burden (TMB) in subgroups of patients measured in number of non-synonymous SNVs per megabase of coding sequence. Boxplots show median, lowe and upper quartiles using Tukey's method. Whiskers show the lower and upper quartile +  $[1.5 \times \text{interquartile range}]$ . Blue dots show individual values, including a single outlier with  $>164$  mutations/Mb from the 'Other' group. (b) Alterations of individual genes used for therapeutic recommendations in different treatment baskets. TK: tyrosine kinase, DDR: DNA damage repair, CC: cell cycle regulation, PAM: PI3K/AKT/mTOR, RME: RAF/MEK/ERK, IE: immune evasion, OTH: other.

not evaluated. Methylation classifier and review pathologist diagnoses concurred in 33/44 cases. DNA quality was related to diagnostic concordance: mean DNA integrity number was 5.4 in concordant cases versus 4.4 for discordant samples (t-test  $p = 0.016$ ). Other reasons for failed matching were low tumour cell content and no equivalent group in the classifier training cohort. There was a diagnostic agreement in 30/31 cases with calibrated diagnostic scores  $>0.9$  and 3/13 (23%) with scores  $<0.9$ . The case with a methylation score  $>0.9$  but discordant methylation classifier and pathology diagnoses was an osteoblastoma-like osteosarcoma where pathology review confirmed the reported diagnosis of osteosarcoma while the methylation classifier diagnosed osteoblastoma. Both WES and RNAseq

contributed to the identification of diagnostic gene fusions. For example, of 21 tumours with pathognomonic gene fusions with FISH evidence, including Ewing sarcoma ( $n = 15$ ), BCOR sarcoma ( $n = 1$ ), synovial sarcoma ( $n = 2$ ), alveolar rhabdomyosarcoma ( $n = 2$ ) and clear cell sarcoma ( $n = 1$ ) gene fusions were identified in 43% by RNAseq (100% when RNA of adequate quality was available) and 33% by WES (37% where DNA of adequate quality was available, [Supplementary Table 3](#)).

### 3.4. Clinically relevant molecular biomarkers

WES was performed in all cases, methylation profiling in 92% and RNA sequencing in 50% (Fig. 2b). Median tumour mutational burden was 0.52 mutations/Mb. A

single outlier angiosarcoma showed somatic hypermutation with 164.6 mutations/Mb. Tumour mutational burden varied slightly between tumour groups (Fig. 3a) and was highest in rhabdomyosarcomas (median 1.2 mutations/Mb, range 0.5–2.6 mutations/Mb).

MTB recommendations relied on a median of one molecular biomarker (range 1–9) and were formulated for 39/48 patients (median 3 recommendations per patient, range 1–6), including recommendations for management (n = 39), genetic counselling (n = 4) and pathologic re-evaluation. All genetic alterations identified in each case are described in Supplementary Table 3. The specific molecular biomarkers contributing to treatment recommendations are described for each patient in Supplementary Table 4. In 6 of 48 cases, nucleic acid quality was too poor to allow meaningful analysis; three additional cases had good quality DNA and RNA but no actionable alterations. Treatment recommendations involved 72 genes, comprising single nucleotide variants, insertions and deletions (SNVs and Indels, n = 25), CNV (n = 96), altered mRNA expression (n = 77) and gene fusions (n = 30).

We grouped recommendations into seven baskets based on genes encoding tyrosine kinases (TK), DNA damage response, cell cycle (CC), PI3K/AKT/mTOR (PAM) and RAF/MEK/ERK (RME) pathways, immune evasion (IE) or to an ‘Other’ basket [5]. Most recommendations were assigned to the TK basket (Figs. 3b and 20 recommendations from 24 biomarkers), DNA damage response pathways (18 recommendations, 16 biomarkers), and the PAM pathway (15 recommendations, 7 biomarkers).

The therapeutic baskets and types of alterations varied by tumour (Supplementary Fig. 2). For example, copy number variants (CNVs) affecting TKs and the PAM pathway were the most common basis for recommendations in osteosarcoma; CNVs and gene expression characteristics affecting TK genes were typical of rhabdomyosarcoma, while the Ewing gene fusions themselves led to recommendations for direct EWSR1/FLI1 inhibition, LSD1 and CDK4/6 inhibition (allocated to the ‘Other’ and CC baskets), and in combination with gene expression characteristics, particularly overexpression of SLFN11, a biomarker of sensitivity to DNA-damaging therapies [16], led to recommendations for poly [ADP-ribose] polymerase (PARP) inhibition (HRD pathway, Supplementary Table 3). Mutational signature analysis provided actionable biomarkers mainly via the SBS3 signature [17] in osteosarcomas (5/8). Other mutational signatures were observed but did not contribute to clinical recommendations in this cohort (Supplementary Table 3). Osteosarcoma was the only entity with an elevated HRD score (median score 11.5).

Most recommendations (n = 82, 74%) were for treatment with a single agent class (Supplementary Fig. 3A). Combination treatments were recommended

in 29 cases (26% of recommendations), the most common being PARP inhibitors and alkylating chemotherapy in Ewing sarcoma (n = 18). Evidence supporting therapeutic recommendations was generally of low quality: 61% of recommendations were based on biomarker-predicted activity in a different tumour (evidence level 2B), case reports with scientific rationale (evidence level 2C), or preclinical evidence (evidence level 3, Supplementary Fig. 3B), the most common example being LSD1 inhibition in Ewing sarcoma in 12 cases (11%).

Alterations in 53 genes contributed to treatment recommendations from initial diagnosis, compared to 37 genes in recurrent disease (Supplementary Fig. 4). CC recommendations were more common at recurrence (12 recommendations from 23 samples) than at initial diagnosis (1 recommendation from 25 samples), albeit without any paired samples.

### 3.5. Incidental germline findings

Pathogenic germline variants were noted in 4 patients: an SDHB (NM\_003000.3) c.72+1G>T (rs587782703) splicing variant in a patient with a BCOR–CCNB3 fusion driven sarcoma, aged 23 at diagnosis; a MUTYH (NM\_001128425.2) c.1437\_1439del(p.Glu480del) (rs587778541) in-frame deletion in a 16-year-old patient with undifferentiated high-grade spindle cell/round cell tumour; an ATM frameshift deletion (NM\_000051.4):c.8292\_8293del(p.Ser2764ArgfsTer4) (rs879254036) in an adolescent with Ewing sarcoma aged 6 at initial diagnosis and a first degree relative with thyroid cancer and an ERCC3 frameshift deletion (NM\_000122.2) c.1757del(p.Gln586ArgfsTer25) (rs753182861) in a 19-year-old with undifferentiated round cell sarcoma.

### 3.6. Treatment implementation

Nine patients died before molecular results were available (three patients were recruited at initial diagnosis who subsequently progressed, six patients were recruited at recurrence); 30 patients had potentially actionable mutations. At database lock, 19 patients had not progressed, and in eight patients, the treating clinician gave a different therapy at recurrence. Treatment was adapted for two patients and the molecular profile supported a third patient’s treatment. The first adaptation was a 13-year-old boy with recurrent Ewing sarcoma who received first line chemoradiotherapy. Three sequential recurrences were treated with temozolomide and irinotecan, an LSD1 inhibitor and etoposide. Analysis revealed EWSR1/ERG fusion, SLFN11 overexpression and focal BRCA1 loss. This combination resulted in a recommendation to receive PARP inhibitors. Given the lack of evidence of activity to single agent PARP inhibitors in Ewing sarcoma, we recommended PARP inhibitors in combination with

alkylating agents, ideally within a clinical trial; he received olaparib and irinotecan (level 1C evidence) and progressed after 3 months. The second case was a 20-year-old man with embryonal rhabdomyosarcoma, recruited during first line therapy. Molecular analysis revealed focal losses of *BRCA1* and *BRCA2* and mutational signature SBS3, indicative of HRD. On progression, he received olaparib with trabectedin (level 2B–2C evidence), progressed after two months and died after two further lines of treatment. The third, an 18-year-old male with desmoplastic small round cell tumour was recruited during first line therapy and had MGMT promoter methylation. At recurrence, he received irinotecan and temozolomide (level 2B evidence), and at database, lock had not progressed.

#### 4. Discussion

In this first international pilot study targeted to AYA patients with cancer for therapeutic molecular profiling, we recruited from seven European countries, including some with national paediatric programmes that AYA patients were unable to access. Other study platforms available for young adults in Europe are Molecularly Aided Stratification for Tumor Eradication Research (MASTER), for adults aged 18 to 50 in Germany with advanced stage cancers [18] and DRUG rediscovery Protocol (DRUP) for adults over 18 in the Netherlands [7], with additional centre-specific studies [19]. Several paediatric programmes allow the recruitment of AYA patients at relapse if the primary diagnosis was during childhood or adolescence [3,4,20,21].

A major challenge for this pilot was the turnaround time from patient recruitment to MTB discussion, the largest component preceding initial sample shipment, and/or sourcing further tissue blocks in cases of sample failure. Two principal remediable factors introduced delays: (1) tissue processing and molecular analysis at separate sites and (2) a monthly MTB. Single-site simultaneous tissue processing for histopathology and molecular analysis, and more frequent MTBs would substantially reduce median turn-around time. Importantly, central pathology review did not delay the release of molecular results to recruiting clinicians and was critical to accurate diagnosis: even in this small pilot, central review identified three patients with molecularly-defined, BCOR-altered sarcomas that were incompletely diagnosed by their local pathologists. Methylation analysis also clearly classified these cases as BCOR-altered sarcomas and contributed to the overall review pathology diagnosis in three quarters of cases. In this pilot, the review pathologists were not blinded to the methylation classifier result: indeed, as in clinical care, the review pathologists took all available clinical and molecular data into account, including the methylation

classifier output, in reaching their consensus diagnosis. Therefore, it was not possible to ascertain the independent diagnostic value of the methylation classifier. However, our findings were in keeping with previous reports of the classifier's performance [10,22].

To maximise recruitment, we limited the pilot study to formalin-fixed tissue, resulting in a high sample failure rate, possibly compounded by a high proportion of biopsies from bone sarcomas requiring decalcification which further reduces the yield of nucleic acids [23], although the sample failure rate was not significantly different between bone and soft tissue sarcomas. Molecular programmes using frozen tissue and study-specific biopsies generally report lower patient failure rates. The Molecular Profiling for Pediatric and Young Adult Cancer Treatment Stratification (MAPPYACTS), Individualized Therapy For Relapsed Malignancies in Childhood (INFORM) and MASTER protocols all required frozen tissue to be couriered nationally or internationally to a central molecular laboratory and reported patient failure rates of 9%–18% [6] and [3,4], respectively. Our 33% patient failure rate was higher than those studies, but was not significantly different to the 29% (53/184) failure rate reported recently in the Netherlands DRUP study in adult cancers, despite its requirement for study-specific fresh frozen biopsies for molecular profiling sourced from a single, well-resourced country. Indeed, the major challenge contributing to sample quality failure for AYA and adult sarcomas is not the transportation of tissue but the lack of infrastructure, ability or desire to store fresh tissue as a routine component of the diagnostic process. Moreover, molecularly useful information was delivered for 81% evaluable patients and 55% of all recruited patients from formalin-fixed tissue. The wide variation in reported patient failure rates across multiple independent platforms, in some cases despite repeated study-specific biopsies, highlights challenges in sourcing adequate tissue from patients with relapsed disease and presents an ongoing logistical challenge for any research study or clinical service that relies on obtaining tumour tissue.

The objective of this pilot was to determine the feasibility of AYA cancer patient recruitment to an international molecular profiling study, with associated sample retrieval, analysis and reporting of results, rather than a comprehensive description of actionable AYA cancer mutations. Therefore, the results are reported with short follow-up, in many cases before patients had relapsed. Nevertheless, we identified critical weaknesses in trial evidence and trial availability for this patient population where actionable mutations were identified. Indeed, the availability of molecularly directed clinical trials—arguably the same for young adults as for older adults—is not the only challenge for AYA patients; there are other well described barriers to clinical trial

recruitment in this age group {Fern, 2014 #30}. The choice of drug regimen may not be appropriate for the sarcoma histologies characteristic of AYAs, trial accessibility may be a challenge for patients who are not financially independent, and cancer services may not be appropriate for AYA patients.

The spectrum of alterations was broadly similar in our study to those reported by the International Cancer Genome Consortium, Cancer Genome Atlas network and other reported sarcoma series [3,5,24–29]. Importantly, recommendations were not only based on point mutations. Moreover, there were differences in both the type of alteration and therapeutic baskets between histotypes. While individual recommendations were typically based on alterations in a limited number of genes (such as high SFLN11 expression in patients with classic Ewing sarcoma gene fusions), molecular analyses capable of directing therapy across multiple sarcomas and multiple patients must be capable of detecting point mutations, CNVs, structural changes and expression changes across the genome. In this series, the expression of 27 genes contributed to therapeutic recommendations in 20 cases (Supplementary Table 3). Reassuringly, given the current interest in multi-targeted TK inhibitors, TK alterations were prevalent both at diagnosis and recurrence across multiple histotypes.

The evidence base to support therapeutic recommendations was weak, reflecting the rarity of sarcomas, the small proportion of sarcoma patients recruited to trials, and the lack of appropriate clinical trials for sarcoma patients at relapse. The majority of recommendations were based on preclinical evidence or biological rationale alone. Trial availability is also the most significant of the well described challenges for AYA clinical trial accrual [30]. A small proportion of all recommended therapies were available via clinical trials, and availability of relevant drugs outside of clinical trials was low and highly variable between countries. Where combination therapies were recommended based on molecular profile, combination toxicity and efficacy data were also generally lacking. It is clear that a meaningful evaluation of the place of molecularly targeted therapies in AYA patients with sarcoma will require both additional preclinical work to build empirical evidence where currently only a biological rationale exists, and clinical trial data far in excess of what currently exists.

Although only three patients from our cohort had molecular biomarker-driven treatment, they represented 15% of patients who were still alive with progressing sarcomas. We could not definitively determine why eight patients with actionable biomarkers had alternative non-biomarker-driven therapies at progression. Anecdotally, barriers in clinical trial access and off-trial drug availability were factors. However, the rapid international recruitment to this study and the small proportion able to join clinical trials despite relevant molecular

targets demonstrate a need for accessible, molecularly stratified clinical trials for this population. The adaptive, multi-arm eSMART basket trial has evaluated multiple combination regimens based on molecular profile, but as a paediatric study only partly fills the gap in trial availability for AYAs. For German patients, INFORM, INFORM 2 and MASTER have shown survival benefits for patients with high-level evidence targets [3,5], and the Netherlands DRUP study has reported clinical benefit, based on imaging response, in 34% of patients [7]. However, there remain significant gaps in trial and drug availability for most European AYA patients with sarcoma, even where paediatric programmes exist.

There is some evidence across adult [31] and paediatric [3,6] molecular profiling programmes that molecular biomarker-driven therapy has limited objective benefit in improving imaging response or survival in the absence of high priority targets. However, a major barrier for AYAs remains a lack of empirical translational and trial data to argue for or against molecular therapies. Our data contribute to the extremely scant evidence base in AYA sarcoma and demonstrate an urgent need to develop more empirical evidence through molecularly directed clinical trial research, e.g. via an AYA sarcoma basket trial. The development of such a trial will involve challenges related to sample acquisition, transport and analysis as we outline here, combined with availability of international funding. With time, the trend of increasing infrastructure to freeze tissue on site should reduce the sample failure rate and growing expertise in ctDNA and ctRNA analysis from circulating blood is a welcome development where tissue is scarce. AYA cancer research is a stated funding priority of the European Commission and major national research funders [32–34]. International data exchange can be facilitated by federated and distributed learning [35] as an alternative to centralised data sharing, and some molecular analyses are possible using existing infrastructure working to a common protocol, retaining international funding to fill gaps. Therefore, the required building blocks are in place to drive progress in the development of a European personalised medicine platform for AYA patients with high-risk disease. The SPECTA AYA pilot sets an important precedent towards the development of a much-needed protocol.

#### Author contributions

##### Study Conceptualisation: MGM.

Methodology: MM, PH, SK, VG, SP, EW, PS, SF, MGM.

Formal analysis: MM, AS, TDR, ED, BH, EW, PS, SF.

##### Investigation and Resources: all authors.

Writing – original draft: MM, PH, SK, EW, PS, SF, MGM.

Writing – review and editing: all authors.

Funding acquisition: VG.

### Conflict of interest statement

The authors declare the following financial interests/ personal relationships which may be considered as potential competing interests: MP has received honoraria for lectures, consultation or advisory board participation from the following for-profit companies: Bayer, Bristol-Myers Squibb, Novartis, Gerson Lehrman Group (GLG), CMC Contrast, GlaxoSmithKline, Mundipharma, Roche, BMJ Journals, MedMedia, Astra Zeneca, AbbVie, Lilly, Medahead, Daiichi Sankyo, Sanofi, Merck Sharp & Dome, Tocagen, AdastrA, Gan & Lee Pharmaceuticals. EW has received honoraria for lectures, consultation or advisory board participation from the following for-profit companies: Bistol-Myers Squibb, Bayer, Roche, PharmaMar. SF: Consulting or advisory board membership: Bayer, Illumina, Roche; honoraria: Amgen, Eli Lilly, PharmaMar, Roche; research funding: AstraZeneca, Pfizer, PharmaMar, Roche; travel or accommodation expenses: Amgen, Eli Lilly, Illumina, PharmaMar, Roche. MGM: Advisory board membership: Amgen, Ipsen. JO: research grant from AstraZeneca; honoraria for lectures, consultation or advisory board participation from GSK, Janssen, Novartis, Roche, Bayer, Merck Sharp & Dohme, Eisai, AstraZeneca, Pierre Fabre Medicament and Bristol-Myers Squibb. SMP reports an IMI-2-funded grant entitled ITCC-P4, which is equally funded by the EU as well as 10 EFPIA companies ([www.itccp4.eu](http://www.itccp4.eu)); in addition, S.M. Pfister has a patent EP 16710700 A 20160311 (methylation-based tumour classification) issued.

All other authors had no relevant conflicts of interest to declare.

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### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ejca.2022.10.020>.

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