REVIEW

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Next-generation sequencing for the management of sarcomas with no known driver mutations

Simon Vyse[a], *Khin Thway*[b], *Paul H. Huang*[a], *and Robin L. Jones*[b]

Purpose of review
Next-generation sequencing (NGS) has enabled fast, high-throughput nucleotide sequencing and has begun to be implemented into clinical practice for genomic-guided precision medicine in various cancer types. This review will discuss recent evidence that highlights opportunities for NGS to improve outcomes in sarcomas that have complex genomic profiles with no known driver mutations.

Recent findings
Global genomic signatures detectable by NGS including tumour mutational burden and microsatellite instability have potential as biomarkers for response to immunotherapy in certain sarcoma subtypes including angiosarcomas. Identification of hallmarks associated with 'BRCAness' and homologous recombination repair defects in leiomyosarcomas and osteosarcomas may predict sensitivity to poly(adenosine diphosphate-ribose) polymerase (PARP) inhibitors. Lastly, the use of NGS for evaluating cancer predisposition in sarcomas may be useful for early detection, screening and surveillance.

Summary
Currently, the implementation of NGS for every sarcoma patient is not practical or useful. However, adopting NGS as a complementary approach in sarcomas with complex genomics and those with limited treatment options has the potential to deliver precision medicine to a subgroup of patients, with novel therapies such as immune checkpoint and PARP inhibitors. Moving forward, molecular tumour boards incorporating multidisciplinary teams of pathologists, oncologists and genomic specialists to interpret NGS data will complement existing tools in diagnosis and treatment decision making in sarcoma patients.

Keywords
BRCAness, microsatellite instability, next-generation sequencing, sarcomas, tumour mutational burden

INTRODUCTION

Advances in next-generation sequencing (NGS) technologies have led to fast, accurate, and inexpensive deoxyribonucleic acid and ribonucleic acid sequencing. In the last decade, NGS has been employed in large-scale discovery efforts that have examined the genomics of different cancer types in unprecedented detail [1]. This has led to a deep annotation of the genomic landscapes of cancers including new genetic drivers, large-scale genomic alterations, and a molecular understanding of intratumoural heterogeneity and tumour evolution [2–5]. Moreover, NGS is being incorporated into clinical practice in some cancer types to inform prognosis and patient management as well as for stratification and therapy selection based on clinically actionable driver mutations or mechanisms of drug resistance, ultimately impacting decision-making for the optimal course of treatment [6].

A strength of NGS is the relative ease at which mutations, insertions, deletions, structural rearrangements, copy number alterations, gene fusions and alternatively spliced isoforms can be detected [7]. This has transformed the companion diagnostic landscape resulting in the Food and Drug Administration (FDA) approval of several NGS-based multigene panel tests for cancer-related genes including the FoundationOne CDx (324 genes) and the MSK-IMPACT (468 genes) tests, as well as more

[a]Division of Molecular Pathology, The Institute of Cancer Research and [b]Sarcoma Unit, The Royal Marsden NHS Foundation Trust and Institute of Cancer Research, London, UK

Correspondence to Robin L. Jones, Sarcoma Unit, The Royal Marsden Hospital, 203 Fulham Road, London SW3 6JJ, UK.
Tel: +44 207 352 8171; e-mail: robin.jones@rmh.nhs.uk

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KEY POINTS

- Polygenic variation in sarcomas is common and at least 40% of sarcomas have no targetable driver mutations.
- NGS can be used to detect genomic signatures including TMB, MSI and 'BRCAness' that function as possible biomarkers for immunotherapy or targeted therapy.
- Detection of high-risk genetic variants in sarcoma patients and families using NGS may be important in early detection and surveillance.
- NGS is not yet cost-effective for every sarcoma patient but will complement existing diagnostic techniques and aid clinical management for specific subtypes with limited treatment options.

focused panels such as the Oncomine Dx Target Test for lung cancer (23 genes) [6,8]. More recently, the European Society for Medical Oncology (ESMO) has recommended routine clinical use of multigene NGS panels as part of its guidelines for advanced nonsmall cell lung cancer (NSCLC), prostate cancer, ovarian cancer and cholangiocarcinoma [9▪].

Beyond individual genetic alterations, NGS can also be used to characterise global genomic features that may predict clinical response, including tumour mutational burden (TMB), microsatellite instability (MSI) and DNA damage repair scores. TMB is defined as the total number of somatic coding mutations within a tumour [10], and has emerged as a potential biomarker for response to immune checkpoint inhibitors primarily in NSCLC [11,12] but with evidence for utility across other cancer types including melanoma [13] and urothelial carcinoma [14]. Although TMB can be readily quantified in panel-based approaches that rely on NGS, a lack of standardisation in methods for TMB quantification and reporting has made implementation of this measure as a clinical biomarker for immunotherapy response challenging [10]. MSI refers to variations in the length of microsatellite sequences in the genome and is associated with defects in DNA mismatch repair genes, accumulation of frameshift mutations and tumours with a distinctive genetic and epigenetic profile [15]. MSI is routinely assessed using standard polymerase chain reaction (PCR)-based assessment of specific DNA markers, however, MSI can also be detected using genome-wide NGS-based approaches which may be implemented in future clinical practice. Similar to TMB, MSI can predict response to immune checkpoint inhibitors in a tumour agnostic manner, with the FDA recently approving the anti-PD1 antibody pembrolizumab for all MSI-high solid tumours regardless of anatomical site [16]. Lastly, NGS can be used to interrogate tumours for specific DNA damage repair signatures such as 'BRCAness' – characteristic genomic features that occur following homologous recombination repair deficiency (HRD), often associated with BRCA1/2 loss [17]. The identification and quantification of BRCAness have been important particularly in breast and ovarian cancers to identify patients that are likely to benefit from poly(adenosine diphosphate-ribose) polymerase (PARP) inhibitor therapy [18,19]. By measuring global genomic signatures, NGS has the potential to be used for clinical decision making in cancers with no known driver mutations.

Sarcomas are a rare and heterogeneous group of malignant mesenchymal tumours that account for 1% of adult malignancies and compass >100 different subtypes [20]. Considerable heterogeneity is present at the molecular level, not only between different histological subtypes but also within subtypes. Furthermore, intra-tumour heterogeneity is well documented in some sarcoma subtypes which presents further clinical challenges to accurate diagnosis and current 'one-size-fits-all' treatment strategies [21]. Jour *et al.* performed a large-scale study using a NGS multigene panel to sequence 194 cancer-related genes in 25 soft tissue sarcoma (STS) tumours [22]. Of these, 60% of cases were found to harbour mutations that were clinically actionable with available clinical trials. A similar proportion of sarcoma patients with targetable mutations was found in a larger NGS-based analysis of 102 patients across a diverse range of subtypes performed by Groisberg *et al.* [23]. Although these analyses have been informative about the genetic landscape of sarcomas, it remains unclear as to how many of these identified mutations are primary drivers of disease. Although a variety of individual mutations are detectable by NGS in sarcomas, it is possible that a large proportion represent secondary mutations acquired later in disease development [24]. Secondary mutations may contribute to advanced disease, but they might not represent a key dependency for early cancer growth and targeting them may have limited impact on overall disease burden. Therefore, while NGS may be useful in identifying sarcoma patients with actionable mutations for enrolment into prospective trials of novel agents, multigene panel-based or whole exome/genome sequencing using NGS to detect targetable drivers is not likely to be not practical nor cost-effective in the diagnostic setting for every sarcoma patient referred to a tertiary centre. Instead, this approach may be limited to specific patients with limited treatment options following expert pathology review. Noting that no targetable driver mutations are detected in ~40% of sarcoma patients using multigene NGS panels [22,23], there remains the outstanding

Table 1. Key NGS and biomarker studies in sarcomas

Key study(s)	Cancer subtypes, *n* pts	Biomarker analysis	Key results	Reference(s)
He *et al.*	SS ($n=21$)	TMB using WES data	1/21 pts with high TMB (212 muts/Mb)	[28▪]
Abeshouse *et al.*	Multiple sarcomas ($n=206$)	TMB using WES/WGS data	Low overall Median TMB (1.06 muts/ Mb)	[29]
SARC028 Petitprez *et al.*	40 STS LMS ($n=10$) UPS ($n=10$) Liposarcoma ($n=10$) SS ($n=10$)	Transcriptomic gene expression analysis	ORR to pembrolizumab: 50% in 'Class E' immune-high group	[26,30▪▪]
Doyle *et al.*	Multiple Sarcomas ($n=304$)	TMB using WES data Sequencing of MMR genes	2.3% sarcomas MMR-D MMR-D sarcomas had higher TMB (16 muts/ Mb) 1/3 pts MMR-D pts treated with pembrolizumab had SD	[31▪▪]
Florou *et al.*	AS ($n=7$)	TMB using WES/Foundation CDx assay	1 CR exceptional responder to anti-CTLA-4, low TMB (0.09 muts/Mb)	[32▪]
Painter *et al.*	AS ($n=47$)	TMB using WES data	Low overall median TMB (3.3 muts/Mb) 9 HNFS pts with high median TMB (20.7 muts/Mb) 2/3 HNFS pts treated with anti-PD1 had exceptional response	[33▪▪]
Campanella *et al.*	Multiple STS subtypes ($n=71$)	MSI using PCR and IHC	All 71 cases were MSS	[48]
Kovac *et al.*	OS ($n=31$)	BRCAness hallmarks (single base substitutions, LOH, genomic instability) using WES data	>80% of OS had BRCAness hallmarks	[50]
Chudasama *et al.*	LMS ($n=49$)	BRCAness hallmarks (HRR gene deletions, structural rearrangements) using WES data	>90% of LMS had BRCAness hallmarks	[49]
International Sarcoma Kindred Study	Multiple sarcomas ($n=1162$)	Germline DNA sequencing	1 in 6 patients' families matched hereditary cancer criteria 1 in 15 patients had actionable germline variants	[55,56]

AS, angiosarcoma; HNFS, head, neck, face and scalp angiosarcoma; HRR, homologous recombination repair; LMS, leiomyosarcoma; LOH, loss-of-heterozygosity; Mb, megabase; MMR, mismatch repair; MMR-D, mismatch repair deficient; MSI, microsatellite instability; MSS, microsatellite stable; ORR, objective response rate; SD, stable disease; SS, synovial sarcoma; STS, soft tissue sarcoma; TMB, tumour mutation burden; UPS, undifferentiated pleomorphic sarcoma; WES, whole exome sequencing; muts, mutations.

question as to the clinical utility of NGS in this patient group. In this review, we discuss studies (summarised in Table 1) that have applied NGS measures such as TMB, MSI and DNA damage repair signatures in sarcomas with no known driver mutations and discuss the opportunities and challenges of introducing NGS-based analyses into the routine clinical management of sarcoma patients.

TUMOUR MUTATIONAL BURDEN AS A BIOMARKER FOR IMMUNE CHECKPOINT INHIBITOR RESPONSE IN SARCOMA

Whole exome sequencing (WES) remains the gold standard of TMB measurement; however, TMB can be extrapolated from NGS-based multigene panels [10]. KEYNOTE-158 is a phase 2 trial in which the TMB status was assessed using the FoundationOne CDx assay and evaluated as a prospective biomarker for the anti-PD1 antibody pembrolizumab response in patients with advanced solid tumours [25▪]. Although only a limited number of cancer types were included in the study, across 790 evaluable patients, patients with TMB high status ($\geq$10 mutations per megabase) had a significantly higher response rate of 29% to pembrolizumab compared with 6% of non-TMB high (<10 mutations per megabase) patients. Although this trial highlights the potential of TMB as a biomarker for predictive benefit from immune checkpoint inhibitor therapy, further studies are needed to investigate the use of TMB in specific cancer types such as sarcomas. For example, in KEYNOTE-158, no TMB high patients were detected in biliary cancer patients and there

was no significant difference in median TMB between responders and nonresponders to pembrolizumab, suggesting TMB may not be a universally applicable biomarker for all cancers.

Several immune checkpoint inhibitor trials have been conducted in sarcomas with mixed results. In 2017, the phase II single arm SARC028 trial assessed treatment with the pembrolizumab in a cohort of 80 advanced STS and bone sarcoma patients (NCT02301039) [26]. Across all subtypes evaluated, 7 out of 40 (18%) STS patients had objective response (OR), which notably was highest in undifferentiated pleomorphic sarcoma (UPS) (4 out of 10 UPS patients; 40% OR), dedifferentiated liposarcoma (DDLPS) (2 out of 10 DDLPS patients; 20% OR) and synovial sarcoma (SS) (1 out of 10 SS patients; 10% OR). These results provide initial evidence that pembrolizumab can provide clinically meaningful responses in STS patients, with 1 UPS patient achieving a complete response. In contrast, no responses were documented in the leiomyosarcoma (LMS) cohort. A phase II trial (NCT02428192) to assess the single-agent nivolumab, a PD1 blocking antibody, in uterine LMS (ULMS) patients similarly demonstrated no clinical benefit in 12 ULMS patients [27]. Due to limited patient cohort sizes in these trials it is difficult to draw definitive conclusions about subtype-specific benefits, although it is clear that predictive biomarkers are required to prospectively identify STS patients that are likely to benefit from immune checkpoint inhibitor therapy.

In sarcomas with defined genetic drivers, the use of TMB as a biomarker for immunotherapy may be limited. A recent study by He *et al.* found that in SS, a subtype characterised by *SS18-SSX* fusions, the majority of cases have low TMB, with only 1 out of 21 cases observed to have a high TMB (212 mutations/Mb) [28▪]. However, it is unknown if this rare subset of SS patients with high TMB corresponds with SS patients that may benefit from to immune checkpoint inhibitors. A report across 206 STS cases from The Cancer Genome Atlas Research (TCGA) found a low overall TMB (average 1.06 mutations/Mb) in these cancers, which suggests that TMB alone as a biomarker may be insufficient in sarcomas [29]. A recent study by Petitprez *et al.* examined gene expression data and tumour microenvironmental features in 608 STS tumours identified 5 distinct molecular subtypes associated with enrichment of specific subsets of immune-related genes [30▪▪]. The immune-high 'class E' subtype was characterised by the presence of B-cell lineage genes and associated with tertiary lymphoid structures (TLS). These molecularly defined subgroups were applied to stratify patients from the SARC028 trial and the class E patients were found to have a significantly higher OR rate (ORR; 50%) to pembrolizumab compared to any other subgroup. These studies indicate that including TLS and gene expression signatures may be helpful in identifying sarcoma patients that may benefit from treatment with immune checkpoint inhibitors. It remains to be investigated if the integration of TMB with TLS scoring and immune-based gene expression signatures described by Petitprez *et al.* will improve the predictive power and robustness as a biomarker of immune checkpoint inhibitor response.

In a large-scale study of 304 sarcomas across multiple subtypes, Doyle *et al.* employed massively parallel sequencing of 447 genes to explore the frequency of mismatch repair defects, a feature associated with response to immune checkpoint inhibitor therapy [31▪▪]. A low proportion (2.3%) of sarcomas were found to be mismatch repair-deficient (MMR-D), which included 1 pleomorphic rhabdomyosarcoma, 1 epithelioid LMS, 1 malignant PEComa and 4 unclassified sarcomas. MMR-D sarcomas had a significantly higher median TMB (16 mutations/Mb) versus mismatch repair-proficient sarcomas (4.6 mutations/Mb). However, TMB in MMR-D sarcomas was generally lower compared to carcinomas with MMR-D (28 mutations/Mb). Of the three MMR-D sarcoma patients who received pembrolizumab, two patients (one malignant PEComa and one suspected angiosarcoma (AS)) progressed and one patient (LMS) had stable disease after 5 months follow-up. This study suggests that a subset of sarcomas are MMR-D accompanied by a higher TMB. However, further studies with larger cohort sizes will be required to determine whether TMB can be used to predict response to pembrolizumab for these MMR-D sarcoma patients.

Of the MMR-proficient sarcomas, Doyle *et al.* found that the three tumours with the highest TMB were all cutaneous in origin and included AS and unclassified sarcoma, with mutations characteristic of ultraviolet (UV) light exposure. There is recent evidence from several studies that suggest that a subset of AS patients benefit from immune checkpoint inhibitor therapy [32▪]. Florou *et al.* identified durable responses to immunotherapy in a case series of 7 AS patients [32▪]. Although intermediate TMB (12–15 mutations/Mb) was detected in two patients with partial response to anti-PD1 or anti-CLTA-4 checkpoint inhibitors, one exceptional responder patient with complete response had extremely low TMB (0.09 mutations/Mb), indicating that TMB alone may not be a reliable predictor for therapy response in AS. The Angiosarcoma Project is a patient-partnered study to collate a compendium of genomic and clinical data across AS patients in the US and Canada [33▪▪]. In the first report arising from

the Project, 47 AS specimens were subjected to WES with a low overall median TMB (3.3 mutations/Mb) reported. However, there were a subset of cases, in particular AS patients of the head, neck, face and scalp (HNFS), where a significantly higher median TMB (20.7 mutations/Mb) compared to the rest of the cohort was found. All of the 9 HNFS tumours examined had mutational signatures strongly associated with UV light exposure. Three HNFS AS patients were treated with anti-PD1 therapies, and of note, two patients with high TMB scores (78.5 and 138.9 mutations/Mb, respectively) demonstrated exceptional, durable responses to pembrolizumab and have remained disease-free for more than 2 years after treatment was discontinued. The third HNFS AS patient received a single dose of anti-PD1 therapy before treatment cessation due to side effects. In contrast, three patients from the non-HNFS AS subgroup which were identified to have received pembrolizumab harboured low TMB (<5 mutations / Mb) and none received clinical benefit. Together, these data suggest that high TMB scores associated with UV light mutational signatures are characteristic of AS of HNFS, a subtype that represents up to 60% of AS [34]. High TMB scores may be predictive of response to immunotherapy in AS, warranting further investigation in prospective subtype-specific clinical trials.

MICROSATELLITE INSTABILITY AS A BIOMARKER FOR IMMUNE CHECKPOINT INHIBITOR RESPONSE IN SARCOMA

Microsatellites are short (1–6 bp), repetitive sequences that exist in the genome, and the length of these sequences can lengthen or shrink during DNA replication [35]. This MSI is normally repaired by the MMR machinery, and therefore cancers with MMR defects are associated with detectable levels of MSI. PCR-based detection of either 5 or 7 specific MSI markers has remained the gold-standard of detection of the MSI signature, and although criteria can vary from study to study, this method allows classification of cancers into microsatellite stable (MSS), MSI-low and MSI-high categories depending on the proportion of markers that show evidence of MSI [35]. Using immunohistochemistry (IHC), the absence of expression of proteins associated with MMR including MLH1, MSH2, MSH6 and PMS2 has also been used to categorise cancers as MSI-positive [36]. There are multiple NGS-based approaches that have been developed to accurately detect MSI [15,37–40] which either utilise genome-wide sequencing or sequencing of smaller gene subsets and offer advantages of throughput, sensitivity and simultaneous analysis of samples for additional genomic signatures rather than performing separate PCR-based analysis. Although NGS-based detection of MSI is poised for clinical implementation moving forwards, many studies to date including those in sarcomas have relied on standard PCR-based methods to investigate the potential of MSI as a clinical biomarker.

An MSI-high signature associated with MMR defects has been found to be associated with response to immune checkpoint inhibitors in several key trials [16,41,42] and has subsequently been approved by the FDA as a predictive biomarker for pembrolizumab for all solid tumours, regardless of anatomical site [16]. Approval was initially based on studies that relied on PCR-based or IHC detection of MSI, however, the FDA expects to consider approval of companion diagnostic approaches to detect MSI, which is likely to encompass NGS-based approaches [43]. In particular, the use of this signature has been validated in colorectal cancers (CRC). Between 4 and 5% of CRC patients are defined as MSI-high and are less susceptible to conventional chemotherapy compared with MMR-proficient CRC, but respond well to immune checkpoint inhibitor therapy [44]. The evidence for the presence of MSI-high signatures in sarcomas has historically been contradictory, with early studies that employed IHC-based methods to look for markers of MSI reporting a range of 0.9–25% MSI-positive cases in STS cohorts [45–47]. A recent study of 71 STS patients across multiple subtypes that used PCR-based detection of 5 MSI markers in combination with protein expression analysis of the MMR proteins MLH1, MSH2, MSH6 and PMS2 by IHC identified all 71 cases as MSS, suggesting MSI may have limited utility in unselected sarcoma cohorts [48]. Notably, in the previously discussed study by Doyle *et al.*, sarcomas defined as MMR-D with intermediate TMB scores (2.3% of 304 sarcoma patients) were confirmed to have MSI by PCR based analysis of marker genes [31▪▪]. Therefore, focused MSI screening may be more effective in specific sarcoma subtypes in which MMR-D are expected to be more prevalent. However, it remains to be investigated whether an MSI high signature is predictive of response to immunotherapy in these sarcomas.

'BRCAness' AS A PREDICTIVE MARKER FOR POLY(ADENOSINE DIPHOSPHATE-RIBOSE) POLYMERASE INHIBITOR SENSITIVITY IN SARCOMA

Characterisation of the genomic landscape of sarcomas using NGS has revealed that tumours from certain sarcoma subtypes including osteosarcoma (OS) and LMS harbour hallmarks that are similar

to those observed in cancers with deficiency in *BRCA1/2* genes [49,50]. Tumours that exhibit these features of 'BRCAness' have hallmarks that include defects in HRD genes, structural rearrangements, and specific mutational signatures associated with errors in double-strand break repair [51]. Importantly, these hallmarks are also strongly linked to a sensitivity to targeted PARP inhibitors, presenting a possible new therapeutic opportunity for targeted therapy in sarcomas. In a study by Kovac *et al.*, WES performed on 31 OS tumours revealed that >80% harboured hallmarks of BRCAness including single-base substitutions, loss-of-heterozygosity and large-scale genomic instability [50]. Interestingly, BRCAness in OS specimens did not correspond to specific somatic mutations in genes typically considered oncogenic drivers or tumour suppressors including *TP53, RB1, RET, FANCA* and *ATRX*. The authors speculate that multiple oncogenic pathways may contribute to chromosomal aneuploidy and instability early in OS development, and that BRCAness may be a shared trait of OS, independent of the pathways which are driving oncogenesis. Similarly, WES and transcriptomic sequencing of a cohort 49 LMS tumours by Chudasama *et al.* identified frequent deletions in homologous recombination repair genes and enrichment of mutational signatures characteristic of BRCAness in almost all cases [49]. In support of these findings, there is preclinical evidence that PARP inhibitors in combination with DNA damaging agents are effective in LMS and OS cell lines [49,50,52]. There are currently several trials evaluating the use of PARP inhibitors NGS in sarcomas [53,54] (NCT03880019, NCT02398058) and translational studies to determine if patients who benefit from this targeted therapy in the trial setting are enriched for 'BRCAness' hallmarks will be crucial for the design of future biomarker-driven prospective clinical trials of PARP inhibitors in sarcomas.

NEXT-GENERATION SEQUENCING FOR EVALUATING CANCER PREDISPOSITION: THE INTERNATIONAL SARCOMA KINDRED STUDY

In addition to identifying predictive biomarkers to aid precision medicine in sarcomas, NGS has the potential to have a major impact on cancer risk assessment and prevention. The International Sarcoma Kindred Study (ISKS) is a large-scale, global research effort to understand the genetic basis of sarcomas in the population [55,56]. The study aims to recruit sarcoma patients and their families to perform NGS of germline DNA using blood samples. Initially, sequencing has focused on 72 genes associated with increased cancer risk with the long-term goal of performing whole-genome sequencing. In an analysis of 1162 patients recruited to the ISKS study, more than half were found to harbour pathogenic genetic variations, with 1 in 6 patients belonging to families that matched criteria for hereditary cancer syndromes including Li-Fraumeni syndrome, hereditary CRC and hereditary breast or ovarian cancer [56]. The study also found that a significant contribution of polygenic effects that could affect sarcoma risk, with a correlation found between increased burden of multiple variants and earlier age of cancer diagnosis. Although preliminary, this data suggests that families of sarcoma patients identified with high-risk genetic variants by NGS may benefit from genetic counselling including surveillance and prevention strategies for disease management. Incorporating NGS into early diagnosis of sarcomas may also have implications for guiding treatment strategies, with one in 15 patients in the ISKS study harbouring germline mutations that could have therapeutic significance. However prospective studies are required to determine whether treatment decisions influenced by the identification of actionable germline mutations from NGS analyses will improve sarcoma patient outcomes.

FUTURE PERSPECTIVES/CONCLUSION

NGS has demonstrated potential for diagnosis and genomic-guided precision medicine in sarcomas in the context of known driver mutations. However, much work needs to be done to evaluate the utility of genomic-wide features such as TMB or MSI as predictive biomarkers for patient stratification. The use of NGS in routine clinical practice is not a cost-effective approach for all sarcomas and in many countries still remains unaffordable [57,58]. Currently, NGS-based approaches should be considered for sarcoma patients with limited treatment options and poor survival outcomes, such as those who are chemorefractory or have unresectable or metastatic disease. In the first instance, NGS will be particularly useful for better patient stratification of sarcoma subtypes that exhibit the greatest genomic complexity and heterogeneity including undifferentiated sarcomas, DDLPS, AS, UPS and LMS [29,31▪▪,59–60]. The finding that some sarcoma patients do not respond to immune checkpoint inhibitors despite the presence of predictive genomic features such as high TMB [28▪], and vice versa– that some patients do respond in the absence of these markers [32▪], indicates that our understanding of biomarkers for immunotherapy in sarcomas is still in its infancy. It is likely that combining current NGS-based biomarkers with emerging findings such as

TLS scores [30▪▪] will be necessary for more accurate and robust prediction of immune checkpoint inhibitor response in sarcomas.

The possibility of using NGS in early detection and to explore the clinical relevance of polygenic variation in sarcomas warrants further investigation. Ultimately, integrating NGS into clinical practice will require careful and coordinated consideration involving molecular tumour boards comprised of multidisciplinary disciplinary teams of pathologists, oncologists and genomic specialists to complement existing tools in diagnosis and treatment decision making in sarcoma.

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