A genetic model for central chondrosarcoma evolution correlates with patient outcome

William Cross1, Iben Lyskjær1,2, Tom Lesluyes3, Steven Hargreaves1, Anna-Christina Strobi4, Christopher Davies1,4, Sara Waise3,5, Shadi Hames-Fathi1, Dahmane Oukrif1, Hongtao Ye5, Fernanda Amary4, Roberto Tirabosco4, Craig Gerrand6, Toby Baker3, David Barnes7, Christopher Steele1, Ludmil Alexandrov8, Gareth Bond3, Genomics England Research Consortium9, Paul Cool10,11, Nischalan Pillay1,4, Peter Van Loo3++ and Adrienne M. Flanagan1,4++

Abstract

Background  Central conventional chondrosarcoma (CS) is the most common subtype of primary malignant bone tumour in adults. Treatment options are usually limited to surgery, and prognosis is challenging. These tumours are characterised by the presence and absence of IDH1 and IDH2 mutations, and recently, TERT promoter alterations have been reported in around 20% of cases. The effect of these mutations on clinical outcome remains unclear. The purpose of this study was to determine if prognostic accuracy can be improved by the addition of genomic data, and specifically by examination of IDH1, IDH2, and TERT mutations.

Methods  In this study, we combined both archival samples and data sourced from the Genomics England 100,000 Genomes Project (n = 356). Mutations in IDH1, IDH2, and TERT were profiled using digital droplet PCR (n = 346), whole genome sequencing (n = 68), or both (n = 64). Complex events and other genetic features were also examined, along with methylation array data (n = 84). We correlated clinical features and patient outcomes with our genetic findings.

Results  IDH2-mutant tumours occur in older patients and commonly present with high-grade or dedifferentiated disease. Notably, TERT mutations occur most frequently in IDH2-mutant tumours, although have no effect on survival in this group. In contrast, TERT mutations are rarer in IDH1-mutant tumours, yet they are associated with a less favourable outcome in this group. We also found that methylation profiles distinguish IDH1- from IDH2-mutant tumours. IDH wild-type tumours rarely exhibit TERT mutations and tend to be diagnosed in a younger population than those with tumours harbouring IDH1 and IDH2 mutations. A major genetic feature of this group is haploidisation and subsequent genome doubling. These tumours evolve less frequently to dedifferentiated disease and therefore constitute a lower risk group.

Conclusions  Tumours with IDH1 or IDH2 mutations or those that are IDHwt have significantly different genetic pathways and outcomes in relation to TERT mutation. Diagnostic testing for IDH1, IDH2, and TERT mutations could therefore help to guide clinical monitoring and prognostication.
Keywords Chondrosarcoma, Sarcoma, Genetics, Genomics, Cancer evolution, IDH1, IDH2, TERT

Background
Central conventional chondrosarcoma (CS) is the most common primary malignant bone tumour in adults. Anatomical location, histopathology, and grading are the current criteria for determining treatment [1, 2], although providing prognoses remains challenging [1–3]; hence, a greater understanding of the disease, and its biomarkers is required to provide patients with a more personalised treatment plan. Well-differentiated cartilaginous tumours are referred to differently depending on where they develop: those presenting at sites, from where they can be excised fully with relative ease, including the phalanges and long bones they have an excellent prognosis, and are referred to as atypical cartilaginous tumours (ACT), the exception being if they are associated with a dedifferentiated (DD) component. In contrast, tumours with features of ACT that occur at sites where complete excision is difficult, such as the axial skeleton and pelvis, are referred to as CS grade (G) 1; these lesions often recur locally and are associated with transformation to higher tumour grade, with many patients eventually succumbing to their disease [4, 5]. G2 disease represents approximately 40% of central CS, and has a 5-year survival of approximately 70–99%, whereas G3 disease, comprising about 10% of all central CS, has a 5-year survival of approximately 30–77% [4, 5]. The most aggressive form of the disease is the DD subtype, which arises on the background of about 10% of all conventional central chondrosarcomas and has a 5-year survival of 7–24% [2, 4, 5]. In contrast high grade disease of the phalanges is uncommon and has little metastatic potential; DD CS rarely occurs at this site [6].

The cytosolic isocitrate dehydrogenase type 1 (IDH1) and mitochondrial isocitrate dehydrogenase type 2 (IDH2) enzymes are key components in the tricarboxylic acid cycle. Specific alterations at the R132 and R172 amino acid residues of these genes respectively occur in CS (amongst other cancers), disrupting normal functions and leading to the accumulation of 2-D-hydroxyglutarate (2HG), a competitive inhibitor of alpha KG-dependent dioxygenases. This results in downstream effects, including hypermethylation of CpG islands [7].

Close to 70% of central CS harbour an IDH1 (60%) or an IDH2 (10%) mutation [8], and these are considered key initiators of disease [8–11]. No recurrent initiating genetic drivers have been reported in the remaining 30% of IDH1/2 wild type (IDHwt) cases [12, 13], although these tumours have been reported to exhibit different methylation profiles compared to IDH1 and IDH2-mutant tumours [14, 15], hereafter referred to as IDH1 and IDH2 tumours. Other key drivers include mutations in COL2A1, CDKN2A/B, and TP53 [12, 13, 16, 17] as well as less common pathogenic alterations in cell cycle-related genes such as RB1 [18] and CDK4/6 [12, 19], alterations in the Indian Hedgehog pathway, and amplifications of MYC [12, 13]. Alterations in CDKN2A and TP53 are more likely to occur in high grade disease (G2, G3, and DD CS) [12, 17, 19]. These alterations have limited value as markers for survival or risk stratification.

Previously, near haploid karyotypes have been reported in CS [20–23], although the relationship with other mutations has not been reported.

The recently identified alterations in the TERT promoter locus (C228T) are thought to result in increased telomerase expression leading to immortalisation. These mutations rarely occur in well differentiated tumours, meaning they are a reliable prognostic marker for CS [24]. In contrast, the impact of IDH1 and IDH2 mutations on survival remains unclear [9, 25, 26] possibly reflecting the relatively small number of cases studied.

The aim of this study was to undertake a comprehensive analysis of a large set of CS genomes, leveraging data from the Genomics England 100,000 Genomes Project [27] combined with digital droplet PCR (ddPCR) and methylation profiling. A particular focus of our efforts was to identify mutations of relevance to patient outcome and identify recurrent driver mutations in those tumours that are wild type for IDH1 and IDH2 mutations (IDHwt).

Methods
Patients and samples
Three hundred fifty-six cases of CS were included in the study. No enchondromas were included. These included 68 tumour-normal paired samples from four clinical sites (Royal National Orthopaedic Hospital Stanmore, Royal Orthopaedic Hospital Birmingham, Nottingham NHS Trust, Queen Elizabeth Hospital Birmingham) that were subjected to whole genome sequencing (WGS) as part of the Genomics England 100,000 Genomes Project (hereby referred to as the 100KGP cohort, Additional file 1: Supplementary Table 1-2). The remaining cases were obtained from the archives of the Royal National Orthopaedic Hospital, Stanmore (Additional file 1: Supplementary Table 3). The pathology was reviewed Adrienne Flanagan, Fernanda Amary, and Roberto Tarabosco. For cases with clinical follow-up, the median surveillance time was 5.6 years (2059 days, range: 5–10,057 days). Nearly all patients received surgery as standard of care;
two died prior to receiving definitive treatment, and 6% (n = 22) of patients received adjuvant treatment in the form of doxorubicin/cisplatin or radiotherapy.

Bioinformatic pre-processing and statistical assessment

Single nucleotide variants (SNVs) and indels were called on WGS data and filtered using a panel of normal (PON) samples via the Genomics England analysis pipeline, which utilises Strelka and other tools [28] (Additional file 2: Supplementary Methods). To quality assess the 100KGP mutation calls, we performed orthogonal verification of hotspot mutations in IDH1 (R132), IDH2 (R172), and TERT (C228T) identified across 64 patients (59 mutations in total) using ddPCR, which yielded a recall rate of 100%. There was one instance (WGS_53) where a mutation was called by ddPCR but not in the WGS data (IDH1 ddPCR, IDHwt WGS, later result used, Additional file 2: Supplementary Methods). Somatic copy number variants were called using Battenberg [29] and structural variants (SVs) were called using Delly (v0.8.5) [30]. Unless otherwise specified, comparisons between groups were performed using Wilcoxon tests for distributions and Fisher exact tests for group counts (i.e. in IDH1/2/WT group comparisons). Survival analysis utilised a Kaplan-Meier standard Cox proportional hazard model.

Identification of driver mutations, genome doubling, partial haploidisation, and analysis of mutational signatures

Driver mutations in SNVs and indels were identified using a combination of known hotspot locations published previously and available in Additional file 2: Supplementary Methods, the SIFT [31] and POLYPHEN [32] tools, plus visual inspection using integrative genomics viewer (IGV) of the IDH1 R132, IDH2 R172, and TERT mutations (Additional file 3: Supplementary Fig. 1, Note 1). Amplification events were designated as copy states of five or in diploid genomes and nine or more in those that are genome doubled. For the purposes of plotting, we classified copy states from Battenberg as either, diploid, trisomy or tetrasomy, copy neutral LOH (cnLOH), and the remaining copy states as ‘other’ (any remaining copy state). Tumours with genome doubling (GD) were identified using a clustering procedure based on the R package Mclust [33]. Cases with more than 50% LOH were marked as exhibiting partial haploidisation. We confirmed the ploidy status in 14 of the 100KGP cases using flow cytometry (Additional file 2: Supplementary Methods, Additional file 3: Supplementary Fig. 2, Note 2). To time the appearance of GD, we used a methodology based on molecular-clock principles [34, 35]. Ninety-six channel single-base substitution (SBS) mutational signatures were extracted from the Strelka-called SNVs using SigProfilerExtractor [36] version 1.1.3 with default parameters.

Methylation data protocol and analysis

We analysed 84 cases using methylation arrays (Additional file 3: Supplementary Tables 4-6, Supplementary Fig. 3). Five hundred nanograms of DNA from frozen tumour samples were bisulphite converted using Zymo EZ DNA methylation Gold kit (Zymo Research Corporation Irvine, CA, USA) and hybridised to the Infinium HumanMethylationEPIC beadchip arrays (Illumina, San Diego, CA). The generated methylation data were analysed using the ChAMP R [37], normalised using BMAIQ, and hierarchical clustering plots were constructed using the ‘pheatmap’ R package [38].

TERT promoter methylation status was determined by the methylation status of the cg11625005 probe as reported previously [39]. Raw DNA methylation data files have been deposited in the ArrayExpress database (https://www.ebi.ac.uk/arrayexpress/experiments/E-MTAB-11031/, accession: E-MTAB-11031).

Statistics and mathematical analysis

In all group comparison situations, such as IDH1, IDH2, and IDH-WT cases, with or without TERT mutations, we used Fisher test statistics as implemented in R (testing both 3x2 and 2x2 contingency tables). Distributions of data, as seen in the tests of timing for GD was performed using Wilcoxon tests, again implemented in R. Linear regressions were also implemented using the standard R methods. The cox proportional hazard model and Kaplan-Meyer, were obtained via the survminer package [40]. For the chromosome arm frequency comparisons, we used Fisher tests and the Bonferroni multiple testing correction. For power calculations, please see Additional file 2: Supplementary Methods.

Results

Driver mutations in central conventional and dedifferentiated chondrosarcomas

Profiling a total of 350 CS cases for IDH1 and IDH2, using ddPCR (n = 282) and WGS (n = 68), we verified previous findings that IDH2 are less frequent than IDH1 mutations (IDH1: 51%, IDH2: 14%, IDHwt: 35%) [16, 26, 41]. In cases with grading information (n = 343), we found that IDH1 mutations were equally frequent across grades (ACT/G1: 51%, G2/3: 53%, DD CS: 55%; p = 0.9) and that IDH2 mutations were more frequent in higher grade disease (ACT/G1: 7%, G2/3: 14%, DD: 25%; p = 0.005). IDHwt was negatively associated with increasing grade (%IDHwt; ACT/G1: 41%, G2/3: 32%, DD CS: 19%; p = 0.01). These data imply that the progression to DD
CS is more common in tumours with IDH2 mutations and least common in IDHwt tumours.

Canonical mutations and structural changes near the TERT promoter have been reported in approximately 20% of CS and found to correlate with high grade disease [24, 26, 42]. We found a similar frequency in our cohort (23%, 74 with C228T, one with C250T, four with structural changes near the TERT promoter (Additional file 3: Supplementary Fig. 1). TERT mutations were rare in well differentiated tumours and increased in frequency across grades (ACT/G1: 3%, G2/3: 22%, DD: 56%; p = 2e-14, Fig. 1A). We found that the TERT promoter was hypermethylated in 19/57 (33%) of cases analysed on methylation arrays, excluding DD CS cases (Fig. 1B). Seven of these cases, all high grade, also harboured TERT C228T promoter mutations. There was no significant difference between the number of cases with both TERT promoter mutations and hypermethylation across IDH1 and IDH2 tumours (Fig. 1B). Rare alterations involving ATRX have been reported previously [26], but in the 100KGP cohort (n = 68), we found no such alterations in this cohort. These data confirm that TERT mutations, and possibly methylation, have distinct roles in CS progression.

We next examined mutations in other key driver genes reported in CS, utilising the 100KGP cohort (Fig. 1A). Our findings were largely similar to those previously reported [13, 43]. Monosomies of 17p and pathogenic SNVs/indels in TP53 were found in 22% of cases in line with previous reports [12]. TP53 mutations were absent from all but one well-differentiated tumour. COL2A1 mutations were common and marginally anti-correlated with increasing grade (ACT/G1: 100%, G2/3: 54%, DD CS: 44%; p = 0.03). As previously reported [17, 19, 21], pathogenic SNVs/indels and/or bi-allelic deletions of CDKN2A and CDKN2B were common in CS and enriched in G2/3 and DD CS, though not significantly in this dataset. Hypermethylation of these genes was not detected. CDK4 and CDK6 gains were found in 12 cases, and a single case had a pathogenic SNV in CDK6. These frequencies are similar to previous reports [12]. MYC amplifications were found in five high-grade tumours establishing its status as a driver of CS [44, 45]. MDM2 alterations were identified in three high grade IDHwt cases, two of which were amplifications (one 8 copies, one 31 copies, confirmed using fluorescence in situ hybridisation), and one was a structural alteration involving intron 7 of MDM2 and an intragenic region on chr4q28.3. The latter did not result in amplification of MDM2 but removal of the zinc finger binding domains, which has been suggested to have an oncogenic effect [46]. All three mutations were mutually exclusive of TP53 mutations. These data support the premise that MDM2 is a potential driver gene in CS [26], though any biological effects require further exploration. Homozygous deletions of PTEN were present in three high grade cases. PTEN promoter hypermethylation was found in 13/57 cases, all high grade. Analysis utilising dNdS [47] returned no previously unknown drivers, implying that all prominent somatically mutated genes driving CS have likely been identified (Additional file 2: Supplementary Methods, Additional file 3: Supplementary Fig. 2).

**IDH1, IDH2, and TERT define key genetic subgroups**

Analysis of all mutation calls (n = 350) revealed that the frequency of TERT mutations was different across IDH1, IDH2, and IDHwt cases (Fig. 1C). IDH2 mutations were strongly associated with TERT mutations (IDHwt: 5%, IDH1: 24%, IDH2: 58%, p = 6e-13; IDH1 vs IDH2: p = 1e-5). This association was observed in G2/3 (IDH1 vs IDH2: p = 7e-6) but not in DD CS (IDH1 vs IDH2: p > 0.99), implying that although TERT is associated with high-grade tumours, this is not equal in the context of IDH mutation status.

**Hypermethylation across IDH1- and IDH2-mutated tumours**

CpG island DNA hypermethylation has been reported to distinguish between cartilaginous IDH and IDHwt tumours [14, 15]. However, utilising the larger numbers available in this study, we found 3468 differentially methylated probes (DMPs) across IDH1 and IDH2 tumours, excluding DD CS (n = 31, p = 0.002, Additional file 3: Supplementary Fig. 2, Supplementary Tables 4-6). The overall methylation level across all probes also revealed significant differences between IDH1 and IDH2 tumours (p = 0.002) indicating that the former are globally hypermethylated compared to IDH2 and IDHwt tumours.

(See figure on next page.)
Fig. 1 (See legend on previous page.)
Partial haploidisation followed by genome doubling is common in IDHwt tumours

We compared the mutational profiles across each IDH group (complete summary of 100KGP data shown in Fig. 1D) and found that the frequency of common drivers, excluding TERT, was similar across IDH1 and IDH2 and IDHwt tumours. We did not find that mutations in CDKN2A/B and TPS3 were enriched in IDH1/2 cases, as previously reported [48] but contrasting another study [26]. The total number of SVs was not statistically different across the IDH groups nor was the number of SVs that fell into gene regions. We did not find any common structural variants affecting the same gene more than 25% of cases, although none of these were cancer-related genes (Additional file 2: Supplementary Methods).

The genetic alterations initiating development of IDHwt CS remains unknown, but previous reports of near-haploid (HP) and hyperhaploidy in CS and in other sarcoma subtypes including undifferentiated sarcomas and malignant peripheral nerve sheath tumours prompted us to investigate this [21, 22, 49, 50]. We found 23 tumours with GD and seven with HP in the 100KGP cohort (n=68, Additional file 3: Supplementary Fig. 4, Additional file 2: Supplementary Methods). Most GD events (16/23, 69%) occurred in the absence of HP, whereas HP always occurred with GD (Fig. 1D). GD was highly enriched in IDHwt tumours (GD%, IDH1: 24%, IDH2: 9%, IDHwt: 63%, IDHwt vs IDH1 p = 0.0005, Fig. 1E), and HP was exclusive to this group (HP%, IDH1: 0%, IDH2: 0%, IDHwt: 37%, IDHwt vs IDH1 p = 8e-5, Fig. 1E). Timing analysis demonstrated that GD events tended to occur at a similar relative time in IDHwt and IDH1 cases implying that it could be an intermediate or late event in evolutionary timelines of both tumour groups (Fig. 1E, Additional file 2: Supplementary Methods). The six cases of IDHwt tumours without HP/GD events, harboured mutations in TPS3 and CDKN2A, although alterations in these genes were not mutually exclusive with the absence of GD and HP (TPS3: 3/6, 50%, CDKN2A/B: 5/6, 83%, Fig. 1D). One of these cases was ACT/G1, pointing to a possible initiating role of TPS3 and CDKN2A in some IDHwt tumours.

Mutational signatures across IDH1, IDH2, and IDHwt groups

Analysis of mutational signatures in the 100KGP cohort (n = 52, Fig. 1F, Additional file 3: Supplementary Fig. 5) revealed nine active signals, with SBS1, SBS5, and SBS8 being ubiquitous and most prominent across IDH1, IDH2, and IDHwt tumours. Five signatures (SBS2, SBS12, SBS13, and SBS17a/b) were principally exclusive to IDHwt tumours. SBS2 and SBS13 have been associated with APOBEC and were simultaneously active in five IDHwt cases (18%). We did not observe any difference in SNV burden in tumours with active SBS2 and SBS13. SBS12 was found in one IDH1 case and three IDHwt cases. SBS17a/b, signatures with unknown aetiology, were found only in IDHwt cases. SBS40, also of unknown aetiology, was found in 28% of IDH1 cases, 25% of IDH2 cases, and 81% of IDHwt. These data demonstrate that IDH1 and IDH2 tumours are comparable in terms of mutational signatures, whereas IDHwt tumours exhibit more heterogeneous mutational processes.

The genetic distinction between central conventional and dedifferentiated chondrosarcoma

We next analysed the DD CS for specific alterations that may explain their histological phenotype and their poor clinical outcomes. We confirmed that metastatic disease was most common in DD CS (60%, compared to 27% in G2/3 and <1% in ACT/G1 (G2/3 vs DD: p = 1e−5). Analysing the 100KGP data (DD: n = 16, G2/3: n = 41), the frequency of identified known drivers in DD CS and G2/3 revealed no difference except for IDH2 and TERT, which were enriched in DD (IDH2: p = 0.05, TERT: p = 3e−6). However, we found differences in total driver burden (p = 2e−8), SNV burden (p = 0.009), number of chromosome segments (p = 0.01), and SV burden (p = 0.01) (Additional file 3: Supplementary Fig. 6). We next explored whether the increased segment counts were attributable to chromothripsis. Using a previously published method [51], we found only one instance of chromothripsis (WGS_21) which overlapped with the SV identified at the TERT loci (Fig. 1D, Additional file 3: Supplementary Fig. 1). Examining the number more broadly, the average number of chromosomes with high breakage was higher in DD CS compared to G2/3 (median, G2/3: 0, DD CS: 2.5, p = 0.03, see Additional file 2: Supplementary Methods). There were no specific chromosome arms enriched amongst those with high fragmentation, although three cases (19%) had fragmentation across chromosome 12q, which has also been reported in dedifferentiated liposarcoma [52]. Previous studies have reported that aberrations of chromosome 5q and trisomy of chromosome 19 distinguish G2/3 from DD CS [53]. Twenty-five percent DD CS harboured 19p/q gains which is less than the 50% previously reported [53]. Examining losses and gains across all chromosome arms revealed no events unique to DD CS although losses at 15q were more common in this subtype (15q loss, G2/3: 10%, DD CS: 38%, p = 0.05). Together, these analyses suggest that the primary genetic difference between G2/3 and DD CS is the number of accrued SNVs and the degree of chromosomal instability.
Age at diagnosis as a clinical factor in chondrosarcoma

Previous studies of CS have treated IDH1 and IDH2 tumours as one group [14, 54]. Our results, leveraging hundreds of cases, provide evidence that IDH1 and IDH2 mutations lead to distinct downstream genetic events, with differences in the frequency of TERT mutations, GD/HP, methylation profiles, and the number and types of mutational signatures.

We examined the effect of the presence or absence of IDH1 and IDH2 mutations on the clinical behaviour of CS (n = 339, Fig. 2). We showed that patient age at diagnosis increased across grades in these groups and that the median age was highest in those with IDH2 tumours (IDH1: 55 year, IDH2: 67 year, IDHwt: 47 year, IDH1 vs IDH2: p = 0.003, IDH1 vs IDHwt: p = 0.0006, Fig. 2A). The age at diagnosis for each IDH group was similar for ACT/G1 and DD CS, and the difference in age of the G2/3 tumours explained the overall difference in ages (median age G2/3, IDH1: 60 year, IDH2: 71 year, IDHwt: 44 year, IDH1 vs IDH2: p = 0.04, IDH1 vs IDHwt: p = 2e−6, Fig. 2B). We considered whether these differences in chronological age at diagnosis were reflected
in the mutational signatures active in each group. The total SNV burden correlated with age at diagnosis, as did SBS5, previously been reported as clock-like [55], SBS8, but not SBS1. In G2/3 tumours, the activity of SBS5 was similar in IDH1 and IDH2, but lower in IDHwt (IDH1 vs IDH2 p = 0.4, IDH1 vs IDHwt: p = 0.03, Fig. 2C). By contrast, SBS5 activity was similar in all DD cases (IDH1 vs IDH2 p = 0.7, IDH1 vs IDHwt: p = 0.8, Fig. 2C). These same results were recapitulated when using SBS8 and total SNV burden (Additional file 3: Supplementary Fig. 7). Together, these data imply further differences in the rate of evolution from G2/3 to DD CS across IDH1, IDH2, and IDHwt tumours.

Divergent outcomes in IDH1, IDH2 and IDHwt tumours
Using all available clinical information (n = 342), we found that IDH2 tumours tended to be larger at time of presentation (IDH1 vs IDH2: p = 0.001, IDH1 vs IDHwt: p = 0.4, Fig. 3A), supporting the premise that these tumours evolve over longer time periods, and present in older people. Development in specific anatomical locations was not significantly different (Fig. 3A).

Using all cases with available follow-up data (n = 328), a Cox proportional hazard model demonstrated that ACT/G1 tumours nearly always had a good outcome with no metastatic events being recorded and only one of 98 patients, with a pelvic tumour, succumbing to disease. No patients with tumours in the small bones of the hands and feet died of disease (Fig. 3B). We found that DD CS had a higher frequency of metastatic disease compared with G2/3 disease (G2/3 vs DD CS, p = 9e−7). There were no significant differences in the frequency of metastatic or recurrent disease across IDH1, IDH2, and IDHwt DD CS tumours. However, metastases or recurrent disease appeared to occur less frequently in patients with IDH2 G2/3 tumours compared to IDH1 and IDHwt tumours (% metastases/recurrence, IDH1: 37%, IDH2: 13%, IDHwt: 23%, IDH1 vs IDH2: p = 0.04, Fig. 3B). We also found that the time interval between diagnosis and detection of metastatic disease six months following presentation of the primary tumour was shorter in IDH2 compared to IDH1 (p = 0.04, Fig. 3B). Finally, we found no differences in outcome related to the different IDH1 mutation contexts (R132C/G/H/L/S) but noted that R132S/L variants found in only a minority number of cases, making statistical analysis difficult (Additional file 3: Supplementary Table 3, Supplementary Fig. 8).

Canonical TERT promoter mutations (g.1295113) had an independent hazard ratio (HR) that was equal to that of grade (TERT: HR = 2.2, p = 0.003, tumour grade: HR = 2.2, p = 2e−13, Additional file 3: Supplementary Fig. 8), pointing to the benefit of TERT as a biomarker. We also found that overall outcomes were worse in patients whose tumours harboured both IDH1 and TERT mutations had significantly worse outcomes than those with an IDH1 mutation alone. TERT mutations had no effect on outcome in patients with IDH2 tumours, even though these mutations are found more frequently in combination with IDH2 mutations (Fig. 3C, Additional file 3: Supplementary Methods). This suggests that TERT mutations are context specific and only relevant to outcome predictions in IDH1 tumours.

Given these findings, identification of tumours with IDH1 and TERT mutations has clinical value.

Discussion
In this study of patients with CS, involving targeted, whole genome, and methylation data, we provide significant insights into the genetic pathways and dynamics underlying disease progression. Here, with the benefit of large sample numbers, we have been able to study tumours with IDH1 and IDH2 mutations independently and shown that they represent distinct genetic and clinical groups.

In addition to confirming that IDH2 tumours represent the minority group with 14% of cases, we report that they are highly represented in DD CS. They also present as larger tumours and on average over a decade later than IDH1 tumours. Despite this, IDH1 and IDH2 tumours have similar molecular ages suggesting that on average, tumours with IDH2 mutations have slower cell division rates. Therefore, we speculate that many of these tumours go into growth arrest and become calcified, representing at least a proportion of calcified enchondromas, a lesion commonly detected, when medical imaging is undertaken for unrelated symptoms. This would account for the comparatively lower frequency of IDH2 tumours. Furthermore, the high incidence of TERT mutations and TERT promoter methylation in high grade IDH2 tumours, suggests that these events, through activation of telomerase, have prevented the senescent phenotype and bring about high-grade IDH2 tumours. This finding could potentially account for the presentation at the relatively late age of these tumours.

No significant differences in the type or number of mutations were identified that accounted for the different clinical findings associated with IDH1 and IDH2. However, we show that IDH1 tumours are globally more methylated at CpG islands compared to both the IDH2 and IDHwt tumours. The small numbers of cases studied to date is likely to account for this being unrecognised previously [14, 15]. Indeed, even IDH1 and IDH2 gliomas,
Fig. 3 Outcomes in chondrosarcoma. A Tumour size of IDH1, IDH2, and IDHwt CS are different at presentation and their anatomical location are largely comparable, although IDHwt tumours develop more frequently in the chest wall and spine (left and middle, and right, respectively). B Kaplan-Meier analysis and hazard ratios (HR) from Cox proportional hazard analysis confirms tumour grade, and anatomical location, as predictors of outcome. The frequency of metastatic/recurrent disease is significantly lower in IDH2 G2/3 disease compared to IDH1 G2/3 disease but is comparable in DD CS. The time interval between diagnosis and discovery of metastatic disease is shortest in IDH2-driven tumours and on average longest in IDHwt (while also being less frequent). Median time in days given right of plot. C TERT mutations are linked to poor outcome, as is methylation of TERT (left plots). In high-grade (G2/3 and DD CS) IDH1 tumours, TERT mutations associate with a poor outcome, but not in IDH2 tumours.
which are considerably more common than CS, are generally studied together because of their small numbers. Nevertheless, although all IDH mutations result in accumulation of 2-HG there is growing evidence that the impact of the different mutations exerts different biological effects. Studies utilising human oligodendroglioma cell lines have shown that the IDH1 R132 mutation leads to higher enzymatic activity than that brought about by IDH2 R172 [56]. Other studies of IDH1 and IDH2 mutations in gliomas point to them as having distinct functional and clinical patterns [57]. Furthermore, different biological effects of 2HG are also seen as a consequence of different IDH2 mutations [58]. As it is known that 2HG exerts diverse biological functions including regulation of DNA hydroxymethylation, it is feasible that the IDH1 and IDH2 mutations explain our different methylation array findings and mediate the different behaviour of the tumour subgroups. However, further research is required to establish this.

The limitation of the study remains the small numbers of cases when broken down by grade, particularly grade 3. Hence, the need to build prospective collaborative studies with detailed clinical outcome over a long period. Previous studies have suggested different effects of IDH mutations on clinical outcome in patients with CS [9, 25, 26]. Here, with the benefit of a large patient cohort, we show that although IDH2 tumours are more commonly associated with TERT mutations, only IDH1 mutations in combination with TERT mutations are associated with significantly reduced survival.

Conclusions
The underlying mutational pathways of tumours with IDH1 or IDH2 mutations, or those that are IDHwt, differ significantly. Based on the finding that TERT leads to different outcomes in IDH1- and IDH2-mutant tumours, we propose that genetic testing for IDH1, IDH2, and TERT promoter mutations in the context of other clinical factors, could be useful in patient stratification.

Supplementary Information
The online version contains supplementary material available at https://doi.org/10.1186/s13073-022-01084-0.

Additional file 1: Supplementary Tables 1-6.
Additional file 2: Supplementary Methods.
Additional file 3: Supplementary Figs. 1-8, Notes 1-3.

Acknowledgements
We are grateful to the Biobank Team at the RNOH and RIAH, and all healthcare workers who cared for the patients. We specifically wish to thank the patients for engaging in our research.
We acknowledge the work of the Genomics England research consortium: John Ambrose1, Prabhu Arumugam1, Roel Bevers1, Marta Bleda1, Freya Boardman-Pretty1,2, Christopher Boustred1, Helen Brittain1, Mark Caufield1,2, Georgia Chan1, Greg Elgar1,2, Tom Fowler1, Adam Giess1, Angela Hamblin1, Shirley Henderson1,2, Tim Hubbard1, Rob Jackson1, Louise Jones1,2, Dalia Kasperaviciute1,2, Melis Kayikci1, Athanasios Kousathanas1, Lea Lahnstein1, Sarah Leigh1, Iovone Leong1, Javier Lopez1, Fiona Maleady-Crowe1, Meriel McEntagart1, Federico Minneci1, Loukas Moutsianas1,2, Michael Mueller1,2, Nirupa Murugaesu1, Anna Need1,2, Peter O’Donovan1, Chris Oldhams1, Christine Patch1,2, Mariana Buongermino Pereira1, Daniel Perez-Gil1, John Pullinger1, Tahrima Rahim1, Augusto Rendon1, Tim Rogers1, Kevin Savage1, Kushmita Sawant1, Richard Scott1, Afshan Siddiq1, Alexander Sieghart1, Samuel Smith1, Alona Sosinski1,2, Alexander Stuckey1, Mélanie Tanguy1, Ana Lisa Taylor Tavares1, Ellen Thomas1,2, Simon Thompson1, Arianna Tucci1,2, Matthew Welland1, Eleanor Williams1, Katarzyna Witkowska1,2, Suzanne Wood1,2.

1. Genomics England, London, UK
2. William Harvey Research Institute, Queen Mary University of London, London, E14 8QH, UK.

Authors’ contributions
Conceptualization AMF. Data curation WC, SH, AS, RT, AM, CD, SW, Genomics England. Investigation WC, IL, TB, CS, DO, HY, TL, LA, SH, SH-F, GB, DB, PC, NP, PVL, AMF. Writing WC, IL, PC, PVL, AMF. All authors read and approved the final manuscript.

Funding
Funding for this study was received from Sarcoma UK (SUKG01.18) and the Bone Cancer Research Trust infrastructure grants to AMF and PC, RNOH NHS R&D grant (AMF). IL is supported by the Lundbeck Foundation (grant: R303-2018-3018). The project was also supported by the National Institute for Health Research, UCLH Biomedical Research Centre, the UCL Experimental Cancer Centre, and the Francis Crick Institute, which receives its core funding from Cancer Research UK (FC001202), the UK Medical Research Council (FC001202), and the Wellcome Trust (FC001202). For the purpose of access, the authors have applied a CC BY public copyright licence to any author accepted manuscript version arising from this submission. PVL is a Win‑ton Group Leader in recognition of the Winton Charitable Foundation’s support towards the establishment of The Francis Crick Institute. We are grateful to the Biobank Team at the RNOH and RIAH, and all healthcare workers who cared for the patients. We specifically wish to thank the patients for engaging in our research. Open Access funding enabled and organized by Projekt DEAL.

Availability of data and materials
All ddPCR results are included in Supplementary Table 3. The SNV, indel, and structural calls produced from the WGS data are available in Additional File 1: Supplementary Table 2. The bam and vcf files of the WGS data are covered by the Genomics England participant consent policy, which has a legally obligatory to protect personal identity. Raw data available on application for access to Genomics England and the Clinical Interpretation Partnership (GeCIP) and reviewed by the Access Review Committee. Please see https://www.genomicsengland.co.uk/patients-participants/data for further information. The raw methylation array files are available via the ArrayExpress database (https://www.ebi.ac.uk/arrayexpress/experiments/E-MTAB-11031/, accession: E-MTAB-11031). Code is available via the Github page: https://doi.org/10.5281/zenodo.6800685.

Declarations
Ethics approval and consent to participate
Informed consent was obtained for all patients either as part of the Genomics England 100,000 Genomes Project (WGS data), as determined by the Genomics England Ethics Advisory Committee, or under the generic biobank consent at the National Research Ethics Committee. The project was also approved by the Royal National Orthopaedic Hospital. The National Research Ethics Committee (RNOH) Biobank is a satellite of the UCL/UCLH Biobank for Health and Disease, which is approved by the National Research Ethics Committee of the Health Research Committee (reference: Integrated Research Application System (IRAS) project identifier: 272816). This project (EC17.14) was approved by the National Research Ethics Committees UCL/UCLH Biobank Ethics Committee. The ethical principles of the Helsinki Declaration for medical research are included as core principles.
Consent for publication
Not applicable

Competing interests
The authors declare that they have no competing interests.

Author details
1 Research Department of Pathology, University College London, UCL Cancer Institute, London, UK. 2 Medical Genomics Research Group, University College London, UCL Cancer Institute, London, UK. 3 The Francis Crick Institute, London, UK. 4 Department of Histopathology, Royal National Orthopaedic Hospital, Stanmore, UK. 5 Cancer Sciences Unit, University of Southampton, Southampton, UK. 6 Bone Tumour Unit, Royal National Orthopaedic Hospital, Stanmore, UK. 7 Institute of Cancer and Genomic Sciences, Birmingham University, Birmingham, UK. 8 University of California, San Diego, USA. 9 Genomics England Limited, London, UK. 10 Robert Jones & Agnes Hunt Orthopaedic Hospital NHS Foundation Trust, Oswestry, UK. 11 Keele University, Keele, UK.

Received: 2 November 2021 Accepted: 7 July 2022 Published online: 30 August 2022

References
1. Griffyra AY, Burgueno JE, Koniaris LG, Gutierrez JC, Duncan R, Scully SP. Chondrosarcoma in the United States (1973 to 2003): an analysis of 2890 cases from the SEER database. J Bone Joint Surg Am. 2009;91(5):1063–72.
2. Bovee J, Flanagan AM, Nielsen G, Akihiko Y, Bloem J. The WHO Classification of Tumours: Soft Tissue and Bone Tumours. IARC Press. 2020.
3. Eefting D, Schrage YM, Geijsneer MJA, Le Cessie S, Taminiau AHA, Bovee JVMG, et al. Assessment of interobserver variability and histologic parameters to improve reliability in classification and grading of central cartilaginous tumors. Am J Surg Pathol. 2009;33(1):50–7.
4. Evans HL, Ayala AG, Romsdahl MM. Prognostic factors in chondrosarcoma of bone: a clinicopathologic analysis with emphasis on histologic grading. Cancer. 1977;40(2):819–31.
5. Fromm J, Klein A, Baur-Melnyk A, Knösel T, Lindner L, Birkenmaier C, et al. Survival and prognostic factors in conventional central chondrosarcoma. BMC Cancer. 2018;18(1):849.
6. Bovee JV, van der Heul RO, Taminiau AH, Hogendoorn PC. Chondrosarcoma of the phalanx: a locally aggressive lesion with minimal metastatic potential: a report of 35 cases and a review of the literature. Cancer. 1999;86(9):1724–32.
7. Nacev BA, Jones KB, Intlekofer AM, Yu JSE, Allis CD, Tap WD, et al. The epigenomics of sarcoma. Nat Rev Cancer. 2019;19(10):638–51.
8. Mandahl N, Johansson B, Merters F, Mittelman F. Disease-associated patterns of disomic chromosomes in hyperhaploid neoplasms. Genes Chromosomes Cancer. 2012 Jun;51(6):536–44.
9. Olsson L, Paulsson K, Bovee JVMG, Nord KH. Clonal evolution through loss of chromosomes and subsequent polyploidization in chondrosarcoma. Plos One. 2011;6(9):e24977.
10. Lin Y, Seger N, Chen Y, Hesla AC, Wejde J, Ghaderi M, et al. hTERT promoter mutations in chondrosarcomas associate with progression and disease-related mortality. Mod Pathol Off J U S Can Acad Pathol Inc. 2018;31(12):1834–41.
11. Bovée JV, van Royen M, Bardelo AF, Rosenberg C, Cornelisse CJ, Clifton-Jansen AM, et al. Near-haploidy and subsequent polyploidization characterize the progression of peripheral chondrosarcoma. Am J Pathol. 2000;157(5):1587–95.
12. Hallor KH, Staal J, Bovée JVMG, Hogendoorn PCW, Clifton-Jansen A-M, Knutslijt S, et al. Genomic profiling of chondrosarcoma: chromosomal patterns in central and peripheral tumors. Clin Cancer Res Off J Am Assoc Cancer Res. 2009;15(8):2685–94.
13. Totoki Y, Yoshida A, Hosoda F, Nakamura H, Hama N, Ogura K, et al. Unique mutation portraits and frequent COL2A1 gene alteration in chondrosarcoma. Nat Genet. 2013;45(8):923–6.
14. Prendergast SC, Strobb AC-C, Cross W, Pillay N, Strauss SJ, Ye H, et al. Sarcoma and the 100,000 Genomes Project: our experience and changes to practice. J Pathol Clin Res. 2020;6(4):297–307.
15. Irvin-Brown T, Siterycz P, Mikula M, Kulecka M, Kulska A, Balabas A, et al. IDH1/2 mutations predict shorter survival in chondrosarcoma. J Cancer. 2018;9(6):998–1005.
16. Zhu GG, Nafa K, Agaram N, Zehir A, Benayed R, Sadowska J, et al. Genomic profiling association of IDH1/IDH2 mutation with longer relapse-free and metastasis-free survival in high-grade chondrosarcoma. Clin Cancer Res Off J Am Assoc Cancer Res. 2020;26(2):419–27.
17. Rausch T, Zichner T, Schlatt A, Stutz AM, Benes V, Korbel JO. DELLY: structural variants on protein function using the SIFT algorithm. Nat Protoc. 2009;4(7):1073–81.
18. Rammohan V, Bork P, Sunyaev S. Human non-synonymous SNPs: server and survey. Nucleic Acids Res. 2002;30(17):3894–900.
19. Scrucca L, Fop M, Murphy TB, Raftery AE. mclust 5: clustering, classification and survey. Nucleic Acids Res. 2017;45(12):905–10.
20. Kumar P, Henikoff S, Ng PC. Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm. Nat Protoc. 2009;4(7):1073–81.
21. Rausch T, Zichner T, Schlatt A, Stutz AM, Benes V, Korbel JO. DELLY: structural variant discovery by integrated paired-end and split-read analysis. Bioinforma Oxf Engl. 2012;28(18):i333–9.
22. Mandahl N, Johansson B, Merters F, Mittelman F. Disease-associated patterns of disomic chromosomes in hyperhaploid neoplasms. Genes Chromosomes Cancer. 2012 Jun;51(6):536–44.
23. Olsson L, Paulsson K, Bovee JVMG, Nord KH. Clonal evolution through loss of chromosomes and subsequent polyploidization in chondrosarcoma. Plos One. 2011;6(9):e24977.
24. Lin Y, Seger N, Chen Y, Hesla AC, Wejde J, Ghaderi M, et al. hTERT promoter mutations in chondrosarcomas associate with progression and disease-related mortality. Mod Pathol Off J U S Can Acad Pathol Inc. 2018;31(12):1834–41.
25. Rausch T, Zichner T, Schlatt A, Stutz AM, Benes V, Korbel JO. DELLY: structural variant discovery by integrated paired-end and split-read analysis. Bioinforma Oxf Engl. 2012;28(18):i333–9.
26. Scrucca L, Fop M, Murphy TB, Raftery AE. mclust 5: clustering, classification and survey. Nucleic Acids Res. 2017;45(12):905–10.
37. Morris TJ, Butcher LM, Feber A, Teschendorff AE, Chakravarty AR, Woj‑
andaz TK, et al. CHAMP: 450k chip analysis methylation pipeline. Bioinforma‑
Oxf Engl. 2014;30(3):428–30.
38. Kolda R. phemaps: Pretty heatmaps [Software]. URL. https://CRAN.R‑Proj‑
packag e=phemaps. 2015.
39. Lee DD, Leoš R, Komosa M, Gally M, Zhang CH, Lipman T, et al. DNA hy‑
permethylation within TERT promoter upregulates TERT expression in can‑
cer. J Clin Invest. 129(4):1801. 
40. Kassambara A, Kosinski M, Beecek P, Fabian S. Survminer: drawing survival
curves using ggplot2. 2021. Available from: https://CRAN.R‑projec‑
tor=package=survminer. (Cited 2021 Sep 15) 
41. Pansuriya TC, Oosting J, Vergeer SHM, Flanagan AM, Sciot R, Kindblom
L‑G, et al. Maffucci syndrome: a genome‑wide analysis using high resolu‑
tion single nucleotide polymorphism and expression arrays on four cases.
Genes Chromosomes Cancer. 2011;50(9):673–9. 
42. Zhang Y, Chen Y, Yang C, Seger N, Hesla AC, Tsagkozis P, et al. TERT pro‑
moter mutation is an objective clinical marker for disease progression in
chondrosarcoma. Mod Pathol. 2021;9:1–8. 
43. Tarpey PS, Behjati S, Young MD, Martincorenna I, Alexandrov LB, Farndon
Si, et al. The driver landscape of sporadic chordoma. Nat Commun.
2017;8 Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/
PMC5638846/. (Cited 2019 Sep 29). 
44. Castelesana JS, Barrios C, Gómez L, Kreicbergs A. Amplification of the
c‑myc proto‑oncogene in human chondrosarcoma. Diagn Mol Pathol Am
J Surg Pathol Part B. 1992;14:235–8. 
45. Morrison C, Radmacher M, Mohammed N, Suster D, Auer H, Jones S,
et al. MYC amplification and polysomy 8 in chondrosarcoma: array com‑
parative genomic hybridization, fluorescent in situ hybridization, and
association with outcome. J Clin Oncol Off J Am Soc Clin Oncol.
2005;23(36):9369–76. 
46. Lindström MS, Jin A, Deisenroth C, White Wolf G, Zhang Y. Cancer‑
associated mutations in the MDM2 zinc finger domain disrupt ribosomal
protein interaction and attenuate MDM2‑induced p53 degradation. Mol
Cell Biol. 2007;27(3):1056–68. 
47. Martincorenna I, Raine KM, Genstung M, Dawson KJ, Haase K, Van Loo P,
et al. Universal patterns of selection in cancer and somatic tissues. Cell.
2017;171(3):1029–1041 e21. 
48. Lucas C‑HG, Grenert JP, Horvai A. Targeted next‑generation sequencing
identifies molecular and genetic events in dedifferentiated chondrosar‑
coma. Arch Pathol Lab Med. 2021;145(8):1009–17. 
49. Lysykjaer I, Lindsay D, Tirabosco R, Steele CD, Lombard P, Strobl A‑C, et al.
H3K27me3 expression and methylation status in histological variants of
malignant peripheral nerve sheath tumours. J Pathol. 2020;252(2):151–64. 
50. Steele CD, Tarabichi M, Oukrif D, Webster AP, Ye H, Fittall M, et al. Undif‑
ferentiated sarcomas develop through distinct evolutionary pathways.
Cancer Cell. 2019;35(3):441–456.e8. 
51. O'Malley DP, Opheim KE, Barry TS, Chapman DB, Emond MJ, Conrad EU,
et al. Chromosomal changes in a dedifferentiated chondrosarcoma:
a case report and review of the literature. Cancer Genet Cytogenet.
2005;156(4):105–11. 
52. Pathmanapan S, Illayeva O, Martin JT, Loi AKH, Zhang H, Zhang G‑F, et al.
Mutant IDH and non‑mutant chondrosarcomas display distinct cellular
metabolomes. Cancer Metab. 2021;9(1):13. 
53. Beird HC, Wu C‑C, Ingram DR, Wang W‑L, Alimohamed A, Gumbs C, et al.
Genomic profiling of dedifferentiated liposarcoma compared to matched
well‑differentiated liposarcoma reveals higher genomic complexity and a
common origin. Mol Case Stud. 2018;4(2):a002386. 
54. O'Malley DP, Opheim KE, Barry TS, Chapman DB, Emond MJ, Conrad EU,
et al. Chromosomal changes in a dedifferentiated chondrosarcoma:
a case report and review of the literature. Cancer Genet Cytogenet.
2005;156(4):105–11. 
55. Porter J, Butcher LM, Feber A, Teschendorff AE, Chakravarty AR, Woj‑
andaz TK, et al. CHAMP: 450k chip analysis methylation pipeline. Bioinforma‑
Oxf Engl. 2014;30(3):428–30.
56. Yan H, Parsons DW, Jin G, McLendon R, Rasheed BA, Yuan W, et al. IDH1
and IDH2 mutations in gliomas. N Engl J Med. 2009;360(8):765–73. 
57. Wang H‑Y, Tang K, Liang T‑Y, Zhang W‑Z, Li J‑Y, Wang W, et al. The com‑
parison of clinical and biological characteristics between IDH1 and IDH2
mutations in gliomas. J Exp Clin Cancer Res. CR. 2016;31(35):86. 
58. Kotredes KP, Razmpour R, Lutteri E, Alfonso‑Prieto M, Ramirez SH, Gamero
AM. Characterization of cancer‑associated IDH2 mutations that differ in
tumorigenicity, chemosensitivity and 2‑hydroxyglutarate production.
Oncotarget. 2019;10(28):2675–92.

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in pub‑
lished maps and institutional affiliations.