An integrated clinical genomic and transcriptomic subgrouping of central chondrosarcoma

Meijer, D.M.; Venneker, S.; Ameline, B.; Erdem, Z.; Cardoso, S.; Ruano, D.; ... ; Bovée, J.V.M.G.

Mod Pathol 38 (2025) 100894

MODERN PATHOLOGY

USCAP UNITED STATES AND CANADIAN ACADEMY OF PATHOLOGY
Advancing Pathology Together

Journal homepage: https://modernpathology.org/

Research Article

An Integrated Clinical Genomic and Transcriptomic Subgrouping of Central Chondrosarcoma

Debora M. Meijer[a,b], Sanne Venneker[a], Baptiste Ameline[c], Zeynep Erdem[a], Sara Cardoso[a,b], Dina Ruano[a], Inge H. Briaire-de Bruijn[a], Brendy E. van den Akker[a], Pauline M. Wijers-Koster[a], Claire H.J. Scholte[d], Veroniek M. van Praag[d], Michiel A.J. van de Sande[d], Marieke L. Kuijjer[a,b,e,f,g], Daniel Baumhoer[c,h], Noel F.C.C. de Miranda[a,b], Judith V.M.G. Bovée[a,b,*]

[a] *Department of Pathology, Leiden University Medical Center, Leiden, The Netherlands;* [b] *Leiden Center for Computational Oncology, Leiden University Medical Center, Leiden, The Netherlands;* [c] *Bone Tumor Reference Center at the Institute of Medical Genetics and Pathology, University Hospital and University of Basel, Basel, Switzerland;* [d] *Department of Orthopaedic Surgery, Leiden University Medical Center, Leiden, The Netherlands;* [e] *Centre for Molecular Medicine Norway (NCMM), Faculty of Medicine, University of Oslo, Oslo, Norway;* [f] *Department of Biochemistry and Developmental Biology, University of Helsinki, Helsinki, Finland;* [g] *iCAN Flagship in Digital Precision Cancer Medicine, University of Helsinki, Helsinki, Finland;* [h] *Basel Research Centre for Child Health, Basel, Switzerland*

ARTICLE INFO

Article history:
Received 25 June 2025
Revised 29 August 2025
Accepted 10 September 2025
Available online 19 September 2025

Keywords:
chondrosarcoma
genome-wide loss of heterozygosity
transcriptomics
subgrouping

ABSTRACT

Central conventional chondrosarcoma, a malignant cartilage-producing bone tumor, is the second most common bone sarcoma. Chondrosarcomas are histologically graded, which is so far the best predictor of survival. Early mutations in isocitrate dehydrogenase 1 (*IDH1*) and *IDH2* genes are frequent, leading to the production of the oncometabolite D-2-hydroxyglutarate, which affects DNA methylation, resulting in a preferred chondrogenic differentiation over osteogenic differentiation of mesenchymal stem cells, which are currently considered the precursor cells of chondrosarcoma. DNA methylation profiling has previously revealed distinct profiles between *IDH*-mutant and *IDH*–wild-type chondrosarcomas, but the presence of further DNA methylation subgroups indicates that classification based solely on *IDH* status is too simplistic. In this study, we aim to identify biological subgroups in a total of 116 chondrosarcomas by integrating clinical data, *IDH* mutation status, gene expression, and genome-wide loss of heterozygosity (LOH). Clinical associations were observed between several factors, including sex and histological grade, as well as tumor site and *IDH* mutation status. RNA sequencing and genome-wide LOH confirmed the distinction between *IDH*–wild-type and *IDH*-mutant chondrosarcomas, where the number of chromosome arms affected by LOH was significantly higher in *IDH*–wild-type tumors than in *IDH*-mutant tumors. However, no clear subgroups emerged within each *IDH* group. Further clustering on RNA expression of differentiation markers identified subgroups characterized by chondrogenic, osteogenic, resting chondrocyte, or dedifferentiated profiles. These different subgroups showed a specific clinical presentation and suggest different precursor cells. Instead of a simple dichotomy between *IDH*-mutant and *IDH*–wild-type, our integrated approach highlights interconnected clinical, genomic, and transcriptomic patterns

* Corresponding author.
E-mail address: j.v.m.g.bovee@lumc.nl (J.V.M.G. Bovée).

https://doi.org/10.1016/j.modpat.2025.100894

that offer a more nuanced view of chondrosarcoma biology and might potentially guide treatment stratification.

Introduction

Chondrosarcoma is a cartilage-producing tumor and the second most common primary bone malignancy after osteosarcoma.[1] Central conventional cartilaginous tumors arise in the medulla of the bone and are considered to form a spectrum, ranging from benign (enchondroma) to grade 3 chondrosarcoma, with multistep progression.[2-4] Although enchondroma and atypical cartilaginous tumor (ACT)/chondrosarcoma grade 1 predominantly carry isocitrate dehydrogenase 1 *(IDH1)* or *IDH2* mutations within an otherwise simple genomic background, high-grade chondrosarcomas are genetically complex, carrying structural variants and copy number alterations.[5] *IDH* mutations occur in approximately half of chondrosarcoma cases and are considered an early event.[6,7] This mutation results in a gain of function, whereby the mutated IDH converts α-ketoglutarate to the oncometabolite D-2-hydroxyglutarate (D2HG), which affects, among others, genome-wide DNA methylation.[8] In mesenchymal stem cells (MSCs), this results in a preference for chondrogenic differentiation over osteogenic differentiation, causing enchondroma formation.[9,10] Pharmacological inhibition of mutant IDH may be beneficial in a subset of chondrosarcomas[11] and is currently under clinical investigation (NCT06127407). Apart from *IDH* mutations, data on alternative and/or additional driver mutations remain limited. However, it has long been noted that subsets of central chondrosarcomas exhibit genome-wide loss of heterozygosity (gwLOH).[12,13] LOH is mostly the consequence of a near-haploid genome, which has been observed exclusively in *IDH*−wild-type (IDH^{WT}) chondrosarcoma, as reported by Cross et al.[14] Furthermore, their study found that genome doubling occurred more frequently in, although not exclusively, IDH^{WT} chondrosarcomas.

Multiple DNA methylation analyses showed that *IDH*-mutant (IDH^{MUT}) central cartilaginous tumors harbor a hypermethylated genome compared with IDH^{WT} tumors.[7,14-16] Although DNA methylation separated central chondrosarcomas into IDH^{WT} and IDH^{MUT} groups, multiple subclusters could be observed within those groups. This divides both the IDH^{WT} and IDH^{MUT} into 2 separate groups, although explanations for this division are still lacking. Moreover, in IDH^{MUT} chondrosarcomas, the number of highly methylated genes correlates with increasing histological grade.[17] In addition, RNA-based analyses identified proliferative and quiescent subgroups within *IDH*-based clusters.[15] This indicates that other biological processes could be active within *IDH* subgroups, supporting the notion that grouping solely on *IDH* mutation status is too simplistic.

In this study, we performed a refined analysis of chondrosarcoma heterogeneity beyond the *IDH* mutation status by integrating genomic and transcriptomic data with clinical features. For a total of 116 central chondrosarcoma samples, subgrouping was performed based on gwLOH and transcriptome profiles. Clinical associations involving sex, grade, *IDH* status, and tumor site were found. gwLOH analysis showed that IDH^{WT} chondrosarcomas were more often affected by gwLOH, in line with previous observation.[14] Although dimensionality reduction on gene expression did not reveal a separation within IDH^{WT} and IDH^{MUT} chondrosarcomas groups, 4 distinct subgroups emerged when focusing on the expression of differentiation markers. These included an osteogenic, chondrogenic, dedifferentiated, and a resting chondrocyte group, which showed specific clinical features and association with disease-free survival. Thus, our integrated approach revealed 4 subgroups, providing a more nuanced understanding of central chondrosarcoma biology with potential implications for classification and treatment stratification.

Materials and Methods

Sample Information

Formalin-fixed, paraffin-embedded tissue and fresh-frozen samples were collected from 116 patients diagnosed with central conventional chondrosarcoma. Cases were reviewed and classified according to the 2020 World Health Organization Classification by an experienced bone tumor pathologist (J.V.M.G.B.), and histological grade was established according to Evans et al.[18] Because all well-differentiated tumors in our cohort were located in the long bones (ACT), no grade 1 chondrosarcomas of the flat bones were included. One case was obtained externally, and clinical information is therefore missing. For the dedifferentiated chondrosarcomas used in this study, only the dedifferentiated component was used for the molecular profiling. Approval of the Institutional Review Board (Medisch-Ethische Toetsingscommissie Leiden Den Haag Delft) was obtained for the use of tissue samples from the Leiden University Medical Center bone and soft tissue tumor biobank (Leiden, the Netherlands) (B17.039, B20.067). Written informed consent from patients was obtained for all samples used for RNA sequencing (RNAseq). All specimens were pseudonymized and handled according to the ethical guidelines described in “Code for Proper Secondary Use of Human Tissue in The Netherlands” of the Dutch Federation of Medical Scientific Societies. Clinical and follow-up data are summarized in Supplementary Table S1.

Mutation Calling

DNA was isolated from all fresh-frozen tissue sections (thickness, 20 μm) using the Wizard Genomic DNA Purification Kit (A1125; Promega), according to manufacturer's instructions. *IDH* status of cases was determined with different methods: 1 by immunohistochemistry of R132H, 1 by the custom Ampliseq Cancer Hotspot v2 Panel (Thermo Fisher Scientific), 2 by the custom Ampliseq Cancer Hotspot v3 Panel (Thermo Fisher Scientific), 8 by Sanger sequencing, 92 by the custom Ampliseq Cancer Hotspot v6 Panel (Thermo Fisher Scientific), and 12 by whole-genome sequencing (WGS). Library construction for WGS was performed by GenomeScan using the NEBNext Ultra II DNA Library Prep Kit, and sequencing was carried out on the NovaSeq

6000 platform, generating 152-bp paired-end reads with a depth of 90× for tumor DNA and 30× for normal DNA. Sequencing of cancer hotspot panels was performed on the Ion Torrent GeneStudio S5 platform (GenomeScan BV). WGS was performed on the NovaSeq 6000 platform (GenomeScan BV) producing 152-bp paired-end reads with 90× depth for tumor DNA and 30× for normal DNA. For cases subjected to the Cancer Hotspot v6 Panel or WGS, mutations in *IDH1*, *IDH2*, *TERT*, and 84 other cancer-related genes were called (Thermo Fisher Scientific). Somatic mutation calling in WGS was performed as previously described.[19]

Genome-wide Loss of Heterozygosity

gwLOH analysis was performed on 82 samples using the custom AmpliSeq Next Generation Sequencing gwLOH v2 Panel, which includes 1500 single-nucleotide polymorphisms that are evenly distributed across all autosomes and the X chromosome. Sequencing was performed on the Ion Torrent GeneStudio S5 platform (GenomeScan BV). For each single-nucleotide polymorphism, the median amplicon read count, normalized median amplicon read count, and variant allele frequency were computed. Subsequently, LOH, imbalance, or heterozygosity was deduced by visual inspection for each chromosome arm.

RNA Sequencing Data Generation and Data Analysis

RNA was isolated from frozen tissue sections (thickness, 20 μm) in TRIzol (15596018; Ambion) according to manufacturer's instructions. RNA purification was performed using the RNeasy Mini Kit (74104; Qiagen), including DNase treatment, according to manufacturer's instructions. RNA concentrations and quality were checked on NanoDrop (Thermo Fisher). RNAseq was performed on 54 samples deemed to have sufficient RNA quality: 3 ACTs, 30 grade 2 tumors, 18 grade 3 tumors, and 3 dedifferentiated chondrosarcomas. Total RNAseq paired end was performed by GenomeScan. The NEBNext Ultra II Directional RNA Library Prep Kit was used for library construction, and sequencing was performed on the NovaSeq 6000 platform. Raw RNAseq data were processed by the RNAseq BioWDL pipeline using default settings developed by the Sequencing Analysis Support Core (https://biowdl.github.io/; Leiden University Medical Center, Leiden, the Netherlands). Reads were aligned against hg38 with Spliced Transcripts Alignment to a Reference (STAR), and expression quantification was performed using HTseq-count.[20] Gene counts were normalized with the "Trimmed mean of M-values" method in the *EdgeR* R package.[21] For exploratory visualization, batch effects between sequencing runs were corrected using ComBat-seq before dimension reduction with t-distributed stochastic neighbor embedding (tSNE).[22] Only genes that passed filtering with filterByExpr in EdgeR were included in the embedding. For differential expression analysis, batch was modeled as a covariate in the EdgeR design matrix, allowing statistical adjustment without modifying raw counts. Significantly differentially expressed genes were defined by Benjamini and Hochberg–corrected *P*s < .05 and $\log_2$FC >1 or <−1. Hierarchical clustering was performed using the Ward.D2 method on *z* score standardized expression values of 22 genes. These genes included markers for MSC osteogenic differentiation (*RUNX2*, *SP7*, *ALPL*, *SPP1*, *BGLAP*, *COL1A1*, *COL1A2*, *IBSP*, *ITGAV*, *ITGB1*, and *FRZB*),[23-31] MSC chondrogenic differentiation (*SOX9*, *COL2A1*, *COL9A1*, *COL11A1*, *ACAN*, *COMP*, and *FGFR3*),[28,32-35] resting chondrocytes (*UCMA*),[36] and hypertrophic chondrocytes (*IHH*, *COL10A1*, and *MMP13*).[37-39]

Statistical Analyses

All statistical analyses were performed in R (version 4.1.0). To test for clinical associations, χ^2 tests were performed between sex, grade, tumor site, and *IDH* status using R function *chi.sq*. Before conducting the χ^2 tests, assumptions were verified to ensure test validity. Student *t* tests or analysis of variance (ANOVA) followed by posthoc Tukey honestly significant difference (HSD) were performed to test clinical associations involving age using R function *t.test* and *aov*, respectively. All clinical associations were corrected for multiple testing using the Benjamini-Hochberg procedure and are reported in Supplementary Table S2. Multinomial logistic regression was used to correct for confounding factors using R function *multinom*. Student *t* tests were performed to assess whether the number of chromosomes affected by LOH differs between IDH^{WT} and IDH^{MUT} cases. Fisher exact test was performed to test for enrichment of clinical features. Univariable and multivariable analyses were conducted in R using the Cox proportional hazards (PH) model using *coxph* function from the survival R package. PH assumptions for Cox regression models were assessed using Schoenfeld residuals via the cox.zph() test. $P < .05$ was considered indicative of violation of the PH assumption. Only analyses for which all model assumptions, including the PH assumption and absence of collinearity, were met are presented. Owing to sparse data and assumption violations in the multivariable model for disease-free survival and univariable analysis for recurrence-free survival, these analyses are not reported.

Results

Clinical Associations

For a total of 116 patients, RNAseq, gwLOH, and/or *IDH* mutation calling was performed, and clinical data were collected (Fig. 1A, Supplementary Table S1). Clinical associations were tested in pairwise comparisons using variables sex, age, grade, tumor site, and *IDH* status (Supplementary Table S2). An association was observed between sex and grade, where men were more frequently diagnosed with high-grade chondrosarcomas compared with women (χ^2 [3, n = 115] = 10; Cramér V = 0.29; false discovery rate [FDR] $P < .05$) (Fig. 1B). After adjusting for age as confounding factor in a multinomial logistic regression model, this relationship was primarily driven by a higher proportion of ACT diagnoses in women than in men (FDR $P < .05$; odds ratio [OR], 0.30; 95% CI, 0.12-0.76). An analysis on the Dutch chondrosarcoma patient population from 1989 to 2013 (n = 1753) confirmed the imbalanced sex distribution by grade ($\chi^2(2, n = 1753) = 39.1$; Cramér V = 0.15; $P < .001$).[40] A significant association between sex and *IDH* status was also found, where women showed a higher proportion of IDH^{MUT} chondrosarcomas than IDH^{WT} chondrosarcomas, and men showed a relatively higher proportion of IDH^{WT} chondrosarcomas ($\chi^2(1, n = 115) = 6.4$; Cramér V = 0.24; FDR $P < .05$). Age was related to histological grade, with a gradual increase in age from patients with ACT to dedifferentiated chondrosarcoma (Fig. 1C; ANOVA, $F(3, 111) = 3.20$; FDR $P < .05$; posthoc Tukey HSD: no significant pairwise differences).

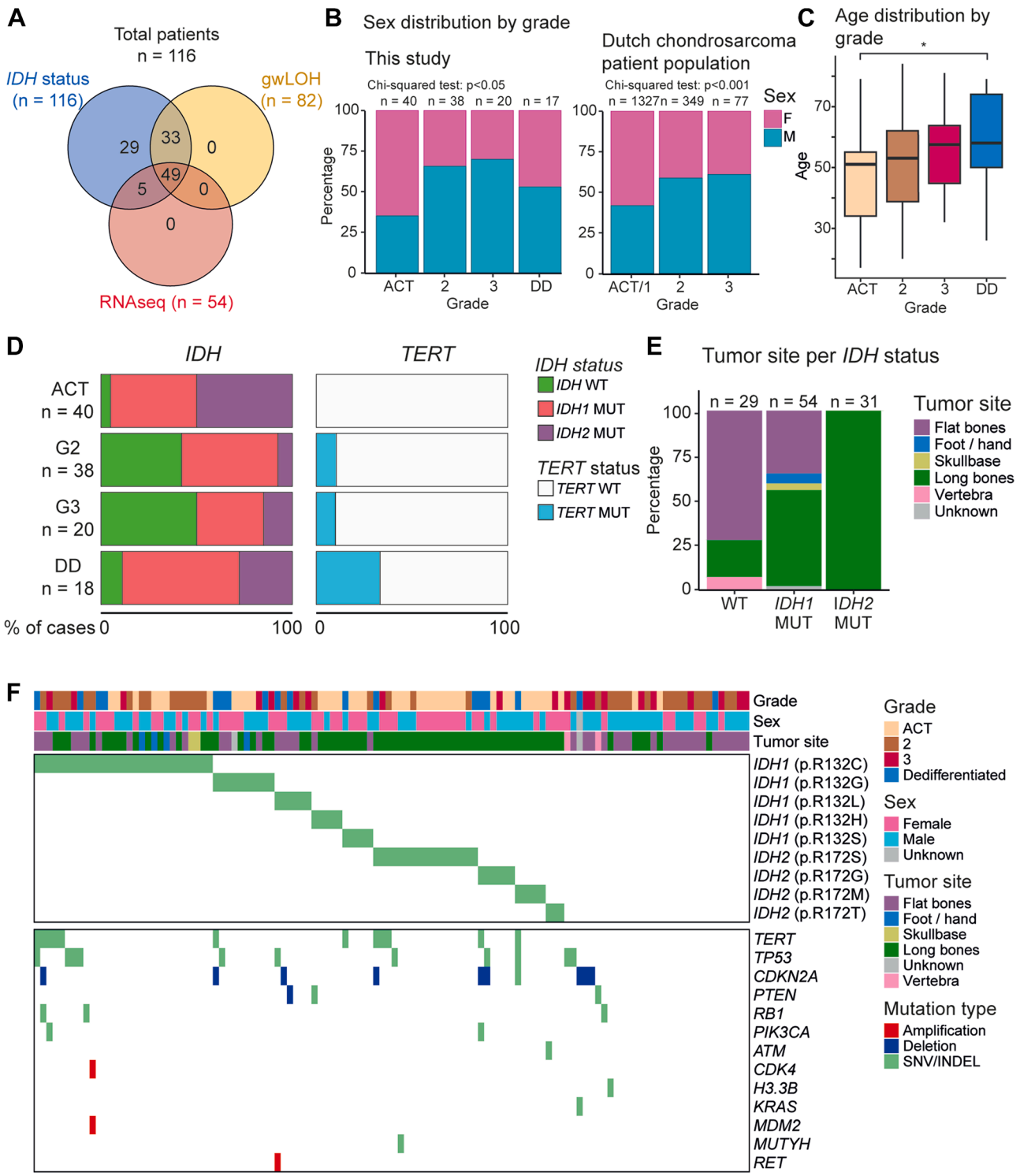

Figure 1.
Clinical and mutational overview of included chondrosarcomas. (A) Venn diagram showing the number of cases for which *IDH* mutation calling, gwLOH, and RNAseq were performed. (B) Barplots showing the sex distributions per grade for samples used in this study (left) and for the Dutch chondrosarcoma patient population (1989-2013). (C) Boxplots showing the age distribution per grade (analysis of variance, $P < .05$; posthoc Tukey honestly significant difference: ACT vs DD; $P = .05$). (D) Barplots showing the percentage of *IDH* mutations and *TERT* mutations per grade. (E) Barplot showing the anatomical location of the tumor against the *IDH* status. (F) Waterfall plot depicting the mutations in *IDH* genes and other class 4 or 5 pathogenic alterations in oncogenes and tumor suppressor genes. Top annotations include grade, sex, and location of the tumor. ACT, atypical cartilaginous tumor; DD, dedifferentiated; gwLOH, genome-wide loss of heterozygosity; *IDH*, isocitrate dehydrogenase; INDEL, insertion-deletion; RNAseq, RNA sequencing; SNV, single-nucleotide variant; WT, wild type.

Looking at *IDH* status by grade revealed that most ACTs and dedifferentiated chondrosarcomas were IDH^{MUT} (Fig. 1D; $\chi^2[3, n = 116] = 22.4$; Cramér V = 0.44; FDR $P < .001$). In contrast, only about half of the grade 2 and 3 chondrosarcomas were IDH^{MUT}. *TERT* promoter mutations were observed more frequently in high-grade chondrosarcomas and dedifferentiated chondrosarcomas. Notably, all $IDH2^{MUT}$ cases were located in long bones, whereas in $IDH1^{MUT}$ cases, tumor sites were more evenly distributed between flat and long bones (Fig. 1E). The majority of IDH^{WT} cases were located in the flat bones (72%). From the perspective of tumor site, IDH^{MUT} and IDH^{WT} cases were evenly distributed in flat bones (47.5% IDH^{MUT} and 52.5% IDH^{WT}), whereas chondrosarcomas in long bones were predominantly IDH^{MUT} (91.0%) ($\chi^2[2, n = 106] = 37.3$; Cramér V = 0.59; FDR $P < .001$; Supplementary Table S2, Supplementary Fig. S1). Next to mutations in the *IDH* genes and *TERT*, other genetic alterations in oncogenes or tumor suppressor genes included, but were not limited to, *TP53*, *CDKN2A*, *PTEN*, *RB1*, and *PIK3CA* (Fig. 1F). These mutations were most prevalent in high-grade chondrosarcomas and dedifferentiated chondrosarcomas, occurring in 7.5% of ACTs, 32.4% of grade 2, 35.0% of grade 3, and 66.7% of dedifferentiated chondrosarcomas.

Distinct IDH Subgroups Based on RNA Sequencing and Genome-wide Loss of Heterozygosity

For further subgrouping of chondrosarcomas, we performed RNAseq (n = 54) and gwLOH (n = 82) and determined the *IDH* status (n = 116) (Fig. 1A). Dimensionality reduction with tSNE on RNAseq revealed a separation between the majority of IDH^{WT} and IDH^{MUT} samples (Fig. 2B). No specific subgrouping based on grade or within IDH^{WT} and IDH^{MUT} tumors could be identified (Fig. 2A, B).

A separation between IDH^{WT} and IDH^{MUT} chondrosarcomas was also observed in the gwLOH data, where the number of chromosome arms affected by LOH was significantly higher in IDH^{WT} tumors (median, 21; SD, 10.4) than in IDH^{MUT} tumors (median, 0; SD, 4.0) (*t* test, $t[33] = 7.31$; 95% CI, 10.57-18.73; $P < .001$). Because most ACTs were IDH^{MUT}, the analysis was repeated for high-grade chondrosarcomas and dedifferentiated chondrosarcomas only, confirming a significant difference: IDH^{WT} tumors (median, 21; SD, 9.9) still exhibited significantly more affected chromosome arms by LOH than IDH^{MUT} tumors (median, 1; SD, 4.4) (*t* test, $t[33] = 6.84$; 95% CI, 9.8-18.11; $P < .001$). One-way ANOVA comparing IDH^{WT}, $IDH1^{MUT}$, and $IDH2^{MUT}$ groups also showed a significant overall effect (ANOVA, $F[2, 79] = 42.2$; $P < .001$; posthoc Tukey HSD, IDH^{WT} vs $IDH1^{MUT}$, $P < .001$; IDH^{WT} vs $IDH2^{MUT}$, $P < .001$) (Fig. 2C, D). Ninety percent (26/29) of IDH^{WT} chondrosarcomas had at least 1 chromosome arm affected by LOH (Supplementary Fig. S2). The number of chromosome arms affected by LOH increased stepwise with grade (Fig. 2C). Chondrosarcomas located in the flat bones and vertebra seemed to be more affected by LOH because those tumors were also more often IDH^{WT} (Fig. 1E, Supplementary Fig. S3). Interestingly, although some chromosomes were more often affected by LOH, such as chromosomes 6 and 13, chromosome 19 did not show any LOH across all analyzed chondrosarcomas (Fig. 2D). This might indicate that important genes for chondrosarcoma cell survival are located on chromosome 19.

Distinct IDH^{WT} and IDH^{MUT} Transcriptome Profiles Reveal a IDH^{WT} Chondrosarcoma Subgroup

The clear separation between IDH^{WT} and IDH^{MUT} chondrosarcomas based on RNAseq and gwLOH prompted us to look deeper into the biological differences between the groups. This revealed additional molecular distinctions that suggest the presence of subgroups beyond *IDH* mutation status. A differential gene expression between IDH^{WT} and IDH^{MUT} tumors revealed *MAGEC2* as differential expressed in the IDH^{MUT} group, although *UCMA* was the most differentially expressed gene in the IDH^{WT} group (Fig. 3A). *MAGEC2* has been identified as a cancer testis antigen (CTA), for example, a gene that is not expressed in healthy tissues (except for the testis) and can be reexpressed in cancer. This makes CTAs a potential target for therapy. However, a detailed look of the *MAGEC* gene family expression showed low expression of *MAGEC* CTAs (Supplementary Fig. S4A). Expression of other known CTAs (http://www.cta.lncc.br/) was low in general, except for specific CTAs that are associated with tumors and still show expression in healthy tissues (Supplementary Fig. S4B). Only 1 dedifferentiated chondrosarcoma showed high expression of *MAGEC1* and *MAGEC2*. Interestingly, UCMA has previously been described as a highly specific marker for resting chondrocytes located in the upper zone of the growth plate.[36] *UCMA* expression was highly enriched in IDH^{WT} tumors. However, its expression was restricted to 41% of IDH^{WT} cases, all of which were located in the flat bones (Fig. 3B). This suggests that the precursor of this specific IDH^{WT} subgroup may be the resting chondrocyte, as opposed to the MSC, which is currently considered the precursor cell of chondrosarcoma. MSCs are characterized by the expression of CD73, CD90, and CD105.[41] However, these MSC genes were expressed across nearly all samples, including those expressing *UCMA* (Supplementary Fig. S5). This may indicate that the expression of these MSC genes either persists following differentiation into resting chondrocytes, which indeed retain stem-like properties, or may be reexpressed in the tumor.

Chondrosarcoma Subclusters Characterized by Gene Expression of Various Mesenchymal Stem Cell Differentiation Lines

To further investigate the potential precursor cell of chondrosarcoma, the expression of genes for specific MSC differentiation lines and chondrocyte types according to existing literature were analyzed. These included genes for MSC osteogenic differentiation (*RUNX2*, *SP7*, *ALPL*, *SPP1*, *BGLAP*, *COL1A1*, *COL1A2*, *IBSP*, *ITGAV*, *ITGB1*, and *FRZB*),[23-31] MSC chondrogenic differentiation (*SOX9*, *COL2A1*, *COL9A1*, *COL11A1*, *ACAN*, *COMP*, and *FGFR3*),[28,32-35] resting chondrocytes (*UCMA*),[36] and hypertrophic chondrocytes (*IHH*, *COL10A1*, and *MMP13*).[37-39] Hierarchical clustering was performed to identify distinct differentiation subgroups. Because the resting chondrocytes were solely defined by *UCMA* expression, *UCMA*-high samples were analyzed separately to prevent this signal from being diluted in a broader clustering. Excluding these samples, hierarchical clustering based on the *z* score standardized expression of multiple precursor cell markers revealed 3 distinct subgroups: (1) MSC osteogenic like, (2) MSC chondrogenic like, and (3) dedifferentiated (Fig. 3C). Together with the separately identified (4) resting chondrocyte group, this resulted in a total of 4 distinct subgroups. Projecting those groups on the RNAseq tSNE did not reveal new clusters (Supplementary Fig. S6). Similar groupings were observed when hierarchical clustering was performed without *z* score standardization (Supplementary Fig. S7).

The MSC osteogenic-like group showed relatively high expression of osteogenic differentiation and hypertrophic chondrocyte markers. These tumors were mostly IDH^{MUT} and enriched for those located in the long bones (IDH^{MUT}, 26/34; Fisher exact test OR, 7.25; 95% CI, 1.9-31.9; $P < .01$; long bones, 19/34; Fisher exact test OR, 7.18; 95% CI, 1.9-27.0; $P < .01$). Disease-free survival

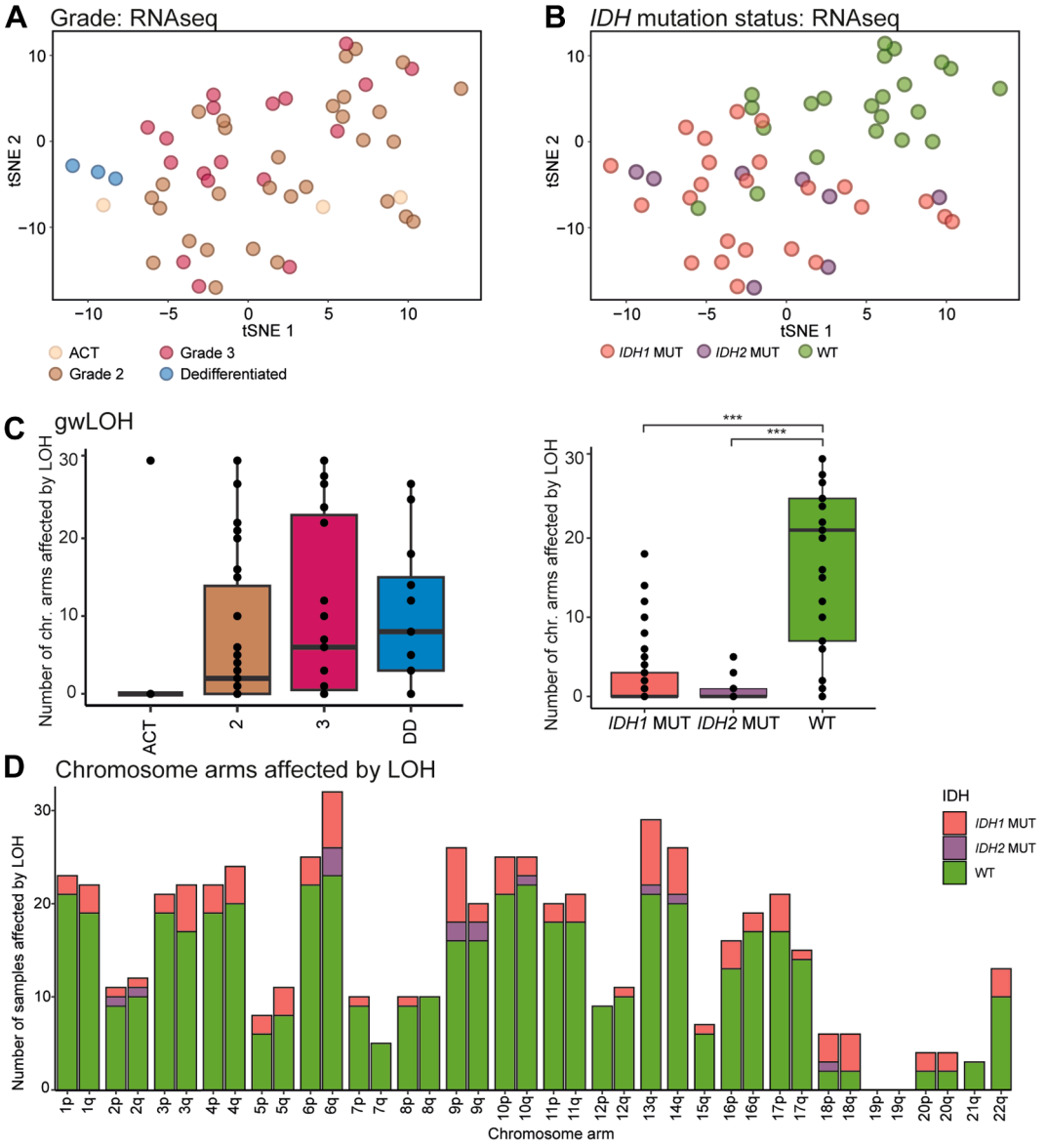

Figure 2.

Molecular variation of chondrosarcoma cases based on RNAseq and gwLOH. tSNE visualizations of RNAseq data colored by (A) grade and (B) IDH mutation status. (C) Boxplots displaying the number of chromosome arms affected by LOH per grade (left) and IDH status (right, analysis of variance: $P < .001$; posthoc Tukey honestly significant difference, IDH^{WT} vs $IDH1^{MUT}$: $P < .001$, IDH^{WT} vs $IDH2^{MUT}$: $P < .001$). (D) Barplot showing the number of samples affected by LOH per chromosome arm colored by IDH status. ACT, atypical cartilaginous tumor; DD, dedifferentiated; gwLOH, genome-wide loss of heterozygosity; *IDH*, isocitrate dehydrogenase; RNAseq, RNA sequencing; tSNE, t-distributed stochastic neighbor embedding; WT, wild type.

differed significantly across all 4 subgroups (log-rank $P < .001$), although this effect was mainly because of the poor survival of the dedifferentiated group (Fig. 3D, Table 1). The MSC osteogenic-like group showed a better survival than the MSC chondrogenic-like group, although the difference was modest.

In contrast, the MSC chondrogenic-like group showed relatively low expression of osteogenic differentiation markers. This group consisted of a balanced mix of grade 2 and 3 chondrosarcomas, with an enrichment for tumors located in the flat bones (flat bones, 8/9; Fisher exact test OR, 8.09; 95% CI, ~0.95 to 386; $P < .05$). Histological differences were not observed between the chondrogenic-like and osteogenic-like subgroups. Survival analysis showed a slightly worse disease-free survival compared with the osteogenic-like group and resting chondrocyte group.

The resting chondrocyte group was characterized by high expression of *UCMA*, although lacking expression of MSC osteogenic differentiation and hypertrophic chondrocyte genes. All cases in this group were IDH^{WT} and located in flat bones. Disease-free survival analysis curve of the resting chondrocyte group followed a similar pattern as the osteogenic-like group.

The dedifferentiated subgroup, although comprising only 3 cases, showed low expression of most differentiation markers consistent with loss of differentiation. Indeed, all chondrosarcomas in this group were dedifferentiated chondrosarcomas: 1 with an undifferentiated pleomorphic sarcoma and 2 with an osteosarcoma in the dedifferentiated component. As expected, this group had the worst disease-free survival,

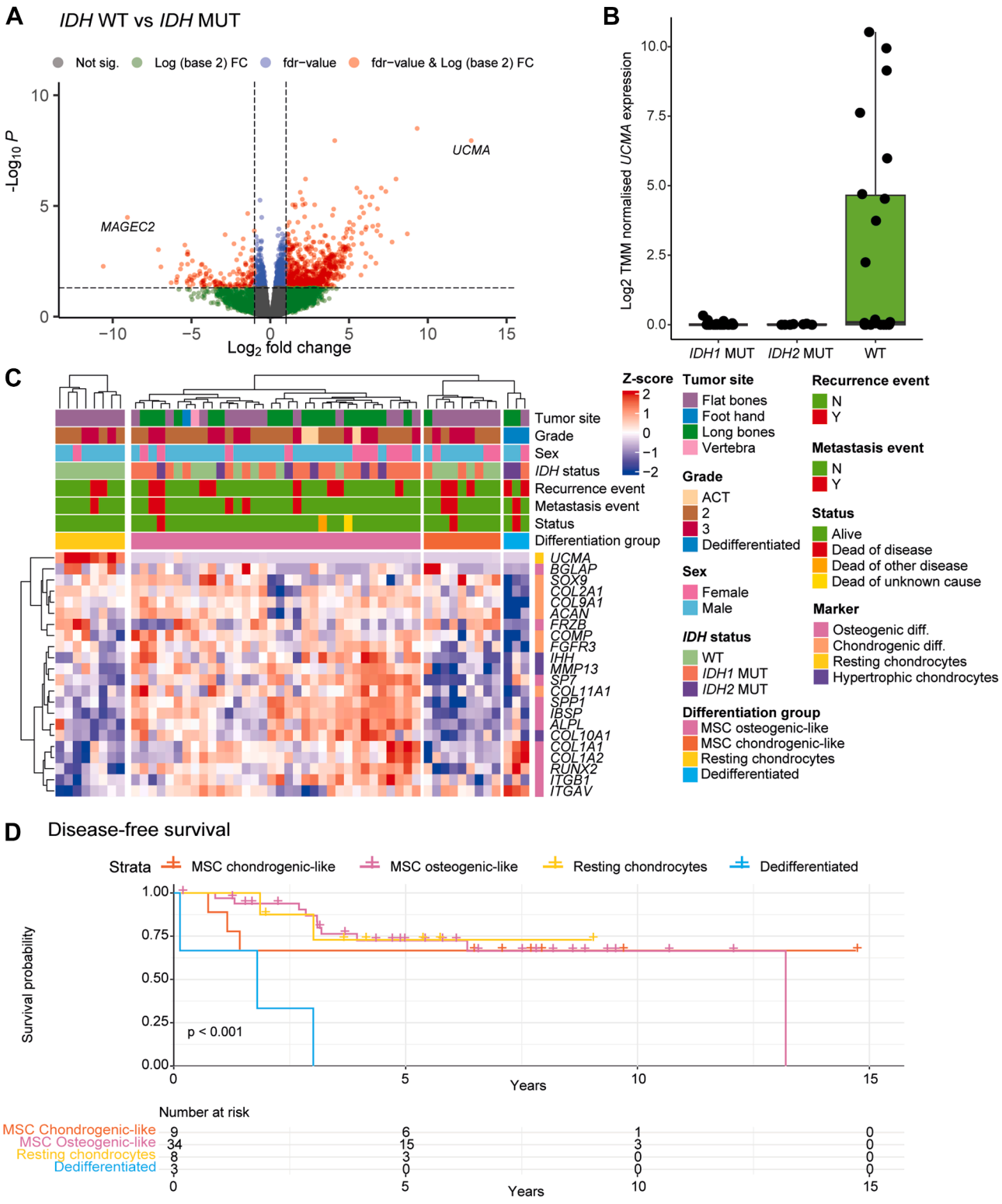

Figure 3.
Gene expression profiles characterized by various MSC differentiation lines. (A) Volcano plot depicting significant genes expressed in IDH^{MUT} cases (left) versus IDH^{WT} cases (right). (B) Barplot showing the gene expression of *UCMA* per *IDH* mutation status. (C) Heatmap showing *z* score gene expression of *UCMA* and MSC osteogenic differentiation, MSC chondrogenic differentiation, and hypertrophic chondrocyte markers. Chondrosarcoma cases and genes are clustered with ward.D2 clustering. (D) Kaplan-Meier curve showing disease-free survival for the chondrosarcoma differentiation subgroups (log-rank $P < .001$). ACT, atypical cartilaginous tumor; FDR, false discovery rate; *IDH*, isocitrate dehydrogenase; MSC, mesenchymal stem cell; WT, wild type.

Table 1
Univariable Cox proportional hazards regression results for disease-free survival in 54 chondrosarcoma cases with available RNA sequencing data

Disease-free survival	Univariable survival analysis		
Variable	HR (95% CI)	*P*	log-rank *P*
Subgroup (dedifferentiated)			
Osteogenic like	0.1 (0.03-0.40)	.001[a]	6e-04[a]
Chondrogenic like	0.1 (0.02-0.57)	.009[a]	
Resting chondrocytes	0.09 (0.06-0.59)	.01[a]	
Grade (2)			
ACT	2.88×10^{-8} (0-inf)	1	5e-05[a]
Grade 3	3.08 (1.06-8.94)	.039[a]	
Dedifferentiated	16.07 (3.63-71.15)	2.55e-04[a]	
IDH status (IDH^{MUT})			
IDH^{WT}	1.44 (0.55-3.74)	.45	.5
Sex (female)			
Sex (Male)	1.53 (0.5-4.68)	.46	.5
Tumor site (flat bones)			
Long bones	0.61 (0.23-1.64)	.33	.6
Foot or hand	3.11e-08 (0-inf)	1	
Vertebra	3.11e-08 (0-inf)	1	

ACT, atypical cartilaginous tumor; HR, hazard ratio; inf, infinite; MUT, mutant; WT, wild type.

performing significantly worse than all other groups (log-rank $P < .001$; Fig. 3D, Table 1).

Statistical significance for overall survival was also achieved for the subgroups, although only 3 deaths because of the disease were recorded (Supplementary Fig. S8). Statistical significance for metastasis-free survival was not achieved in the subgroups (Supplementary Fig. S8, Supplementary Table S3).

Thus, these findings suggest the existence of different biological chondrosarcoma subgroups that are associated with tumor site, histological grade, and *IDH* mutation status. Although the differentiation subgroups display a modest prognostic value, this is, however, not superior to traditional histological grading. An overview of the subgroups with clinical associations is provided in Table 2.

Discussion

In this study, we aimed to go beyond the *IDH* status and performed a refined subgrouping of chondrosarcomas, integrating genomic and transcriptomic data with clinical features to define biological subgroups of chondrosarcoma. An interesting association between sex and grade was found, where men more often are diagnosed with high-grade chondrosarcomas than women. This association was found in our own cohort and confirmed in an independent large Dutch registration cohort and is to our knowledge previously unreported. Sex significantly influences the incidence, disease prognosis, and mortality rates across various types of cancer.[42] This could suggest the possibility of hormonal influences. Indeed, active estrogen signaling has previously been observed in chondrosarcomas, and inhibiting estrogen signaling did not show decreased tumor growth.[43,44] The higher frequency of high-grade chondrosarcoma in men may suggest a protective effect of estrogen in women against chondrosarcoma progression. Nonetheless, other biological or environmental factors could also contribute to this sex difference, and further studies are needed to elucidate the underlying mechanisms.

An analysis of gwLOH events showed that chromosome arms of IDH^{WT} chondrosarcomas are more often affected by LOH compared with those of IDH^{MUT} chondrosarcomas. This is in line with previous observations of LOH and near-haploidy in IDH^{WT} chondrosarcomas.[12,14] Additionally, our study now revealed that specific chromosomes are more often affected compared with others. For example, chromosomes 6, 13, and 14 were often affected by LOH. Because a genetic driver in IDH^{WT} chondrosarcomas is lacking, it is possible that specific LOH events can affect tumor suppressor genes, thereby contributing to the tumorigenesis in those tumors. Patterns of LOH have also been observed in other cancers and can be used as molecular markers, such as the combined 1p and 19q LOH in IDH^{MUT} gliomas.[45,46] Although the biological implications of this LOH pattern remain unclear, glioma patients exhibiting this pattern show a better prognosis than those without it.[47] However, in our study, it is noteworthy that LOH in chromosome 19 was not observed in any of the chondrosarcomas. Chromosome 19 is the most gene-dense chromosome, containing multiple large clustered gene families.[48] This might suggest that chromosome 19 contains genes that are essential for the survival and growth of chondrosarcomas. A similar pattern can be seen in oncocytic follicular thyroid carcinomas, which can display homozygosity across all chromosomes except for chromosome 7.[49] Indeed, several genes located on chromosome 7 were essential for cell survival in oncocytic follicular thyroid carcinomas.[50] Our data also show that the number of chromosome arms affected by LOH increased with increasing histological grade, probably reflecting increased genomic complexity in higher-grade tumors. The detection of gwLOH may assist bone tumor pathologists in challenging differential diagnoses, particularly when morphology is atypical, and radiological features raise suspicion, but no *IDH* mutation is present. However, in the absence of gwLOH, the test does not provide an additional diagnostic value.

IDH-mutant and *IDH*–wild-type tumors also showed a specific anatomical distribution. Although IDH^{WT} tumors were more common in the flat bones, all $IDH2^{MUT}$ chondrosarcomas were found in the long bones. An association between *IDH* status and tumor site has previously been reported in a series of 88 head and neck chondrosarcomas: chondrosarcomas of the facial bones and

Table 2
Overview of chondrosarcoma differentiation subgroups

Feature	MSC osteogenic like	MSC chondrogenic like	Resting chondrocytes	Dedifferentiated
Markers	Osteogenic differentiation and hypertrophic chondrocytes	Chondrogenic differentiation	Resting chondrocyte (*UCMA*)	Low expression of differentiation markers
Grade	ACT, G2, and G3	Mixed G2 and G3	Mixed G2 and G3	Dedifferentiated chondrosarcoma
IDH mutation status	Enriched for IDH^{MUT}	Mixed IDH^{WT} and $IDH1^{MUT}$	IDH^{WT}	IDH^{MUT}
Tumor site	Enriched for long bones	Enriched for flat bones	Flat bones	Flat and long bones
Sex	Mixed	Mixed	Mixed	Mixed
Outcome	Good	Medium	Good	Poor

ACT, atypical cartilaginous tumor; IDH, isocitrate dehydrogenase; MUT, mutant; MSC, mesenchymal stem cell; WT, wild type.

laryngotracheal region showed low frequencies of *IDH1* mutations (0% and 12%, respectively), whereas skull base chondrosarcomas exhibited high *IDH1* mutation rates (86%).[51] In our cohort, both skull base chondrosarcomas were *IDH*-mutant. We now also show that 91% of chondrosarcomas in long bones are IDH^{MUT} compared with only 47.5% in flat bones. This is in line with a previous analysis of central and periosteal cartilaginous tumors reporting that IDH^{MUT} tumors occurred more frequently in tubular bones of the hands and feet (90%) compared with those in long bones (53.2%) and flat bones (35.1%).[6] Long and short tubular bones grow longitudinally through endochondral ossification, involving a growth plate. In contrast, flat bones develop through intramembranous (desmal) ossification, a process that does not involve growth plates. This may reinforce the hypothesis that IDH^{MUT} and IDH^{WT} may have distinct cells of origin. Tumor location is therefore an important clinical parameter when evaluating the frequency of *IDH* mutations in chondrosarcoma cohorts.

Although the *IDH* mutation is known to promote chondrogenic differentiation in MSCs, pointing to MSCs as the cell of origin and explaining the formation of enchondromas in the developing long bone,[9,10] the oncogenesis of IDH^{WT} chondrosarcoma remains so far unclear. Differential gene expression analysis between IDH^{WT} and IDH^{MUT} in the present study revealed *UCMA*, a highly specific marker for resting chondrocytes,[36] to be exclusively expressed in a subgroup of IDH^{WT} chondrosarcomas. This finding suggests that *UCMA*-positive IDH^{WT} chondrosarcomas may originate from resting chondrocytes rather than MSCs. Unfortunately, validation of UCMA protein expression using immunohistochemistry was not feasible because the only commercially available UCMA antibody did not show reliable results in our hands. Notably, a recent study found higher *UCMA* expression in the growth plates of IDH^{WT} than IDH^{MUT} mice.[52] Because flat bone growth does not involve growth plates, it still remains to be determined whether resting chondrocytes are hidden in these bones and can serve as the precursor of IDH^{WT} central chondrosarcoma.

In addition to the resting chondrocyte subgroup, further exploration of differentiation lineage markers revealed 3 distinct groups of chondrosarcomas: MSC osteogenic-like group, MSC chondrogenic-like group, and dedifferentiated group (Table 2). These groups differed in molecular profiles, tumor location, and *IDH* mutation status. Although differences in disease-free survival were observed across subgroups, these likely reflect underlying histological grade or genetic factors rather than the differentiation state alone. Nevertheless, the 4 distinct subgroups seem to reflect different mechanisms of tumorigenesis with corresponding clinicoanatomical presentation and behavior.

One limitation of the study is the potential bias in cohort composition, including the relatively small number of IDH^{WT} ACTs, the limited representation of dedifferentiated chondrosarcomas in the survival analysis, and possible institutional selection bias. This imbalance may introduce confounding factors that affect the interpretation of associations. Expanding the cohort in future studies might strengthen the generalizability of the findings.

In conclusion, our study revealed distinct molecular and clinical patterns in chondrosarcoma, including associations between sex and grade, as well as *IDH* mutation status and tumor site. Furthermore, we observed distinct LOH profiles between IDH^{MUT} and IDH^{WT} tumors, with IDH^{WT} tumors showing more extensive gwLOH. This specific pattern may help the pathologist in case of suspicion of chondrosarcoma and lack of IDH^{MUT}. Beyond *IDH* mutation status, gene expression analysis revealed distinct subgroups based on differentiation markers, which may reflect different mechanisms of tumorigenesis with distinct precursor cells, with corresponding clinicoanatomical presentation and behavior. These findings suggest a more refined subclassification, offering potential for improved treatment stratification combining molecular and clinical features.

Author Contributions

D.M.M. was involved in conceptualization, methodology, project administration, and data curation, provided software, performed formal analysis, validation, investigation, and visualization, and wrote the original draft, reviewed, and edited the manuscript. S.V. was involved in conceptualization, methodology, project administration, and data curation, performed investigation, and wrote, reviewed, and edited the manuscript. B.A., Z.E., S. C., D.R., I.H.B.-d.B., B.E.v.d.A., P.M.W.-K., and C.H.J.S. performed investigation and wrote, reviewed, and edited the manuscript. M. A.J.v.d.S. provided resources and wrote, reviewed, and edited the manuscript. M.L.K. was involved in conceptualization, methodology, and supervision and wrote the original draft, reviewed, and edited the manuscript. D.B. was involved in conceptualization and methodology and wrote, reviewed, and edited the manuscript. N.F.C.C.d.M. was involved in conceptualization, methodology, and supervision and wrote, reviewed, and edited the manuscript. J.V.M.G.B. was involved in conceptualization, methodology, and supervision and wrote the original draft, reviewed, and edited the manuscript.

Data Availability

RNA sequencing data generated in this study are publicly available on the Gene Expression Omnibus (GSE299759).

Funding

This work was financially supported by the intramural Leiden Center for Computational Oncology Strategic Fund and the Bone Cancer Research Trust (grant number 8922). M.L.K. is funded by the Norwegian Research Council, Helse Sør-Øst and the University of Oslo through the Centre for Molecular Medicine Norway (grant number 187615), the Norwegian Research Council (grant number 313932), the Norwegian Cancer Society (grant numbers 214871 and 273592), and the iCAN Flagship in Digital Precision Cancer Medicine. N.F.C.C.d.M. is funded by the European Research Council under the European Union's Horizon 2020 Research and Innovation Program (grant agreement number 852832) and by the Vidi Zorgonderzoek en Medische Wetenschappen (ZonMW) (project number 09150172110092).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethics Approval and Consent to Participate

Approval of the Institutional Review Board (Medisch-Ethische Toetsingscommissie Leiden Den Haag Delft) was obtained for the

use of tissue samples from the Leiden University Medical Center bone and soft tissue tumor biobank (Leiden, the Netherlands) (B17.039, B20.067). Written informed consent from patients was obtained for all samples used for RNA sequencing.

Supplementary Material

The online version contains supplementary material available at https://doi.org/10.1016/j.modpat.2025.100894.

References

1. Bovee JVMG, Bloem JL, Flanagan AM, et al. Central chondrosarcoma, grades 2 and 3. In: WHO Classification of Tumours Editorial Board, ed. *WHO Classification of Tumours Soft Tissue and Bone Tumours*. 5th ed. IARC Press; 2020: 400-402.
2. Brien EW, Mirra JM, Kerr R. Benign and malignant cartilage tumors of bone and joint: their anatomic and theoretical basis with an emphasis on radiology, pathology and clinical biology. I. The intramedullary cartilage tumors. *Skeletal Radiol*. 1997;26(6):325–353. https://doi.org/10.1007/s002560050246
3. Weber KL, Raymond AK. Low-grade/dedifferentiated/high-grade chondrosarcoma: a case of histological and biological progression. *Iowa Orthop J*. 2002;22:75–80.
4. Kristensen IB, Sunde LM, Jensen OM. Chondrosarcoma. Increasing grade of malignancy in local recurrence. *Acta Pathol Microbiol Immunol Scand A*. 1986;94(2):73–77.
5. Zhu GG, Nafa K, Agaram N, et al. Genomic profiling identifies association of IDH1/IDH2 mutation with longer relapse-free and metastasis-free survival in high-grade chondrosarcoma. *Clin Cancer Res*. 2020;26(2):419–427. https://doi.org/10.1158/1078-0432.CCR-18-4212
6. Amary MF, Bacsi K, Maggiani F, et al. IDH1 and IDH2 mutations are frequent events in central chondrosarcoma and central and periosteal chondromas but not in other mesenchymal tumours. *J Pathol*. 2011;224(3):334–343. https://doi.org/10.1002/path.2913
7. Pansuriya TC, van Eijk R, d'Adamo P, et al. Somatic mosaic IDH1 and IDH2 mutations are associated with enchondroma and spindle cell hemangioma in Ollier disease and Maffucci syndrome. *Nat Genet*. 2011;43(12): 1256–1261. https://doi.org/10.1038/ng.1004
8. Dang L, White DW, Gross S, et al. Cancer-associated IDH1 mutations produce 2-hydroxyglutarate. *Nature*. 2009;462(7274):739–744. https://doi.org/10.1038/nature08617
9. Suijker J, Baelde HJ, Roelofs H, Cleton-Jansen AM, Bovée JV. The oncometabolite D-2-hydroxyglutarate induced by mutant IDH1 or -2 blocks osteoblast differentiation in vitro and in vivo. *Oncotarget*. 2015;6(17):14832–14842. https://doi.org/10.18632/oncotarget.4024
10. Jin Y, Elalaf H, Watanabe M, et al. Mutant IDH1 dysregulates the differentiation of mesenchymal stem cells in association with gene-specific histone modifications to cartilage- and bone-related genes. *PLoS One*. 2015;10(7): e0131998. https://doi.org/10.1371/journal.pone.0131998
11. Tap WD, Villalobos VM, Cote GM, et al. Phase I study of the mutant IDH1 inhibitor ivosidenib: safety and clinical activity in patients with advanced chondrosarcoma. *J Clin Oncol*. 2020;38(15):1693–1701. https://doi.org/10.1200/JCO.19.02492
12. Bovée JV, Cleton-Jansen AM, Kuipers-Dijkshoorn NJ, et al. Loss of heterozygosity and DNA ploidy point to a diverging genetic mechanism in the origin of peripheral and central chondrosarcoma. *Genes Chromosomes Cancer*. 1999;26(3):237–246.
13. Hallor KH, Staaf J, Bovée JV, et al. Genomic profiling of chondrosarcoma: chromosomal patterns in central and peripheral tumors. *Clin Cancer Res*. 2009;15(8):2685–2694. https://doi.org/10.1158/1078-0432.CCR-08-2330
14. Cross W, Lyskjær I, Lesluyes T, et al. A genetic model for central chondrosarcoma evolution correlates with patient outcome. *Genome Med*. 2022;14(1):99. https://doi.org/10.1186/s13073-022-01084-0
15. Nicolle R, Ayadi M, Gomez-Brouchet A, et al. Integrated molecular characterization of chondrosarcoma reveals critical determinants of disease progression. *Nat Commun*. 2019;10(1):4622. https://doi.org/10.1038/s41467-019-12525-7
16. Koelsche C, Schrimpf D, Stichel D, et al. Sarcoma classification by DNA methylation profiling. *Nat Commun*. 2021;12(1):498. https://doi.org/10.1038/s41467-020-20603-4
17. Venneker S, Kruisselbrink AB, Baranski Z, et al. Beyond the influence of mutations: exploring epigenetic vulnerabilities in chondrosarcoma. *Cancers*. 2020;12(12). https://doi.org/10.3390/cancers12123589
18. Evans HL, Ayala AG, Romsdahl MM. Prognostic factors in chondrosarcoma of bone: a clinicopathologic analysis with emphasis on histologic grading. *Cancer*. 1977;40(2):818–831. https://doi.org/10.1002/1097-0142(197708)40:2<818::aid-cncr2820400234>3.0.co;2-b
19. Meijer DM, Ruano D, Briaire-de Bruijn IH, et al. The variable genomic landscape during osteosarcoma progression: insights from a longitudinal WGS analysis. *Genes Chromosomes Cancer*. 2024;63(7):e23253. https://doi.org/10.1002/gcc.23253
20. Anders S, Pyl PT, Huber W. HTSeq—a Python framework to work with high-throughput sequencing data. *Bioinformatics*. 2015;31(2):166–169. https://doi.org/10.1093/bioinformatics/btu638
21. Robinson MD, McCarthy DJ, Smyth GK. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics*. 2010;26(1):139–140. https://doi.org/10.1093/bioinformatics/btp616
22. Zhang Y, Parmigiani G, Johnson WE. ComBat-seq: batch effect adjustment for RNA-seq count data. *NAR Genom Bioinform*. 2020;2(3):lqaa078. https://doi.org/10.1093/nargab/lqaa078
23. Ducy P, Zhang R, Geoffroy V, Ridall AL, Karsenty G. Osf2/Cbfa1: a transcriptional activator of osteoblast differentiation. *Cell*. 1997;89(5):747–754. https://doi.org/10.1016/s0092-8674(00)80257-3
24. Nakashima K, Zhou X, Kunkel G, et al. The novel zinc finger-containing transcription factor osterix is required for osteoblast differentiation and bone formation. *Cell*. 2002;108(1):17–29. https://doi.org/10.1016/s0092-8674(01)00622-5
25. Jaiswal N, Haynesworth SE, Caplan AI, Bruder SP. Osteogenic differentiation of purified, culture-expanded human mesenchymal stem cells in vitro. *J Cell Biochem*. 1997;64(2):295–312.
26. Morinobu M, Ishijima M, Rittling SR, et al. Osteopontin expression in osteoblasts and osteocytes during bone formation under mechanical stress in the calvarial suture in vivo. *J Bone Miner Res*. 2003;18(9):1706–1715. https://doi.org/10.1359/jbmr.2003.18.9.1706
27. Ducy P, Karsenty G. Two distinct osteoblast-specific cis-acting elements control expression of a mouse osteocalcin gene. *Mol Cell Biol*. 1995;15(4): 1858–1869. https://doi.org/10.1128/MCB.15.4.1858
28. van der Rest M, Garrone R. Collagen family of proteins. *FASEB J*. 1991;5(13): 2814–2823.
29. Kim RH, Shapiro HS, Li JJ, Wrana JL, Sodek J. Characterization of the human bone sialoprotein (BSP) gene and its promoter sequence. *Matrix Biol*. 1994;14(1):31–40. https://doi.org/10.1016/0945-053x(94)90027-2
30. Hoang B, Moos M Jr, Vukicevic S, Luyten FP. Primary structure and tissue distribution of FRZB, a novel protein related to Drosophila frizzled, suggest a role in skeletal morphogenesis. *J Biol Chem*. 1996;271(42):26131–26137. https://doi.org/10.1074/jbc.271.42.26131
31. Ross FP, Chappel J, Alvarez JI, et al. Interactions between the bone matrix proteins osteopontin and bone sialoprotein and the osteoclast integrin alpha V beta 3 potentiate bone resorption. *J Biol Chem*. 1993;268(13): 9901–9907.
32. Lefebvre V, Huang WD, Harley VR, Goodfellow PN, deCrombrugghe B. SOX9 is a potent activator of the chondrocyte-specific enhancer of the pro alpha1 (II) collagen gene. *Mol Cell Biol*. 1997;17(4):2336–2346. https://doi.org/10.1128/MCB.17.4.2336
33. Han Y, Lefebvre V. L-Sox5 and Sox6 drive expression of the aggrecan gene in cartilage by securing binding of Sox9 to a far-upstream enhancer. *Mol Cell Biol*. 2008;28(16):4999–5013. https://doi.org/10.1128/MCB.00695-08
34. Hedbom E, Antonsson P, Hjerpe A, et al. Cartilage matrix proteins. An acidic oligomeric protein (COMP) detected only in cartilage. *J Biol Chem*. 1992;267 (9):6132–6136.
35. Lazarus JE, Hegde A, Andrade AC, Nilsson O, Baron J. Fibroblast growth factor expression in the postnatal growth plate. *Bone*. 2007;40(3):577–586. https://doi.org/10.1016/j.bone.2006.10.013
36. Tagariello A, Luther J, Streiter M, et al. Ucma—a novel secreted factor represents a highly specific marker for distal chondrocytes. *Matrix Biol*. 2008;27 (1):3–11. https://doi.org/10.1016/j.matbio.2007.07.004
37. Akiyama H, Shigeno C, Iyama K, et al. Indian hedgehog in the late-phase differentiation in mouse chondrogenic EC cells, ATDC5: upregulation of type X collagen and osteoprotegerin ligand mRNAs. *Biochem Biophys Res Commun*. 1999;257(3):814–820. https://doi.org/10.1006/bbrc.1999.0494
38. Zheng Q, Zhou G, Morello R, Chen Y, Garcia-Rojas X, Lee B. Type X collagen gene regulation by Runx2 contributes directly to its hypertrophic chondrocyte-specific expression in vivo. *J Cell Biol*. 2003;162(5):833–842. https://doi.org/10.1083/jcb.200211089
39. Wang Y, Middleton F, Horton JA, Reichel L, Farnum CE, Damron TA. Microarray analysis of proliferative and hypertrophic growth plate zones identifies differentiation markers and signal pathways. *Bone*. 2004;35(6): 1273–1293. https://doi.org/10.1016/j.bone.2004.09.009
40. van Praag Veroniek M, Rueten-Budde AJ, Ho V, et al. Incidence, outcomes and prognostic factors during 25 years of treatment of chondrosarcomas. *Surg Oncol*. 2018;27(3):402–408. https://doi.org/10.1016/j.suronc.2018.05.009
41. Boxall SA, Jones E. Markers for characterization of bone marrow multipotential stromal cells. *Stem Cells Int*. 2012;2012:975871. https://doi.org/10.1155/2012/975871
42. Siegel RL, Giaquinto AN, Jemal A. Cancer statistics, 2024. *CA Cancer J Clin*. 2024;74(1):12–49. https://doi.org/10.3322/caac.21820
43. Meijer D, Gelderblom H, Karperien M, Cleton-Jansen AM, Hogendoorn PC, Bovée JV. Expression of aromatase and estrogen receptor alpha in chondrosarcoma, but no beneficial effect of inhibiting estrogen signaling both

in vitro and in vivo. *Clin Sarcoma Res*. 2011;1(1):5. https://doi.org/10.1186/2045-3329-1-5
44. Cleton-Jansen AM, van Beerendonk HM, Baelde HJ, Bovée JV, Karperien M, Hogendoorn PC. Estrogen signaling is active in cartilaginous tumors: implications for antiestrogen therapy as treatment option of metastasized or irresectable chondrosarcoma. *Clin Cancer Res*. 2005;11(22):8028–8035. https://doi.org/10.1158/1078-0432.CCR-05-1253
45. Reifenberger J, Reifenberger G, Liu L, James CD, Wechsler W, Collins VP. Molecular genetic analysis of oligodendroglial tumors shows preferential allelic deletions on 19q and 1p. *Am J Pathol*. 1994;145(5):1175–1190.
46. Riemenschneider MJ, Jeuken JW, Wesseling P, Reifenberger G. Molecular diagnostics of gliomas: state of the art. *Acta Neuropathol*. 2010;120(5):567–584. https://doi.org/10.1007/s00401-010-0736-4
47. Cairncross JG, Ueki K, Zlatescu MC, et al. Specific genetic predictors of chemotherapeutic response and survival in patients with anaplastic oligodendrogliomas. *J Natl Cancer I*. 1998;90(19):1473–1479. https://doi.org/10.1093/jnci/90.19.1473
48. Grimwood J, Gordon LA, Olsen A, et al. The DNA sequence and biology of human chromosome 19. *Nature*. 2004;428(6982):529–535. https://doi.org/10.1038/nature02399
49. Corver WE, Ruano D, Weijers K, et al. Genome haploidisation with chromosome 7 retention in oncocytic follicular thyroid carcinoma. *PLoS One*. 2012;7(6):e38287. https://doi.org/10.1371/journal.pone.0038287
50. Boot A, Oosting J, de Miranda NF, et al. Imprinted survival genes preclude loss of heterozygosity of chromosome 7 in cancer cells. *J Pathol*. 2016;240(1):72–83. https://doi.org/10.1002/path.4756
51. Tallegas M, Miquelestorena-Standley É, Labit-Bouvier C, et al. IDH mutation status in a series of 88 head and neck chondrosarcomas: different profile between tumors of the skull base and tumors involving the facial skeleton and the laryngotracheal tract. *Hum Pathol*. 2019;84:183–191. https://doi.org/10.1016/j.humpath.2018.09.015
52. Puviindran V, Shimada E, Huang Z, et al. Single cell analysis of Idh mutant growth plates identifies cell populations responsible for longitudinal bone growth and enchondroma formation. *Sci Rep*. 2024;14(1):26208. https://doi.org/10.1038/s41598-024-76539-y