

Chromosome Instability Accounts for Reverse Metastatic Outcomes of Pediatric and Adult Synovial Sarcomas

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ABSTRACT

Purpose

Synovial sarcoma (SS) occurs in both children and adults, although metastatic events are much more common in adults. Whereas the importance of the t(X;18) translocation in SS oncogenesis is well established, the genetic basis of SS metastasis is still poorly understood. We recently reported expression (CINSARC; Complexity Index in Sarcoma) and Genomic Index prognostic signatures related to chromosome integrity in sarcomas and GI stromal tumors. Here we investigate whether these signatures can also predict outcomes in SS.

Patients and Methods

One hundred patients who had primary untreated SS tumors were selected for expression and genomic profiling in a training/validation approach.

Results

CINSARC and Genomic Index have strong independent and validated prognostic values ($P < .001$). By comparing expression profiles of tumors with or without metastasis, 14 genes that are common to the CINSARC signature were identified, and the two top-ranked genes, *KIF14* and *CDCA2*, were validated as prognostic markers in an independent cohort. Comparing genomic profiles of adult versus pediatric SS, we show that metastasis is associated with genome complexity in both situations and that the adult genome is more frequently rearranged. Accordingly, pediatric patients with an even genomic profile do not develop metastasis.

Conclusion

Metastasis development in SS is strongly associated with chromosome complexity, and CINSARC and Genomic Index are validated independent prognostic factors. The differences in metastasis frequency between adults and children are associated with genome instability, which is much more frequent in adults. Genomic Index is potentially the best overall biomarker and clearly the most clinically relevant, considering that genome profiling from formalin-fixed samples is already used in pathology.

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INTRODUCTION

Synovial sarcomas (SSs) are high-grade soft tissue tumors accounting for 5% to 10% of all soft tissue sarcomas.¹ They occur in adolescents as well as in adults.² Two histologically distinct subtypes of SS can be distinguished: biphasic tumors containing both epithelial-like and spindle cells and monophasic lesions containing only spindle cells. They are characterized by a specific translocation t(X;18) (p11.2;q11.2) that occurs in more than 95% of patients and leads to two main chimeric fusion genes, *SYT-SSX1* and *SYT-SSX2*.^{3,4} A strong association between fusion type and morphology has been reported, with the majority of *SYT-SSX2* tumors showing a monophasic phenotype and almost all

biphasic tumors containing an *SYT-SSX1* rearrangement.⁵ Reported 5-year survival rates vary from 40% to 60%.⁶ Various factors have been proposed as prognostic factors^{7,8}; however, there is currently no consensus. Three series report chromosomal instabilities by comparative genomic hybridization (CGH) in SS.⁹⁻¹¹ Genetic changes are more complex and common in monophasic tumors than in biphasic tumors.^{10,11} Yet there has been no validation of the relationship between chromosomal instabilities and clinical outcomes. Pediatric patients are known to have excellent clinical outcomes in comparison with adults, whereas both harbor exactly the same histologic features and translocations. To the best of our knowledge, no explanation has been proposed so far to explain this clinical observation.

We recently established a 67-gene prognostic signature related to chromosome integrity, mitotic control, and genome complexity in sarcomas (CINSARC: Complexity Index in Sarcoma)¹² and reported its application in different types of sarcomas, especially in GI stromal tumors, in which we subsequently identified a genomic biomarker—the Genomic Index—that is based on the number and type of chromosomal aberrations.¹³ To assess the effectiveness of these biomarkers in SS and to identify the underlying mechanisms leading to high CINSARC scores, as well as to search for an explanation for the distinct pediatric and adult outcomes, we performed genome-wide DNA copy number and gene expression analyses in a training/validation approach on 100 patients with fully annotated records regarding primary untreated SS.

PATIENTS AND METHODS

Tumor Samples

Two series of primary untreated frozen SS samples, 60 from the French Sarcoma Group (training set) and 40 tumors from Leuven Cancer Institute (validation set), were selected from CONTICABASE (the European Connective Tissue Cancer Database). Dates of diagnosis were from September 1990 to September 2009. Patient and tumor information is summarized in Table 1.

The samples included in the training set are part of the Biologic Resources Center of Institut Bergonié (CRB-IB). In accordance with the French Public Health Code (articles L. 1243-4 and R. 1243-61), the CRB-IB has received authorization from the French authorities to deliver samples for scientific research (No. AC-2008-812, February 2011). These samples originate from patient care and are requalified for research. The project was approved by the French Committee for the Protection of Individuals. The samples included in the validation set originate from patient care and were requalified for research. The study was approved by Ethical Committee of Katholieke Universiteit Leuven.

Array-CGH Analysis

DNA was hybridized either to 8 × 60K whole-genome Agilent arrays (G4450A) for the training set or to 4 × 180K whole-genome Agilent arrays (G4449A) for the validation set (Agilent Technologies, Santa Clara, CA), according to the manufacturer's protocol. The ADM-2 algorithm of the CGH Analytics, v4.0.76, software (Agilent Technologies) was used to identify DNA copy number anomalies at the probe level. A low-level copy number gain was defined as a log₂ ratio more than 0.25, and a copy number loss was defined as a log₂ ratio less than -0.25. A high-level gain or amplification was defined as a log₂ ratio more than 1.5, and a homozygous deletion was suspected when the log₂ ratio was below -1. The Genomic Index was calculated for each profile as follows: Genomic Index = A²/C, where A is the total number of alterations (segmental gains and losses) and C is the number of involved chromosomes.

Gene Expression Profiling

Total RNA was extracted from frozen tumor samples by using the miR-Neasy Mini Kit (Qiagen, Germantown, MD) and was purified by using the RNeasy Min Elute TM Cleanup Kit (Qiagen), according to the manufacturer's instructions. RNA quality was checked on an Agilent 2100 bioanalyzer (Agilent Technologies). Gene expression analysis was carried out by using Agilent Whole Human 44K Genome Oligo Array (G4112F and G4845A, for training and validation sets, respectively; Agilent Technologies), according to the manufacturer's protocol. For each series, all microarrays were simultaneously normalized by using the Quantile algorithm. *t* tests were performed by using GeneSpring GX software (Agilent Technologies), and *P* values were adjusted by using the Benjamini-Hochberg procedure. Gene ontology (GO) analysis was performed to establish statistical enrichment in GO terms by using GeneSpring GX software.

Table 1. Patient and Tumor Characteristics for Patients With Synovial Sarcoma

| Characteristic | French Sarcoma Group Series (training set; n = 60) | | | Leuven Cancer Institution Series (validation set; n = 40) | | |
|-------------------------------------------|----------------------------------------------------|-----------|--------------|-----------------------------------------------------------|-----------|--------------|
| | No. | % | 95% CI | No. | % | 95% CI |
| Median follow-up, months | 34.2 | | 25.2 to 43.2 | 39.9 | | 24.7 to 55.2 |
| Age, years | | | | | | |
| Median | 24 | | | 38 | | |
| SD | 15.26 | | | 19.14 | | |
| Male sex | 35 | 58 | | 24 | 60 | |
| FNCLCC grade | | | | | | |
| 2 | 17 | 28 | | 18 | 45 | |
| 3 | 28 | 47 | | 14 | 35 | |
| N/D | 15 | 25 | | 8 | 20 | |
| Fusion transcript type | | | | | | |
| SYT-SSX1 | 42 | 70 | | 24 | 60 | |
| SYT-SSX2 | 18 | 30 | | 16 | 40 | |
| Location | | | | | | |
| Extremities | 43 | 72 | | 25 | 63 | |
| Trunk wall | 11 | 18 | | 13 | 32 | |
| Head and neck | 5 | 8 | | 2 | 5 | |
| N/D | 1 | 2 | | | | |
| Size, cm | | | | | | |
| > 5 | 40 | 67 | | 21 | 53 | |
| < 5 | 17 | 28 | | 14 | 35 | |
| N/D | 3 | 5 | | 5 | 12 | |
| Histologic subtype | | | | | | |
| Monophasic | 28 | 47 | | 27 | 67 | |
| Biphasic | 7 | 12 | | 13 | 33 | |
| N/D | 25 | 41 | | | | |
| Age ≤ 18 years at diagnosis and treatment | 27 | 45 | | 6 | 15 | |
| Chemotherapy | 24 | 89 | | 3 | 50 | |
| Neoadjuvant | 10 | 37 | | | | |
| Adjuvant | 7 | 26 | | 3 | 50 | |
| Neoadjuvant plus adjuvant | 7 | 26 | | | | |
| Radiotherapy | 16 | 60 | | 5 | 80 | |
| Age > 18 years at diagnosis and treatment | 33 | 55 | | 34 | 85 | |
| Chemotherapy | 21 | 64 | | 7 | 21 | |
| Neoadjuvant | 7 | 21 | | 2 | 6 | |
| Adjuvant | 12 | 36 | | 5 | 15 | |
| Neoadjuvant plus adjuvant | 2 | 6 | | | | |
| Radiotherapy | 24 | 73 | | 12 | 35 | |
| Metastatic events | 29 | 48 | | 22 | 55 | |

NOTE. Bold indicates total number and rate of patients with chemotherapy. Abbreviations: FNCLCC, Fédération Nationale des Centres de Lutte Contre le Cancer; N/D, not determined; SD, standard deviation.

Quantitative Reverse Transcription and Real-Time Polymerase Chain Reaction

Quantitative reverse transcription and quantitative real-time polymerase chain reaction for *CDCA2* and *KIF14* were carried out as previously described¹⁴ by using the TaqMan Gene Expression Assays Hs00299250_m1 and Hs00208408_m1, respectively (Applied Biosystems, Foster City, CA).

A reference Ct (threshold cycle) for each sample was defined as the average measured Ct of the two reference genes, *ACTB* and *RPLP0*. Relative mRNA level in a sample was defined as $\Delta Ct = Ct(\text{gene of interest}) - Ct(\text{mean of the two reference genes})$.

SS Prognosis Related to Chromosome Instability

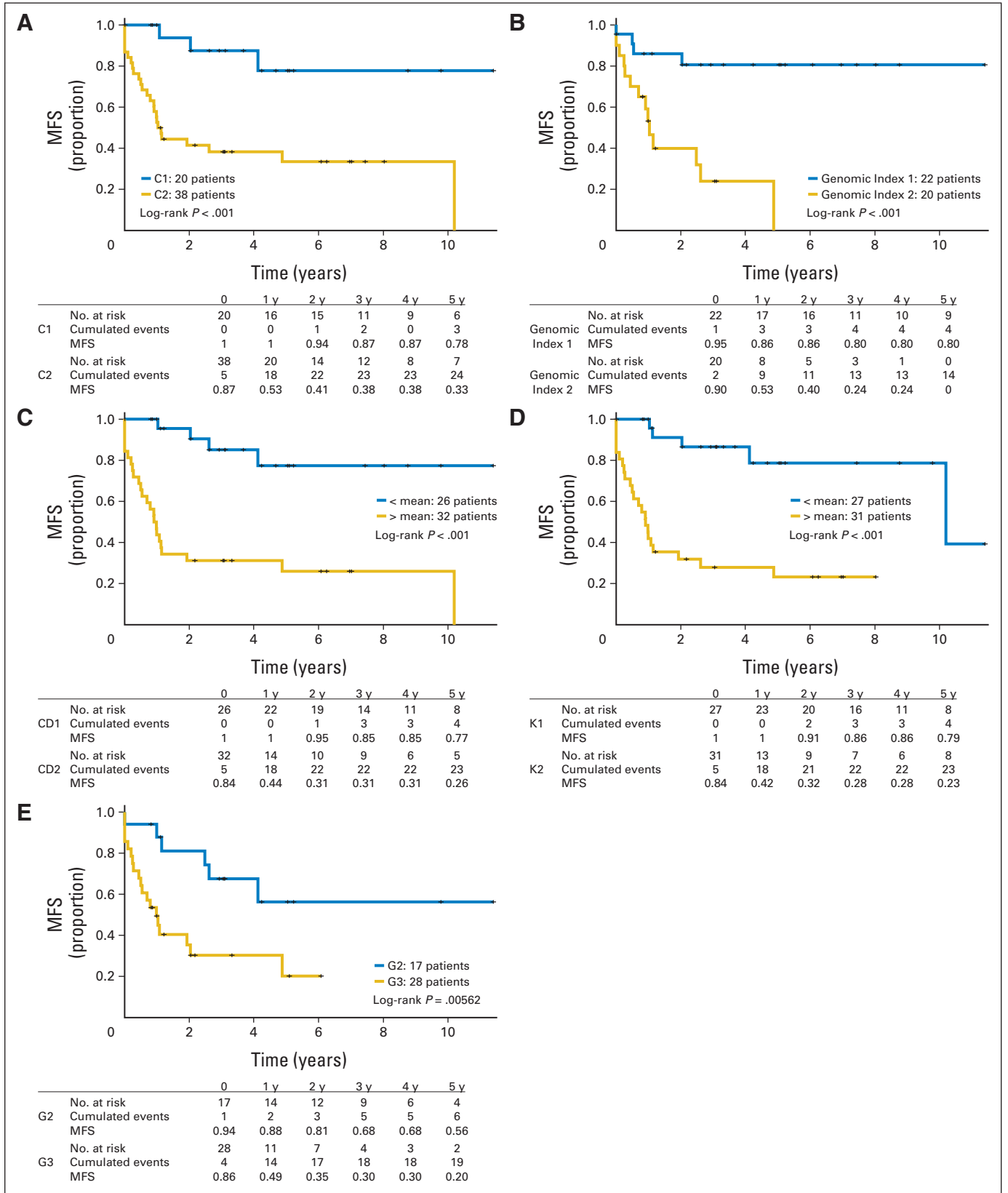


Fig 1. Kaplan-Meier analysis of metastasis-free survival (MFS) in the training set of synovial sarcoma according to (A) Complexity Index in Sarcoma (CINSARC; C1 and C2), (B) Genomic Index (Genomic Index 1 and Genomic Index 2), (C) cell division cycle A2 (*CDCA2*) expression (CD1 and CD2), (D) kinesin family member 14 (*KIF14*) expression (K1 and K2), and (E) Fédération Nationale des Centres de Lutte Contre le Cancer (FNCLCC) grade (G2 and G3). y, year.

Statistical Analysis

The CINSARC reference centroids are mean-centered profiles, determined and fixed from the CINSARC signature gene expression in the 310 metastatic and nonmetastatic sarcomas from our previous study.¹² Each individual SS was allocated to the prognostic class with the highest Spearman correlation to the reference centroids. Metastasis-free survival was calculated by the Kaplan-Meier method from the date of initial diagnosis to the date of first metastasis, last follow-up, or death for all patients without diagnosis of metastasis. Survival curves were compared with the log-rank test. All survival analyses were performed by using R software, version 2.14.1 (R Development Core Team, Vienna, Austria, 2009), and a survival package (A Package for Survival Analysis in S; Terry Therneau, February 2002; R package, version 2.36-14). Multivariate analysis was done by using Cox regression with Firth's correction (R by Meinhard Ploner and Fortran by Georg Heinze, 2011; coxphf: Cox regression with Firth's penalized likelihood. R package, version 1.08. <http://CRAN.R-project.org/package=>

RESULTS

Are CINSARC and Genomic Index Significant Prognostic Factors for Synovial Sarcomas?

To test whether the CINSARC signature has prognostic value in SS, we carried out gene expression profiling on 58 SS tumors from the training series (Table 1) with mRNA of sufficient quality. We assigned these tumors to prognostic groups (Data Supplement) on the basis of correlations with the published CINSARC centroids from our previous series of 310 sarcomas.¹² Survival analysis (Fig 1A) revealed that the CINSARC classification split the tumors into two groups with different metastasis-free survival (MFS; $P = 6.3 \times 10^{-4}$).

We performed genome profiling on 42 SS tumors from the training series and observed that the profiles of tumors that did not undergo metastasis had few or no losses or gains and, when present, generally involved whole chromosomes, whereas the tumors that developed metastases more frequently harbored segmental alterations (Data Supplement). We therefore tested whether genome complexity could predict metastatic outcome. To take into account the number and the type of changes, we applied the previously described Genomic Index¹³ with scores ranging from 0 to 56 across the entire series. The proportion of patients with metastasis increased with increasing Genomic Index score. Patients with metastasis predominated when the Genomic Index was above 1 (Data Supplement). Stratification by Genomic Index at a cutoff of 1 split the tumors into two groups with different outcomes (MFS $P = 2.5 \times 10^{-4}$; Fig 1B; Data Supplement).

Is It Possible to Derive a Better Signature Specific for SS?

The CINSARC signature was established on several different types of sarcoma. To test whether it is possible to derive a better signature that is specific for SS, we analyzed the SS gene-expression profiles to identify genes differentially expressed by metastatic and nonmetastatic tumors. Among the 64 differentially expressed genes (Data Supplement), five (seven probe sets) were downregulated and 59 (65 probe sets) were upregulated in patients with metastasis (fold change > 2 ; $P < .001$). GO analysis identified no significantly enriched pathways for the five downregulated genes. In contrast, GO analysis revealed that 71 (41%) of the 172 pathways containing upregulated SS genes were also identified by GO analysis with the CINSARC genes (Data Supplement). Indeed, 14 (24%) of the 59 upregulated SS genes belonged directly to the CINSARC signature (Data Supplement).

Table 2. Prognostic Value of CINSARC Signature, Genomic Index, and CDCA2 and KIF14 Expression Evaluated Against the FNCLCC Grading System

| Variable | Univariate Analyses | | | Multivariate Analyses | | |
|---------------|---------------------|----------------------|----------------------------------------|-----------------------|----------------------|--------------|
| | HR | 95% CI | P | HR | 95% CI | P |
| CINSARC | 6.2 | 1.88 to 20.71 | 6.3×10^{-4} | 6.58 | 1.57 to 61.01 | .007 |
| FNCLCC | 3.4 | 1.36 to 8.9 | 5.6×10^{-3} | 2.19 | 0.72 to 8.70 | .17 |
| Genomic Index | 21.34 | 2.08 to 20.68 | 2.8×10^{-4} | 4.20 | 1.49 to 14.26 | .006 |
| FNCLCC | 3.4 | 1.36 to 8.9 | 5.6×10^{-3} | 3.38 | 1.12 to 13.41 | .03 |
| CDCA2 | 7.6 | 2.63 to 22 | 1.1×10^{-5} | 4.83 | 1.49 to 20.37 | .007 |
| FNCLCC | 3.4 | 1.36 to 8.9 | 5.6×10^{-3} | 1.66 | 0.51 to 7.03 | .42 |
| KIF14 | 8.04 | 2.77 to 23.37 | 5.9×10^{-6} | 6.58 | 1.92 to 34.27 | .0016 |
| FNCLCC | 3.4 | 1.36 to 8.9 | 5.6×10^{-3} | 1.98 | 0.63 to 7.97 | .25 |

NOTE. Analyses used Cox regression model. Significant results are indicated in bold.
Abbreviations: CDCA2, cell division cycle A2; CINSARC, Complexity Index in Sarcoma; FNCLCC, Fédération Nationale des Centres de Lutte Contre le Cancer; HR, hazard ratio; KIF14, kinesin family member 14.

Among the top-ranked differentially expressed genes identified by *t* tests, CDCA2 (cell division cycle A2) and KIF14 (kinesin family member 14) were the highest ranked genes (fold change > 3 ; Data Supplement) belonging to the CINSARC signature (Data Supplement). Validation by quantitative real-time polymerase chain reaction showed that there was a high correlation between the microarray and polymerase chain reaction data for both CDCA2 and KIF14 (Pearson correlation coefficient $r = 0.8$; $P < 1 \times 10^{-6}$ and $r = 0.81$; $P < 1 \times 10^{-6}$, respectively). We then stratified samples according to CDCA2 and KIF14 expression by using the mean expression levels (9.9 for CDCA2 and 5.3 for KIF14) as cutoffs in the subsequent two analyses (Data Supplement). Survival analysis showed that both CDCA2 expression and KIF14 expression split the tumors into two groups with different outcomes (CDCA2 MFS $P = 1.13 \times 10^{-5}$ and KIF14 MFS $P = 5.93 \times 10^{-6}$; Figs 1C and 1D).

Independency and Validation of CINSARC, Genomic Index, and CDCA2 and KIF14 Expression

Multivariate analyses comparing each molecular signature to the Fédération Nationale des Centres de Lutte Contre le Cancer (FNCLCC) grading system (Fig 1E; Table 2) demonstrated that the CINSARC signature, Genomic Index, and CDCA2 and KIF14 expression are all independent from FNCLCC prognostic factors, which split FNCLCC G2 or G3 tumors into good and bad prognostic tumor groups (Data Supplement). Among these molecular markers, the risk of metastasis was the highest in tumors with high KIF14 expression (hazard ratio, 6.58; 95% CI, 1.92 to 34.27; Table 2). To validate the prognostic value of CINSARC, Genomic Index, CDCA2, and KIF14, we performed genomic and expression profiling in a second independent series of 40 primary untreated SS tumors (Data Supplement). MFS analyses confirmed that all these factors split SS tumors into groups with large and significant differences in MFS (Fig 2).

Are Pediatric and Adult SS the Same Disease?

SS can arise in pediatric or adult contexts. Even if tumors in both contexts share the same histologic features, translocations, and types of fusion transcripts, they have totally different metastatic outcomes. Thus, we asked whether this difference could be explained by genes

SS Prognosis Related to Chromosome Instability

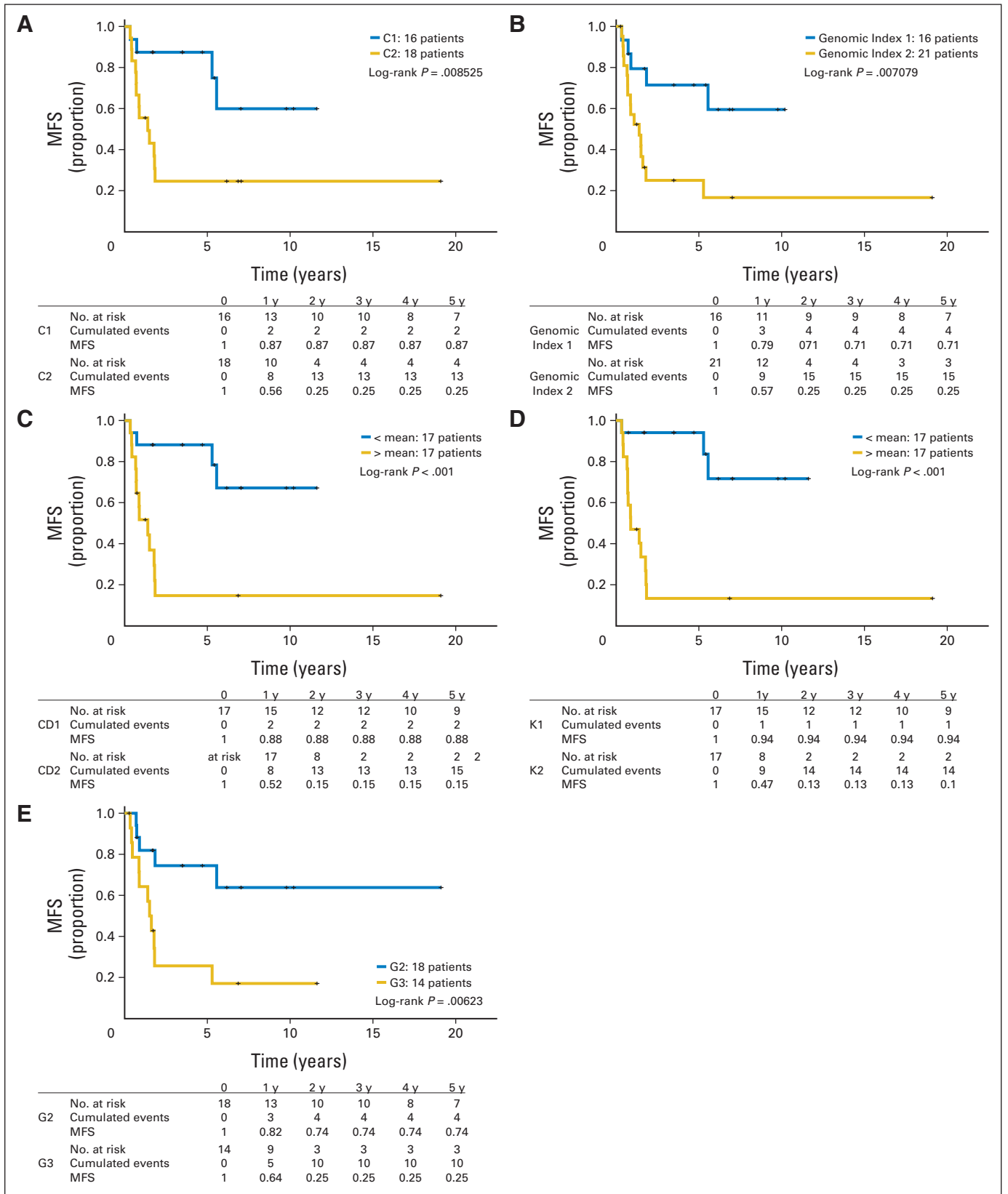


Fig 2. Kaplan-Meier analysis of metastasis-free survival (MFS) in the validation set of synovial sarcoma according to (A) Complexity Index in Sarcoma (CINSARC; C1 and C2), (B) Genomic Index (Genomic Index 1 and Genomic Index 2), (C) cell division cycle A2 (*CDCA2*) expression (CD1 and CD2), (D) kinesin family member 14 (*KIF14*) expression (K1 and K2), and (E) Fédération Nationale des Centres de Lutte Contre le Cancer (FNCLCC) grade (G2 and G3).

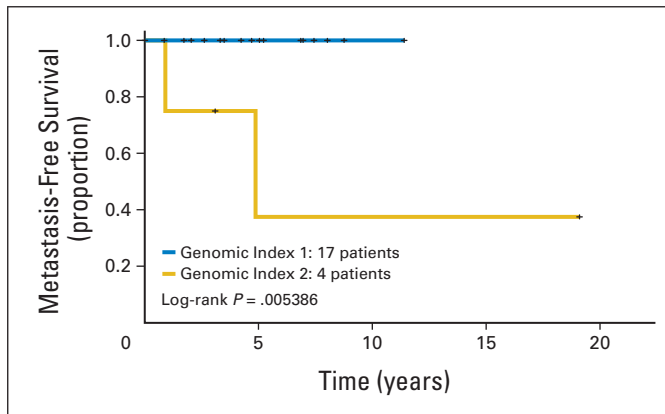


Fig 3. Kaplan-Meier analysis of metastasis-free survival according to Genomic Index (Genomic Index 1 and Genomic Index 2) in the pediatric subpopulation of synovial sarcoma.

differentially expressed between these two entities, but clustering analysis did not demonstrate any specific genes that would enable us to discriminate pediatric and adult patient tumors, and by using a supervised approach, we could not identify any differentially expressed genes ($P < .001$). On the contrary, we observed a strong link between the level of genome complexity and the metastatic outcome, especially in pediatric patients. Across pediatric patients from both series, we observed that all patients without any detectable quantitative rearrangements (even CGH profile) did not develop any metastasis, whereas of the four (four [19%] of 21) patients with a rearrangement, two recurred with a metastasis (Fig 3). The situation contrasts with the adult context, in which a much higher proportion of patients harbor rearrangement profiles (37 of 58; 64% v 19% of pediatric patients), of whom 28 (76%) developed metastasis.

DISCUSSION

The development of a valid and reliable investigator-independent method of SS prognostication is essential for the proper clinical management of patients, especially in the context of pediatric disease in which all patients are considered to have highly aggressive sarcoma.²

One of the main conclusions of this study is that it offers a biologic explanation for the roughly opposite outcomes of pediatric and adult patients with SS. Results show that metastatic outcomes are strongly associated with chromosomal complexity in both age strata and that this instability is frequent in adult but not in pediatric SS. Given that the initial genetic driver event (the t(X;18) translocation) is the same for both situations, this is likely to mean that an independent, still unknown mechanism is permissive to chromosome instability in adult patients and resistant in pediatric patients. We did not identify any genomic alterations or significantly differentially expressed genes indicative of such mechanisms. We hypothesized that this discrepancy could be the result of somatic events not detectable by using array-CGH or that permissiveness to chromosomal complexity could be more likely related to the possible patient predilection, age, or genome aging.

Only a few studies have reported extensive SS genome characterization^{9,11,15}; they all describe SSs as frequently having tumors with rearranged genomic profiles but they fail to demonstrate a link be-

tween these rearranged genomic profiles and outcomes. Reported results of two studies that did not observe any association between chromosomal complexity and metastasis outcome^{11,15} contradict results of a third study that reported the link but only in a small subset of tumors (22 patients) in which the link was not validated.⁹ Applying the recently published Genomic Index,¹³ we demonstrate and validate in a training/validation approach (79 SS tumors were collected) that chromosome complexity as evaluated by CGH is strongly associated with metastatic outcome. Given that multivariate analysis shows that Genomic Index is a stronger prognostic factor than histologic grade and that CGH is a technique applicable to formalin-fixed paraffin-embedded (FFPE) samples, which is already used routinely,¹⁶ we are working on prospectively validating the Genomic Index to make it a molecular prognostic marker that could drive therapeutic management. This is particularly necessary in the pediatric context in which virtually all patients are currently given radiotherapy and/or chemotherapy, although our results indicate that most of them (patients with an even genomic profile and thus no metastatic risk) would not benefit from this care. An alternative hypothesis could be that these patients with an even profile are indeed good responders to chemotherapy compared with patients with rearranged profiles. Regarding this hypothesis, we note that in our series, pediatric patients were more frequently treated with chemotherapy (81.8% of pediatric patients [27 of 33 in both series with chemotherapy] v 41.7% of adult patients [28 of 67 in both series with chemotherapy]; Table 1) and received twice as much chemotherapy as adults (mean doxorubicin dose, 233 v 117 mg/m²; mean ifosfamide dose, 35 v 17 g/m² for pediatric and adult patients, respectively; Data Supplement). Thus, one can ask whether Genomic Index is more predictive of response to chemotherapy than a prognostic biomarker, as has been proposed for CINSARC.¹⁷ To assess this issue, we are setting up a larger cohort of patients with SS to be profiled by using CGH from FFPE blocks. The question of drug response after neoadjuvant treatment according to Genomic Index will be addressed by evaluating histologic response.

The second conclusion from this study is that the CINSARC score is a strong and validated predictor of metastasis in patients with SS. None of the previously published gene expression studies in SS has reported any prognostic expression signatures nor have they sought to understand tumor aggressiveness and progression; instead, they have focused their approaches on deciphering SS biology, the impact of the fusion transcript, or tumor differentiation.¹⁸⁻²³ Likewise, none of these studies compared pediatric patients with adult patients. One explanation could be the rarity of SS, especially pediatric SS, the related limited size of cohorts, and the difficulty of obtaining fully annotated patient records. Here, by using a training/validation approach gathering 92 tumors from patients with SS, we validate the CINSARC signature as a new prognostic marker in SS.

Prognostic expression signatures have demonstrated their experimental efficacy in several other tumor types, but their clinical application has been complicated by technical issues such as weak reproducibility across array platforms. Importantly, we show that CINSARC scoring is platform-independent; the signature we developed on Affymetrix data was applied and validated here on Agilent data and previously validated in other sarcomas.¹³ All together, the CINSARC score is prognostic for SS, for the non-translocation-related sarcomas on which it was originally developed,¹² and for GI stromal tumors.¹³ The CINSARC signature comprises 67 genes (Data Supplement) involved in the maintenance of chromosome integrity

and mitotic control,¹² indicating that these processes play a crucial role in the development of metastasis in sarcomas from the three different groups of genetics: sarcomas characterized by a specific translocation, a point mutation, or a complex karyotype.

Supervised analysis comparing SS with or without metastatic outcomes showed that 14 of 59 genes prognostic in SS were common to the CINSARC signature. The top-ranked genes common to both approaches were *CDCA2* and *KIF14*, and each of them was also an independent prognostic factor for SS. *KIF14* belongs to the large family of kinesin proteins and appears to be a well-known oncogene located in 1q32.1 that is overexpressed and associated with metastatic outcomes in lung,²⁴ breast,²⁵ ovary,²⁶ and liver²⁷ carcinomas. *KIF14* targets the central spindle via its interaction with protein regulating cytokinesis 1 (PRC1) and has an essential function in cytokinesis.²⁸ *KIF14* depletion leads to cytokinesis failure and cell cycle arrest.^{28,29} The impact of *KIF14* overexpression is still unknown, but it is of interest to note that DNA content, notably aneuploidy, which can occur as a result of cytokinesis failure, has been recently shown to be associated with metastatic outcome in SS,³⁰ as reported here for *KIF14* high expression. *CDCA2* (also known as Repo-Man) has been initially identified as a nuclear protein that is a specific regulatory subunit for PP1 γ .³¹ *CDCA2* is recruited to mitotic chromatin at anaphase where it promotes chromosome decondensation by dephosphorylation of histone H3 and coordinates nuclear envelope reformation.³² Recently Wurzenberger et al³³ reported the involvement of *CDCA2* in the regulation of the microtubule-kinetochore interface during anaphase for faithful chromosome segregation. *CDCA2* is located in 8p21.2 and has not yet, to the best of our knowledge, been directly implicated in oncogenesis. Overall, the two most significant genes in SS prognostication are deeply involved in the control of DNA content and chromosome integrity by their action in the late phase of mitosis, suggesting a potentially interesting biologic link between chromosome instability and metastatic outcome.

To conclude, this study offers results that are meaningful for understanding the metastatic potential of sarcomas in general, and particularly in SS for which this study provides a biologic explanation for the distinct aggressiveness of pediatric and adult SS. The four signatures have high sensitivity and specificity, but multivariate analysis shows that the Genomic Index is the best overall prognostic factor (hazard ratio, 4.4; 95% CI, 1.19 to 4.95; $P = .026$), and Genomic Index is applicable to FFPE SS samples (Data Supplement). Altogether, this prompts us to now validate in a prospective study the Genomic Index as decision criteria for the clinical management of patients with SS.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

AUTHOR CONTRIBUTIONS

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Data analysis and interpretation: Pauline Lagarde, Joanna Przybyl, Céline Brulard, Gaëlle Pérot, Antoine Italiano, Frédéric Chibon

Manuscript writing: All authors

Final approval of manuscript: All authors

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