Journal of Molecular Biomarkers & Diagnosis

Christelle, et al., J Mol Biomark Diagn 2015, 6:2
http://dx.doi.org/10.4172/2155-9929.1000229

Research Article Open Access

Molecular Reclassification in Pediatric Osteosarcomas at Surgical Resection is a Potential Helpful Prognostic Marker

Lasthaus Christelle[1], **Litzler Marie**[1], **Marcellin Luc**[2], **Chenard Marie-Pierre**[2,3], **Marec-Berard Perrine**[4], **Tabone Marie-Dominique**[5], **Pacquement Hélène**[6], **Brugières Laurence**[7], **Guenot Dominique**[1] **and Entz-Werlé Natacha**[1,8,9*]

[1]*EA3430 –Progression tumorale et microenvironnement. Approches translationnelles et épidémiologie - 3 Avenue Molière, Strasbourg, France*

[2]*Laboratoire d'Anatomie Pathologique - CHU Hautepierre - Avenue Molière, Strasbourg, France*

[3]*Centre de Ressource Biologique - CHU Hautepierre - Avenue Molière, Strasbourg, France*

[4]*Service d'Oncologie Pédiatrique – Centre Anticancéreux Léon Bérard,Lyon, France*

[5]*Service d' Hémato-Oncologie pédiatrique – Hôpital Trousseau, Paris, France*

[6]*Service de Pédiatrie Oncologique – Institut Curie, Paris, France*

[7]*Service de Cancérologie de l'enfant et de l'adolescent –Gustave Roussy Cancer Campus, Villejuif, France*

[8]*Service de Pédiatrie Onco-Hématologie - CHU Hautepierre - Avenue Molière, Strasbourg, France*

[9]*FMTS, Fédération de Médecine Translationnelle de Strasbourg – Faculté de Médecine –Université de Strasbourg – 4 rue Kirschleger, Strasbourg, France*

[*]**Corresponding author:** Natacha Entz-Werlé, EA3430 –Progression tumorale et microenvironnement. Approches translationnelles et épidémiologie - 3 Avenue Molière - 67000 - Strasbourg, France, Tel: 00 33 88 12 83 96; Fax: 03 88 12 80 92; E-mail: Natacha.Entz-Werle@Chru-Strasbourg.Fr

Rec date: Feb 11, 2015; **Acc date:** Mar 28, 2015; **Pub date**: Mar 31, 2015

Abstract

Introduction: The management of pediatric high grade osteosarcoma is lacking new approaches to classify closely the patients and adapt thereafter the treatment initially or post-operatively. The objective of this study was to estimate the impact of the tumor molecular response comparatively to initial biopsy and to see if this molecular analysis was correlated to the histological response to neoadjuvant chemotherapy and/or prognosis.

Material and methods: 33 patients were included and allelotyping analyses using 23 micro satellites were performed on biopsy's and tumor's DNA versus normal blood DNA. Allelic imbalances were detected in all biopsy samples and the number of persistent AIs or not were quantified on tumors after pre-operative chemotherapy.

Results: We identified 4 subgroups with a significant impact on survival. The first group presents a complete disappearance of the AIs and a complete response independently from the histopathologic measure for tumor necrosis. The second group showed a partial response with persistence of some rearrangements after treatment. The third and the last one were characterized by the same molecular profile even more rearrangements, allowing to considered those subgroups as highly resistant osteosarcomas. This molecular re-stratification was associated with a significant impact on survival and provides evidence that this new approach on tumor resection might be a complementary and useful tool combined with histological response assessment.

Keywords: Osteosarcoma; Response to chemotherapy; Prognostic marker; Allelotyping

Introduction

As the prognosis and survival seem to be stable among all protocols since almost two decades, new approaches to re-classify closely pediatric patients diagnosed for high grade osteosarcoma at diagnosis and on primitive tumor grading are highly needed. The only stratification marker at diagnosis is the presence of metastases, which are diagnosed in almost 20% of patients and seem to contribute to a worse prognostic [1-4]. Multiple molecular studies were performed to obtain new diagnostic markers but, for instance and to our knowledge, no surrogate biomarker is used in the current and ongoing management of pediatric osteosarcomas at diagnosis in the world. Even in the more recent publications, those markers can cover a specific biomarker, as well as multiple bio-profiles studied by innovative techniques but without any immediate use in the patient routine [5-8]. Furthermore, the high genome instability of osteosarcomas is not facilitating the establishment of one or several markers, which could be routinely used. The second prognostic marker is the histological response or Rosen's grading, to neoadjuvant chemotherapy, calculated on the surgical resection. An estimation of less than 10% of tumor viable cells is considered as a good response to chemotherapy (GR) and significantly linked to a better prognosis throughout the multiple international protocols [3,4,9-12]. Even though, among those GR patients, 10 to 15% will relapse in a metastatic setting and/or with a rarer local recurrence. No histological features or biomarkers were known to predict in the good responders to treatment those relapses. In parallel, in the poor responders to chemotherapy, no analyses highlighted the markers of patients who will relapse or have a long-term survival. Current therapeutic strategies for osteosarcoma patients relied on this stratification of patients into risk categories, but, ideally, minimizing therapy for some, while expanding treatment for others, this management should optimize osteosarcoma treatments and reduce histological grading limits. Therefore, multiple histological and molecular analyses were

performed, but no potential marker for instance can be used in a prognostic manner to screen more accurately children with high grade osteosarcomas. The difficulties encountered with such studies are the tumor collections, where the paired biopsic and surgical samples should be available for comparison. The major problem to refine this histological classification is to find the biomarkers able to do this re-stratification on primitive tumors if the necrotic cells are highly represented. In the Lab, since 10 years, we developed molecular analyses based on simple and routinely done allelotyping technique for the study of Lynch syndromes. This sensitive method is based on the comparison between the paired DNA of the tumor and a normal tissue, usually patient's blood. A first publication by our group in 2003 [13], on 13 patients, was showing preliminary data comparing the genetic profiling by allelotyping on paired biopsies and post-chemotherapeutic tumor specimens. These preliminary analyses revealed in case of persistence or appearance of rearrangements in good responders to chemotherapy a lower survival rate and an increase of relapse. To confirm those data, we progressively increased the number of paired samples to finally obtain a cohort of 33 patients homogeneously treated with the French OS94 protocol. Comparing normal and tumor DNAs, the allelotyping analyses are identifying directly the presence of chromosomal alterations and is able to detect either chromosomal (allelic imbalance or AI) or microsatellite instabilities (MSIs). In the entire previously published cohort of 105 pediatric osteosarcomas [14], no MSIs, identifying usually a repair error phenotype, were detected, but only AIs were observed on biopsy's DNA. Twenty-three different microsatellites were analyzed on all specimens to screen tumor response to chemotherapy at a molecular level based on the presence or not of diagnostic AIs. Subsequently, the objective of our study was to determine at a DNA level the tumor response and to see if this molecular analysis and response were correlated to the histological response to neoadjuvant chemotherapy and its prognosis.

Materials and Methods

Patients' data

33 pediatric patients (≤18 years) were included in this study from November 1994 to December 2004. They were treated homogeneously with the French OS94 protocol (included in the protocol or treated based on the OS94 guideline after 2001) [4]. 23 patients underwent their tumor surgery after neoadjuvant chemotherapy in the University Hospital of Strasbourg. The 10 remaining patients were treated in the Curie Institute in Paris (3 patients), Leon Berard Centre in Lyon (3 patients), Hospital of Trousseau in Paris (2 patients) and GustaveRoussy Institute (2 patients). This study was conducted in accordance with the Declaration of Helsinki. Among the 33 children, 16 were good responders and 17 poor responders to chemotherapy. The histological grading was done as required in the SFOP-OS94 protocol [4], where a good response was defined as an estimation of less than 5% of viable tumor cells. Clinical details (age, sex and metastatic status) and histological response characteristics of this population are detailed in (Table 1). Overall survival (OS) was calculated as the period from diagnosis to death or last follow-up and event-free survival (EFS) was calculated as the period from diagnosis to first relapse or progression.

Tumors and biopsie's collection

After the histological assessment by the pathologist, 33 paired samples, where blood DNA, biopsy DNA and tumor DNA were provided for the same patient, were included in the study. All diagnostic biopsies and all tumors after local surgery were fresh-frozen and stored at −80°C. Control tissues were obtained from peripheral blood conserved on Whatman paper at room temperature.

Patients Characteristics		
Age in Years		13.5 (4-18)
Sex	Female	13
	Male	20
Histological response to chemotherapy		
	GR	16
	Grade IV	4
	Grade III	12
	PR	17
Metastatic Status		2 with lungs metastases
survival		21 alive
		12 dead (2 metastatic diseases)
OS	Median in months	85
	At 5 Years	72%
	At 10 Years	64%
EFS	Median in months	79
	At 5 Years	61%
	At 10 Years	54%

Table 1: patient's clinical characteristics

Tumor tissues and blood paired DNAs were purified as already described [13]. Biopsy and tumor genomic DNA concentrations were quantified by fluoro spectrometry (NanoDrop 3300, Thermoscientific, Wilmington, USA), ranging from 25 to 400 ng/µL, whereas blood DNA concentrations were ranging from 1 to 10 ng/µL. The quantification is following the PicoGreen® assay protocol. The good-quality DNA was assessed by the fluorometer ratio (A260/A280) and those ratios were between 1.6 and 2.0 for all biopsies and tumors' DNAs.

Microsatellite analyses

Twenty-three different microsatellites were analyzed on blood, biopsy and tumor resection DNA of each patient. These microsatellites were as follows: *TP53, RB1, D2S2176, D2S2348, D3S1283, D3S700, D4S2996, D4S428, D5S346, D5S492, D7S486, D7S667, D7S2495, D7S2559, D7S2532, D7S1683, D8S1778, D8S1018, D9S171, D17S800, D17S1818, D20S107, D20S855 (*see primer description at http://www.ncbi.nlm.nih.gov/genemap99 and http://www.gdb.orgwebsites) *and are targeting the following and respective loci:17q13, 13q14, 2q37, 3p24, 4q12, 5q21-31, 7q31, 7p21.2, 8q22, 9p21, 17q21 and 20q12. DNAs*

originated from blood, biopsy and tumor samples (10 ng) were amplified by PCR in a total volume of 25 µL using 0.125 µL of Taq polymerase and 4 pmol of both forward and Cy5 labeled reverse primers. PCR was carried out in an Omnigen Hybaid Thermocycler (Hybaid Ldt, Ashford, UK) using the protocols already described in our previous publications [13-17]. The PCR products were analyzed by capillary electrophoresis on ABI PRISM® Genetic Analyzer 3100 (Applied Biosystems, Foster City, CA, USA). The data were analyzed with the Genemapper Software (Applied Biosystems). This technique detects two types of rearrangements: a modification of the allele ratio in tumor or biopsy DNA compared to the paired blood DNA, which is described as an allelic imbalance (AI), and the microsatellite instability (MSI), which was not described in the entire French osteosarcoma cohort [13-17]. The AI is the witness of a deletion or an amplification of the targeted locus. An allelic variation above a cut-off of 20% is defining the presence of a significant AI [13]. Each alteration was confirmed by a duplicate PCR.

Statistics

Data were computed using SPSS 16.0 for Windows (SPSS, Inc., Chicago, IL, USA). The chi2 test was performed to analyze correlations between subgroups based on presence or absence of allele typing rearrangements and clinical data. Survival analysis was estimated using the Kaplan-Meier test for overall survival and event free survival calculations. Multivariate survival analysis was conducted using Cox regression proportional hazards and a 95% confidence interval (CI). A p value of less than 0.05 was considered to indicate statistical significance.

Results

To find out if the study is suitable with the samples obtained from the biopsies and surgical resections, we validated, first, the DNA's quality and, thereafter, the consistency of these patients with the SFOP-OS94 population. Based on these pre-requisites, we went further with the analyses to identify 4 subgroups of patients, who were significantly linked to prognosis and outcome.

The DNAs' quality and patients' clinical data were relevant for further analyses

The 33 patients were selected on the availability of fresh-frozen samples concomitantly at diagnosis and at surgical resection after neoadjuvant chemotherapy. Reproducible PCR products (from 73 to 300 base pairs) were detected on electrophoretic gels in all resected tumors' samples. These reproducible analyses showed, even on high necrotic specimens, the possibility to amplify correctly tumor DNA in those samples and allowed to compare in each patient the blood, biopsy and tumor's DNAs, if the DNA concentrations were low and when bigger PCR products were amplified. Those results were especially conclusive in the four patients presenting a grade IV without any macroscopic residual osteosarcoma viable cells observed at histological assessment and, consequently, a very high percentage of necrosis. All the 23 microsatellites could be PCR-amplified in all samples and the products were separated on gels at the expected sizes, described by the manufacturer and usually observed in high quality DNA. The microsatellites were considered as informative as they are providing heterozygous results and, consequently, characterized by the presence of 2 interpretable peaks, representing the two amplified alleles of the targeted locus. All samples across osteosarcomas included in this study were informative for at least six rearranged heterozygote microsatellites and, thus, allowed a contributive comparison between biopsy and tumor resection. Twenty-six out of the thirsty-three biopsies presented 10 to 18 rearranged microsatellites. All those findings demonstrated, firstly, that all DNAs were interpretable and, secondly, that our selection of microsatellites represented a reliable and sensitive tool in the tumor samples, even when they were highly necrotic specimens.

For the cohort validation, it was difficult to compare the entire population of the SFOP-OS94 protocol [4] to this selection of patients, based on the availability of specific tumor samples. Nevertheless, the clinical data were revealing an equivalent proportion of 16 GRs and 17 PRs to chemotherapy. Among the GRs, 4 patients were diagnosed as grade IV and 12 were presenting a modified grade III response to chemotherapy (less than 5% of tumor viable cells after neoadjuvant chemotherapy). The overall survival (OS) in our reduced population of 33 patients was 75% at 5 years and 67% at 10 years after diagnosis. The five-year event-free survival (EFS) was 61% and decreased to 56% at 10 years. The median follow-up was 97 months. These outcome results were similar to the findings described in the published SFOP-OS94 trial [4]. The only discordant result was the absence of tumor response impact in the selected population of 33 patients. There was in this small cohort no statistical significant difference in terms of OS and EFS based on histological response stratification (p=0.41 and p=0.56, Figures 1A and 1B). Among the entire cohort, the metastatic status was considered as a significant prognostic clinical marker: only 2 patients had a lung metastatic disease and both died from metastatic relapses.

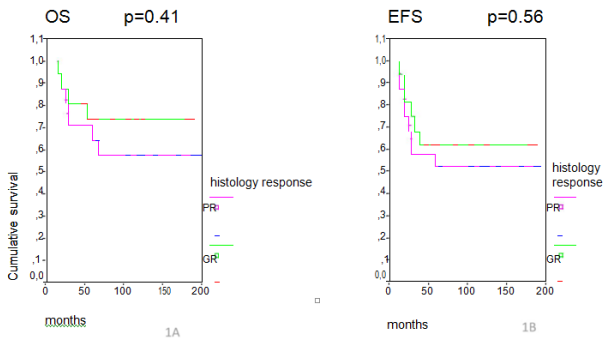


Figure 1: (A and B) are showing the absence of prognostic impact of histological response on tumor after treatment in pediatric osteosarcomas in this population of 33 patients.

The allelotyping analyses re-stratified the 33 tumors after chemotherapy in 4 subgroups

Comparing the rearrangements present or absent in the biopsy's DNA and those in the paired-resected tumor's DNA, we identified 4 subgroups of osteosarcomas independently from the histological response assessed on the completely resected primary tumors.

The first subgroup (Table 2) comprised four patients (3 GRs and 1 PR). These children had a complete disappearance in their tumors after treatment of all allelic imbalances (AIs) present at diagnosis on the biopsy samples. The unique PR patient was a histological grade II and did not relapse with a follow-up of 194 months. The three GRs

were divided in one grade IV and two patients with a modified grade III. All four patients were long term survivors with an OS and EFS of 100% and might be considered as a group of complete responders to chemotherapy based on molecular analyses of the resected tumors.

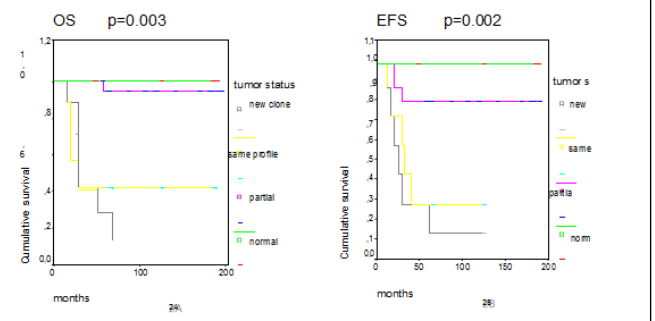
The second subgroup of fifteen patients (Table 2) showed on the resected tumors' DNAs a partial disappearance of the AI present at diagnosis on the biopsies' DNAs. The eight GRs and seven PRs of this group had a 5-year OS of 92% and 5-year EFS of 80%. Two children (one grade III GR and one PR with initial metastatic disease) died after a metastatic relapse and one GR is still in second complete remission. The number of residual AI in this partial normalized group was not significantly linked to survival or relapse risk. The only trend observed in the statistical analyses was the presence of residual AI located on chromosome 7(*D7S486, D7S667, D7S2495, D7S2559, D7S2532, D7S1683*) in most of the 13 alive patients (7/13) (p=0.08). This subgroup might comprise patients, who would be considered as partial molecular responders on tumor analyses or good molecular responders.

The third subgroup (Table 2) was composed of seven patients, who were maintaining the same rearrangements in the tumor's DNA comparatively to the biopsy's DNA. The persistent AIs after chemotherapy were, then, observed in five PRs and two grade III GRs, among which five patients relapsed and four out of five died after a second line therapy. The 5-year OS was 43% and the 5-year EFS was 29%. The two long-term survivors were one GR and one PR, with a respective follow up of 159 and 111 months. Those osteosarcomas might be stratified as resistant cancers at the molecular level independently from the histological response assessment or considered as molecularly poor responders to chemotherapy.

4 Subgroups	**Subgroup 1**	**Subgroup 2**	**Subgroup 3**	**Subgroup 4**	
Molecular status	Complete normalisation	Partial normalisation	Same profile	New rearrangements	
33 patients	4 patients 3 GRs 1 PR	15 patients 8 GRs 7 PRs	7 patients 2 GRs 5 PRs	7 patients 3 GRs 4 PRs	
5-year OS 5-year EFS	100% 100%	92% 80%	43% 29%	15% 15%	p= 0.0003 p=0.002
Alive Dead	4 0	13 2	3 4	1 6	p=0.001 p=0.0001
Median survival (months) event	155 no event	119 2 events	39.5 4 events	24.5 6 events	p=0.0001

Table 2: Characteristics of the 4 subgroups identified with the allelotyping analyses comparing biopsy and tumor molecular rearrangements.

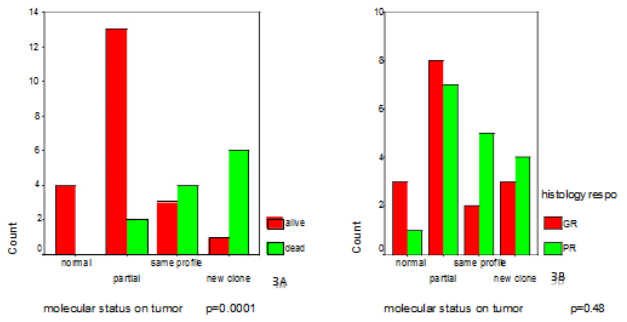
The last subgroup clustered seven patients (Table 2). Their tumor samples after chemotherapy were characterized by the detection of new DNA rearrangements, which was not detected on the diagnostic biopsy. All tumors, except one, had at least three new detected AI comparatively to diagnosis status. Six, 3 GRs and 3 PRs, out of seven patients were relapsing and, finally, died after second line therapy. The only remaining patient is still in complete remission 125 months after diagnosis, was initially a PR with a localized osteosarcoma and presented only one new AI in her resected osteosarcoma. The 5-year OS and EFS are dramatically low at 15%. In this small population, no statistical link was highlighted based on the location of the newly rearranged loci, except for 5q21-31, where a trend was observed (p=0.07). Three out of seven tumors were adding a new AI in the 5q21-31 locus (D5S346, D5S492) comparatively to the initial panel of rearrangements in the paired biopsy. In this group, the neoadjuvant treatment seems to select a new and resistant tumor cell clone. Nevertheless, the long term survivor was only showing one new single AI and presented a partial response on the others AIs, as described in the second subgroup of partial molecular responders. No other patient of this subgroup had this molecular profile. The 6 dead patients retained all previous AIs, which were described on the biopsy sample and accumulated at least 3 new AIs. This subgroup should be considered as a selection of highly resistant osteosarcomas, especially in case of maintenance of all previous genomic abnormalities plus new DNA AIs.



Figures 2: (A and B), showing the statistical significance was present for overall survival (OS, p=0.003) and for event-free survival (EFS, p=0.002) and linked the 4 molecular subgroups to a prognostic significance. (Green line=complete molecular response, pink line= partial molecular response, yellow line=poor molecular responders and black line=highly resistant tumors with appearance of new cellular clones).

This re-stratification by molecular allelotyping was significantly related to survival, but not to histological response to chemotherapy

The correlation between the different molecular subgroups of resected tumors and overall and event-free survival was statistically significant (p=0.003 and p=0.002, respectively) (Figures 2A and 2B). An increase of relapses was significantly linked to the subgroups of molecularly poor responders(third subgroup) and in case of new cell clone appearance and correlated with the increase of deaths (Table 2 and Figure 3A, Khi-deux test, p=0.0001). This molecular re-stratification was significantly linked to survival and had a prognostic impact in univariate analyses, as well as the metastatic disease status at diagnosis. The variables, identified as independent factors affecting patient survival, were including the presence of metastasis (HR, 5.589; 95% CI, 1.561–20.013; p=0.008) and the molecular re-stratification (HR, 3.709; 95% CI, 1.770–7.774; p=0.001). Surprisingly, this molecular response was not statistically related to the histological response assessment (Figure 3B, p=0.48), but seems to reclassify the PRs with better outcome in the good molecular responders and the GRs with worst outcome in the poor molecular responders.



Figures 3: (A) is presenting the statistical correlation between the 4 subgroups of molecular response on tumor resection and the percentage of deaths in each subgroup of molecular response. In the (B), no correlation was highlighted between the molecular responses and the histological response on tumor resection. (Normal=complete molecular tumor response, partial=partial molecular response in the surgical resection, same profile=poor molecular responders with the persistence of same molecular rearrangements and new clone=resistant tumors with the appearance of new cellular clones after neoadjuvant treatment).

Discussion

This study aimed to identify how the molecular analyses of the resected tumor's DNA could help to re-stratify the tumor histological grading, as GRs and PRs, and whether its results might add additional prognostic information. Interestingly, this molecular evaluation is based on a simple, rapid and routinely done technique, which can be used on samples with a low percentage of cells, like on tumor surgeries after neoadjuvant chemotherapy, and still remains informative. The PCR product amplifications were reproducible with applicability to surgical samples after treatment. All samples could be analyzed accurately. Badly, even the survival statistical analyses were consistent with the survival analyses of the SFOP-OS94 trial [4], the histological response to chemotherapy was not significantly linked to patient prognosis in our small group of 33 pediatric osteosarcomas. This discrepancy is probably due to the random choice of the 33 patients only based on the availability of the fresh-frozen samples at diagnosis and surgery and could have induced a statistical bias.

However, this molecular analysis, comparing the diagnostic allelotyping profile to the resected tumor's status after treatment, allowed identify 4 subgroups: two groups with a complete or partial molecular response and two highly resistant subgroups to chemotherapy, for whom the prognostic outcome was especially bad. These subgroups based on the molecular response after pre-operative chemotherapy were not correlated with measurement of histopathologic tumor necrosis on the same sample. Whereas PRs can be complete or partial responders at molecular level and have a good prognosis, GRs can also be at molecular level considered as resistant osteosarcomas and relapse rapidly in those cases.

To our knowledge, this is one of the first studies analyzing molecular status on surgery samples of pediatric osteosarcomas to understand further, why PR may have a better prognosis and why some of the GRs to treatment will relapse and died. Most of the studies, even in our Lab, were focusing on diagnostic samples to determine really early the chemoresistant osteosarcomas [5-8,13-17]. Nevertheless, this risk re-stratification is necessary to adapt the treatment but, for instance, was mostly based on clinical features [18-21]. The first explanation in the difficulty to find one or multiple biomarkers useful in osteosarcoma re-stratification and risk assessment is the complexity and the heterogeneity of this cancer [22,23].

Therefore, our molecular analyses on the surgical samples are overpassing such tumor cell complexity as they are simple, rapid and independent from the diagnostic rearranged biomarkers. We have, then, provided evidence that these simple and routinely done analyses were feasible even in patients for whom a complete necrosis was measured at tumor histopathology after neoadjuvant treatment. It appeared as a useful tool to refine the histopathological response and to determine potential PRs, who will be long term survivors, and the GRs with a worse prognosis. So, this approach via tumor surgery analyses seemed to allow complementary prognostic assessment with histology, but this molecular re-stratification has to be confirmed in larger cohorts and has to be tested on paraffin-embedded samples, even preliminary data on paraffin-embedded versus fresh-frozen specimens seemed to be promising.

Acknowledgement

We are thankful for funding support of Infosarcome association, Ligue contre le Cancer and Hospital of Strasbourg (API). We are also thankful to the community of the pathologists involved in bone cancers in the Société Française des Cancers de l'Enfant.

References

1. Kager L, Zoubek A, Pötschger U, Kastner U, Flege S, et al. (2003) Cooperative German-Austrian-Swiss Osteosarcoma Study Group. Primary metastatic osteosarcoma: presentation and outcome of patients treated on neoadjuvant Cooperative Osteosarcoma Study Group protocols. J Clin Oncol 21: 2011-2018.
2. Mialou V, Philip T, Kalifa C, Perol D, Gentet JC, et al. (2005) Metastatic osteosarcoma at diagnosis: prognostic factors and long-term outcome--the French pediatric experience. Cancer 104: 1100-1109.

3. Bielack SS, Kempf-Bielack B, Delling G, Exner GU, Flege S, et al. (2002) Prognostic factors in high-grade osteosarcoma of the extremities or trunk: an analysis of 1,702 patients treated on neoadjuvant cooperative osteosarcoma study group protocols. J Clin Oncol 20: 776-790.
4. Le Deley MC, Guinebretière JM, Gentet JC, Pacquement H, Pichon F, et al. (2007) Société Française d'Oncologie Pédiatrique (SFOP). SFOP OS94: arandomised trial comparingpreoperative high-dose methotrexate plus doxorubicin to high-dose methotrexate plus etoposide and ifosfamide in osteosarcoma patients. Eur J Cancer 43: 752-61.
5. Jentzsch T, Robl B, Husmann M, Bode-Lesniewska B, Fuchs B (2014) Worse prognosis of osteosarcoma patients expressing IGF-1 on a tissue microarray. Anticancer Res 34: 3881-3889.
6. Martin JW, Chilton-MacNeill S, Koti M, Van Wijnen AJ, Squire JA, et al. (2014) Digital expression profiling identifies RUNX2, CDC5L, MDM2, RECQL4, and CDK4 as potential predictive biomarkers for neo-adjuvant chemotherapy response in paediatricosteosarcoma. PLoS One 9: 95843.
7. Rosenblum JM, Wijetunga NA, Fazzari MJ, Krailo M, Barkauskas DA, et al. (2015) Predictive properties of DNA methylation patterns in primary tumor samples for osteosarcoma relapse status. Epigenetics 1: 31-39.
8. Kubota D, Mukaihara K, Yoshida A, Tsuda H, Kawai A, et al. (2013) Proteomics study of open biopsy samples identifies peroxiredoxin 2 as a predictive biomarker of response to induction chemotherapy in osteosarcoma. J Proteomics 91: 393-404.
9. Lewis IJ, Nooij MA, Whelan J, Sydes MR, Grimer R, et al. (2007) MRC BO06 and EORTC 80931 collaborators; European Osteosarcoma Intergroup. Improvement in histologic response but not survival in osteosarcoma patients treated with intensified chemotherapy: a randomized phase III trial of the European Osteosarcoma Intergroup. J Natl Cancer Inst 99: 112-128.
10. Meyers PA, Schwartz CL, Krailo MD, Healey JH, Bernstein ML, et al. (2008) Osteosarcoma: the addition of muramyltripeptide to chemotherapy improves overall survival--a report from the Children's Oncology Group. J Clin Oncol 26: 633-638.
11. Janeway KA, Barkauskas DA, Krailo MD, Meyers PA, Schwartz CL, et al. (2012) Outcome for adolescent and young adult patients with osteosarcoma: a report from the Children's Oncology Group. Cancer 118: 4597-4605.
12. Whelan JS, Bielack SS, Marina N, Smeland S, Jovic G, et al. (2015) EURAMOS-1, an international randomised study for osteosarcoma: results from pre-randomisation treatment. Ann Oncol 26: 407-414.
13. Entz-Werle N, Schneider A, Kalifa C, Voegeli AC, Tabone MD, et al. (2003) Genetic alterations in primary osteosarcoma from 54 children and adolescents by targeted allelotyping.Br J Cancer 88: 1925-1931.
14. Nguyen A, Lasthaus C, Guerin E, Marcellin L, Pencreach E, et al. (2013) Role of Topoisomerases in Pediatric High Grade Osteosarcomas: TOP2A Gene Is One of the Unique Molecular Biomarkers of Chemoresponse. Cancers (Basel) 5: 662-675.
15. Entz-Werlé N, Marcellin L, Gaub MP, Guerin E, Schneider A, et al. (2005) Prognostic significance of allelic imbalance at the c-kit gene locus and c-kit overexpression by immunohistochemistry in pediatric osteosarcomas.J ClinOncol 23: 2248-2255.
16. Entz-Werlé N, Stoetzel C, Berard-Marec P, Kalifa C, Brugiere L, et al. (2005) Frequent genomic abnormalities at TWIST in human pediatric osteosarcomas. Int J Cancer 117: 349-355.
17. Entz-Werle N, Lavaux T, Metzger N, Stoetzel C, Lasthaus C, et al. (2007) Involvement of MET/TWIST/APC combination or the potential role of ossification factors in pediatric high-grade osteosarcoma oncogenesis. Neoplasia 9: 678-88.
18. Kim MS, Lee SY, Cho WH, Song WS, Koh JS, et al. (2008) Initial tumor size predicts histologic response and survival in localized osteosarcoma patients. J Surg Oncol 97: 456-61.
19. Lau CC (2009) Molecular classification of osteosarcoma.Cancer Treat Res. 152: 459-465.
20. Lee JA, Kim MS, Kim DH, Lim JS, Park KD, et al. (2009) Risk stratification based on the clinical factors at diagnosis is closely related to the survival of localized osteosarcoma. Pediatr Blood Cancer 52: 340-345.
21. Niswander LM, Kim SY (2010) Stratifying osteosarcoma: minimizing and maximizing therapy. Curr Oncol Rep 12: 266-270.
22. Sadikovic B, Park PC, Selvarajah S, Zielenska M (2013) Array comparative genomic hybridization in osteosarcoma. Methods Mol Biol 973: 227-247.
23. Martin JW, Squire JA, Zielenska M (2012) The genetics of osteosarcoma. Sarcoma: 627254.