

## **Search for predictive biomarkers of response to induction chemotherapy (doxorubicin, ifosfamide and cisplatin) treatment in patients with locally advanced soft tissue sarcomas.**

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### **Introduction**

Soft tissue sarcomas are solid tumors that encompass a heterogeneous group of cancers, with approximately 50 different histological subtypes, which vary greatly in natural history and response to treatment. Use of neoadjuvant chemotherapy treatment for locally advanced soft tissue sarcomas (LASTS) is still controversial; theoretical reasons of its use are: a) tumor size reduction allows margin negative (R0) resections or less radical resections that allow preservation of function; b) systemic benefits from early delivery of cytotoxic agents such as elimination of micrometastatic disease, and c) induction therapy permits rapid assessment of tumor response *in situ* and dispense the use of postoperative chemotherapy and its adverse toxic effects in patients who do not respond to therapy. Antitumoral responses are extremely variable and so far there are no histological or molecular criteria to predict the response to induction chemotherapy.

We assumed that chemo resistance (intrinsic or acquired) against various LASTS neoplasm with distinct genomic expressions would be very likely sustained by common specific abnormalities, characterized by a specific protein expression profile.

### **Objectives**

To identify genomic markers using genomic large scale analysis as well as the role of ERCC1 and Topoisomerase II-alpha (Topo2A) protein expression involved in the response to neoadjuvant chemotherapy treatment in LASTS and to correlate them with histological response and clinical outcomes after induction chemotherapy treatment.

### **Methods**

Retrospective study based on archival tumoral tissue from LASTS patients treated at the IGR during 1990-2005. The 78 patients included in the study received the standard neoadjuvant chemotherapy schema consisting of 2 cycles of API-AI: doxorubicin (60 mg/m<sup>2</sup> d1 and d15), cisplatin (100 mg/m<sup>2</sup> d1) and ifosfamide (I) 5 g/m<sup>2</sup> (d2 and d15) every 4 weeks. All patients had pathological necrosis (PN) evaluation after surgical tumor removal. Clinical data

characteristics such as age, sex, date of diagnosis, tumor characteristics (size, grade, deepness and localization), and pathological (histology, tumor grade as defined by the FNCLCC, percentage of necrosis in the post surgical pathological specimen and status of surgical margins), starting at diagnosis and until last follow up or last known status as of January 31st 2007 were collected and pooled in a single database. Biological material was recovered before initiation of induction treatment in order to undertake immuno-histochemical protein expression analysis (paraffin embedded tumoral tissue) for ERCC1 and Topo2A in 59 and 60 patients respectively, and cDNA extraction (frozen tumoral tissue) for 244 K CGH array analysis was done in 19 samples (13 primitive tumors and 6 post chemotherapy samples). Standard protocols according to manufacturers were followed for each of the laboratory techniques used.

## **Results**

The median age of the 43 males and 36 females was 43 years (range 18-68) and the median tumor size at diagnosis was 109 mm (range 10-250 mm). Six percent of patients progressed under induction chemotherapy, 57% achieved SD and 32% PR. Eighty-two percent of patients were able to undergo R0 surgery. All patients but one received postoperative radiotherapy. Four patients had local relapse and 35 patients distant relapse (mostly to the lungs). After a median follow-up of 6.5 years (range 1.6-10.5 years) 49 patients were alive. The 5 year DFS and OS were 47% and 62% respectively.

The 78 patient population was divided in 3 classes according to their PN in order to put one third of the population in each group. Class 1: 0-50% PN (25 patients), class 2: 50-90% PN (26 patients) and class 3: 90-100% PN (27 patients). The annual risk of progression was calculated for each class as the number of events divided by the number of patient years (the addition of participating time in years). Globally the risk of progression decreased as the percentage of PN increases but it was not possible to establish a cutoff point which will allow to determine an important difference or a good histological response (GHR) thus >70% PN was taken as cutoff for defining GHR. When dividing the 78 patients in 2 groups according to GHR (39 patients in each group) the median DFS was of 3.1 years in the group of  $\leq 70\%$  PN as compared to 10.5 years in the group with >70% PN ( $p \leq 0.01$ ) with a risk of progression 2.3 times higher in patients with  $\leq 70\%$  PN (HR=2.3, 95%IC= [1.17-4.34]).

ERCC1 IHC (59 tumors, figure 8) median protein expression (H score) was 1. The PN percentage distribution did not differ with expression of ERCC1 ( $p \leq 0.476$ ). Protein expression had no impact on patients DFS ( $p \leq 0.19$ ), however the median DFS in patients

whose tumors had low expression of ERCC1 is 2.5 in comparison to 7 years in patients with high ERCC1 expressing tumors.

Topo2A IHC median percentage of protein expression was 27.5%. The PN percentage distribution of high expressing TopoII tumors was different to that of low expressing tumors ( $p \leq 0.0004$ ) and a high expression of Topo2A was associated to a high PN (Spearman  $r=0.416$ ). Median DFS in patients with low Topo2A was 2.7 years and not reached in patients with high Topo2A (HR=1.95, IC=0.94-4.14). The annual risk of progression diminished when Topo2A percentage increased.

CGH array (aCGH) identified several copy numbers anomalies in all samples studied, the median number of copy number alterations in all 19 samples were 53 (range 7-114), gains and losses were present in high numbers. Only one sample had less than 10 alterations and corresponded to a primitive myxoid liposarcoma (tumor harboring a specific translocation). All STS samples had copy number alterations but not all the samples had aberrations in all chromosomes. The chromosome with the less number of abnormalities was chromosome 18 with 22 and the one with the most was chromosome 1 with 62 aberrations. Interestingly primitive tumors known to harbor specific balanced translocations (not detected by aCGH) showed many other alterations; the 2 myxoid liposarcomas 7 and 87 anomalies respectively, having both copies altered in 3q26.31 (gain) and in 8p11.23, 9p24.1 and 16p11.2 (gain or loss). The 5 primitive synoviosarcomas had 19, 25, 39, 46 and 54 copy abnormalities respectively, these tumors had some chromosomal regions altered in common: 11q11 (2 gains and 3 losses), 7p22.3 (gains in 4 samples), 15q11.4 (3 gains and 1 loss), 17q21.31 (1 loss and 3 gains) and 21q22.3 (1 loss and 3 gains), in 3 of 5 samples like 1p36.33 (3 gains), 8q24.3 (2 gains and 1 loss) and 15q11.2 (3 gains) and 4q35.2 and 7q11.33 (2 losses and 2 gains respectively).

Unsupervised analysis grouped the 19 samples according to their genomic aberrations, the hierarchical clustering dendrogram presented two apparent branches, the first corresponding to tumors with anomalies and the second globally without aberrations, it is of note that only 3 coupled samples (primitive-post CT) were found in the same group and could potentially show progression of tumors during treatment. After induction CT treatment 10 different genomic regions (size varies between 5.5 Mb to 4 kb) were found to differ ( $p \leq 0.05$ ) of those half contained polymorphisms and were difficult to interpret, of the other 5 found 3 have not been noted to participate in cancer biology thus they might correspond to polymorphisms not yet described. In relation to GHR, the analysis of all samples found several regions to differ significantly ( $p \leq 0.01$ ) being of relevance those found on chromosomes 1, 8,

10, 11, 13, 14 and 18. When the analysis was done taking into account only the primitive tumors we found some interesting regions ( $p \leq 0.05$ ) that have still to be validated with more tumoral samples. CGH array analysis is still ongoing.

Finally *TOP2A* gene locus in 17q21.2 chromosome showed 3 gains and 1 loss of genetic material in primitive tumors while 1 post CT showed a loss. Analysis is still ongoing.

### **Conclusions**

Topo2 protein expression seems to be a relevant early predictive marker for better EFS in pts with LASTS treated with a doxorubicin-containing induction CT. In low Topo2 expressing LASTS the use of doxorubicin as induction CT might be questioned. Nowadays due to STS heterogeneity, treatment of this family of tumors has started to be individualized according to histology but the presence of common copy abnormalities harboring gene locus is possible thus the same biological pathways and proteins expression involved in LASTS development might be susceptible of being targeted, it will allow also choosing those patients that will obtain real benefit of neoadjuvant chemotherapy treatment and diminishing unnecessary treatments toxicities in the others.

**Perspectives:** Topo2 gene amplification analysis by FISH will be started soon. These observations will be validated in prospective multi institutional studies (CONTICANET program).

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