

Alveolar rhabdomyosarcoma with regional nodal involvement: results of a combined analysis from two cooperative groups

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Abstract

Background: Treatment of children and adolescents with alveolar rhabdomyosarcoma (ARMS) and regional nodal involvement (N1) have been approached differently by North American and European cooperative groups. In order to define the better therapeutic strategy, we analyzed two studies conducted between 2005 and 2016 by the European paediatric Soft tissue sarcoma Study Group (EpSSG) and Children's Oncology Group (COG). **Methods:** We retrospectively identified patients with ARMS N1 enrolled in either EpSSG RMS2005 or in COG ARST0531. Chemotherapy in RMS2005 comprised IVADo (ifosfamide, vincristine, dactinomycin, doxorubicin), IVA and maintenance (vinorelbine, cyclophosphamide); in ARST0531 it consisted on either VAC (vincristine, dactinomycin, cyclophosphamide) or VAC alternating with VI (vincristine, irinotecan). Local treatment was similar in both protocols. **Results:** The analysis of the clinical characteristics of 239 patients showed some differences between study groups: in RMS2005, advanced IRS Group and large tumors predominated. There were no differences in outcomes between the two groups: 5-year event-free survival (EFS), 49% (95%CI=39-59) and 44% (95%CI=30-58), and overall survival (OS), 51% (95%CI=41-61) and 53.6% (95%CI=40-68), in RMS2005 and ARST0531, respectively. In RMS2005, EFS

of patients with FOXO1-positive tumors was significantly inferior to those FOXO1-negative (49.3% vs 73%, $p=0.034$). In contrast, in ARST0531, EFS of patients with FOXO1-positive tumors was 45% compared with 43.8% for those FOXO1-negative. Conclusions: The outcome of patients with ARMS N1 was similar using different schemas of chemotherapy. However, patients with FOXO1 fusion-negative tumors enrolled in RMS2005 showed a significantly better outcome, suggesting that this subgroup may benefit from the EpSSG strategy which included maintenance chemotherapy.

1. INTRODUCTION

Rhabdomyosarcoma (RMS) is the most common soft tissue sarcoma in children and adolescents and comprises two major histologic subtypes: alveolar RMS (ARMS) and embryonal RMS (ERMS) [1-3].

The prognosis of RMS has improved considerably over time due to numerous clinical trials conducted by collaborative groups working in North America: Intergroup Rhabdomyosarcoma Study Group (IRS) and Children's Oncology Group (COG), and in Europe: International Society of Paediatric Oncology-Malignant Mesenchymal Tumour Group (SIOP-MMT), Italian Soft Tissue Sarcoma Committee-STSC, Gesellschaft Cooperative Soft Tissue Sarcoma Study Group-CWS and more recently, EpSSG (European paediatric Soft tissue sarcoma Study Group). Despite this successful history, the improvement in the prognosis of patients with RMS has not been uniform. While the probability of cure in pediatric patients with localized disease is over 70%, the prognosis of those with distant metastatic disease remains poor [4-8], and the presence of disseminated disease at diagnosis continues to be the most powerful prognostic factor in this neoplasm.

For patients with localized RMS, clinical and tumor characteristics are used to classify RMS in different risk categories and to determine treatment intensity. These characteristics include histology, tumor invasiveness, tumor location, nodal involvement, tumor size and patient age [9-11] and they constitute the basis for the risk stratification system used in North America by COG and in Europe by EpSSG. Patients with ARMS and regional lymph node involvement represent approximately 5-10% of all cases of RMS in children and adolescents. Previous experience suggested that these patients represent a group with particularly poor prognosis [9].

In the EpSSG RMS2005 study, we stratified these patients into a very high risk (VHR) category and treated them with an intensified regimen of chemotherapy that included doxorubicin added to the standard schema of IVA (ifosfamide, vincristine, and dactinomycin) in the first 4 cycles, followed by 5 cycles of IVA and six cycles of intravenous vinorelbine and daily oral cyclophosphamide [12]. During the same period, COG conducted the ARST0531 study for intermediate risk RMS patients that included those with non-metastatic ARMS at any primary site without distant metastases; these patients were randomly assigned to receive the standard schema VAC (vincristine, dactinomycin, and cyclophosphamide) or VAC alternating with VI (vincristine, irinotecan) for 42 weeks [13].

Herein we report the results of a combined analysis of patients with ARMS and regional nodal involvement enrolled in the aforementioned studies, and we compare these results to determine which therapy optimizes survival.

2. PATIENTS AND METHODS

2.1. Patients

For the purpose of this analysis eligible patients were those with:

- a. Alveolar rhabdomyosarcoma confirmed by histology **and**
- b. Regional nodal involvement defined by clinical **or** radiological **or** pathologic criteria, without distant metastases, **and**
- c. Enrolled in protocol EpSSG RMS2005, between November 1, 2005 and December 31, 2016 **or**
- d. Enrolled in protocol COG ARST0531, between December 1, 2006 and December 31, 2012.

Date of data cutoff for the analysis were: Dec 31, 2019 for RMS2005 and Dec 31, 2018 for ARST0531.

2.2. Treatment

2.2.1. *EpSSG RMS2005*

Patients received intensified induction chemotherapy and additional maintenance chemotherapy with systematic local treatment to primary and nodal sites. Induction chemotherapy comprised four 21-day cycles of IVADo (ifosfamide 3 g/m² on days 1-2 with MESNA, vincristine 1.5 mg/m² [max dose 2 mg] on days 1, 8 and 15 in the first 2 cycles and on day 1 in cycles 3 and 4, dactinomycin 1.5 mg/m² [max dose 2 mg] on day 1, and doxorubicin 30 mg/m² on days 1-2) followed by five 21-day cycles of IVA and six 28-day cycles of maintenance chemotherapy comprising continuous daily oral cyclophosphamide 25 mg/m² and intravenous vinorelbine 25 mg/m² on days 1, 8 and 15 of each cycle. The total duration of chemotherapy was 50 weeks.

Response was evaluated at weeks 9, 27 and at the end of treatment. If free margins were achievable without organ or functional impairment, local treatment after the initial 4 cycles of IVADo (week 13) included delayed surgical resection. External beam radiotherapy was administered to the primary tumor area and the affected lymph node region, delivered using a daily dose per fraction of 1.8 Gy. Doses varied according to chemotherapy response and surgical results. The total dose to the primary tumor following delayed surgery with complete resection was 41.4 Gy. For IRS Group III with incomplete resection, or when delayed surgery was not feasible, total dose was 50.4 Gy with an optional additional boost of 5.4 Gy in 3 fractions for large tumors with poor response to chemotherapy. Radiotherapy (RT) was recommended for involved lymph nodes at a dose of 41.4 Gy regardless of the extent of surgical resection.

2.2.2. *COG ARST531*

Patients were randomly assigned to receive either VAC (vincristine 1.5 mg/m² [max dose 2 mg], dactinomycin 0.045 mg/kg [max dose 2.5 mg], cyclophosphamide 1.2 g/m² with MESNA) or VAC alternating with VI (vincristine 1.5 mg/m², irinotecan 50 mg/m² on 5 consecutive days) intravenously. During the first 12 weeks, the two treatment arms were identical in duration and schedule, with the exception of substituting irinotecan for dactinomycin and cyclophosphamide at week 4 and for cyclophosphamide at week 7 in VAC/VI. During the subsequent 30 weeks of therapy, irinotecan replaced dactinomycin and cyclophosphamide at weeks 16, 19, 25, 31, and 37 in VAC/VI. The schedule of vincristine differed slightly between the two treatment regimens, allowing for its administration during the weeks that followed all courses of irinotecan, but the total number of vincristine doses was the same in both regimens. The total duration of chemotherapy was 42 weeks.

Patients were evaluated for response at weeks 15 and 30 and at the end of therapy. For patients older than 24 months, definitive RT was the planned local control modality. Delayed primary resection was allowed but not encouraged. For patients younger than 24 months, individualized local control approaches, including delayed primary excision and response-adapted RT, were permitted. RT started at week 4, and the dose was determined by clinical group and histology at study entry: IRS Group II ARMS with regional lymph node involvement, 41.4 Gy; IRS Group III ARMS with orbital primary site, 45 Gy; and non-orbital primary sites, 50.4 Gy. RT was delivered using megavoltage photon, proton, and/or electron beams. For tumors with a rapid substantial decrease in tumor size, a volume reduction by cone down after 36 Gy was permitted, particularly for tumors with pushing rather than infiltrating margins.

2.3. Statistical analysis

Survival time was calculated from the date of diagnosis to the time of event or last follow-up. Tumor progression, relapse, occurrence of second malignancy, or death due to any cause were considered for event free survival (EFS). Overall survival (OS) was measured from the date of diagnosis to death from any cause. Patients still alive at the end of the study were censored at the date of the last observation. Survival probability was calculated by the Kaplan-Meier method and heterogeneity in survival among strata of selected variables was assessed with the Log-Rank test [14]. Five-year EFS and OS were reported along with their 95% confidence intervals (CI) as computed using the Peto-Peto method [15]. The Cox proportional hazards models were fit to compare EFS and OS while adjusting for potential confounders. Categorical variables

were reported as counts and percentages and compared using Fisher’s Exact test. All data analyses were performed using the SAS statistical package (SAS, release 9.4; SAS Institute Inc., Cary, NC, USA).

3. RESULTS

3.1. Patient characteristics

The clinical characteristics of 154 patients enrolled in EpSSG RMS2005 and 85 patients enrolled in COG ARST0531 are presented in **Table 1**.

Adverse prognostic factors including tumor size > 5 cm, advanced IRS Group and tumor location at unfavorable sites, predominated in both cohorts. Large tumors (>5 cm) were more frequent in EpSSG than in COG patients: 71% vs 53%, respectively, (p-value 0.0029). IRS Group III tumors were also more frequent in the EpSSG than in the COG cohort: 93% vs 80%, respectively, (p-value 0.0027). Tumor location at unfavorable sites (parameningeal, limbs, bladder-prostate, other sites) (79% in EpSSG and 84% in COG) and invasiveness (T-stage 2, 60% in EpSSG and 65% in COG), were similar in both cohorts.

Fusion status of tumors, determined by the presence of *FOXO1* translocation (regardless of the *PAX* fusion partner), was analyzed in 81% of patients in EpSSG and in 93% in COG. *FOXO1* translocation, when *FOXO1* fusion status was known (76% in EpSSG and 79% in COG), was similar in both series.

Histological analysis of nodal involvement was performed less frequently (50%) in patients enrolled in EpSSG (nodal biopsy was not mandatory in RMS2005, except for tumors arising in extremities), as compared to COG (81%) (p-value 0.0035).

3.2. Treatment

Systemic and local treatment given to patients in both protocols are detailed in **Table 2**. One hundred and forty-five (145) patients who were enrolled in RMS2005 (94%) received chemotherapy according to protocol, 8 patients received IVA without doxorubicin, and 1 patient received IVA followed by VA. All patients included in ARST0531 received chemotherapy according to protocol: 44 received VAC and 41 VAC/VI.

Radiotherapy to the site of primary tumor and nodes was administered in 132 (86%) and 82 (97%) patients enrolled in RMS2005 and ARST0531, respectively (Table 2). A significantly higher proportion of patients in EpSSG did not receive RT for the following reasons: parent refusal (2), early progression (6), amputation (2), very young age (1) and treatment center decision (7). Four patients received nodal RT only. The most common type of radiotherapy in both series was photon external beam, and a minority of patients in both protocols received proton beam radiotherapy.

In the EpSSG cohort, 75 out of 154 patients (49%) underwent surgical resection of the primary tumor: up-front primary resection in 10/75 (13%) and delayed primary resection in 65/75 (87%). Results of surgery included complete local resection (R0) in 55 (73%), microscopic residual disease (R1) in 14 (19%), and macroscopic residual (R2) in 6 (8%). In the COG cohort, 22 out of 85 patients (26%) underwent surgical resection of the primary tumor: up-front primary resection in 17/22 (77%) and delayed primary resection in 5/22 (23%). R0 was achieved in 20 (91%) and R1 in 2 (9%).

3.3. Outcome and prognostic factors

The median follow-up of patients was 5.2 years in COG study and 5.4 years in EpSSG. At the time of the analysis, 45 patients in the COG cohort had relapsed, of whom, 38 died (37 by disease progression and 1 by an unrelated disease). Seventy-eight patients in the EpSSG cohort had relapsed, 69 of whom died (66 by disease progression, 2 by toxic deaths and 1 by a second neoplasm). Outcomes of patients (EFS and OS) included in the study are detailed in **Table 3**. The 5-year EFS of the COG cohort was 44% (95% CI: 30.0-58.1) compared with 49% (95% CI 38.8-59.3) of the EpSSG. The 5-year OS was 53.6% in COG (95% CI 39.6-67.7) and 51% in EpSSG (95% CI 40.9-60.9) (**Figure 1**). There were no differences in EFS or OS between the analytic cohort of RMS2005 and ARST0531. These results held when EFS (p=0.2) and

OS ($p=0.28$) were compared while adjusting for tumor size, nodal status and IRS Group (I/II *vs* III). In the combined analysis of both cohorts, outcomes by *FOXO1* fusion status showed 5-year EFS of 63% for patients with fusion-negative tumors (95% CI 44.6-81.0) *vs* 47.6% for fusion-positive (95% CI 37.2-58.1). The 5-year OS for patients with fusion-negative tumors was 69% (95% CI 51.7-86.3) compared with 52.7% (95% CI 42.6-62.9), for fusion-positive.

However, results from each cohort showed striking differences on the impact of the presence of *FOXO1* fusion on prognosis. In EpSSG, the 5-year EFS of patients with *FOXO1* fusion-positive tumors (49.3%, 95% CI 35.8-62.8) was significantly inferior to those with *FOXO1* fusion-negative ones (73%, 95% CI 53.0-92.8) ($p=0.034$). In contrast, COG patients showed no differences between these two groups: The EFS of COG patients with *FOXO1* fusion-positive tumors was 45.1% (95% CI 28.8-61.5) compared with 43.8% (95% CI 6.6-80.9%) of *FOXO1* fusion-negative (**Figure 2**).

Five-year OS of patients with *FOXO1* fusion-positive tumors in the EpSSG cohort was 51.6%, compared with 76% of *FOXO1* fusion-negative and approached significance ($p=0.069$). In contrast, there were no differences in the outcomes between COG patients with fusion-positive or fusion-negative tumors (53.7% and 56.2%, respectively) (**Figure 3**).

The pooled COG and EpSSG 5-year EFS and OS did not show statistically significant differences by location of primary tumor.

The site of the first relapse was also similar in both cohorts (*Supplementary Table S4*). Metastatic relapses with or without local recurrence represented half of the relapses in both protocols. Loco-regional relapses represented 35% and 29% of all recurrences in COG and EpSSG cohorts, respectively. Tumor progression during treatment occurred in 13% of the patients in COG and 18% in EpSSG.

4. DISCUSSION

The combined analysis of the results of these two cooperative studies suggests that different strategies of chemotherapy may have an impact in the outcome of a subgroup of patients with ARMS and regional nodal involvement.

The prognosis of patients with ARMS N1 has been reported to be poor in the historical series of European co-operative studies. In the CWS-86 study, 3-year EFS was 25% and OS 29% [11]. In the SIOP MMT84 study, 5-year EFS was 31% comparable to that of stage IV disease [16].

The experience of similar patients in North American cooperative groups has been better than that of the European studies. Of 125 patients with localized RMS and nodal involvement enrolled in the IRS-IV study, Rodeberg et al [17] reported 43% five-year failure-free survival of patients with ARMS and nodal involvement. The overall outcome of patients with alveolar histology and N1 disease reported in that study was similar to that of patients with metastatic disease at a single site.

We recently reported the experience of EpSSG RMS2005 [12] in patients with ARMS N1 and showed results similar to the IRS-IV study. The reasons for this apparent improvement in the outcome of these patients, compared to those treated within previous European studies, could be due in part to better risk stratification, more adequate treatment with intensified chemotherapy, systematic local treatment and/or improvement in supportive care.

Failures among patients reported in the present study were predominantly with metastatic disease and was similar in both cohorts. Their tumors frequently presented with advanced IRS Group and unfavorable location, characteristics related to an increased risk of distant metastatic disease [18-20]. Local recurrences represented one-third of all relapses in both protocols and local treatment in RMS2005 and in ARST0531 was identical. However, Casey et al [21] reported more loco-regional relapses in patients enrolled in ARST0531 compared with the previous COG intermediate-risk RMS study, D9803. The reasons for this increase in local relapses were not clear but may have been related to changes in cyclophosphamide dosing, changes in RT administration and fewer surgical procedures.

The most important difference between EpSSG and COG protocols was the type of chemotherapy. Patients received three different regimens based on the use of the standard combination (IVA in EpSSG and VAC in COG) to which was added doxorubicin and 6 months of maintenance chemotherapy with vinorelbine and cyclophosphamide in the European study or irinotecan in the North American study. In RMS2005, doses of alkylating agents included 54 g/m^2 of ifosfamide plus 4.5 g/m^2 of cyclophosphamide. In contrast, COG patients received 16.8 g/m^2 (VAC) or 8.4 g/m^2 of cyclophosphamide (VAC/VI). Moreover, patients in EpSSG received doxorubicin at a total dose of 240 mg/m^2 . One-half of the patients in COG received irinotecan. Total duration of chemotherapy was 42 weeks in COG protocol *vs* 50 weeks in EpSSG.

There is no evident advantage of adding doxorubicin or irinotecan to the standard chemotherapy in these patients. Hawkins et al [13] have previously reported a lack of improvement with the addition of irinotecan to VAC in the same of group of patients as the present analysis. The European experience demonstrated that the addition of doxorubicin to IVA in patients with high-risk disease also failed to improve outcome when compared to standard chemotherapy [22]. Moreover, previous experiences in Europe demonstrated that the addition of carboplatin, etoposide, and epirubicin did not improve the outcome for patients with high-risk disease enrolled in the SIOP MMT-95 study [8]. In RMS2005, two toxic deaths and one secondary neoplasm occurred, so that when designing future studies, we consequently should search for a balance between the total burden of chemotherapy and the risk of toxicity.

The impact of *FOXO1* fusion status in the outcome of patients in both cohorts showed important differences. In the European study, fusion-positive tumors had significantly worse EFS as well as a trend toward inferior OS, while the EFS/OS of patients enrolled in the COG study was the same whether they had fusion-positive or fusion-negative tumors. These contradictory results are intriguing. Numerous studies have reported the impact of fusion status on the outcome of patients with ARMS [23-25]. However, this impact could be different in patients with other adverse prognostic factors. For example, in patients classified as low or intermediate risk in COG studies, the presence of *FOXO1* fusion has a strong impact in prognosis [25,26] while in patients of high risk with metastatic disease, the clinical prognostic factors have a stronger impact than the fusion status [27]. However, these observations do not explain the differences we observed between our two homogeneous cohorts. In the European study the EFS of patients with fusion-negative tumors was 73%, similar to that of patients considered as high risk in the same protocol [22].

We can hypothesize that those patients with fusion-negative tumors benefit from a more intense chemotherapy. Patients in ARST0531 received a lower cumulative dose of cyclophosphamide than in previous COG RMS studies, with a possible negative impact on outcome [13]. In contrast, patients with ARMS N1 in RMS2005 could have had a benefit from the addition of maintenance chemotherapy, similar to the results of high-risk patients [28].

However, our study presents some limitations, such as the relatively small number of patients with fusion-negative tumors in both cohorts, and the higher proportion of patients with fusion status unknown in the EpSSG cohort, which could reduce the precision of the EFS estimations.

In conclusion, there are some lessons learned from the present combined analysis that should be further explored in the upcoming studies of both cooperative groups: First, very different treatment strategies in two concurrent clinical trials generated similar outcomes for patients with ARMS and regional lymph node involvement. Second, among patients with *FOXO1* fusion-negative ARMS and regional lymph node involvement, the cumulative dose of alkylators and/or maintenance chemotherapy may influence outcome and, third, a need persists for innovative therapeutic strategies for those patients with fusion-positive tumors.

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Author's contribution:

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Collection and assembly of data, data analysis and interpretation	Yueh-Yun Chi, Gian Luca de Salvo, M. J. Garcia
Approval of the final version of the manuscript	All authors

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FIGURE LEGENDS

Figure 1 Kaplan-Meier curves representing 5-year EFS (A) and 5-year OS (B), by protocol.

Figure 2 Kaplan-Meier curves representing 5-year **EFS by fusion status** in separated cohorts, (A) EpSSG RMS2005 and (B) COG ARST0531.

Figure 3 Kaplan-Meier curves representing 5-year **OS by fusion status** in separated cohorts (A) EpSSG RMS2005 and (B) COG ARST0531.

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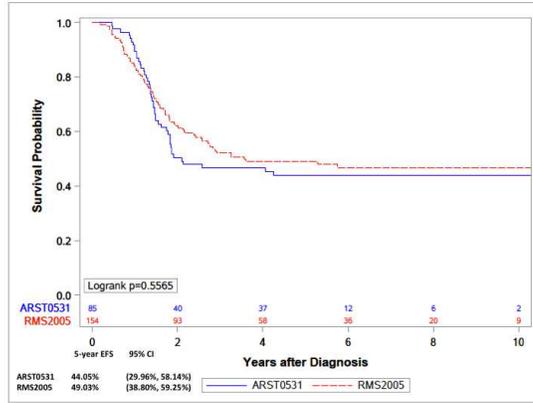
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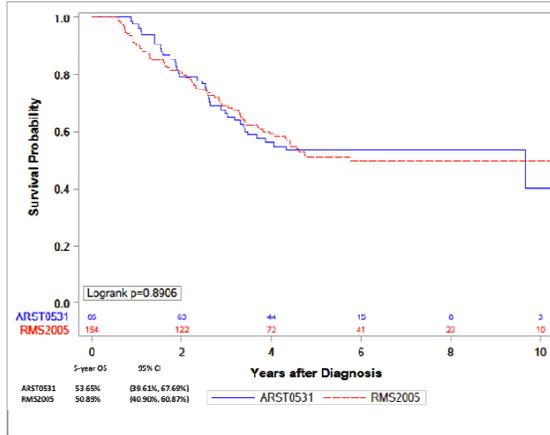
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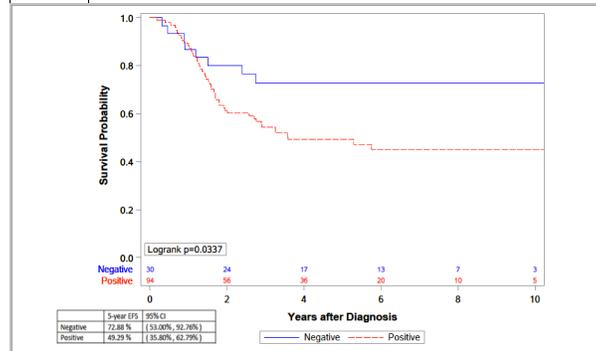


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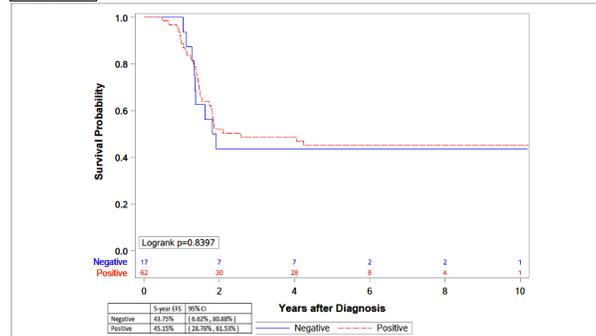
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RMS2005

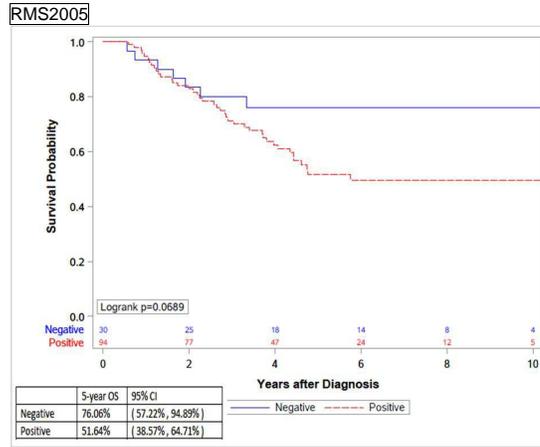


B

ARST0531



A



B

