Reappraisal of prognostic factors used in the EpSSG RMS 2005 study for localized rhabdomyosarcoma to optimize risk stratification and generate a prognostic nomogram

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Short title

A prognostic nomogram for localized Rhabdomyosarcoma patients

Condensed abstract

Traditional clinical factors together with FOXO1 fusion status in non-metastatic rhabdomyosarcoma were investigated to develop a predictive model for event-free survival and provide a rationale for risk stratification for future trials.

The most important result is the replacement of histology with fusion status and this model was utilized for the patient stratification in the new FaR RMS trial.

Keywords

Rhabdomyosarcoma; Nomograms; Proportional Hazards Models; Survival Analysis; Pediatrics; FOXO1 protein, human.

Authors' contributions.

Conceptualization: GLDS, PDB, GB, JHMM, VMC, JC, MJ
Methodology: GLDS, PDB
Data curation: VMC, JC, MJ, GG, CD, DO, AF, MCM, JHMM, GB
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ABSTRACT

PURPOSE

The objective of this study was to investigate the role of clinical factors together with FOXO1 fusion status in non-metastatic rhabdomyosarcoma to develop a predictive model for event-free survival and provide a rationale for risk stratification for future trials.

PATIENTS AND METHODS

This study used data from patients enrolled in the E*p*SSG RMS 2005 study. The following baseline variables were considered for the multivariable model: age at diagnosis, sex, histology, primary tumor site, IRS group, tumor size, nodal status, and FOXO1 fusion status.

Main effects and significant second-order interactions of candidate predictors were included in a multiple Cox proportional hazards regression model. A nomogram was generated for predicting 5-year Event Free Survival (EFS) probabilities.

RESULTS

The event-free and overall survival rates at 5 years were 70.9% (95% CI 68.6-73.1) and 81.0% (95% CI 78.9-82.8), respectively. The multivariable model retained 5 prognostic factors including age at diagnosis interacting with tumor size, tumor primary site, IRS group, and FOXO1 fusion status. Based on each patient's total score in the nomogram, patients were stratified into four groups. The 5-year EFS rates were 94.1%, 78.4%, 65.2%, and 52.1%, respectively, in low-, intermediate-, high-, and very high-risk groups. The corresponding 5-year OS rates were 97.2%, 91.5%, 74.3%, and 60.8%.

CONCLUSION

Results presented here have provided the rationale to modify the E*p*SSG stratification with the most significant change represented by the replacement of histology with fusion status. This classification was adopted in the new international trial launched by the EpSSG.

INTRODUCTION

Survival of patients with rhabdomyosarcoma (RMS) has improved in the past 30 years owing to the application of a multimodality approach that includes chemotherapy with surgery and/or radiotherapy.

Clinical trials coordinated by national and international cooperative groups have helped to refine the treatment and to identify the most active multidrug regimens through randomized studies.

A major advance has been the capacity to tailor the treatment strategy according to a series of prognostic factors found to be associated with different levels of risk of treatment failure[1-3]. The most powerful adverse risk factor for RMS patients is the presence of metastases at diagnosis. In this group, the outcome tends to be much poorer, with only one-third of patients surviving 3 years after diagnosis[4,5].

In the absence of metastatic dissemination, the search for prognostic factors is made difficult by the clinical and biological heterogeneity of RMS: patients vary in age (with two peaks of incidence: less than 6 years and adolescence), the primary tumor arises in many different sites across the body, and its disease extent and involvement of nearby organs and lymph nodes show considerable variation with consequences for accessibility to local therapy. Two main histological subtypes are distinguished: embryonal (ERMS; 70% of all RMS) and the poorer prognosis alveolar RMS (ARMS; 20-30%), characterized by the presence of PAX3/7- FOXO1 translocations.

All this information was used by the European *paediatric* Soft tissue sarcoma Study Group (E*p*SSG) to elaborate a risk stratification that has been used in the RMS 2005 protocol for non-metastatic RMS. The E*p*SSG risk stratification was based on six prognostic factors: histology, post-surgical stage according to IRS grouping, primary tumor site, nodal involvement, tumor size, and patient age as reported in Table 1.

Since the design of the RMS 2005 protocol, the association between PAX3/7-FOXO1 translocation and poorer prognosis has been recognized, overruling the impact of histological classification and leading to the replacement of histology by the FOXO1 fusion status in the current risk stratification

system used by the Children's Oncology Group[3], and the new E*p*SSG Frontline and Relapse RhabdoMyoSarcoma (FaR-RMS) study (NCT04625907).

The objective of the study presented here was to investigate the role of clinical factors together with FOXO1 fusion status in non-metastatic rhabdomyosarcoma patients treated in the RMS 2005 protocol to develop a predictive model for event-free survival and provide a rationale for risk stratification for future trials.

METHODS

Patients and treatments

This study used data from 1733 non-metastatic rhabdomyosarcoma patients enrolled in the E*p*SSG RMS 2005 study (EudraCT Number: 2005-000217-35) from October 1, 2005 to December 31, 2016. The data cut for last follow-up was 15th November 2022. Only patients with complete data were eligible for analyses. As FOXO1 was not always investigated a priori for patients with a favorable histology (Botryoid, Embryonal, Spindle cells/Leiomiomatous) since it was assumed to be negative, we performed a "clinical" and fixed imputation considering FOXO1 fusion status as negative for the 361 patients with favorable histology RMS without FOXO1 fusion data[6]. Patients with unfavorable histology (ARMS, Solid Alveolar, and Not Otherwise Specifed) without FOXO1 fusion data (n=47) and those with missing clinical data (n=9 without record of nodal involvement and n=16 with missing tumor size) were excluded yielding 1661 evaluable patients (Table S1). Ninety-four ARMS patients out of 362 (26%) were fusion negative, and this result is consistent with literature.

The protocol encouraged histology to be centrally reviewed and 73% of patients had the diagnosis reviewed by a national reference pathologist and/or by the international E*p*SSG Pathology Panel.

FOXO1 fusion status assessment was performed in different laboratories according to national arrangements. It was not mandatory and treatment usually was given based on the histopathology diagnosis. The analysis presented here has been performed according to the final diagnosis, i.e. the diagnosis reviewed centrally or, if this was missing, the local diagnosis.

Patients were assigned to one of the four RMS 2005 risk groups and treated according to the protocol guidelines that have been already described in detail[7-10] and summarized in Table 1.

The protocol included two randomized trials for high-risk patients that evaluated 1) two regimens of chemotherapy in the first part of treatment: IVA vs the IVADo regimen (IVA plus doxorubicin 30 mg/m^2 on days 1 and 2 in the initial 4 cycles of chemotherapy followed by 5 cycles of IVA) 2) the addition of a maintenance treatment with low dose cyclophosphamide and vinorelbine in patients in clinical complete remission after initial standard treatment.

Delayed surgery and/or radiotherapy were planned after assessing tumor response to the 3 initial cycles of chemotherapy. When a residual mass was identified, surgical resection was encouraged if clear margins were achievable without organ or functional impairment. Marginal resection at sites, where complete resection was deemed unfeasible, was acceptable, provided it was followed by radiotherapy. Radiotherapy was the only local treatment for patients not suitable for secondary surgery due to the tumor's location (i.e. parameningeal RMS). Radiotherapy doses were delivered according to histology, chemotherapy response and surgical results: 41·4 Gy were given to patients with alveolar RMS in IRS Group I or II, for patients in IRS Group III who achieved a complete remission after secondary surgery, and to patients with embryonal RMS that achieved a complete remission with initial chemotherapy; 50·4 Gy for cases of incomplete or unfeasible secondary resection.. A boost of 5.4 Gy to the residual tumor was recommended for large tumors responding poorly to chemotherapy. Radiotherapy to the involved lymph node sites was recommended at a dose of 41·4 Gy independently of histology and surgical resection. Treatment was delivered with megavoltage photons, one fraction per day, five days per week, with conventional fraction sizes of 1·8 Gy per day.

This study was approved by the ethics committee of the participating centers and informed consent was obtained from all patients according to the Declaration of Helsinki.

Statistical analysis

The primary endpoint was Event Free Survival (EFS) assessed by the investigator at each center and defined as the time from date of study enrolment to the date of the first event including death from any cause, progression of disease (in cases for which complete tumor remission was never achieved), relapse after previous complete remission, appearance of a new tumor, or time of the latest follow-up.

The following baseline variables were considered for the multivariable model: age at diagnosis, sex, histology, primary tumor site, IRS group, tumor size, nodal status, and FOXO1 fusion status.

For the purpose of this analysis, histology was maintained with its original clinical classification: favorable (Embryonal, Spindle cell) and unfavorable (Alveolar and not otherwise specified), while the categorization for age, tumor size, and primary tumor site was re-evaluated in order to confirm or to establish new groups with favorable and unfavorable prognosis.

Treatment received was not included as an independent prognostic factor since it was administered according to patient clinical characteristics considered in the multivariable model.

No formal sample size was calculated, since we used event per candidate variable for the derivation of the model[11]. Patient characteristics were summarized as median and interquartile range for continuous variables, or as count and percentage for categorical variables. To evaluate the functional form of age and size, these continuous variables were plotted against martingale residuals of null Cox proportional hazards model. Cut-off values were determined based both on a visual evaluation of martingale residual distribution and on cut-points corresponding to the most significant relation with the risk of event, estimated by maximally selected log-rank statistic for values between the 10% and 90% quantiles using the upper bound of the p-value by Hothorn and Lausen[12], as well as on optimal equal-HR method to discretize a continuous variable that has a U-shaped relationship with log relative hazards in survival data[13]. The classification for primary tumor site was defined both on a visual evaluation of martingale residual distribution and on pairwise logrank test with Benjamini-Hochberg correction.

Median follow-up time was computed using the reverse Kaplan-Meier method.

The occurrence of second-order interactions was verified using a likelihood ratio test comparing models with and without the interaction terms.

Main effects and significant second-order interactions of candidate predictors were included in a multiple Cox proportional hazards regression model. No deviation from the proportional hazards assumption was found by the test statistic of Grambsch and Therneau. A backward elimination with the Akaike information criterion (AIC) was applied for selecting all independent prognostic variables. A nomogram of the final reduced Cox regression model was generated for predicting 3-year and 5-year EFS probabilities. Model performance was evaluated by examining measures of discrimination and calibration. Discrimination, i.e. the ability of the model to differentiate between high-risk patients and low-risk patients, was calculated with Harrell's concordance (C) index, adjusted through 1000 bootstrap resamples. Bias-corrected calibration plots at 3-year and 5-year EFS rates were produced by a bootstrap procedure (1000 resamples) to account for consistency between observed and estimated survival probabilities.

Patients were stratified into 4 risk groups based on their individual score in the nomogram corresponding to optimal cut-points[12] and the log-rank test was used to compare groups. The survival probabilities were estimated using the Kaplan-Meier method and were reported with their 95% confidence interval (CI) calculated according to log-log transformation.

Statistical analyses were performed using R version 4.0.2, and packages rms, survival, survminer, and ggplot2.

RESULTS

Patient characteristics and outcome

Patient characteristics are summarized in Table 2. Median follow-up was 6.3 years (IQR [interquartile range] 4.5-8.6). During follow-up, 491 patients had an event and 326 died. Overall, 245 (50%) loco-regional relapses, 128 (26%) metastatic progressions, 84 (17%) progressive diseases, 24 (5%) second malignancies, 5 (1%) deaths due to disease and 5 (1%) failures due to toxicity were

registered. The pattern of treatment failures appeared different across age groups, with higher local failures in younger children: 24% in patients younger than 3 years, 18% in the group 3-10 years and 19% in the older patients (p=0.0156, Table S2).

The event-free and overall survival rates at 5 years were 71% (95% CI 69-73) and 81% (95% CI 79-83), respectively.

Continuous variable categorization

Considering age as a continuous variable, the risk of event was higher for patients younger than 3 years, it decreased moving towards 10 years, plateau from 10 to 14 years, and was higher again in older patients (Figure S1). We therefore categorized age at diagnosis as follows: less than 3 years, 3 to 9, 10 years or more.

When tumor size was considered as a continuous variable, the risk of event increased when the longest diameter was > 5 cm (Figure S1). Similarly, the single estimated cut-point that corresponded to the most significant relationship with outcome was 5 cm.

The analysis of the primary tumor site identified 3 groupings with different risks of an event: 1) bile ducts, genitourinary non bladder/prostate; 2) orbit, head and neck non parameningeal, bladder/prostrate; 3) extremity, parameningeal, and other sites. (Figure S1)

Multivariable model

Interactions involving age at diagnosis with tumor primary site, tumor size and FOXO1 fusion status, sex with tumor primary site and IRS group with tumor primary site (Table S3) were identified as significant and were included in the multivariable Cox regression model.

Backward elimination procedure based on AIC (6924.83) in the multivariable modeling retained 5 prognostic factors including age at diagnosis interacting with tumor size, tumor primary site, IRS group, and FOXO1 fusion status (Table 3). The nomogram predicting 3-year and 5-year EFS is presented in Figure 1.

The calibration plot for internal validation (Fig. S2) showed a good agreement of 3-year and 5-year event-free survival probabilities between the estimated outcomes and actual observations. The C-

index was 0.66 in the original data and the optimistic-corrected C statistics with 1000 bootstrap replications was 0.65.

Based on each patient's total score in the nomogram, patients were stratified into four groups: low-risk group (176/1661, 11%; total score <68), intermediate-risk group (701/1661, 42%; 68≤ total score <182.4), high-risk group (423/1661, 26%, 182.4≤ total score <232), and very high-risk group (361/1661, 22%, total score ≥ 232). The 5-year EFS rates were 94%, 78%, 65%, and 52%, respectively, in low-, intermediate-, high-, and very high-risk groups (Table 4 and Figure 2A). The corresponding 5-year OS rates were 97%, 92%, 74%, and 61% (Figure 2B).

DISCUSSION

There is a continuous need to refine risk classification for pediatric tumors to confirm prognostic variables used in the past and incorporate new findings as they are discovered and to help decide the best possible treatment for each patient. This analysis represents an effort to review the E*p*SSG classification that has been in use since 2005 and has served as a basis for the current E*p*SSG FAR-RMS trial. It is particularly important also to try to incorporate molecular findings in a classification system that has been so far based essentially on clinical factors.

A recent study validated the clinic-pathologic factors used by the Children Oncology Group studies and confirmed that patients age (>10 years), unfavorable tumor site, and tumor dimension (>5 cm) are associated with an inferior outcome. Clinical group, nodal involvement and histology were also confirmed as prognostic factors[3].

E*p*SSG adopted the same factors in the RMS 2005 study, but they were combined in a different way determining a different treatment allocation for at least a proportion of RMS patients. Our analysis confirms the prognostic value of most of the factors we used previously but also presents important new information.

The role of patient age as a prognostic variable is difficult to establish because biological characteristics and treatment modalities applied may change depending on age[14]. The unfavorable alveolar fusion positive RMS is more common in older children, the favorable spindle cell VGLL2/NCOA2 positive RMS is typical of infants. On the other hand, the treatment of younger children is challenging, and the RMS 2005 protocol recommended age-dose adaptation of chemotherapy for infants and a discussion case by case to decide the use of radiotherapy in children <3 years. Therefore, it is not surprising to find children <3 years old having a relatively poorer prognosis. The relatively higher proportion of local failures in this group may be determined by the difficulties to implement an aggressive local treatment and in particular radiotherapy for the concern about late sequelae.

The upper cut-off of ≥10 years is currently used both in E*p*SSG and COG trials and it is confirmed by our analysis. As the risk of failure is similar in patients 10 to 14 years old, a 14 years age limit could also be considered to identify patients at higher risk.

A tumor size of 5 cm in its largest diameter is confirmed as the optimal cut-off to separate children with differing risk. This variable is easy to use and it is not clear if considering 2 or 3 dimensions of the tumor may be more appropriate (but it is certainly more complicated)[15].

However, in our analysis, and as shown previously, age and tumor size outcomes were interdependent confirming that older children having large tumors represent the population at higher risk of treatment failure.

In comparison to patients with RMS arising in extremities, parameningeal sites or in the so-called other sites, those located in bladder/prostate and bile ducts had a better outcome. The latter sites were included in the unfavorable group in the RMS 2005 study, but in the light of the good results obtained in RMS 2005, we decided to move them into the more favorable standard risk group in the FaR-RMS trial. For biliary site, this is in contrast with the results presented by the COG group that recently decided to include biliary RMS in the unfavorable category due to suboptimal outcomes of patients treated in low-risk studies[16]. This difference may partially be explained by the different dose of alkylating agents administered to this group of patients in the E*p*SSG and COG studies and demonstrates the necessity of a common analysis and classification.

Clinical group has been identified as a major prognostic determinant since the initial cooperative studies on RMS[2] and it has always retained its value.

FOXO1 fusion status has been indicated as an independent prognostic factor by several retrospective studies. In a recent analysis published by COG only the presence of metastases surpassed FOXO1 fusion status as prognostic factor[6]. This led COG to include FOXO1 status in their stratification system. We present here similar results, further supporting the use of FOXO1 rather than histology to assign treatment to the patients. The inclusion of PAX3/7-FOXO1 fusion in risk stratification in place of histology in the FaR-RMS protocol represents a first attempt to include tumor molecular characteristics in the risk stratification. Additional prognostic biologic factors have been identified in

RMS. *MYOD1* and *TP53* mutations have been associated with a worse prognosis, while NCOA2/*VGLL2*-associated gene fusions have a very good prognosis. The RMS 2005 study was not designed to collect these data in our population so their inclusion in the E*p*SSG stratification is under debate.These and other new biological factors could have a very important role in stratifying patients.

The independent role of nodal involvement has been controversial. A lower survival has been reported both in alveolar and embryonal node-positive RMS included in the RMS 2005 study[8,17]. It is possible however that other tumor characteristics may be more important when a more intensive treatment is adopted. In addition, the gradual introduction of more sensitive imaging methods like FDG-PET may have changed the evaluation of nodal involvement, possibly upstaging patients with a lower tumor load and a better prognosis.

The impact of treatment has not been included in our model and this represents a limitation of our study. However treatment is determined by the risk groups assigned to the patient on the basis of the initial disease and patient characteristics. So we mainly aimed to identify prognostic factors that can stratify patients at diagnosis.

Besides identifying the role of different prognostic factors, the merit of this analysis is the production of a nomogram that may be used to calculate the prognosis for each patient based on currently known risk factors.

This methodology is in use for adult patients with sarcoma. Validated nomograms can be used to predict overall survival and distant metastases in patients after surgical resection of soft-tissue sarcoma of the extremities[18]. A nomogram has been developed to estimate the chance of salvage for individual children with relapsed rhabdomyosarcoma treated according to the International Society of Pediatric Oncology Malignant Mesenchymal Tumor (SIOP-MMT) protocols to direct therapy appropriately toward cure, use of experimental therapies, and/or palliation[19].

The nomogram we propose is based on a study that analyzes a large international multicenter population that has been treated homogeneously and with data prospectively collected. It can be used at diagnosis to assist clinicians to guide treatment. At the same time, it should be recognized we have not yet undertaken an external validation which could be facilitated through an international

collaboration. Moreover, it should be noted that this analysis used data from a prospective study where the assessment of the fusion status was not mandatory.

In conclusion, the results presented here have provided the rationale to modify the E*p*SSG stratification for adoption in the current FaR-RMS trial, confirming the adverse prognostic value of 10 years of age and 5 cm as tumor size. It also supported reconsideration of the role of primary tumor site. Bladder/prostate and biliary tree RMS are now included in the favorable category in the FaR-RMS trial. The most significant change is probably represented by the replacement of histology with fusion status. This makes the E*p*SSG stratification more similar to the COG system and will facilitate data comparison in the future.

In the meantime, the international community has recognized the need to adopt a common stratification system. This represents the main goal of the recently established INSTRuCT consortium[20] and will help establishing a common language and hopefully risk stratification in RMS treatment and research.

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Figure 1. A nomogram for the 3-year and 5-year event-free survival (EFS) probability prediction.

Figure 2. Kaplan-Meier curves for event free survival (A) and overall survival (B) stratified by risk group.

Figure S1. Assessment of functional form for continuous variables. Smoothed martingale residual plot from a null Cox PH model versus age at diagnosis, tumor size, and tumor site.

Figure S2. Performance of the prognostic model. Calibration curve showing predicted and actual 3-year and 5-year event free survival probabilities. The diagonal line indicates the perfect correspondence between the Kaplan-Meier observed probability (y-axis) and average nomogram-predicted probability (x-axis) for each equally sized subgroup of 150 patients. The 95% confidence intervals of the Kaplan–Meier estimates are indicated with vertical lines.

Table 1. Risk grouping stratification and therapy in E*p*SSG RMS 2005 study

Risk Group	Subgroups	Pathology	Post-surgical Stage (IRS Group)	Site	Node Stage	Size & Age	Chemotherapy	Delayed surgery	Radiation therapy
Low Risk	**A**	Favourable	I	Any	N0	Favourable	8 x VA	Not necessary	No
Standard Risk	***B***	Favourable	I	Any	N0	Unfavourable	4 x IVA + 5 x VA	Not necessary	No
	C	Favourable	II, III	Favourable	N0	Any	9 IVA **or** 5 x IVA + 4 x VA if radiotherapy	Yes, if not mutilating	Optional
	D	Favourable	II, III	Unfavourable	N0	Favourable	9 IVA	Yes, if not mutilating	Yes
High Risk	***E***	Favourable	II, III	Unfavourable	N0	Unfavourable	9 x IVA vs 4 IVADo + 5 IVA ± 6 x maintenance	Yes	Yes
	F	Favourable	II, III	Any	N1	Any			
	G	Unfavourable	I, II, III	Any	N0	Any			
Very High Risk	***H***	Unfavourable	II, III	Any	N1	Any	4 IVA Do + 5 IVA + 6 x maintenance	Yes	Yes

- **Pathology (histology):**

Favourable= all embryonal, spindle cells, botryoid RMS
Unfavourable= all alveolar RMS (including the solid-alveolar variant)

- **Post-surgical stage** (according to the IRS grouping, see appendix A.2):

Group I= primary complete resection (R0);
Group II= microscopic residual (R1) or primary complete resection but N1;
Group III= macroscopic residual (R2);

- **Site:**

Favourable= orbit, GU non bladder prostate (i.e. paratesticular and vagina/uterus) and non PM Head & neck
Unfavourable= all other sites (parameningeal, extremities, GU bladder-prostate and "other site")

- **Node stage** (According to the TNM classification, see appendix A1 and A.5):

N0= no clinical or pathological node involvement
N1= clinical or pathological nodal involvement

- **Size & Age:**

Favourable= Tumour size (maximum dimension) ≤5cm ***and*** Age <10 years
Unfavourable= all others (i.e. Size >5 cm ***or*** Age ≥10 years)

- **Chemotherapy**:

VA= Vincristine-Dactinomycin; IVA= Ifosfamide-Vincristine-Dactinomycin; IVADo= IVA-Doxorubin

Table 2. Patients' characteristics

Variable	Categories	Total (N=1661)
Age at diagnosis, years	Median (Q1, Q3)	5.3 (2.7, 10.1)
Sex, No. (%)	Female	655 (39%)
	Male	1006 (61%)
Histology, No. (%)	Favorable	1286 (77%)
	Embryonal/Botryoid	*1218 (73%)*
	Spindle cells	*68 (4%)*
	Unfavorable	375 (23%)
	Alveolar	*362 (22%)*
	Not Otherwise Specify	*13 (1%)*
Tumor site, No. (%)	Extremities	184 (11%)
	Bile ducts	26 (2%)
	Bladder/prostrate	196 (12%)
	Genitourinary - non bladder/prostate	322 (19%)
	Head and neck - non parameningeal	159 (10%)
	Parameningeal	393 (24%)
	Orbit	179 (11%)
	Other sites	202 (12%)
IRS, No. (%)	IRS I	204 (12%)
	IRS II	204 (12%)
	IRS III	1253 (75%)
Tumor size, No. (%)	a: ≤5 cm	815 (49%)
	b:>5 cm	846 (51%)
Tumor size*, cm	Median (Q1, Q3)	5.2 (3.4, 7.2)
Lymph-node status, No. (%)	N0	1392 (84%)
	N1	269 (16%)
FOXO1 fusion status, No. (%)	Negative	1393 (84%)
	Positive	268 (16%)

* data available for 1445 patients

Table 3. Multiple Cox regression model for event free survival and nomogram coefficients.

		E/N	HR (95%CI)	p-value	HR (95%CI) after bootstrapping	p-value	points
Tumor size	**a: ≤5 cm**						
	Age at diagnosis [0,3) years	76/233	1.44 (1.06, 1.97)	*0.0201*	1.41 (1.04, 1.92)	*0.0294*	70
	Age at diagnosis [3,10) years	86/394	Ref				25
	Age at diagnosis [10,max] years	33/188	0.81 (0.54, 1.22)	0.3122	0.82 (0.55, 1.23)	0.3438	0
	b:>5 cm						
	Age at diagnosis [0,3) years	76/234	1.15 (0.86, 1.54)	0.3381			57
	Age at diagnosis [3,10) years	124/377	Ref				40
	Age at diagnosis [10,max] years	96/235	1.58 (1.19, 2.09)	*0.0014*			96
Primary site	Extremities/HNPM/Other sites	312/779	Ref		Ref		75
	GUBP/HNnoPM/Orbit	125/534	0.60 (0.48, 0.75)	*<0.0001*	0.62 (0.49, 0.77)	*<0.0001*	12
	GUnoBP/Bile ducts	54/348	0.54 (0.38, 0.77)	*0.0006*	0.56 (0.40, 0.80)	*0.0014*	0
IRS group	IRS I	24/204	Ref		Ref		0
	IRS II	37/204	1.29 (0.76 - 2.21)	0.3512	1.27 (0.74 - 2.17)	0.3825	31
	IRS III	430/1253	2.27 (1.41 - 3.66)	*0.0008*	2.15 (1.33 - 3.48)	*0.0017*	100
Fusion status	Negative	367/1393	Ref		Ref		0
	Positive	124/268	1.45 (1.16, 1.80)	*0.0011*	1.41 (1.13, 1.76)	*0.0022*	45

Table 4. Patients' characteristics according to the risk groups.

		Low (N=176)	Medium (N=701)	High (N=423)	Very high (N=361)
Age at diagnosis	[0,3) years	6 (3%)	256 (36%)	23 (5%)	182 (50%)
	[3,10) years	102 (58%)	277 (40%)	331 (78%)	61 (17%)
	[10,max] years	68 (39%)	168 (24%)	69 (17%)	118 (33%)
Tumor size	a: ≤5 cm	147 (83%)	438 (62%)	144 (34%)	86 (24%)
	b:>5 cm	29 (17%)	263 (38%)	279 (66%)	275 (76%)
Primary site	Extremities/HNPM/Other sites		80 (11%)	346 (82%)	353 (98%)
	GUBP/HNnoPM/Orbit	21 (12%)	451 (64%)	54 (13%)	8 (2%)
	GUnoBP/Bile ducts	155 (88%)	170 (24%)	23 (5%)	
IRS group	IRS I	128 (73%)	73 (10%)	3 (1%)	
	IRS II	48 (27%)	142 (20%)	12 (3%)	2 (1%)
	IRS III		486 (69%)	408 (96%)	359 (99%)
Fusion status	Negative	176 (100%)	661 (94%)	360 (85%)	196 (54%)
	Positive		40 (6%)	63 (15%)	165 (46%)
Age & tumor	[0,3) years, b:>5 cm	6 (3%)	112 (16%)	3 (1%)	113 (31%)
size	[0,3) years, a:≤5 cm		144 (20%)	20 (5%)	69 (19%)
	[3,10) years, a:≤5 cm	79 (45%)	194 (28%)	104 (25%)	17 (5%)
	[3,10) years, b:>5 cm	23 (13%)	83 (12%)	227 (54%)	44 (12%)
	[10,max] years, a:≤5 cm	68 (39%)	100 (14%)	20 (5%)	
	[10,max] years, b:>5 cm		68 (10%)	49 (12%)	118 (33%)
EFS probability	3 years (95%CI)	94.1 (89.4, 96.8)	80.6 (77.4, 83.3)	68.4 (63.7, 72.6)	54.3 (48.9, 59.3)
	5 years (95%CI)	94.1 (89.4, 96.8)	78.4 (75.1, 81.3)	65.2 (60.4, 69.6)	52.1 (46.8, 57.2)
Type of event	Dead		2 (1%)	3 (2%)	
	Local-regional	6 (55%)	96 (64%)	66 (44%)	77 (43%)
	Metastases progression	4 (36%)	24 (16%)	40 (26%)	60 (34%)
	Other	1 (9%)	10 (7%)	13 (9%)	5 (3%)
	Progressive disease		19 (13%)	29 (19%)	36 (20%)
OS probability	3 years (95%CI)	98.8 (95.2, 99.7)	94.0 (92.0, 95.6)	78.7 (74.5, 82.4)	72.5 (67.5, 76.8)
	5 years (95%CI)	97.2 (92.7, 99.0)	91.5 (89.0, 93.4)	74.3 (69.7, 78.3)	60.8 (55.4, 65.9)

Table S1. FOXO1 fusion status distribution according to histology

	Negative	Positive	Missing	Total
Favorable	**925 (72%)**	**0**	**361 (28%)**	**1286**
Embryonal/Botryoid	871 (72%)	0	347 (28%)	1218
Spindle cells	54 (79%)	0	14 (21%)	68
Unfavorable	**107 (25%)**	**268 (64%)**	**47 (11%)**	**422**
Alveolar	94 (23%)	268 (66%)	44 (11%)	406
Not Otherwise Specify	13 (81%)	0	3 (19%)	16
Total	**1032 (60%)**	**268 (16%)**	**408 (24%)**	**1708**

Table S2. Pattern of treatment failures across age groups

Age (years)	**Local relapse only**	**Metastases[&]**	**Other***	**No**	**Total**	**p value**
<3	112 (24)%	29 (6%)	11 (2%)	315 (67%)	467	*0.0156*
3-10	138 (18%)	54 (7%)	18 (2%)	561 (73%)	771	
>10	79 (19%)	45 (11%)	5 (1%)	294 (70%)	423	
Total	**329 (20%)**	**128 (21%)**	**34 (2%)**	**1170 (70%)**	**1661**	

[&]87 metastases only, 41 combined local and distant relapse

*24 second malignancies, 5 deaths due to disease and 5 failures due to toxicity

Table S3. Anova table of second order interactions

Interacting variable 1	**Interacting variable 2**	**p value**
age at diagnosis	sex	*0.3589*
age at diagnosis	histology	*0.4576*
age at diagnosis	tumor primary site	*0.0059*
age at diagnosis	IRS group	*0.2173*
age at diagnosis	tumor size	*0.0005*
age at diagnosis	nodal status	*0.1841*
age at diagnosis	FOXO1 fusion status	*0.0266*
sex	histology	*0.2070*
sex	tumor primary site	*0.0020*
sex	IRS group	*0.0893*
sex	tumor size	*0.0786*
sex	nodal status	*0.1093*
sex	FOXO1 fusion status	*0.2642*

histology	tumor primary site	*0.4117*
histology	IRS group	*0.2660*
histology	tumor size	*0.7694*
histology	nodal status	*0.5489*
histology	FOXO1 fusion status	*1.0000*
tumor primary site	IRS group	*0.0249*
tumor primary site	tumor size	*0.1058*
tumor primary site	nodal status	*0.8801*
tumor primary site	FOXO1 fusion status	*0.7790*
IRS group	tumor size	*0.2331*
IRS group	nodal status	*0.0624*
IRS group	FOXO1 fusion status	*0.1613*
tumor size	nodal status	*0.1048*
tumor size	FOXO1 fusion status	*0.8547*
nodal status	FOXO1 fusion status	*0.9580*