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Genomic identification of sarcoma radiosensitivity and the clinical implications for radiation dose personalization

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ABSTRACT

Background: Soft-tissue sarcomas (STS) are heterogeneous with variable response to radiation therapy (RT). Utilizing the radiosensitivity index (RSI) we estimated the radiobiologic ratio of lethal to sublethal damage (α/β), genomic-adjusted radiation dose(GARD), and in-turn a biological effective radiation dose (BED).
Methods: Two independent cohorts of patients with soft-tissue sarcoma were identified. The first cohort included 217 genomically-profiled samples from our institutional prospective tissue collection protocol; RSI was calculated for these samples, which were then used to dichotomize the population as either highly radioresistant (HRR) or conventionally radioresistant (CRR). In addition, RSI was used to calculate α/β ratio and GARD, providing ideal dosing based on sarcoma genomic radiosensitivity. A second cohort comprising 399 non-metastatic-STS patients treated with neoadjuvant RT and surgery was used to validate our findings.
Results: Based on the RSI of the sample cohort, 84% would historically be considered radioresistant. We identified a HRR subset that had a significant difference in the RSI, and clinically a lower tumor response to radiation (2.4% vs. 19.4%), 5-year locoregional-control (76.5% vs. 90.8%), and lower estimated α/β (3.29 vs. 5.98), when compared to CRR sarcoma. Using GARD, the dose required to optimize outcome in the HRR subset is a $BED_{\alpha/\beta=3.29}$ of 97 Gy.
Conclusions: We demonstrate that on a genomic scale, that although STS is radioresistant overall, they are heterogeneous in terms of radiosensitivity. We validated this clinically and estimated an α/β ratio and dosing that would optimize outcome, personalizing dose.

Research in context:

Current preoperative radiotherapy in soft-tissue sarcoma (STS) does not take into account potential differences in intrinsic radiosensitivity. The radiosensitivity index (RSI) has identified STS as overall radioresistant. We hypothesize that RSI can be used to distinguish highly radioresistant STS histologies, which may subsequently allow the individualization of radiotherapy dose using the genomic adjusted radiation dose (GARD).

Added value of this study

We demonstrate that RSI can be used to group STS histologies into conventionally and highly radioresistant histologies. We demonstrate a difference in locoregional control between these groups.

Implications

We demonstrate the utility of RSI in identifying radiosensitivity of STS histologies and propose this as the framework for personalized

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radiotherapy dose in STS.

Introduction

Soft tissue sarcomas (STS) constitute a heterogeneous group of rare solid tumors of mesenchymal cell origin with distinct histological and clinical features. Due to the rarity of STS and the diverse histopathological subtypes, the study and treatment of STS has been challenging [1]. STS has generally been classified into subtypes according to their histological resemblance to normal tissue. Common subtypes of STS include undifferentiated pleomorphic sarcoma (UPS), liposarcoma (LPS), and leiomyosarcoma (LMS) [2]. The mainstay in the treatment of STS is surgery, but the addition of perioperative radiation treatment (RT) has allowed for improved limb preservation and improved locoregional control (LRC) [3,4]. The RT volume, dose, and timing of treatment are clinical decisions primarily based on clinical features (e.g. size, tumor location, etc.).

Since there is evidence that favorable pathologic response (fPR≥90–95%) can predict for LRC and OS [5,6], neoadjuvant dose escalation has been considered but is limited by toxicity and long-term sequelae. Currently, retroperitoneal (RP) STS is one of the only subtypes considered for selective dose-escalation, but this decision is based on expected high microscopic residual tumor burden (e.g. anatomic challenges preventing clear margins) rather than specific tumor biology [7-9]. To date, this is the first exploration of utilizing biology to personalize RT-based treatment for soft tissue sarcomas.

Improvements in understanding the intrinsic radiation sensitivity of STS can potentially guide the evolution of more effective RT. Our group has previously described an algorithm to assess the intrinsic radiosensitivity of tumors using a genome-based approach - the radiosensitivity index (RSI) [10-12]. RSI is a 10-gene signature that has been clinically validated in over 2200 RT- receiving patients across multiple malignancies, including: glioblastomas, breast, colon, pancreas, and lung tumors [13-20]. In radiobiology, the intrinsic radiosensitivity of a cell has been commonly referred to as the alpha (α), whereas the beta (β) reflects a cell's ability for cellular repair [21]. Therefore, patients with a low α/β ratio, as seen in sarcoma (2 to 6), require higher radiation dose per fraction to have a biologically equivalent dose [22-24]. Further, RSI can be used to estimate a tumor specific α value creating a personalized α/β [18,25]. In addition, our group developed a model which incorporates RSI and RT dose to estimate the genomically based effective dose, referred to as the genomic adjusted radiation dose (GARD), which can be utilized to personalize RT dose prescription for individual patients [18].

Although soft-tissue sarcoma (STS) is thought to be resistant to radiation, we hypothesize that there will be significant variability in the gene expression-based radiosensitivity index (RSI). Our goal will be to identify radioresistant subsets of STS and evaluate for differences in α/β ratio, and whether this translates to a difference in a response to neoadjuvant radiation, as measured by favorable pathologic response (FPR) and local control (LC), We will then utilize GARD to identify a biological effective dose required to optimize patient outcome.

Materials and methods

Identification of clinical correlates for RSI in STS: The study was institutional review board (IRB) approved by the University of South Florida and Moffitt Cancer Center (MCC). This cohort was extracted from Total Cancer Care (TCC), a prospective IRB-approved data and tissue collection protocol active at Moffitt and 18 other institutions since 2006[26]. Tumors from patients enrolled in TCC protocol were arrayed on Affymetrix Hu-RSTA-2a520709 (Affymetrix, Santa Clara, CA), which contains approximately 60,000 probesets representing 25,000 genes. Chips were normalized using iterative rank-order normalization (IRON) [27]. Batch-effects were reduced using partial-least squares (PLS). To identify clinical correlates for RSI in STS, we abstracted patient information from the TCC database.

For clinical correlation, we retrospectively reviewed a cohort of 399 STS patients treated with neoadjuvant RT and surgery from 11/1987 to 1/2016. Neoadjuvant therapy consisted of preoperative radiation to a median dose of 50 Gy in 25 fractions in 2 Gy daily fractions – treatment technique used was 3D-conformal RT or intensity modulated RT (IMRT). The patient and clinical characteristics were obtained by the TCC protocol and reviewed from the clinical chart. Osteosarcoma, Ewing sarcoma, and chondrosarcoma histologies were confirmed as extraskeletal in origin. Pathologists specializing in soft tissue sarcoma confirmed all histologies and determined response at the time of surgery, by quantifying percentage of viable cells remaining in concert with tumor necrosis, when possible.

Individual tumor intrinsic radiosensitivity assessment

RSI score was calculated with the previously published algorithm listed below[10-12] where the lower the RSI, the more radiosensitive the tumor.

$$RSI = -0.0098009 * AR + 0.0128283 * cJun + 0.0254552 * STAT1 - 0.0017589 * PKC - 0.0038171 * RelA + 0.1070213 * caBL - 0.0002509 * SUMO1 - 0.0092431 * PAK2 - 0.0204469 * HDAC1 - 0.0441683 * IRF1$$

A previously described RSI cut-off of ≥0.375 was identified as radioresistant [18]. This cut-point was utilized to determine the proportion of sarcoma histologies deemed radioresistant when compared to other tumor types similarly assessed by RSI. To dichotomize our clinical cohort into highly radioresistant sarcomas (HRR) or conventionally radioresistant sarcomas (CRR), we evaluated the 75th percentile RSI value for each histology, against the RSI distribution for the whole cohort. Histologies with a median at or above the 75th percentile RSI value for the whole cohort were classified as highly radioresistant.

Estimating personalized α/β and genomic-adjusted radiation dose in sarcoma

A patient-specific α was derived by substituting the radiosensitivity index for survival (S) in $S = e^{-nd(\alpha + \beta d)}$, where dose (d) is 2 Gy, n=1, and β was derived from sarcoma cell line experiments (0.045/Gy) [23,24,28]. The algorithm of GARD has been previously described [18]. GARD was derived using the linear quadratic model and the individual gene-expression-based RSI, defined as GARD=nd(α + βd). GARD was modeled for the neoadjuvant radiation dose in this study (50 Gy in 25 fractions). The calculation for GARD is similar to the biologically effective dose (BED) but is genomically determined with a patient-specific α. A higher GARD predicts a higher radiation therapeutic effect on the tumor.

To identify a GARD value that distinguishes CRR and HRR sarcomas, receiver operator characteristic (ROC) analysis was utilized to guide recommendations for dose which maximized sensitivity and specificity. Using the specific α/β, we determined the dose required for highly radioresistant sarcomas (HRR) to achieve effective therapeutic doses as the conventionally radioresistant sarcomas (CRR), with respect to total number of fractions (n), and daily dose (d).

Statistical analysis

Descriptive statistics were administered to assess the clinical cohort for continuous and categorical variables with comparisons using Pearson Chi Square and Mann-Whitney U test as appropriate. Kaplan-Meier curves with log-rank tests were calculated from the start of RT to the date of event or last follow-up. Locoregional control was defined as absence of failure within gross disease in the primary site. Univariate and multivariate analyses (UVA and MVA, respectively) were conducted with Cox proportional-hazard models. Multivariate analysis included variables associated with locoregional control, such as age (≥50 vs.

<50), margin status, primary site, and clinical tumor classification. Grade of disease was omitted from MVA due to difficulties with consistent grading after neoadjuvant therapy.

SPSS25 (IBM corporation, Armonk, NY) and JMP 15(SAS Institute, Cary NC), were used for statistical analyses and generation of figures and graphs. A two-tailed p <0.05 was considered statistically significant. Mathematical calculations were performed using Excel 2016 (Microsoft, Redmond WA).

Results

Highly radioresistant sarcomas as a clinical correlate for radioresistance

There were a total of 217 resected sarcoma samples available. The most common histologies were leiomyosarcoma (n=53, 24.2%) and non-myxoid liposarcoma (n=44, 20.2%) (Table 1). Table 1 depicts the distributions of RSI by histology.

There was a significant overall difference in RSI when comparing all histologies (ANOVA test p=0.011) (Fig. 1a), especially HRR, when compared to CRR. (Fig. 1b). One hundred ninety-five (84%) of the samples had RSI ≥0.375, and would be considered radioresistant when compared to other cancers [15]. The most radiosensitive histologies were angiosarcoma (median 0.44, range 0.2–0.64) and extraskeletal Ewing sarcoma (median 0.47, range 0.41–0.49),whereas the least radiosensitive histology was non-myxoid liposarcoma (median 0.62, 0.25–1.0).

The 25th and 75th percentile of RSI were 0.42 and 0.62 – using the 75th percentile we designated the histologies with median RSI values above this as highly radioresistant sarcoma (HRR), in contrast to histologies which fell below this threshold, termed conventionally radioresistant sarcoma (CRR). Non-myxoid liposarcoma was the sole histology within the HRR cohort (n=44), with the remaining sarcoma histologies comprising the CRR group (n=173).

Based on the RSI data from resected specimens, HRR histology was used as a clinical surrogate for radioresistance in STS. Fig. 1b shows the difference in RSI of STS when dichotomized with this approach (Mann-Whitney U p<0.001).

The clinical characteristics of the STS cohort (n=399) are presented in Table 2. The cohort was most commonly HRR (n=67, 16.7%), age ≥50 (n=313, 78.4%), cT2b (n=280, 70.2%), high grade (n=223, 55.9%), negative surgical margins (n=274, 68.7%), and extremity site (n=241, 60.4%) (Table 2a). There is a significant difference in characteristics by histology with HRR having more unknown grade (p=0.01), retroperitoneal primary site (p<0.01), and higher portion of positive surgical margins (p<0.01) (Table 2a). The histology specific breakdown are illustrated in Table 2b.

Response to therapy

In the entire cohort, the median tumor response to RT was 52%, with 16.4% achieving a ≥95% pathologic response rate. HRR patients had a lower rate of treatment response to RT (median 35% vs. 60%, p=0.01), with a significant difference in favorable pathologic response (≥95%: 2.4% vs. 19.4%, p<0.01).

At a median follow-up of 58.9 months in surviving patients, the 5-year LRC was 88.1%. On UVA, HRR had a significant decrease in 5-year LRC (76.5% vs. 90.8%, p=0.01) (Fig. 2) compared to CRR. On MVA, HRR histology was an independent predictor for worse LRC, with an HR of 2.54 (95% CI 1.23-5.22 p=0.01) (Table 3). There was no significant difference in 5-year OS between groups (p=0.08).

GARD modeling

Using specimen specific-RSI to calculate a patient-specific α and sarcoma-specific β (0.045), we calculated the median α/β of the entire cohort as 5.42, which was significantly lower in HRR (3.29 IQR 2.1-5.0) when compared to CRR (5.98 IQR 4.0-7.7, p<0.01).

When the GARD was modeled for a total delivered dose of 50 Gy in 2 Gy fractions we observed a wide range of GARD distributed amongst sarcoma histologies. The median delivered GARD was 16.7 (Fig. 3), which was significantly lower for HRR (11.9), when compared to CRR (18.0) (p<0.01).

Given the clinically significant differences in LRC between HRR and CRR, we modeled the radiation dose-escalation required based on the GARD for CRR. This increase in GARD reflects the differences in intrinsic radiosensitivity between HRR and CRR. A threshold GARD of 14.37 was estimated as the ideal cut-point that significantly differentiated between HRR and CRR sarcomas (sensitivity of 70.5%, specificity of 71.7%, and an area under curve (AUC) of 0.71, p<0.01).

A small proportion of HRR patients (7/44, 16%) achieved the GARD threshold (14.37), reflecting a range of $BED_{\alpha/\beta=3.29}$ from 57 to 178 Gy after neoadjuvant radiation (50 Gy) was delivered. In contrast, majority of CRR patients (124/173, 71.6%) achieved above the GARD threshold, with a higher $BED_{\alpha/\beta=5.98}$ range (54.1 to 310.5 Gy).

When modeling GARD, the additional number of fractions needed by HRR (α/β= 3.29) to achieve the GARD threshold (14.37) is 5.2 fractions, at 2 Gy per fraction, totaling 60.4 Gy. Derivation of dose per fraction needed to achieve a GARD of 14.37 in 25 fractions resulted in a daily dose of 2.3 Gy, or 57.3 Gy in 25 fractions. The $BED_{\alpha/\beta=3.29}$ estimated to optimize outcome was ≥97 Gy.

Discussion

Clinically, soft-tissue sarcomas are largely considered radioresistant and treated homogenously. On a genomic level, our study confirms this,

Table 1
Specimen Cohort Characteristics – the most radiosensitive histologies are angiosarcoma, Ewing sarcoma, and chondrosarcoma. The most radioresistant histology is non-myxoid liposarcoma, with a median RSI value above 0.60 and above the 75th percentile of all RSI values. Abbrev: malignant peripheral nerve sheath tumor (MPNST), not otherwise specified (NOS)

Histology	Count N	%	Mean	Median	RSI Minimum	RSI Maximum
Angiosarcoma	14	6.4%	0.43	0.44	0.20	0.64
Ewing Sarcoma	3	1.4%	0.46	0.47	0.41	0.49
Chondrosarcoma	11	5.0%	0.47	0.47	0.19	0.63
MPNST	3	1.4%	0.47	0.48	0.23	0.70
Sarcoma NOS	35	16.1%	0.49	0.49	0.12	0.80
Pleomorphic Sarcoma	29	13.3%	0.50	0.52	0.25	0.75
Leiomyosarcoma	53	24.3%	0.49	0.51	0.09	0.69
Osteosarcoma	7	3.2%	0.52	0.53	0.42	0.63
Rhabdomyosarcoma	1	0.5%	0.54	0.54	0.54	0.54
Fibrosarcoma	7	3.2%	0.55	0.48	0.38	0.86
Synovial Sarcoma	11	5.0%	0.57	0.55	0.29	0.84
Non-myxoid Liposarcoma	44	20.2%	0.60	0.62	0.25	1.00

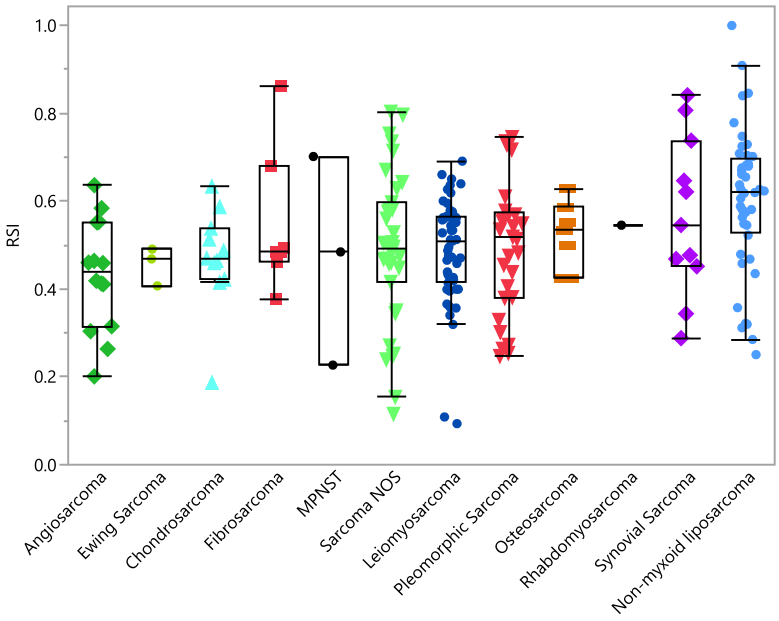

Fig. 1a. RSI plot by individual sarcoma histology.

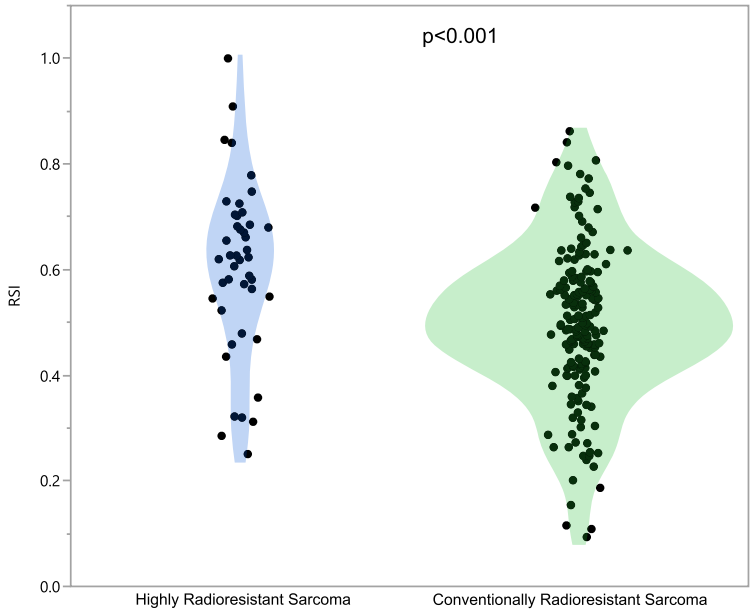

Fig. 1b. Highly Radioresistant Sarcoma and Conventionally Radioresistant sarcoma violin plot for RSI.

3.2. HRR histology is associated with worse pathological response and clinical outcome.

but also shows that this resistance can range both within and between histologies. Our study is the first large scale assessment of radiosensitivity in soft tissue sarcomas, utilizing a radiation-specific biomarker to identify a highly radioresistant subset. We demonstrated that the HRR subset has a significantly different treatment response to neoadjuvant radiation, which was independently associated with a detriment in local control. We also genomically estimated an α/β, that although fell into the range of historically reported α/β in sarcoma (2 to 6), was significantly different between radioresistant subsets. This provided the first genomic specific dose personalization, which coincides with the dosing utilized in current prospective studies from Massachussetts General Hospital [9].

Current national guidelines for STS include preoperative radiotherapy prior to definitive resection as a category one recommendation for operable patients [2], with a trend in its utility over postoperative RT, due to key advantages including: lower radiation dose (50 Gy vs. >60 Gy), smaller treatment volumes, and improved R0 resection rate [29]. These differences may translate to decreased long-term toxicity including fibrosis [30], joint stiffness [30], edema [30,31], and pain [31]. In addition, meta-analysis data suggest that this approach can also lead to improved oncologic outcomes (e.g. LC, DM, an OS) [4,32,33], which may be due to improved tumor oxygenation, radiographically definable lesion with preoperative therapy, prevention of tumor spillage or seeding during resection, and immune modulatory effects.

Favorable pathologic response (fPR) rates in STS, is ~ 8% after

Table 2a
Clinical Cohort Characteristics – patients were balanced with regards to age and clinical tumor stage. Highly radioresistant sarcomas had a large proportion of low grade disease, retroperitoneal primary site, and higher positive margin rate. Institutional practice considers a positive margin to include disease suspicious for well-differentiated liposarcoma at ink. Of the HRR patients with positive margins, only 7 had high-grade/dedifferentiated liposarcoma at the surgical margin.

	Characteristic	Overall		Highly Radioresistant (HRR)		Conventionally Radioresistant (CRR)		Chi Square (*p* value)
		N	%	*N*	%	*N*	%	
Age	Age <50 years	86	21.6%	15	22.4%	71	21.4%	0.86
	Age ≥50 years	313	78.4%	52	77.6%	261	78.6%	
Tumor Stage	cT1a	7	1.8%	1	1.5%	6	1.5%	0.61
	cT1b	39	9.8%	5	7.5%	34	8.5%	
	cT2a	30	7.5%	3	4.5%	27	6.8%	
	cT2b	280	70.2%	51	76.1%	229	57.4%	
	Unknown stage	43	10.8%	7	10.4%	36	9.0%	
Grade	Low grade	16	4.0%	10	14.9%	6	1.8%	<0.01
	High grade	223	55.9%	36	53.7%	187	56.3%	
	Unknown	160	40.3%	21	31.3%	139	41.9%	
Primary Site	Extremity	241	60.4%	23	34.3%	218	65.7%	<0.01
	Retroperitoneal	88	22.1%	32	47.8%	56	16.9%	
	Other	70	17.5%	12	17.9%	58	17.5%	
Surgical Margins	Negative	274	68.7%	31	46.3%	243	73.2%	<0.01
	Positive	115	28.8%	36	53.7%	79	23.8%	
	Unknown	10	2.5%	0	0.0%	10	3.0%	

Table 2b
Sarcoma Histology Subtypes. The most common HRR subtypes were de-differentiated and well-differentiated liposarcoma. The most common CRR subtypes were pleomorphic sarcoma and giant cell sarcoma.

Non-myxoid liposarcoma histology subtypes (*n*=67)	*N*	%
Liposarcoma, NOS	11	16.4%
Well-Differentiated Liposarcoma	17	25.4%
Pleomorphic Liposarcoma	11	16.4%
De-differentiated Liposarcoma	28	41.8%
Other sarcoma histology subtypes (*n*=332)	***N***	**%**
Alveolar soft part sarcoma	1	0.3%
Angiosarcoma	19	5.7%
Extraskeletal chondrosarcoma	9	2.7%
Dermatofibrosarcoma, NOS	1	0.3%
Epitheloid sarcoma	1	0.3%
Extraskeletal Ewing sarcoma	5	1.5%
Fibromyxosarcoma	32	9.6%
Fibrosarcoma, NOS	7	2.1%
Leiomyosarcoma	29	8.7%
Malignant solitary fibrous tumor	2	0.6%
Malignant peripheral nerve sheath tumor	7	2.1%
Myxoid sarcoma (NOS)	2	0.6%
Extraskeletal osteosarcoma	3	0.9%
Undifferentiated pleomorphic sarcoma	88	26.5%
Primitive neuroectodermal tumor	2	0.6%
Rhabdomyosarcoma	6	1.8%
Sarcoma, NOS	32	9.6%
Spindle cell Sarcoma	32	9.6%
Stromal sarcoma	9	2.7%
Synovial sarcoma	24	7.2%
Undifferentiated sarcoma	21	6.3%

standard neoadjuvant radiation [34]. The fPR in our study was markedly different between HRR and CRR (2.4% vs. 19.4%), highlighting genomically identified differences in the innate radiobiology. Achieving a fPR has been proposed as a potential surrogate for response to radiation, as it has been associated with improved R0 resection rate, LC, DM, and OS [5,6,35]. In addition, specific histologies that are commonly treated with definitive RT alone, (e.g. Ewing sarcoma and angiosarcoma), were estimated to have the highest radiosensitivity in our study, further confirming the validity of our genomic signature [36-38]. This also suggests that there may be a subset of sarcoma patients that could achieve adequate local control with a definitive radiation approach.

Therefore, identifying a population who would selectively benefit from intensified therapy in the form of radiation dose escalation, could potentially improve treatment response and in-turn disease outcome. Based on the GARD, an ideal threshold to improving outcome for HRR subset includes achieving an adequate equivalence dose ($BED_{3.29}$ ≥97 Gy), which translates to 57.3 Gy in 25 fractions or >60 Gy (2 Gy/fraction). Current dose escalation strategies target areas at high risk for residual microscopic disease (e.g. posterior rim in retroperitoneum sarcoma) [39]. Interestingly, this work and retrospective work leading up to these trials have utilized doses ≥57.5 Gy in 25 fractions [8,40]. In a recent phase I trial, with a high proportion of HRR sarcoma (64%)[9], the 18 month follow up with a dose escalated approach reported no failures in the 9 patients that underwent resection, which surpasses the historic local control rates of 60–80%[41,42]. These preliminary findings may be due to the higher portion of HRR sarcoma with a higher potential for microscopic residual disease. The recently reported STRASS trial utilized a composite abdominal-recurrence free survival endpoint in evaluating preoperative RT in retroperitoneal sarcomas – although this was a negative trial overall, subset analyses focusing on liposarcoma histology appears to support the notion that these tumors may benefit from upfront RT [43].

Our study was the first to genomically estimate the α/β ratio in sarcoma, which coincides with the historic in vitro studies, which showed that the α/β ratio in response to radiation was 2 to 6 [44]. Although our model estimated that both HRR and CRR were within this historic range (3.29 vs. 5.98), they were significantly different. Therefore, determining a personalized α/β ratio can help determine ideal dosing, fractionation, and potentially the ideal therapeutic window to improve disease response and patient toxicity. This is particularly an area of interest as recent studies have investigated more hypofractionated approaches to neoadjuvant radiation [45], and the dosing schema chosen can be tailored to the patient.

As our efforts to identify radiosensitivity in STS evolves, our goal is personalize treatment based on the innate biology of their disease, rendering care histologically agnostic. This evolution could further individualize patients based on their intrinsic tumor genomics and recommend an optimal GARD, dose, and potentially fractionation. Just as landmark prognostic indices have identified which breast cancer patients derive the most benefit from chemotherapy, RSI/GARD can shape how we personalize RT in the future.

Limitations

Despite the novel analysis that RSI and GARD have to offer for sarcoma, our study contains some limitations. The primary limitation remains that the individual RSI values are not available for our clinical

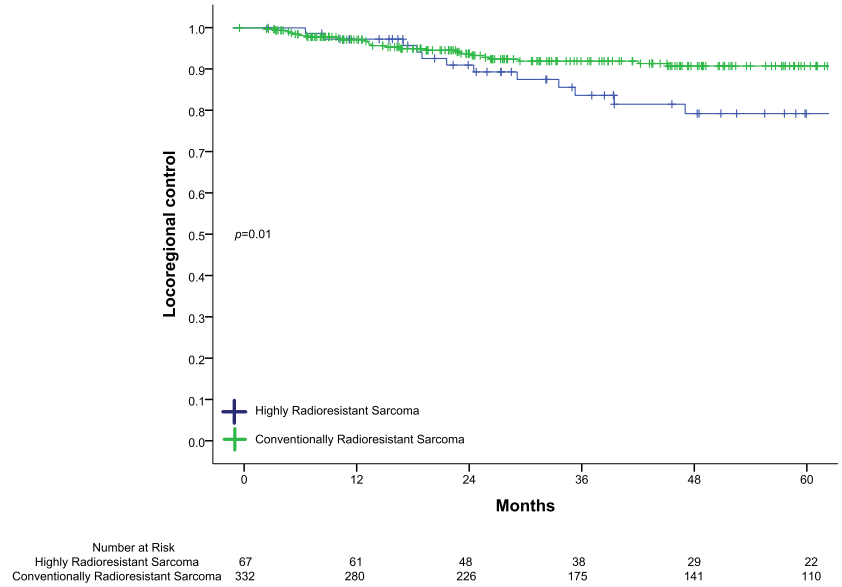

Fig. 2. Highly Radioresistant Sarcoma and Conventionally Radioresistant Sarcoma violin plot for RSI.

Table 3
Multivariate Analysis for locoregional control demonstrated HRR histology as an independent predictor of worse locoregional control, even when evaluating for other features that are traditionally considered as risk factors for recurrence such as age, margin status, clinical T-stage, and primary site.

Characteristic	Hazard ratio	95% Confidence Interval	*P*-value
Age <50 years	1.00	Ref	-
Age ≥50 years	1.35	0.59–3.10	0.48
Margin status			
Negative surgical margins	1.00	Ref	-
Positive surgical margins	1.87	0.93–3.80	0.08
Clinical T-stage			
T1	1.00	Ref	-
T2	0.70	0.33–2.10	0.84
Histology			
Highly Radioresistant	2.54	1.23–5.22	0.01
Conventionally Radioresistant	1.00	Ref	-
Primary site			
Extremity	1.00	Ref	-
Retroperitoneum/Pelvis	0.64	0.27–1.53	0.32
Other	1.09	0.46–2.53	0.85

correlation cohort, as the RSI was derived from resected surgical tissue from our institution's biorepository. Myxoid liposarcomas were not included in this analysis, given their unusual radiosensitivity and unique genetic features, with over 90% of myxoid liposarcomas containing the 12q13 and 16q11 translocation, leading to *FUS-DDITR* (*TLS-CHOP*) fusion [46,47]. This translocation family may be responsible for these unusually radiosensitive characteristics, along with distinct dense vascular patterns in response to radiation [46], which sets this histology apart from all other soft tissue sarcomas and is now an area of interest for treatment de-escalation [48]. Unfortunately, this unique and not yet elucidated mechanism for unusual radiosensitivity is not adequately captured with this genomic signature [48].

The treatment regimens utilized in our study were standard preoperative courses of radiotherapy, and although it offers a homogeneous treatment paradigm, it is difficult to determine varying effects dose-escalation or altered fractionation would have on tumor response. There is no consensus agreement regarding measuring effective neoadjuvant therapy response in STS, especially as necrosis is a component of grading and a potential confounder to treatment response. This limitation was mitigated by using pathologists that specialize in STS, who quantified percent of viable cells remaining in concert with necrosis, when possible. The use of neoadjuvant therapy allowed us to evaluate tumor response but made pathologic grading less reliable. This contributes to the high percentage of unknown grade in our study. There was also a difference in the clinical characteristics between the HRR and CRR cohorts. Namely, there were a higher percentage of retroperitoneal sarcoma and positive margins in the HRR group. Truly negative margins are difficult to obtain in RPS, as our institutional practice considers a positive margin to include disease suspicious for well-differentiated liposarcoma at ink. Of the 67 HRR patient, 32 (47.8%) had RPS, and only 7 had high-grade/dedifferentiated liposarcoma at the surgical margin. Even after accounting for this on the MVA, HRR histology independently predicted for >2 times the risk of developing a locoregional failure. The use of RSI/GARD has been derived from photon-based treatment with unknown correlation to particle therapy. However, further studies investigating the relationship of the relative biological effectiveness (RBE) of proton/particle therapy and RSI could be promising. Due to the limitations of this retrospective study, a large prospective study evaluating tumor response, potentially accounting for tumor volume/heterogeneity and patient outcome utilizing GARD is required. These future trials could help answer further questions on the impact of altered/hypofractionation for normal tissue effects, which are currently unclear and primarily extrapolated from other disease entities [49].

Conclusions

Sarcomas are radioresistant by nature. This study is the first to demonstrate that within this heterogeneous group lies a broad range of radiosensitivities, most notably for non-myxoid liposarcoma histology. With the use of the radiosensitivity index (RSI), we were able to genomically estimate a broad range of α/β within the range previously described in the literature. The innate tumor radiosensitivity is reflected in both the radiation treatment response (favorable pathologic response) and locoregional control. With the use of genomics, we estimate an effective dose (GARD) that could improve patient outcomes. In highly radioresistant subpopulations, the ideal dose needed to optimize outcome was a $BED_{3.29}$ ≥97 Gy or >60 Gy (2 Gy/fraction).

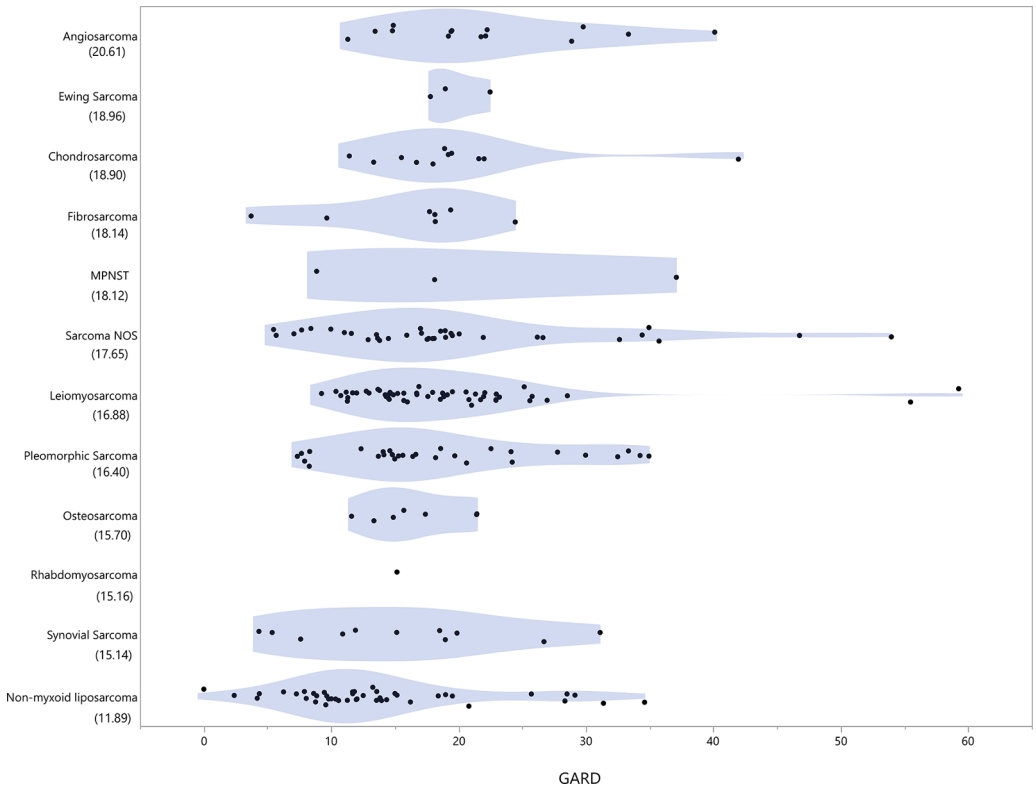

Fig. 3. Violin plot of GARD by histology with median GARD values. Sarcoma histologies are listed in decreasing GARD, with increasing GARD representing more effective radiation dose based on individual tumor RSI value. Median GARD of the entire cohort was 16.7, with HRR (non-myxoid liposarcoma) median GARD of 11.9, and CRR histologies median GARD of 18.0.

Declaration of Competing Interest

Javier F. Torres-Roca MD reports stock and leadership in Cvergenx, Inc. and royalty and patents on RSI. Jacob G. Scott MD PhD reports a patent in GARD and stock in Cvergenx, Inc.

All remaining authors have declared no conflicts of interest.

Data sharing statement

Data in this study is available upon reasonable request from the corresponding author at Arash.Naghavi@moffitt.org

Contributors

Conceptualization, G.Y, Z.Y, A.N., K.A., J.G. J.T.; methodology, G.Y., E.W., W.J., A.N..; software, G.Y., E.W..; validation, M.B., G.Y., A.N., J. G..; formal analysis, G.Y., E.W., A.N. data curation, G.Y., Z.Y., E.W., A. N..; writing—original draft preparation, G.Y., Z.Y., A.N, .; writing—review and editing, K.A., J.T., J.G., R.G., L.H., J.E, D.L,; funding acquisition, J.G., A.N., J.T.. All authors have read and agreed to the published version of the manuscript.

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