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HLA Genotyping in Synovial Sarcoma: Identifying HLA-A*02 and its Association with Clinical Outcome

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Abstract

Purpose: To determine if a targeted exome panel utilizing matched normal DNA can accurately detect germline and somatic HLA genes in synovial sarcoma (SS) patients and whether select HLA-A*02 genotypes are prognostic or predictive of outcome in metastatic SS.

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Experimental Design: Metastatic SS patients consented to HLA typing by a Clinical Laboratory Improvement Amendments (CLIA)-certified test to determine eligibility for a clinical trial of NY-ESO-1-specific engineered T cells restricted to carriers of HLA-A*02:01, A*02:05, or A*02:06 (HLA-A*02-eligible). HLA genotype was determined from MSK-IMPACT, where feasible, and somatic loss-of-heterozygosity (LOH) in HLA alleles was identified. Overall survival (OS) was estimated and stratified by HLA-A*02-eligibility.

Results: 23 patients had HLA genotyping by a CLIA-certified lab and MSK-IMPACT. 98% (108/110) of the sequenced alleles were concordant between IMPACT and the outside lab. LOH of HLA genes was detected in 3 tumors, one had loss of HLA-A*02:01. In total, 66 patients were screened for T cell therapy and 20 (30%) were HLA-A*02-eligible on outside testing. Univariate analysis of OS from the time of metastasis found HLA-A*02-eligibility was marginally associated with shorter OS (HR 1.95, 95% CI 0.995 – 3.813, P = 0.052). On multivariate analysis, older age and larger tumor size, but not HLA-A*02-eligibility, were significantly associated with decreased OS. HLA-A*02-eligibility did not impact OS after chemotherapy or pazopanib in the metastatic setting.

Conclusion: Targeted gene panels like MSK-IMPACT may accurately report HLA type and identify loss of somatic HLA alleles. In a multivariable model, HLA-A*02-eligibility was not significantly associated with OS in patients with metastatic SS.

Keywords

Synovial sarcoma; HLA; clinical outcome; sarcoma; pazopanib

Introduction

SS is a rare malignancy of mesenchymal origin representing approximately 5 to 10% of soft tissue sarcomas, with an incidence of 1.42 per million U.S. adults [1,2]. It frequently arises in the extremities of young adults in the third and fourth decade and is characterized by a pathognomonic translocation, t(X;18)(p11.2;q11.2) leading to a fusion of *SS18* with *SSX* [3,4]. The prognosis of SS varies depending on the primary tumor site, patient age at diagnosis, tumor grade, size, and diagnosis stage. The estimated 5-year survival of SS patients is between 55% and 75% [2,5,6,1,7,8]. While SS may be responsive to chemotherapy, the median OS for patients with locally advanced or metastatic disease is only 15 months, according to one estimate [2].

The standard of care chemotherapy in the first-line setting for patients with unresectable or metastatic SS is an anthracycline or an alkylator [9]. In a retrospective study of more than 1000 patients with advanced SS, the median progression-free survival (PFS) after ifosfamide with or without doxorubicin was 30 weeks, with a median OS of 64 weeks and an overall response rate (ORR) of 34% [10]. Patients who progress on cytotoxic chemotherapy are eligible to receive pazopanib, a multitargeted tyrosine kinase inhibitor with activity against the vascular endothelial growth factor and platelet-derived growth factor receptors. In a randomized phase III trial versus placebo, the median PFS of synovial sarcoma patients receiving pazopanib was 4.1 months, compared to 1.0 months in the placebo arm [11]. The median OS of Japanese patients with SS treated with pazopanib was 11.2 months [12].

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Efforts are underway to develop novel therapies that can induce durable responses in patients with advanced SS refractory to available agents. One promising approach consists of targeting cancer-testis antigens such as NY-ESO-1, an antigen not normally expressed in healthy tissue outside the testis, but heterogeneously expressed in a minority of cancers including SS [13,14]. Approximately 80% of SSs express NY-ESO-1, possibly due to the X-linked translocation that defines the disease [15,16]. Similarly, 88% of SS tumors in one study expressed the MAGE cancer-testis antigen [17].

Select CD8+ T cells can recognize a fragment of NY-ESO-1 bound to the HLA class I molecule on the surface of cancer cells and trigger immune-mediated cancer cell death. High-affinity variants of the T cell receptor (TCR) that can recognize the immunodominant HLA-A*02-restricted peptide of NY-ESO-1 (amino acids 157–165) have been engineered for use as an adoptive immunotherapy strategy against tumors expressing NY-ESO-1 [18–22]. The engineered TCR binds with high affinity to the following HLA class I subtypes when bound to NY-ESO-1: HLA-A*02:01, HLA-A*02:05, and HLA-A*02:06 (herein referred to as HLA-A*02-eligible subtypes). A similar strategy is being utilized to target other cancer-testis antigens, such as MAGE-A4 [23].

The adoptive T cell therapy trials reported to date are single-arm studies and therefore subject to confounding. One possible confounder is an HLA*02-eligible genotype, which in theory could predispose patients to have disparate outcomes compared to a ‘real-world’ population of SS patients with heterogenous HLA genotypes. This study seeks to determine whether HLA-A*02-eligible genotypes, a sine qua non for those treated with engineered T cells, impacts clinical outcome in patients with SS. In addition, we report that a targeted genomic sequencing panel (MSK-IMPACT) can successfully be utilized to determine HLA genotype. This is of interest, as the screening process to enroll on adoptive T cell protocols may require a lengthy wait time to determine eligibility based on HLA testing. As targeted next generation sequencing panels are often utilized in patients with advanced SS, we reason that leveraging this data to determine HLA genotype can facilitate identification of appropriate patients for enrollment on adoptive T cell protocols.

Methods

Patient Selection

This study was approved by the Memorial Sloan Kettering Cancer Center (MKSCC) institutional review board. Patients with histologically confirmed SS who provided informed consent to screen for a clinical trial of genetically engineered NY-ESO-1-specific T cells (NCT01343043) at MSKCC were included in this retrospective study. The design of NCT01343043, key eligibility criteria, and the results of the initial cohorts that received adoptive T cells have been previously described [21,22]. The study was conducted in compliance with the Declaration of Helsinki and in accordance with local legal and regulatory requirements and written informed consent was obtained for all participating patients. As part of the trial screening procedures, patients underwent high resolution HLA testing in a CLIA-certified laboratory to determine if they were eligible to receive adoptive T cell therapy per protocol. Patients who were treated with NY-ESO-1 Specific engineered T cells were excluded from this analysis.

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Study Design

The objectives of this study were to measure HLA genotype utilizing the Memorial Sloan Kettering Integrated Molecular Profiling of Actionable Cancer Targets (MSK-IMPACT) assay and to determine the prognostic potential of HLA-A*02 status on clinical outcomes in a subset of advanced SS patients who were not treated with HLA-A*02-specific therapy. Demographic, pathologic, and clinical information were retrieved from the medical record for each patient. The following variables were included: age at diagnosis, sex, SS subtype (monophasic or biphasic), primary tumor location, primary tumor size, use of neoadjuvant or adjuvant chemotherapy and/or radiation, date of unresectability, first recurrence or metastasis, date of initiation of systemic chemotherapy in the metastatic setting, number of systemic therapies used in the metastatic setting, start date of pazopanib, HLA typing via high resolution testing, and date of death or last contact. The cutoff date for clinical follow-up was August 15, 2019.

HLA Typing

A select number of patients included in this study provided informed written consent to participate in MSK-IMPACT, a prospective tumor sequencing initiative that has been described in detail elsewhere [24,25]. MSK-IMPACT is a hybridization capture-based matched tumor-normal sequencing platform that profiles up to 468 genes (depending on the assay version[25]) for mutations, copy number alterations and select structural variants. HLA genotyping was performed in a research setting using POLYSOLVER [26]. Briefly, POLYSOLVER first extracts all reads that are putatively aligned to the HLA locus and then performs a multi-step inference that accounts for the aligned reads, base qualities, and observed insert sizes to predict the HLA Class I genotypes.

Statistical Analyses

Patients were divided into subgroups based on the presence of an HLA-A*02-eligible genotype. Survival analyses were performed on three overlapping sets of patients: the first included all patients from the time of metastatic disease, the second included patients who received doxorubicin or ifosfamide-based treatment in the first-line metastatic setting, and the third included only patients who were treated with pazopanib in the metastatic setting. For the first patient set, OS was defined as the time the patient was considered to have unresectable, recurrent, or metastatic disease until the date of death or last contact; patients who were alive at the time of last contact were censored. A sensitivity analysis was performed within the first patient set that only included patients who consented to screen for the NY-ESO-1-specific T cell trial within one year of developing unresectable, recurrent, or metastatic disease. In the latter two patient sets, OS was defined as the date of first chemotherapy or pazopanib administration until the date of death or last contact; patients who were alive at the time of last contact were censored.

Summary statistics, median and interquartile range, were used to describe continuous variables and count and percent for categorical variables. Wilcoxon rank sum test and Fisher’s exact test were used to compare continuous and categorical variables, respectively, between groups. Survival outcomes were analyzed using Kaplan-Meier methods and log rank test were used to associate factors. Univariable and multivariable analysis were

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performed with Cox proportional hazard regression models for progression free and OS. Multivariable models were selected using backward selection with inclusion criteria of being significant at 0.10 in the univariable analysis. SAS version 9.4 SAS institute Inc., Cary, NC was used for all analysis. All tests were two-sided and $P < 0.05$ was considered significant.

Results

Patient Characteristics

Between March 24, 2014 and November 21, 2017, 75 patients consented to screen for a clinical trial of NY-ESO-1-specific engineered T cells. Of the screened patients, 39% (n = 29) tested positive for HLA A*02:01 (n = 18), HLA A*02:05 (n = 1), or HLA A*02:06 (n = 1) by an outside CLIA-certified test. The time to receive outside HLA results, a measure available in all but 2 patients, ranged between 3 and 27 days (median: 8). Nine patients ultimately received treatment with engineered T cells on trial and were excluded from this analysis (Figure 1; their characteristics are outlined in Supplemental Table 1). Of the 20 patients who were HLA-eligible, 5 were not treated because of clinical deterioration (n = 3) or death (n = 2). The remaining patients were not treated for reasons other than a change in clinical status (Supplemental Table 2).

The characteristics of the 66 patients not treated with engineered T cells are presented in Table 1. Patient and tumor characteristics were well-balanced between groups. The median age at diagnosis was 35 (range: 8 – 84) and the median primary tumor size was 7.8 cm (range: 1.5 – 19.0). Most patients (70%) were male, had an extremity (46%) or thoracic (24%) primary tumor, and a monophasic tumor histology (73%). Twenty-three patients (35%) had unresectable or metastatic disease at the time of diagnosis. The median time from diagnosis until the date of recurrence or metastasis was 12 months (range: 0 – 189). Of the 43 patients with potentially resectable disease at diagnosis, 51% (n = 22) were treated with neoadjuvant chemotherapy and/or radiation. Of those who did not receive neoadjuvant therapy, the majority (57%; n = 12) were treated with adjuvant chemotherapy and/or radiation. 9 patients (21% of those with resectable disease) received no perioperative therapy.

HLA Genotype Detection from MSK-IMPACT

32 patients underwent prospective sequencing with MSK-IMPACT during their treatment course. 75% of sequenced tumors were from metastatic sites, rather than primary tumors. 2 patients had no matched-normal control samples and 6 had an early version of IMPACT that precluded analysis of HLA genotype. The remaining 24 patient samples were eligible for analysis of HLA genotype and are reported in Supplemental Table 3. Of these 24 patients, 16 had their complete HLA class I genotype (two alleles each of HLA-A, HLA-B, and HLA-C) determined at an outside CLIA-certified laboratory and 7 had HLA-A gene analysis only. The outside HLA genotype of one patient was unavailable, but was documented to be HLA-A*02-negative in the medical record and thus ineligible for the clinical trial.

Of the 23 patients who had HLA genotyping by IMPACT and an outside CLIA-certified laboratory, 21 (91%) had matching HLA genotypes (including the 7 patients with outside

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testing of HLA-A only). The two patients that were not a complete match had 5 of 6 matching HLA alleles. At an allele-specific level, 110 HLA alleles sequenced by an outside lab were available for comparison (2 alleles each of HLA-A, HLA-B, and HLA-C for 16 patients, and 2 HLA-A alleles for 7 patients). 98% (n = 108) of all HLA alleles matched the IMPACT analysis and 100% of HLA-A*02 alleles were a match.

Somatic loss-of-heterozygosity (LOH) status was available for the 24 patients who had HLA genotyping by IMPACT. Three patients demonstrated LOH of at least one HLA gene (Figure 2). Two were HLA-A*02-eligible; one had LOH of HLA*02:01 in the primary tumor. This patient was treated with 3 cycles of neoadjuvant doxorubicin plus ifosfamide prior to initial surgical resection of the primary tumor. None of the HLA-A*02-eligible patients who had LOH were treated with engineered T cells.

OS Independent of Treatment Modality

To determine whether HLA-A*02-eligible status was prognostic of clinical outcome independent of treatment modality, survival was estimated from the time of unresectable or metastatic disease in patients not previously treated with HLA-A*02-specific T cells (n = 66, excluding 9 HLA*02-eligible patients treated with SPEAR T cells). Median follow-up for survivors was 48 months. On univariate analysis, larger primary tumor size (HR 1.2, 95% CI 1.12 – 1.34) and longer time from diagnosis until metastatic disease (HR 0.99, 95% CI 0.976 – 0.999) were significantly associated with OS ($P < 0.001$ and $P = 0.032$, respectively), the former with a shorter survival and the latter with longer survival. HLA-A*02-eligible status (HR 1.95, 95% CI 0.96 – 3.81) and age at diagnosis (HR 1.021, 95% CI 0.99 – 1.04) were slightly above the threshold for statistical significance ($P = 0.052$ and $P = 0.061$, respectively) (Table 2). The median OS of HLA-A*02-eligible patients was 25.1 months (95% CI 10.8 – 35.7), compared to 43.9 months (95% CI 25.7 – 69.5) in the HLA-A*02-ineligible group (Figure 3A).

In a multivariate analysis accounting for HLA status, age at diagnosis, and primary tumor size, the significance of HLA-A*02-eligible status was above the threshold for significance (HR 1.8, 95% CI 0.94 – 3.6; $P = 0.076$). In contrast, older age at diagnosis (HR 1.03, 95% CI 1.002 – 1.049) and larger tumor size (HR 1.2, 95% CI 1.1 – 1.4) were associated with a significantly shorter OS ($P = 0.037$ and $P < 0.001$, respectively) (Table 3).

Given that providing informed consent for the clinical trial was a key inclusion criterion and that time from diagnosis of metastatic disease until informed consent varied, a sensitivity analysis was performed in the 39 patients (59% of the study population) who consented to screen for the trial within 1 year of developing metastatic disease. This excluded patients whose prolonged time to consent could have influenced the OS analysis. The median OS of HLA-A*02-eligible patients was 13.6 months (95% CI 16.3 – 38.9) versus 25.7 months (95% CI 8.5 – 15.2) for ineligible patients ($P = 0.016$). On univariate analysis, HLA-A*02 status was the only variable that significantly impacted survival (HR 2.79, 95% CI 1.18 – 6.61; $P = 0.020$) (Supplemental Table 4).

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OS After First-line Chemotherapy

Two-thirds of the above cohort (n = 44) received either doxorubicin or ifosfamide-based therapy in the first line metastatic setting. The date of treatment initiation was not known for 8 patients. Therefore, a total of 36 patients were eligible for inclusion in survival analyses, 25% (n = 9) of whom were HLA A*02- eligible. OS did not significantly differ between the HLA-A*02-eligible and ineligible groups (32.8 months [95% CI 3.8 – 39.6] versus 38.4 [95% CI 21.2 – 72.3] months, respectively; P = 0.217) (Figure 3B). On univariate analysis, only primary tumor size was significantly associated with a shorter OS (HR 1.2, 95% CI 1.1 – 1.4; P = 0.003) (Table 2). The remaining clinicopathologic factors tested did not significantly associate with OS.

OS After Pazopanib

39 patients were treated with pazopanib at some point during their treatment course. OS data from the date of pazopanib initiation was available for 37 patients, 32% of whom (n = 12) were HLA-A*02-eligible. OS did not significantly differ between the HLA-A*02-eligible and ineligible groups (11.2 [95% CI 6.6 – 23.7] and 14.1 [95% CI 4.5 – 17.7] months, respectively; P = 0.884) (Figure 3C). No clinicopathologic variables were associated with a significant difference in OS (Table 2).

Discussion

This study reports our experience using a targeted genetic sequencing panel (MSK-IMPACT) to identify HLA genotype. The MSK-IMPACT assay had a high concordance rate with HLA genotyping performed at a CLIA-certified laboratory outside our institution. This has the potential to become an important tool as we continue to learn about the impact of HLA and antigen presentation machinery on response to cancer therapies such as immune checkpoint blockade [27–29]. It may also help expedite the screening process for patients interested in participating in adoptive T cell therapy trials at our institution, whose HLA type is unknown. At present, the screening process can be lengthy, which is compounded by the time it takes to manufacture the NY-ESO-1 SPEAR T cells. Using next-generation sequencing data that is readily available could save the patient time as he or she considers the next line of systemic treatment.

Furthermore, LOH analysis of SS tumors may be a useful tool to clarify mechanisms of resistance to NY-ESO-1 SPEAR T cells. We identified a patient with the HLA-A*02:01 genotype who would have been eligible for a clinical trial of engineered T cells based on this HLA type, who lost this critical HLA gene in his tumor after treatment with neoadjuvant chemotherapy. This may represent immunoediting as a form of resistance to innate anti-tumor T cell activity [30]. Investigation of somatic LOH at HLA loci on serial biopsy specimens in patients who receive engineered T cells on trial may further clarify whether LOH is a potential mechanism of resistance.

This study reports that SS patients with an HLA-A*02 genotype had a shorter OS when survival was analyzed from the time of metastasis. This finding did not reach the threshold for significance in a multivariable model that accounted for additional clinical variables.

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Retrospective studies in other malignancies, including ovarian cancer [31], non-small cell lung cancer [32], prostate cancer [33], and HPV-positive tonsillar squamous cell carcinoma [34] report an HLA-A*02 genotype as a negative prognostic factor. A retrospective analysis of 453 melanoma patients treated with ipilimumab with or without a peptide vaccine identified a trend toward a worse OS in a subgroup of HLA-A*02:01-positive patients treated with 10 mg/kg of ipilimumab. The authors attributed this finding to statistical variability between groups and consider discordant findings between subgroups an expected limitation in retrospective studies [35].

Theoretical mechanisms for an adverse prognosis in HLA-A*02-eligible patients with SS compared to other HLA genotypes may include decreased expression of HLA-A*02 in the tumor due to transcriptional downregulation, hypermethylation, or alteration of key cytokine signaling in the tumor microenvironment required for immune activation [36]. However, given the small sample size of this cohort we interpret these findings with caution, as do Wolchok et al in ipilimumab-treated melanoma patients. Confirmatory studies are warranted to further investigate the effect of HLA haplotype on prognosis in SS.

Adoptive T cell therapy is now being utilized in SS clinical trials with encouraging early results. Robbins et al utilized genetically engineered autologous T cells in a small study of 18 SS patients and reported a 61% objective RR [20]. In another pilot study, D'Angelo and colleagues treated 12 patients with NY-ESO-1-expressing SS with genetically engineered NY-ESO-1-targeted T cells (NY-ESO-1^{c259} SPEAR T cells). The ORR was 50%, with 1 complete response, 5 partial responses, and a median PFS of 15 weeks [21]. A recent update reported that 15 of 42 patients (36%) achieved a confirmed objective response [22]. Given the rarity of SS as a diagnosis, conducting a large, randomized, phase III trial of adoptive T cell therapy will be a challenge. Thus, defining the prognostic effect of eligible HLA-A*02 genotypes on clinical outcome may serve as an important historic control.

The clinicopathologic characteristics of patients in this study are comparable to previously published studies of SS, suggesting that our population may be a representative sample. The median age of diagnosis was in the third decade, primary tumors were most commonly identified in the extremities, and monophasic histology was more common than biphasic. The median time from diagnosis until the date of metastasis was 12 months and OS from date of metastasis was 29 months. For comparison, a Royal Marsden Hospital retrospective of 104 SS patients found a time from diagnosis until metastasis of 16 months and a median OS of 22 months from the time of metastasis [37]. Additionally, among Caucasians in this study – the largest ethnic group in this cohort - 32% (15 of 47) tested positive for HLA-A*02:01. This is on par with the allele frequency of 29.6% found among US adults of European ancestry [38]. In addition, our OS analyses identified older age and larger tumor size to be two prognostic factors of outcome, which corroborates the results of a previous analysis by Singer and colleagues [39].

The strengths of this study include its relatively large cohort size for a single-center retrospective in a rare disease, its investigation of a genomic biomarker, and its focus on patients screened on a prospective study. Its retrospective nature and the selection bias that follows is a potential weakness. Specifically, patients treated with SPEAR T cells were

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excluded, while HLA-eligible patients who were not treated on trial because of clinical deterioration were included. These two groups may have different outcomes, highlighting the difficulties of defining a uniform cohort for analysis among a group of patients that have disparate clinicopathologic, molecular, and treatment characteristics. Thus, validation of these findings in a future study is warranted.

In summary, HLA genotype is a necessary predictive biomarker for SS patients interested in receiving genetically engineered T cells. Our findings indicate that select HLA-A*02 genotypes may be prognostic of poor outcome in the advanced setting. While this study is restricted to an analysis of HLA-A*02:01, HLA-A*02:05, or HLA-A*02:06, it would be of interest to learn whether other HLA alleles are prognostic or predictive in this disease. Utilizing a targeted genomic sequencing panel to determine HLA genotype could help facilitate patient screening for SS and other clinical trials, clarify mechanisms of resistance to therapy, and allow future study of HLA gene on clinical outcomes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Translational Relevance

The MSK-IMPACT assay, a targeted next-generation sequencing (NGS) panel utilizing matched-normal DNA, was utilized in a real-world cohort of metastatic synovial sarcoma patients to report HLA genotype. Results were highly concordant with HLA typing performed by a CLIA-certified laboratory outside our institution. HLA typing via NGS can potentially expedite the screening process for patients interested in participating in adoptive T cell therapy trials restricted to select HLA genotypes. Targeted panels may also detect somatic loss-of-heterozygosity of HLA-A*02:01, a potential mechanism of resistance to adoptive T cell therapies that rely on HLA-A*02 expression. As randomized trials of adoptive T cell strategies are unlikely in a rare disease like synovial sarcoma, we sought to determine the prognostic potential of HLA*02-eligible patients who are not treated with engineered T cells.

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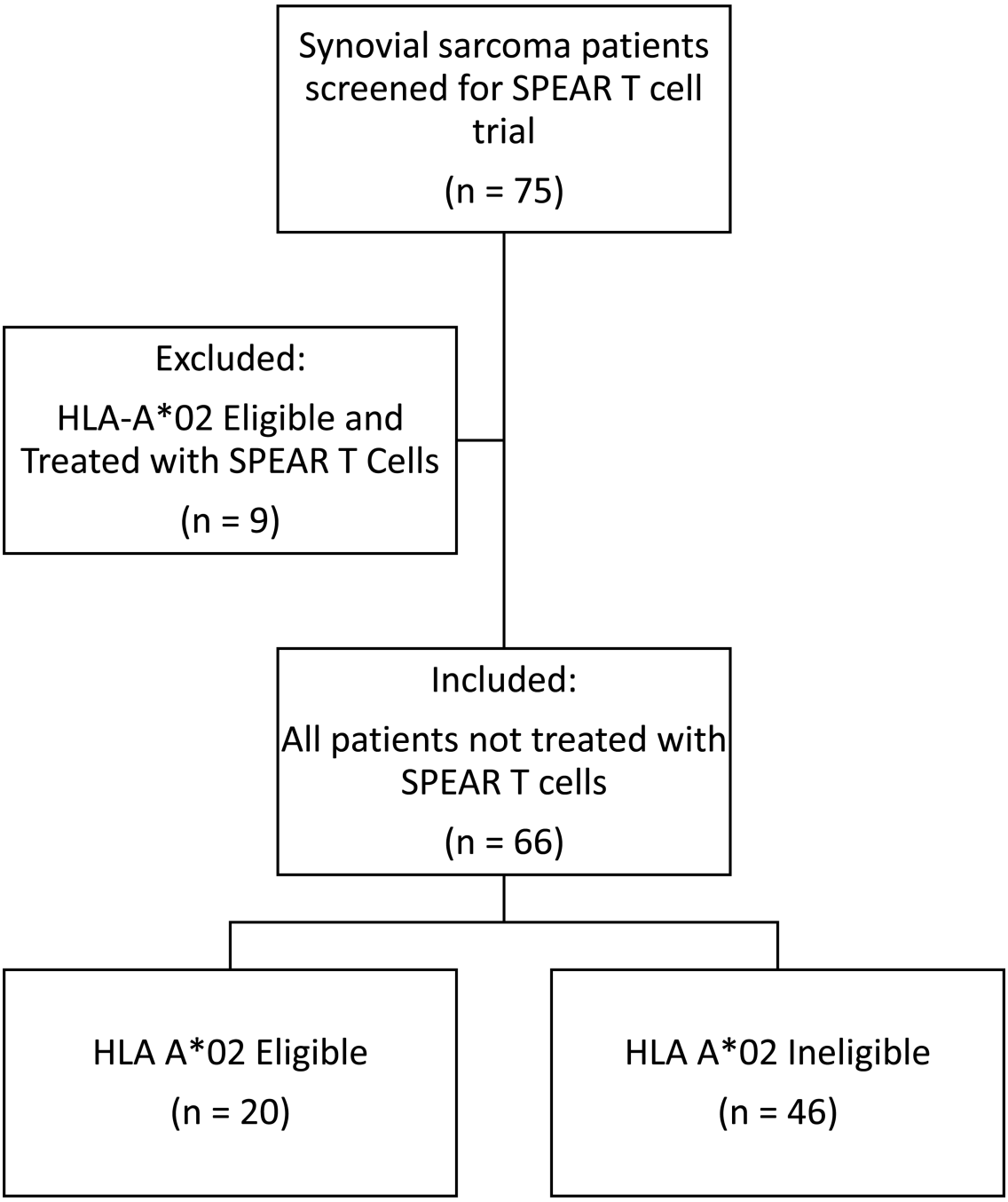

Figure 1:
Schematic of synovial sarcoma patients included and excluded from this retrospective study

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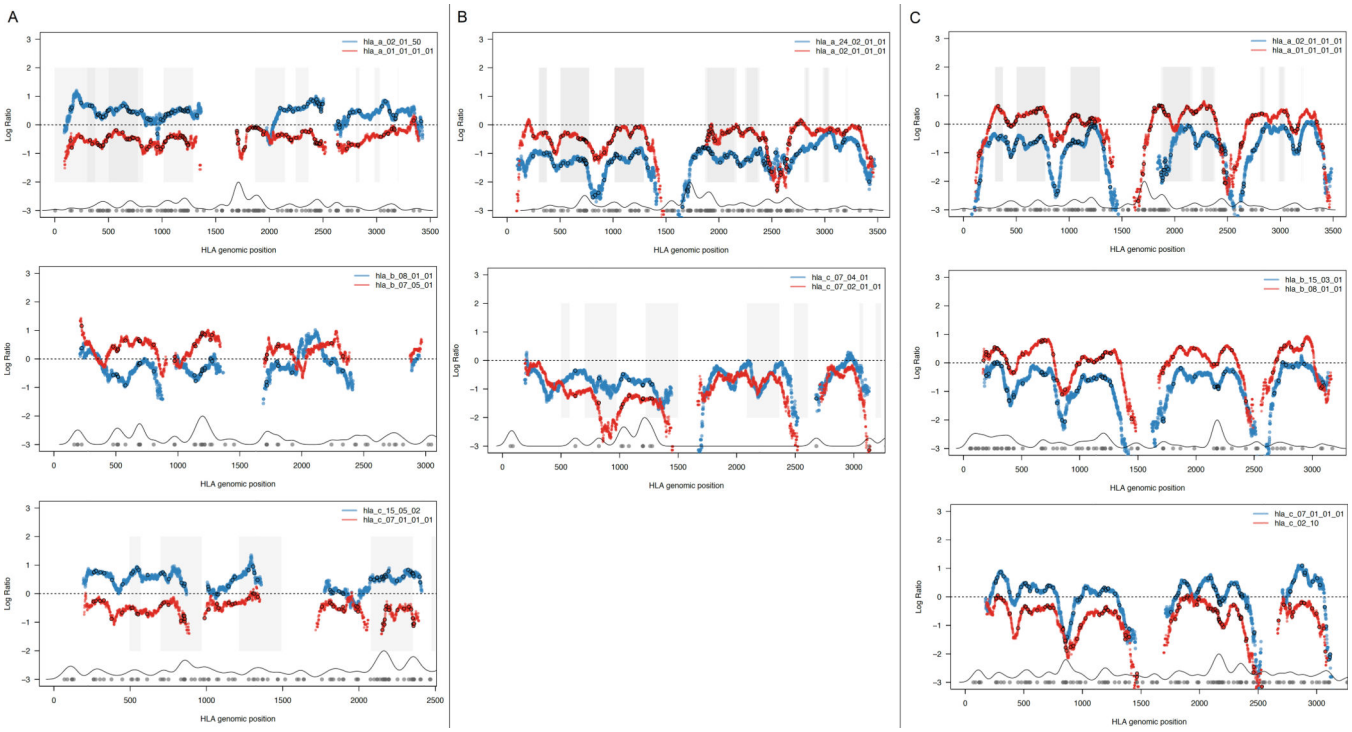

Figure 2:

Loss-of-heterozygosity at HLA alleles in three patients with metastatic synovial sarcoma (panels **A**, **B**, and **C,** respectively) who underwent MSK-IMPACT testing. The log ratio of depth of coverage in the tumor and normal tissue is displayed along HLA alleles, highlighting loss of HLA gene coverage depth in tumor tissue, compared to normal.

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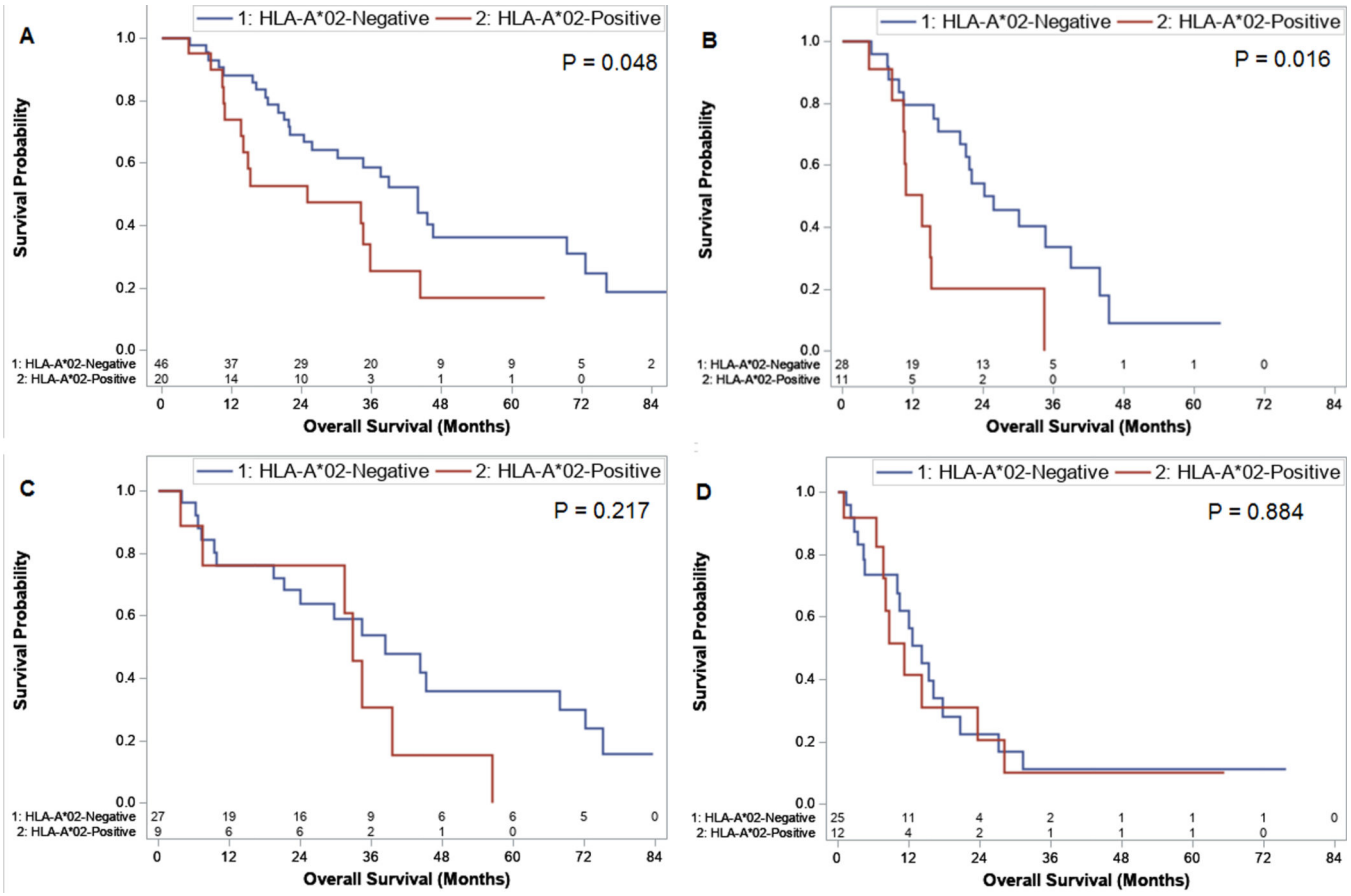

Figure 3:

Overall survival by HLA-A*02 status from the time of metastasis (**A**), from time of metastasis in those who signed consent within one year of metastasis (sensitivity analysis) (**B**), from start of chemotherapy in the metastatic setting (**C**), and from start of pazopanib in the metastatic setting (**D**). Patients treated with SPEAR T cells were not included in these analyses.

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Table 1:

Patient Characteristics

			HLA-A*02-Negative (n=46)	HLA-A*02-Positive (n=20)	P value
Age at Diagnosis	Median (range)	35 (8–84)	35 (8–84)	35 (14–75)	0.601
Sex	F	20 (30.3)	14 (30.4)	6 (30)	>0.95
	M	46 (69.7)	32 (69.6)	14 (70)	.
Race	Unknown	5 (.)	5 (.)	0 (.)	>0.95
	Other	14 (23)	10 (24.4)	4 (20)	.
	White	47 (77)	31 (75.6)	16 (80)	.
Ethnicity	Hispanic	6 (9.1)	4 (8.7)	2 (10)	>0.95
	Non-Hispanic	60 (90.9)	42 (91.3)	18 (90)	.
Location	Abdominal/Visceral	6 (9.1)	2 (4.3)	4 (20)	0.170
	Bone	1 (1.5)	0 (0)	1 (5)	.
	Extremity	30 (45.5)	23 (50)	7 (35)	.
	Head & Neck	3 (4.5)	3 (6.5)	0 (0)	.
	Pulmonary/Thoracic	16 (24.2)	11 (23.9)	5 (25)	.
	Truncal Soft Tissue	10 (15.2)	7 (15.2)	3 (15)	.
Primary Tumor Size (cm)	Median (range)	7.80 (1.50–19.0)	7.20 (1.50–19.0)	8.00 (2.80–14.5)	0.887
Histology	Unknown	6 (.)	5 (.)	1 (.)	0.754
	Biphasic	16 (26.7)	12 (29.3)	4 (21.1)	.
	Monophasic	44 (73.3)	29 (70.7)	15 (78.9)	.
Neoadjuvant Chemotherapy	No	48 (72.7)	34 (73.9)	14 (70)	0.770
	Yes	18 (27.3)	12 (26.1)	6 (30)	.
Neoadjuvant RT	No	55 (83.3)	40 (87)	15 (75)	0.287
	Yes	11 (16.7)	6 (13)	5 (25)	.
Adjuvant Chemotherapy	No	48 (72.7)	34 (73.9)	14 (70)	0.770
	Yes	18 (27.3)	12 (26.1)	6 (30)	.
Adjuvant RT	No	51 (77.3)	35 (76.1)	16 (80)	>0.95
	Yes	15 (22.7)	11 (23.9)	4 (20)	.
Time from Diagnosis to Metastasis (Months)	Median (range)	12 (0–189)	12 (0–189)	11 (0–59)	0.498
Survivor Follow up (Months)	Median (range)	47.96 (3.82–307.93)	65.81 (3.82–307.93)	37.38 (17.96–47.17)	

Values are n (%), unless otherwise indicated

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Table 2:

Univariable analyses of overall survival

	Univariate Analysis											
Study Group	**Chemotherapy**				**Pazopanib**				**All Patients**			
Parameter	**HR**	**95% CI**		**P value**	**HR**	**95% CI**		**P value**	**HR**	**95% CI**		**P value**
HLA-A*02-eligible	1.763	0.706	4.404	0.225	1.062	0.471	2.393	0.884	1.948	0.995	3.813	0.052
Age at diagnosis	1.021	0.986	1.058	0.242	1.013	0.985	1.041	0.362	1.021	0.999	1.044	0.061
Sex	1.140	0.448	2.898	0.783	1.141	0.522	2.494	0.742	1.203	0.626	2.313	0.579
Primary tumor size	1.224	1.073	1.396	0.003*	1.068	0.974	1.170	0.162	1.229	1.123	1.345	<.001*
Histology (monophasic/ biphasic)	2.025	0.753	5.446	0.162	1.030	0.383	2.766	>0.95	1.040	0.484	2.234	0.920
Neoadjuvant chemotherapy	0.882	0.258	3.009	0.840	0.902	0.406	2.003	0.801	1.201	0.605	2.385	0.601
Neoadjuvant radiotherapy	2.847	0.816	9.929	0.101	0.644	0.192	2.156	0.475	1.255	0.523	3.011	0.611
Adjuvant chemotherapy	0.808	0.300	2.175	0.673	1.062	0.444	2.538	0.893	0.714	0.339	1.504	0.375
Adjuvant radiotherapy	0.492	0.146	1.661	0.253	1.226	0.512	2.935	0.648	0.719	0.342	1.514	0.385
Time from diagnosis to metastasis	0.990	0.979	1.002	0.113	0.995	0.984	1.005	0.294	0.987	0.976	0.999	0.032*

*Statistically significant, as defined by prespecified threshold of $P < 0.05$

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Table 3:

Multivariable analysis of overall survival in all patients from the time of metastasis

Parameter	HR	95% CI		P value
HLA-A*02-eligible	1.840	0.939	3.606	0.076
Age at diagnosis	1.025	1.002	1.049	0.037 *
Primary tumor size	1.243	1.127	1.370	<.001 *

* Statistically significant, as defined by prespecified threshold of $P < 0.05$