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Tumor Radiosensitivity is Associated with Immune Activation in Solid Tumors

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Abstract

Purpose—Our goal was to determine whether tumor radiosensitivity is associated with activation of the immune system across all tumor types as measured by two gene expression signatures (GES).

Methods—We identified 10,240 genomically profiled distinct solid primary tumors with gene expression analysis available from an institutional de-identified database. Two separate GES were included in the analysis, the radiosensitivity index (RSI) GES (a 10-gene GES as a measure of radiosensitivity), and the 12-chemokine (12-CK) signature (a 12-gene GES as a measure of immune activation). We tested whether the RSI and 12-CK were associated with each other across all tumor samples, and in an exploratory analysis, their prognostic significance in predicting distant metastasis-free survival (DMFS) among a well-characterized, independent cohort of 282 early-stage breast cancer cases treated with surgery and post-operative radiation alone without systemic therapy. The lower the RSI score, the higher the tumor radiosensitivity; whereas, the higher the 12-CK score the higher the immune activation.

Results—Using an RSI cut-point of ≤0.3745, RSI-low tumors (n=4,291, 41.9%) had a significantly higher median 12-CK GES value (0.54 [range −0.136,1.095]) compared with RSI-high tumors (−0.17 [−0.82,0.42]; p<0.001) across all tumor samples, indicating that

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radiosensitivity is associated with immune activation. In an exploratory analysis of early stage breast cancer cases, a multivariable model with patient age, RSI and 12-CK provided a strong composite model for DMFS (p=0.02), with RSI (HR 0.63 [95%CI 0.36,1.09]) and 12-CK (HR 0.66 [0.41,1.04]) each providing comparable contributions.

Conclusions—Tumor radiosensitivity is associated with immune activation as measured by two GES.

Keywords

immune; cancer; radiation; radiosensitivity; survival

Introduction

Over the past few decades, it has been well established that specific cancers are exquisitely radiosensitive, resulting in high locoregional control rates following treatment. HPV-positive oropharyngeal squamous cell carcinoma is a radiosensitive tumor, which exhibits a high cure rate following radiotherapy with or without concurrent chemotherapy.(1,2) Using daily or weekly 3D image guidance, HPV-positive oropharyngeal tumors often demonstrate a complete radiologic response shortly after completing definitive radiation therapy.(3) However, the pre-treatment identification of radiosensitive tumors and host factors associated with radiosensitivity remains a fragmented story for other tumor types.

The tumor microenvironment, including the immune system and function of the host, has been shown to play an important role in mediating both tumor growth and responses to radiation therapy.(4) Tumors frequently down-regulate the host's adaptive immune system to avoid cell-mediated death and, in response, immune modulators have become a promising tool to combat this effect. Radiation therapy has been shown to have both enhancing and inhibitory effects on host immune function depending largely on the radiation dose and target.(5) A large radiation field targeting multiple vertebral bodies as palliative therapy for spinal metastases, for instances, can reduce a patient's white blood cell count and potentially act as an immunosuppressive agent.(5,6) However, when radiation is highly conformal, with the use of modern treatment planning and delivery, and when it is delivered in higher doses per fraction, radiation has the potential to act as an immune stimulatory agent.(5)

Additionally, when combined with immune modulators, pre-clinical studies and case reports have shown a potential synergy between the radiation and immunotherapy via the abscopal effect. (7–9) How often the abscopal effect occurs and whether it can be triggered by a pre-defined strategy via combining radiotherapy and immunotherapy remains a critical clinical question.(9) Radiation has been shown to have a mixed response on PD-L1 expression on the surface of tumor cells, by upregulating, or even down-regulating PD-L1 expression(10,11), with differing responses possibly driven by tumor site, histology, and/or radiation dose per fraction.(5) Thus the identification of biomarker-based approaches is central to the development of clinical strategies to combine radiation therapy and immunotherapy.

To address this, our group recently developed two gene-expression signatures (GES) of radiosensitivity and immune-activation. The radiosensitivity index (RSI) is a 10-gene based signature developed as a marker of cellular radiosensitivity that has been independently validated as pan-tissue biomarker of radiosensitivity in multiple disease sites.(12–16) The 12-Chemokine (12-CK) GES is based on 12 chemokine genes (CCL2, CCL3, CCL4, CCL5, CCL8, CCL18, CCL19, CCL21, CXCL9, CXCL10, CXCL11, and CXCL13) chosen from a metagene grouping of immune-related and inflammation-related genes.(17) The 12-CK GES has been shown to be associated with the presence of tumor localized ectopic lymph node like structures (TL-ELNs) in both colorectal cancer and metastatic melanoma patients and was associated with improved survival outcomes in both patient populations.(17–19)

In the present study, we hypothesize that RSI and 12-CK are associated and, when combined, will provide an improved prognostic tool for patient outcomes. Combining RSI and 12-CK also serves as a possible clinical strategy to explore the relationship between tumor radiosensitivity and patient immune activation across many unique tumor types.

Methods

Gene Profile Analysis of Archived Tumors -Total Cancer Care (TCC) Database

We identified 10,240 genomically profiled distinct solid, primary, non-metastatic tumors from the TCC database, a prospective IRB-approved tissue collection protocol active at the Moffitt Cancer Center and 17 other institutions since 2006.(20) Tumors from patients enrolled in the TCC protocol were arrayed on Affymetrix Hu-RSTA-2a520709 Gene Chips (Affymetrix, Santa Clara, CA). The chip contains roughly 60,000 probesets representing 20,155 unique genes. Iterative Rank-Ordered Normalization (IRON) was used to normalize all samples and log2 values were calculated.(21). A RNA quality batch effect was removed using Partial Least Squares. Both the normalized and de-batched expression values for 10,240 tumor samples from the ten RSI genes and 12 chemokine genes were extracted from the TCC database.

Radiosensitivity Index (RSI) GES

RSI was previously trained in 48 cancer cell lines to predict cellular radiosensitivity as determined by survival fraction at 2 Gy (SF2). Each of ten genes in the algorithm was ranked based on gene expression (highest expressed gene is ranked at 10 and lowest at 1) as previously described by Eschrich et al.(12). To make RSI comparisons across the entire dataset of 10,240 tumors across 62 disease sites, we used RSI=0.3745 as the cut-point, as it represents the value for the local minimum in the bimodal RSI density function (Figure 1). RSI-low tumors (i.e. more sensitive) were defined as having an RSI GES <0.3745 and RSI-high tumors (i.e. less sensitive) as having an RSI GES ≥0.3745.

12-Chemokine (12-CK) GES

The 12-CK GES was defined as the first principal component (PC1) from a PCA model using all 12 genes in all 10,240 samples. The sample scores from the PCA model were scaled to have a variance of 1.

Erasmus Breast Cancer Cohort

The Erasmus Cancer Cohort has been previously described.(13) It includes 282 lymph node negative breast cancer patients treated with loco-regional therapy (surgery and RT) and no adjuvant systemic therapy (i.e. chemotherapy or hormonal therapy).(22) Patient exclusion characteristics and tumor RNA preparation and gene expression profiling were previously described.(22–24) Raw gene expression data from this cohort are publicly available in GEO (Erasmus – GSE2034, GSE5327). A robust multi-array (RMA) normalization method was previously applied to the Affymetrix U133A CEL files.(25–27) The study was approved by the Medical Ethics Committee of the Erasmus Medical Center. Radiation dose to the tumor cavity in the cohort ranged from 40–74 Gy delivered in standard fractionation (1.8–2 Gy per fraction). The endpoint defined by the Erasmus investigators was distant metastasis free survival, defined as an early distant recurrence in the first five years following completion of primary treatment, or death. RSI was previously generated for this cohort and RSI-low and RSI-high were previously defined and specific to this dataset.(13) The RSI cut point previously used with the ERASMUS dataset (13) and in the present analysis was 0.34; this is similar to the RSI cut point of 0.3745 for the overall analysis although the normalization methods were different making it difficult to directly compare with the two RSI cut points. We explored five alternative cut-points for dichotomization of the 12-CK GES, including 12-CK-high (immune-active) vs. 12-CK low (immune-inactive) in the Erasmus cohort. The cut-point used for testing outcomes was the top 75% of 12-CK patients (12-CK-high, 212 patients) vs. the bottom 25% (12-CK-low, 70 patients). This was the optimal cut-point of the five tested on univariate analysis for DMFS (p=0.08).

Statistical analysis

The primary endpoint of the study was to assess the association of RSI GES with 12-CK GES among 10,240 solid primary tumors in the TCC de-identified database. A secondary endpoint was to test whether the RSI and 12-CK GES could be prognostically important for distant metastasis free survival outcomes, using the Erasmus cohort of 282 breast cancer patients treated with breast conservation therapy as the validation dataset.

Statistical analysis was performed using SPSS® version 22.0 (IBM®, Chicago, IL) and R. For the TCC analysis of 10,240 solid tumors, differences in 12-CK values by RSI group were assessed using the Wilcoxon rank-sum test among all patients, and between individual tumor types with ≥10 samples in both RSI groups (n=17 tumor types compared). The associations between RSI and 12-CK GES were assessed using the non-parametric Spearman correlation coefficients in the full TCC cohort and within individual tumor types with ≥10 samples (n=42 tumor types included).

For the Erasmus dataset analysis, clinicopathologic differences between the RSI-low (radiosensitive)/RSI-high (less-radiosensitive) and 12-CK-high/12-CK-low patient populations were compared using the Wilcoxon rank-sum test and Pearson’s Chi-square test for continuous and discrete variables, respectively. Distant metastasis free survival rates were then calculated using the Kaplan-Meier method and compared using the log-rank test. Cox regression main-effects models for DMFS were performed to assess the prognostic significance of RSI, and the 12-CK score individually and together adjusted for age.

Results

Association between tumor radiosensitivity and immune-activation

Table 1 and figure 2 show the association between tumor radiosensitivity and immune activation across the TCC cohort (n=10,240). Among 10,240 unique non-metastatic human tumors, RSI-low tumor samples (more radiosensitive) had a significantly higher median 12-CK GES (0.537 [range −0.136, 1.095]) compared with RSI-high tumors (−0.167 [range −0.816, 0.415]; p<0.001) across all tumor samples. This suggests that tumors with increased radiosensitivity also have increased immune activation and visa-versa. This observation was confirmed for the majority of unique tumor types on subset analysis (Table 1).

The 12-CK and RSI GES were then tested by Spearman correlation (Supplemental figure 1 and Supplemental Table 1). Across all tumors types, there was a negative correlation between RSI and 12-CK GES (R=−0.355, p<0.001), indicating that tumors with a high relative radiosensitivity also often have a high level of immune activation, while radioresistant tumors tend to have a lower level of immune activation. When assessed by unique tumor site and histology, this negative correlation was consistent across the majority of tumors. The degree of the negative correlation varied between tumor types.

An exploratory combined model including both RSI and 12-CK phenotype predicts clinical outcome in the Erasmus Dataset

The prognostic value of the RSI and 12-CK GES were then explored in an independent dataset of 282 breast cancer patients treated with breast conservation therapy. No differences were observed in the RSI score when compared between patient clinicopathologic and tumor characteristics (Supplemental Table 2). Patients with a high 12-CK score (immune activated) were significantly younger (median 51 vs. 57 years, respectively; p=0.002), more often pre-menopausal (60.8% vs. 42.9%, respectively; p=0.008) and more frequently had ER-/PR-tumors (34.3% vs. 9.1%, respectively; p<0.001) compared to patients whose tumors had low 12 CK scores. Similar to the TCC analysis, the dichotomized RSI and 12-CK GES in the Erasmus dataset were associated with each other (p=0.002, data not shown).

When each GES was assessed separately with patient outcomes, both an RSI-low (more radiosensitive tumor) status (Figure 3, Table 2; HR 0.58 [95% CI 0.34, 1.00]; p=0.05) and a 12-CK-high immune-active status (HR 0.61 [95% CI 0.39, 0.96]; p=0.03) were independently associated with improved distant metastasis-free survival. In addition, a composite model including all three variables (p=0.02) outperformed both age-adjusted individual models for RSI and 12-CK (AIC for composite model one unit lower than the best individual model).

Discussion

We demonstrate a clear association between tumor radiosensitivity and immune activation among a large cohort of patients using two separate microarray GES. Each signature was designed for a unique purpose and previously studied in different patient populations. RSI was designed to detect intrinsic tumor radiosensitivity using 10 genes that play a role in DNA damage response, histone deacetylation, cell-cycle regulation, apoptosis, and

proliferation.(12,28,29) RSI has been shown to predict outcomes among patients treated with radiation therapy with breast cancer(13,30), head and neck cancer, rectal cancer, esophageal cancer(12) pancreatic cancer,(15), glioblastoma(16), and metastatic colorectal cancer(14). In contrast, 12-CK was designed using 12 immune-related and inflammation-related genes with the purpose of detecting intra-tumoral lymphoid cell aggregates as a marker of immune activation.(17,19) Twelve CK was previously found to be associated with the presence of TL-ELNs and was able to predict patient outcomes among patients with both colorectal cancer(17) and metastatic melanoma.(18,19) By combining the two gene signatures, we provide evidence for the presence of a clinical interplay between radiosensitivity and immune activation across a wide-variety of tumor types that has not previously been shown in the clinical setting.

There are clinical implications to the observed association between radiosensitivity and immune-activation in solid tumors. We hypothesize that this association could have clinical implications. In one scenario it is possible that the immune system primes the tumor for improved response to radiation. Conversely it could be that radiation therapy primes the tumor for increased immune activation, or potentially both processes take place in a synergistic manner. Understanding the clinical impact of this association is critical given the emerging immunotherapy options that could be utilized in combination with RT.

In the absence of radiation therapy, it is now well-established that tumors have a variety of innate mechanisms by which they can suppress the body's natural immune response directed towards them.(31) Fortunately, many of these mechanisms provide therapeutic targets for immune modulation.(32) Two more recently studied targets of tumor-directed immune modulation include the CTLA4 axis, which causes immune tolerization, and the PD1-PDL1 axis, which causes T-cell exhaustion.(4) Immune modulators including the anti-CTLA4 antibody Ipilimumab (Yervoy, Bristol-Myers Squibb), and the anti-PD1 antibodies Nivolumab (Opdivo, Bristol-Myers Squibb) and Pembrolizumab (Keytruda, Merck), effectively remove the tumor brake-pedal, increasing the tumor-directed immune activation. These targeted immunotherapies have been shown to positively affect tumor response and survival initially among melanoma patients(33–39), and subsequently among many other tumors including squamous cell and non-squamous non-small-cell carcinoma of the lung, renal cell carcinoma, and lymphoma.(40–43) Interestingly, cutaneous melanoma ($r=-0.56$), lung adenocarcinoma ($r=-0.50$) and renal cell carcinoma ($r=-0.56$) each had a relatively high degree of correlation between immune activation and radiosensitivity in the current study (Supplemental Table 1), suggesting that combined immunotherapy and radiation therapy treatment might also be beneficial for these tumors. Results from immunotherapy trials thus far have been compelling and exciting, yet there remains a lack of biomarkers to more appropriately select patients for immunotherapy trials(44). Additionally, it remains unclear as to whether immunotherapy-specific biomarkers, if successfully developed and implemented, would also predict for response to combined modality treatment if radiation is added as an immune-stimulatory mechanism. (45)

The ability of radiation therapy to influence tumor-directed immune responses in the pre-clinical setting has been supported by many disease site-specific studies.(5) Radiation has been shown to stimulate the immune system by upregulating MHC class I molecules(46),

enhancing FAS surface expression(47), activating dendritic cells(48), and by increasing the concentration of tumor infiltrating lymphocytes(49). Radiation also modulates the expression of immune checkpoint molecules in both favorable and unfavorable ways(5), which are less predictable and less well understood. Additionally, radiation has been shown to increase the concentration of regulatory T-cells in tumors(50–52), which acts to suppress the benefits of tumor-directed immune activation. Because of the multifactorial influences of radiation therapy on the immune system, an abundance of clinical trials are underway in an attempt to harness the potential synergy of immunomodulators and radiation therapy of various disease sites.(4,53,54) In support of the combined radiation and immunotherapy trials, Twyman-Saint Victor et al(55) demonstrated in a preclinical model that radiation therapy and immune checkpoint blockade activated non-redundant immune mechanisms in cancer. However, there remains a dearth of pre-treatment and tumor-specific biomarkers to identify patients who might, and more importantly might not, benefit from combined immunotherapy and radiation therapy. Since the composite model outperforms both individual RSI and 12-CK models, we hypothesize that these signatures may serve as biomarkers to identify patients that may benefit from combined RT and immunotherapy.

Moving forward, we hypothesize that the combined 12-CK/RSI phenotype could provide a pre-treatment biomarker to identify patients who will have increased response and outcomes to combined immunotherapy and radiation therapy treatment. For instance, patients with immune active/radiosensitive tumors could be selected for combined immunotherapy and radiation therapy trials, while those with immune inactive/less radiosensitive tumors could either be treated without radiation therapy, or selected for dose-escalated radiation trials with or without immunotherapy. Recently, we have developed the genomic-adjusted radiation dose (GARD) a genomic-based model that provides for the first time the opportunity to adjust the dose of radiation to match the individual radiosensitivity of the tumor. It is possible that the immune active/radiosensitive GES phenotype from the present study could be used to more accurately identify patients who experience the seemingly elusive abscopal effect.(9) To date, the abscopal effect remains a clinically rare and relatively unpredictable event(56,57), where radiation of a single metastatic site causes systemic regression of unirradiated distant metastases. When the abscopal effect does take place, it can render patients with a high metastatic disease burden, free of disease.(9) If a GES could improve the identification of these patients, even by a few percent, it would likely be deemed a success and as such, we believe that this is worth pursuing. A likely first-step to evaluate this would be to assess the 12-CK/RSI phenotype among a cohort of patients treated with combined modality treatment.

In conclusion, tumor radiosensitivity is associated with immune activation across solid tumors as measured by two unique gene expression signatures. Radiosensitive tumors are more frequently present in patients with a phenotype of immune-activation and the combined RSI and 12-CK GES has the potential to improve the prognostic ability of patients undergoing combined radiation therapy and immunotherapy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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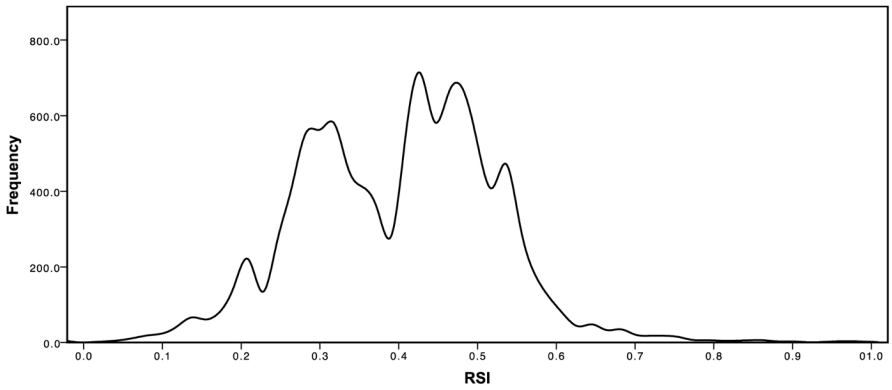


Figure 1.
Histogram demonstrating RSI values across 10,240 solid tumors.

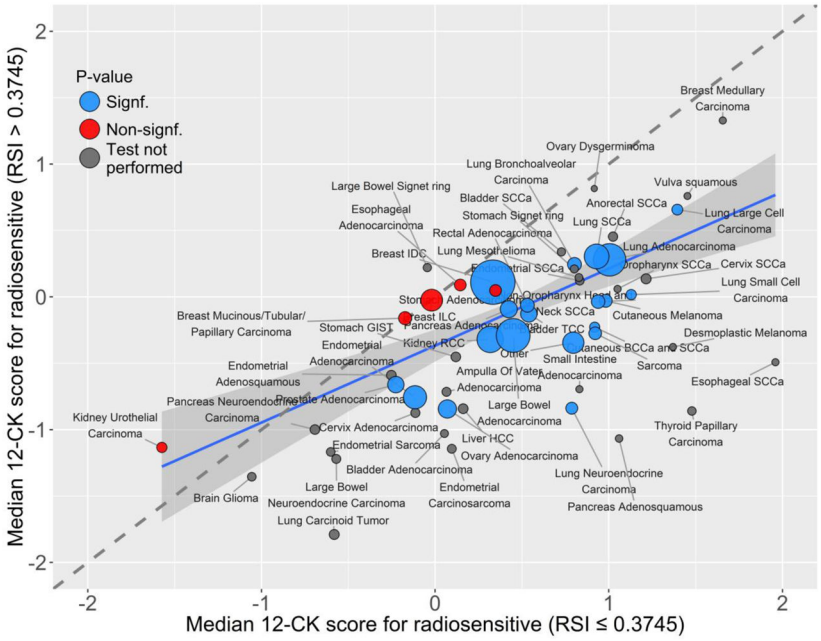
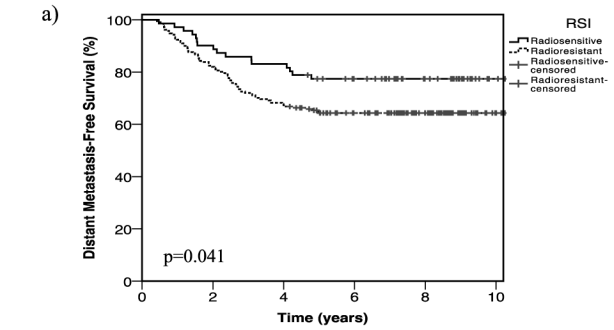
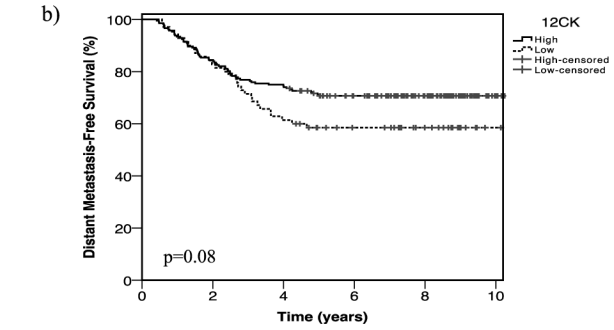


Figure 2.

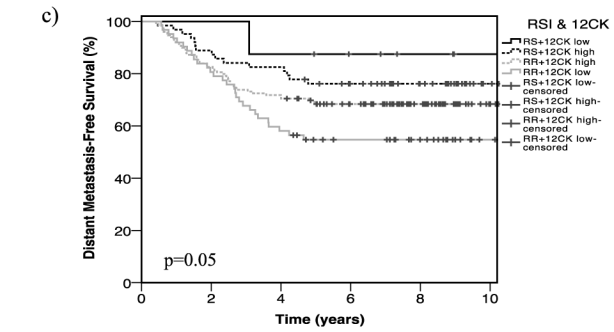
Tumor radiosensitivity and immune activation are associated across human tumor samples.



RS # at risk	71	64	59	48	33	12
RR # at risk	211	173	142	117	79	35



12CK-high # at risk	212	179	158	132	89	38
12CK-low # at risk	70	58	43	33	23	9



RS/12CK-H # at risk	63	56	52	43	30	11
RS/12CK-L # at risk	8	8	7	5	3	1
RR/12CK-H # at risk	149	123	106	89	59	27
RR12CK-L # at risk	62	50	36	28	20	8

Figure 3.

Kaplan Meier plots demonstrating distant metastasis free survival among 282 patients with breast cancer treated with radiotherapy compared by (a) radiosensitive (bottom 25% RSI) and less radiosensitive (upper 75% RSI) gene expression profiles, (b) 12-chemokine (12-CK) high and low gene expression profiles, and (c) by combined RSI and 12-CK expression profiles.

Table 1

Comparison of tumor types and 12-CK gene expression signature (GES) scores by RSI-low (more radiosensitive, RSI≤0.3745) and RSI-high (less radiosensitive, RSI>0.3745) groups. Tumor types are sorted from highest to lowest 12-CK GES (most to least immune-active) within the RSI-low group. Tumor types with <10 samples in either RSI group were not compared.

Tumor site and histology	RSI GES ≤ 0.3745		RSI GES > 0.3745		p
	N (%)	Median 12-CK GES (IQR)	N (%)	Median 12-CK GES (IQR)	
Esophageal SCCa	1 (16.7)	1.959 (1.959, 1.959)	5 (83.3)	−0.492 (−0.696, 0.217)	-
Breast Medullary Carcinoma	7 (87.5)	1.656 (1.517, 1.963)	1 (12.5)	1.328 (1.328, 1.328)	-
Thyroid Papillary Carcinoma	3 (10.7)	1.478 (1.336, 1.779)	25 (89.3)	−0.860 (−1.266, −0.127)	-
Vulva squamous	1 (25.0)	1.453 (1.453, 1.453)	3 (75.0)	0.759 (0.556, 0.895)	-
Lung Large Cell Carcinoma	28 (66.7)	1.394 (1.014, 1.605)	14 (33.3)	0.657 (−0.293, 1.352)	0.017
Desmoplastic Melanoma	1 (20.0)	1.370 (1.370, 1.370)	4 (80.0)	−0.378 (−0.674, −0.051)	-
Cervix SCCa	22 (68.8)	1.215 (0.854, 1.475)	10 (31.2)	0.136 (−0.136, 0.464)	-
Lung Small Cell Carcinoma	11 (31.4)	1.129 (0.690, 1.428)	24 (68.6)	0.017 (−0.490, 0.576)	0.002
Pancreas Adenosquamous	3 (60.0)	1.059 (0.292, 1.362)	2 (40.0)	−1.067 (−1.256, −0.877)	-
Oropharynx SCCa	9 (90.0)	1.051 (0.919, 1.612)	1 (10.0)	0.059 (0.059, 0.059)	-
Anorectal SCCa	7 (35.0)	1.024 (0.212, 1.274)	13 (65.0)	0.454 (−0.614, 0.758)	-
Lung Adenocarcinoma	584 (50.1)	1.005 (0.627, 1.358)	581 (49.9)	0.279 (−0.187, 0.716)	<0.001
Cutaneous Melanoma	25 (21.7)	0.983 (0.584, 1.326)	90 (78.3)	−0.030 (−0.611, 0.641)	<0.001
Non-Oropharynx Head and Neck SCCa	43 (45.3)	0.939 (0.546, 1.383)	52 (54.7)	−0.036 (−0.388, 0.548)	<0.001
Lung SCCa	311 (53.4)	0.930 (0.575, 1.272)	271 (46.6)	0.305 (−0.161, 0.716)	<0.001
Sarcoma	23 (22.5)	0.922 (0.510, 1.523)	79 (77.5)	−0.274 (−0.917, 0.596)	<0.001
Cutaneous BCCa and SCCa	11 (35.5)	0.919 (0.423, 1.503)	20 (64.5)	−0.228 (−0.731, 0.009)	<0.001
Ovary Dysgerminoma	1 (50.0)	0.916 (0.916, 0.916)	1 (50.0)	0.815 (0.815, 0.815)	-
Endometrial SCCa	7 (58.3)	0.833 (0.201, 1.070)	5 (41.7)	0.121 (0.034, 0.161)	-
Small Intestine Adenocarcinoma	7 (87.5)	0.831 (0.588, 0.913)	1 (12.5)	−0.695 (−0.695, −0.695)	-
Lung Mesothelioma	2 (16.7)	0.828 (0.496, 1.160)	10 (83.3)	0.146 (−0.111, 0.608)	-
Lung Bronchoalveolar Carcinoma	45 (44.1)	0.803 (0.428, 1.130)	57 (55.9)	0.244 (−0.141, 0.497)	<0.001
Stomach Signet ring	3 (33.3)	0.801 (0.451, 1.177)	6 (66.7)	0.210 (0.103, 0.267)	-
Other	143 (32.4)	0.796 (0.090, 1.271)	298 (67.6)	−0.345 (−1.086, 0.291)	<0.001
Lung Neuroendocrine Carcinoma	22 (34.9)	0.787 (0.444, 1.103)	41 (65.1)	−0.838 (−1.884, 0.074)	<0.001

Tumor site and histology	RSI GES ≤ 0.3745		RSI GES > 0.3745		p
	N (%)	Median 12-CK GES (IQR)	N (%)	Median 12-CK GES (IQR)	
Bladder SCCa	5 (55.6)	0.727 (−0.104, 0.916)	4 (44.4)	0.339 (−0.006, 0.507)	-
Bladder TCC	69 (35.8)	0.540 (−0.261, 1.266)	124 (64.2)	−0.128 (−0.775, 0.526)	<0.001
Rectal Adenocarcinoma	37 (32.7)	0.531 (−0.324, 0.810)	76 (67.3)	−0.063 (−0.753, 0.306)	<0.001
Large Bowel Adenocarcinoma	600 (46.0)	0.449 (−0.100, 0.972)	704 (54.0)	−0.291 (−0.811, 0.184)	<0.001
Pancreas Adenocarcinoma	66 (23.7)	0.424 (−0.086, 0.884)	212 (76.3)	−0.092 (−0.556, 0.328)	<0.001
Stomach Adenocarcinoma	25 (47.2)	0.348 (−0.227, 0.816)	28 (52.8)	0.048 (−0.508, 0.551)	0.192
Breast IDC	1147 (46.1)	0.333 (−0.335, 1.076)	1340 (53.9)	0.111 (−0.491, 0.626)	<0.001
Kidney RCC	291 (40.6)	0.317 (−0.125, 0.798)	425 (59.4)	−0.321 (−0.833, 0.267)	<0.001
Liver HCC	41 (85.4)	0.161 (−0.512, 0.416)	7 (14.6)	−0.842 (−1.289, −0.527)	-
Esophageal Adenocarcinoma	28 (52.8)	0.145 (−0.422, 0.682)	25 (47.2)	0.090 (−0.167, 0.506)	0.951
Stomach GIST	7 (22.6)	0.119 (−0.253, 0.870)	24 (77.4)	−0.452 (−1.235, 0.047)	-
Endometrial Carcinosarcoma	4 (14.8)	0.096 (−0.217, 0.524)	23 (85.2)	−1.145 (−1.449, −0.754)	-
Ovary Adenocarcinoma	108 (42.7)	0.070 (−0.646, 0.599)	145 (57.3)	−0.845 (−1.565, −0.164)	<0.001
Ampulla Of Vater Adenocarcinoma	10 (62.5)	0.065 (−0.142, 0.220)	6 (37.5)	−0.715 (−1.297, 0.032)	-
Bladder Adenocarcinoma	3 (42.9)	0.054 (−0.778, 0.966)	4 (57.1)	−1.029 (−1.275, −0.458)	-
Kidney Urothelial Carcinoma	23 (67.6)	−1.574 (−1.792, −0.851)	11 (32.4)	−1.134 (−2.411, −0.369)	0.913
Brain Glioma	3 (1.7)	−1.056 (−1.244, −0.548)	171 (98.3)	−1.355 (−1.827, −0.901)	-
Pancreas Neuroendocrine Carcinoma	6 (11.3)	−0.692 (−0.846, −0.564)	47 (88.7)	−0.999 (−1.585, −0.695)	-
Endometrial Sarcoma	4 (28.6)	−0.601 (−0.859, −0.056)	10 (71.4)	−1.168 (−1.685, −0.517)	-
Lung Carcinoid Tumor	7 (8.9)	−0.581 (−1.233, 0.279)	72 (91.1)	−1.790 (−2.105, −1.218)	-
Large Bowel Neuroendocrine Carcinoma	7 (46.7)	−0.569 (−1.284, −0.277)	8 (53.3)	−1.220 (−1.404, −0.984)	-
Endometrial Adenosquamous	7 (23.3)	−0.252 (−0.789, 0.532)	23 (76.7)	−0.590 (−1.355, −0.289)	-
Prostate Adenocarcinoma	73 (39.2)	−0.226 (−1.007, 0.158)	113 (60.8)	−0.661 (−1.065, −0.282)	<0.001
Breast Mucinous/Tubular/Papillary Carcinoma	35 (42.7)	−0.173 (−0.669, 0.310)	47 (57.3)	−0.160 (−0.772, 0.450)	0.723
Endometrial Adenocarcinoma	180 (32.5)	−0.116 (−0.760, 0.496)	374 (67.5)	−0.756 (−1.196, −0.197)	<0.001
Cervix Adenocarcinoma	10 (58.8)	−0.114 (−0.436, 0.517)	7 (41.2)	−0.873 (−1.109, −0.742)	-
Large Bowel Signet ring	3 (27.3)	−0.045 (−0.169, 0.197)	8 (72.7)	0.220 (−0.095, 0.522)	-
Breast ILC	172 (40.3)	−0.020 (−0.605, 0.540)	255 (59.7)	−0.025 (−0.468, 0.424)	0.547
Ovary Granulosa Cell Carcinoma	0 (0.0)	NA	10 (100.0)	−1.603 (−1.911, −1.076)	-

Table 2

Age-adjusted risk of distant metastasis free survival in the Erasmus breast cancer cohort compared using Cox multivariate modeling with clinicopathologic and treatment variables and a) RSI gene expression signature (GES) alone, b) 12-CK GES alone, and (c) RSI and 12-CK GES combined.

Variable		HR	CI (low)	CI (high)	P
RSI	Age (years)	0.99	0.97	1	0.11
	RSI-S (ref: RSI-R)	0.58	0.34	1	0.05
12-CK	Age (years)	0.98	0.96	1.00	0.04
	12-CK- high (ref: 12-CK- low)	0.61	0.39	0.96	0.03
RSI and 12-CK	Age (years)	0.98	0.97	1.00	0.05
	RSI-S (ref: RSI-R)	0.63	0.36	1.09	0.10
	12-CK- high (ref: 12-CK- low)	0.66	0.41	1.04	0.07