



Inconsistent Associations Between Risk Factor Profiles and Adjuvant Radiation Therapy (ART) Treatment in Patients with Cutaneous Squamous Cell Carcinoma and Utility of the 40-Gene Expression Profile to Refine ART Guidance

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ABSTRACT

Introduction: National Comprehensive Cancer Network (NCCN) recommendations for adjuvant radiation therapy (ART) use are similar for High Risk and Very High Risk cutaneous squamous cell carcinoma (cSCC) with negative post-surgical margins. Although studies report reductions in disease progression following ART treatment, ART use is likely inconsistent when guided by available risk factors. This study evaluated the association of ART with clinical risk factors in ART-treated and untreated patients and showed the clinical utility of the 40-gene expression profile (40-GEP) for guiding ART.

Methods: A multicenter study of 954 patients was conducted with institutional review board

(IRB) approval. The 40-GEP test was performed using primary tumor tissue from patients with either a minimum of 3 years of follow-up or a documented regional or distant metastasis. Unsupervised hierarchical cluster analysis identified patterns of clinical risk factors for ART-treated patients, then identified untreated patients with matching risk factor profiles. Results were cross-referenced to 40-GEP test results to determine utility of the test to guide ART.

Results: Analysis demonstrated inconsistent implementation of ART for eligible patients. Cluster analysis identified four patient profiles based on clusters of risk factors and, notably, matching profiles in ART-treated and untreated patients. Further, the analysis identified patients who received but could have deferred ART on the basis of 40-GEP test result and biologically low risk of metastasis, and untreated patients who likely would have benefitted from ART on the basis of their 40-GEP test result.

Conclusions: ART guidance is not determined by the presence of specific clinicopathologic factors, with treated and untreated patients sharing the same risk factor profiles. cSCC risk determination based on NCCN recommendations for clinical factor assessment results in inconsistent use of ART. Including tumor biology-based prognostic information from the 40-GEP refines risk and identifies patients who are most appropriate and likely to benefit from ART, and those that can consider deferring ART.

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Key Summary Points

Clinical use of adjuvant radiation therapy (ART) for cutaneous squamous cell carcinoma (cSCC) is effective for many patients but applied inconsistently as a result of vague guideline recommendations and subjective clinicopathologic factors. Better prognostic and predictive factors are needed to accurately identify regional and distant metastasis risk, and to identify patients who are likely or unlikely to benefit from ART.

The study aimed to evaluate whether clinical decisions for the use of ART were applied consistently within a large multicenter cohort of high risk patients with cSCC, and the utility of 40-GEP test results as an additional criterion to guide decisions for the use of ART.

The study demonstrated that 70% of untreated patients had the same patterns of clinicopathologic risk factors compared to ART-treated patients. Further, the 40-GEP test was able to refine risk to identify patients who were more likely to benefit from ART and a subset of ART-treated patients who could have safely deferred ART treatment on the basis of 40-GEP result and low risk of metastasis.

The study demonstrates the clinical challenge of assessing the metastatic risk potential of cSCC tumors to determine which are appropriate for treatment with ART and the utility of the 40-GEP to improve risk assessment and better guide decisions about ART.

INTRODUCTION

National Comprehensive Cancer Network (NCCN) recommendations for follow-up surveillance of patients diagnosed with cutaneous squamous cell carcinoma (cSCC) are different but overlapping for the NCCN-defined Low Risk, High Risk, and Very High Risk groups, with recommended history and physical examination frequencies increasing relative to risk across the three groups [1]. Patients with residual tumor identified after definitive surgical treatment and patients who are not candidates for surgery are advised to consider adjuvant radiation therapy (ART) regardless of NCCN risk group. For patients with no residual tumor noted after surgical treatment of the primary tumor, ART is recommended for consideration equally in patients with High Risk and Very High Risk disease. Both groups are advised to pursue multidisciplinary consultation and to consider ART in the presence of a few specific clinical and pathologic features (e.g., perineural invasion, PNI), in cases with “other poor prognostic features,” or for tumors assessed to have a “high risk for regional or distant metastasis” [1]. The benefit of ART in patients with high risk cSCC is consistent with that observed in other radiation responsive cancers [2, 3].

The 40-gene expression profile test (40-GEP; DecisionDx-SCC) was validated and is clinically indicated for use in patients with cSCC who have at least one high-risk feature (e.g., poor differentiation, PNI); most clinically tested patients have High Risk or Very High Risk tumors. The test is performed using formalin-fixed, paraffin-embedded (FFPE) tissue from the primary biopsy, wide-local excision, or Mohs debulk sample to assess the expression of 34 prognostic genes and six stably expressed control genes. One of three potential results for risk of disease progression are reported: Class 1 (lowest risk), Class 2A (higher risk), and Class 2B (highest risk). Similar, clinically actionable risk-stratification tests are available and widely used for management of patients diagnosed with cutaneous melanoma, uveal melanoma, breast cancer, thyroid cancer, and prostate cancer [4–8].

Validation and performance studies designed to evaluate the ability of the 40-GEP to stratify risk of metastatic disease progression have proven that the test provides independent prognostic information when compared to commonly assessed clinicopathologic factors [9, 10]. The significant improvement in risk stratification provided by the 40-GEP has also been demonstrated in the context of all available risk assessment tools and staging systems, including the NCCN guidelines and the Brigham and Women's Hospital (BWH) and 8th Edition American Joint Committee on Cancer (AJCC8) staging systems [10, 11]. In addition to the clinical validity of the test, clinical utility has been confirmed in studies reporting that clinicians use the 40-GEP results to inform decisions about nodal assessment, imaging, ART, and surgical or therapeutic intervention in a manner that is aligned with the biological risk profile of the tumor [12–14]. Overall, data show that 40-GEP test results increase the accuracy of predicted risk of disease progression which subsequently enables treating clinicians to refine and improve their risk-aligned clinical management decisions. Of note, recently reported data show that the 40-GEP test is able to distinguish between ART-eligible patients with cSCC who are likely to have improved survival following ART treatment and those who are not [15]. In a study comparing matched cohorts of ART-treated and untreated patients, metastasis-free survival (MFS) analysis showed that 40-GEP Class 2B patients who received ART had an approximately 50% increase in MFS rates compared to untreated Class 2B patients. In contrast, Class 1 and 2A patients had similar survival rates regardless of ART treatment status. Recently, it was shown that utilizing the 40-GEP test can reduce unnecessary ART treatments and associated complications, resulting in a more cost-effective and resource-efficient approach to cSCC disease management [16].

The current study first evaluates the association between ART treatment and the clinical risk factors of ART-treated and untreated patients in the context of the current guidance provided by NCCN Low Risk, High Risk, and Very High Risk categories in a large cohort of patients with cSCC ($n = 954$). The data suggest

that clinicians do not strictly adhere to NCCN recommendations for ART, do not consistently apply ART treatment on the basis of risk factor assessment, and instead modify treatment algorithms to guide decisions about the use of ART. Second, we report on data demonstrating that the 40-GEP test can identify patients who are most or least likely to benefit from ART, highlighting the clinical impact for patient care when the 40-GEP test is used to guide ART.

METHODS

Study Cohort and Data Acquisition

As part of a multicenter study that included 59 participating institutions, FFPE cSCC tumor tissue and associated clinicopathologic data were obtained for patients diagnosed with cSCC between February 2006 and October 2020. The appropriate institutional review board (IRB) ethical approval via Western IRB (approval number 20162697) was obtained and a waiver of consent was obtained. The study adhered to the Declaration of Helsinki and its subsequent amendments. Patients who had a prior history of cSCC, cutaneous basal cell carcinoma, or melanoma in situ could contribute samples if these non-cSCC prior malignancies were considered cured by the enrolling physician. Inclusion criteria for the study required availability of the original paraffin-embedded tissue block from primary tumor and at least 3 years of documented follow-up or report of a metastatic event. Sample selection was made with no consideration or stipulation regarding treatments. Inclusion/exclusion criteria have been previously described and only patients with at least one high risk clinical feature were included in analyses [9, 10]. Eligibility for this analysis is shown in the flow diagram in Fig. 1 and demographics of the cohort are presented in Table 1. Clinical site personnel and sponsor monitors were blinded to 40-GEP test results. Sponsor laboratory personnel were blinded to clinicopathologic and outcomes data. Importantly, the 40-GEP risk status of enrolled patients was unknown by the treating clinicians at the time of ART treatment decisions.

In short, two independent validation cohorts were merged for the analysis presented here (Fig. 1). A total of 34 patients were censored from analysis because of unknown ART status ($n = 6$), radiation therapy that occurred after a disease progression event ($n = 6$), or documented nodal metastatic event within 3 months of primary cSCC diagnosis given the questionable time window within which ART could have been completely administered after definitive surgical treatment ($n = 22$). All exclusion criteria were established prior to any data assessment or analysis.

Data Analysis

Risk factors associated with ART-treated and untreated patients were compiled and tabulated relative to NCCN guidelines. Further exploration of generalizable patterns of clinicopathological risk factors associated with ART-treated patients was performed using hierarchical cluster analysis (hclust function from the stats package, R version 4.3.1). Clinicopathological data relative to cluster assignment was tabulated to demonstrate the general patterns

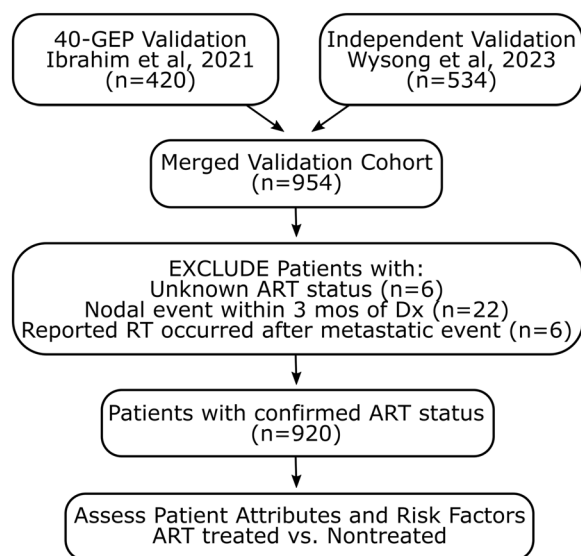


Fig. 1 Path of merged validation cohorts as data source for the current study examining the use of ART within NCCN guidelines, risk factors associated with ART-treated patients and untreated patients, and impact of 40-GEP testing on treatment decisions regarding ART

of factors observed in ART-treated patients, which was used to match ART-untreated patients fitting the same pattern of risk factors as ART-treated patients. The clusters of risk factors observed between ART-treated and untreated patients were cross-referenced to 40-GEP class result to determine the potential impact of using the test to guide ART treatment decisions.

RESULTS

Study Cohort Demographics

As detailed in Table 1, the study cohort included 920 patients with tumors classified as NCCN-defined Low Risk ($n = 15$), High Risk ($n = 568$) or Very High Risk ($n = 337$). Of the 113 nodal and distant metastatic events in this cohort, 29% occurred in the High Risk group and 71% occurred in the Very High Risk group. Though predicted by NCCN guidelines to have the highest risk for nodal or distant metastasis, only 24% of the patients in the very high risk group experienced a nodal or distant metastasis. In the overall cohort, 56/920 patients were confirmed to have received ART. Table 1 shows risk factors reported to be associated with a poorer prognosis in cSCC, which alone or in combination could qualify a High Risk or Very High Risk patient for ART.

ART Treatment Decisions Reflect Inconsistent Consideration of Clinicopathologic Factors

Analysis of this cohort showed that, despite being eligible for ART, use of ART was inconsistently implemented across the cSCC disease spectrum as indicated by presence of risk factors. For instance, there were 14 patients with residual tumor, and 25 additional patients with reported PNI of a nerve caliber > 0.1 mm who did not receive ART treatment despite recommendation in NCCN guidelines to consider ART treatment in these patients. The patients with no residual tumor who did not receive ART had between 0 and 5 highest level of risk factors

Table 1 Patient demographics

NCCN risk groups	Low risk (<i>n</i> = 15)	High risk (<i>n</i> = 568)	Very high risk (<i>n</i> = 337)	All (<i>n</i> = 920)
Non-local metastasis event, <i>n</i> (%)				
Yes	0 (0%)	33 (29.2%)	80 (70.8%)	113 (12.3%)
No	15 (1.9%)	535 (66.3%)	257 (31.8%)	807 (87.7%)
Adjuvant radiation treated, <i>n</i> (%)				
Yes	0 (0%)	6 (10.7%)	50 (89.3%)	56 (6.1%)
No	15 (1.7%)	562 (65.1%)	287 (33.2%)	864 (93.9%)
Perineural invasion, <i>n</i> (%)				
< 0.1 mm nerve involvement	0 (0%)	24 (25.0%)	72 (75.0%)	96 (10.4%)
≥ 0.1 mm nerve involvement	0 (0%)	0 (0%)	42 (100%)	42 (4.6%)
Absent/not reported	15 (1.9%)	544 (69.6%)	223 (28.5%)	782 (85.0%)
Lymphovascular invasion, <i>n</i> (%)				
Present	0 (0%)	0 (0%)	17 (100%)	17 (1.8%)
Absent/not reported	15 (1.7%)	568 (62.9%)	320 (35.4%)	903 (98.2%)
Immunosuppression status, <i>n</i> (%)				
Immunosuppressed	0 (0%)	169 (72.5%)	64 (27.5%)	233 (25.3%)
Not immunosuppressed	15 (2.2%)	399 (58.1%)	273 (39.7%)	687 (74.7%)
Tumor diameter, <i>n</i> (%)				
≤ 2 cm	15 (2.7%)	381 (69.8%)	150 (27.5%)	546 (59.3%)
> 2 to ≤ 4 cm	0 (0%)	138 (57.5%)	102 (42.5%)	240 (26.1%)
> 4 to ≤ 6 cm	0 (0%)	0 (0%)	34 (100%)	34 (3.7%)
> 6 cm	0 (0%)	0 (0%)	24 (100%)	24 (2.6%)
Not reported	0 (0%)	49 (64.5%)	27 (35.5%)	76 (8.3%)
Tumor location, <i>n</i> (%)				
Lower risk (extremities, trunk)	15 (7.9%)	115 (60.5%)	60 (31.6%)	190 (20.7%)
Special site (anogenital area, acral, pretibial)	0 (0%)	110 (83.3%)	22 (16.7%)	132 (14.3%)
Head or neck	0 (0%)	343 (57.4%)	255 (42.6%)	598 (65.0%)
Histological differentiation, <i>n</i> (%)				
Well differentiated	7 (2.1%)	239 (72.6%)	83 (25.2%)	329 (35.8%)
Moderately differentiated	1 (0.5%)	94 (51.1%)	89 (48.4%)	184 (20.0%)
Poorly or undifferentiated	0 (0%)	0 (0%)	121 (100%)	121 (13.1%)
Not addressed or Unable to assess	7 (2.4%)	235 (82.2%)	44 (15.4%)	286 (31.1%)
Invasion beyond subcutaneous fat, <i>n</i> (%)				
Present	0 (0%)	6 (10.7%)	50 (89.3%)	56 (6.1%)
Absent/not reported	15 (1.7%)	562 (65.0%)	287 (33.2%)	864 (93.9%)

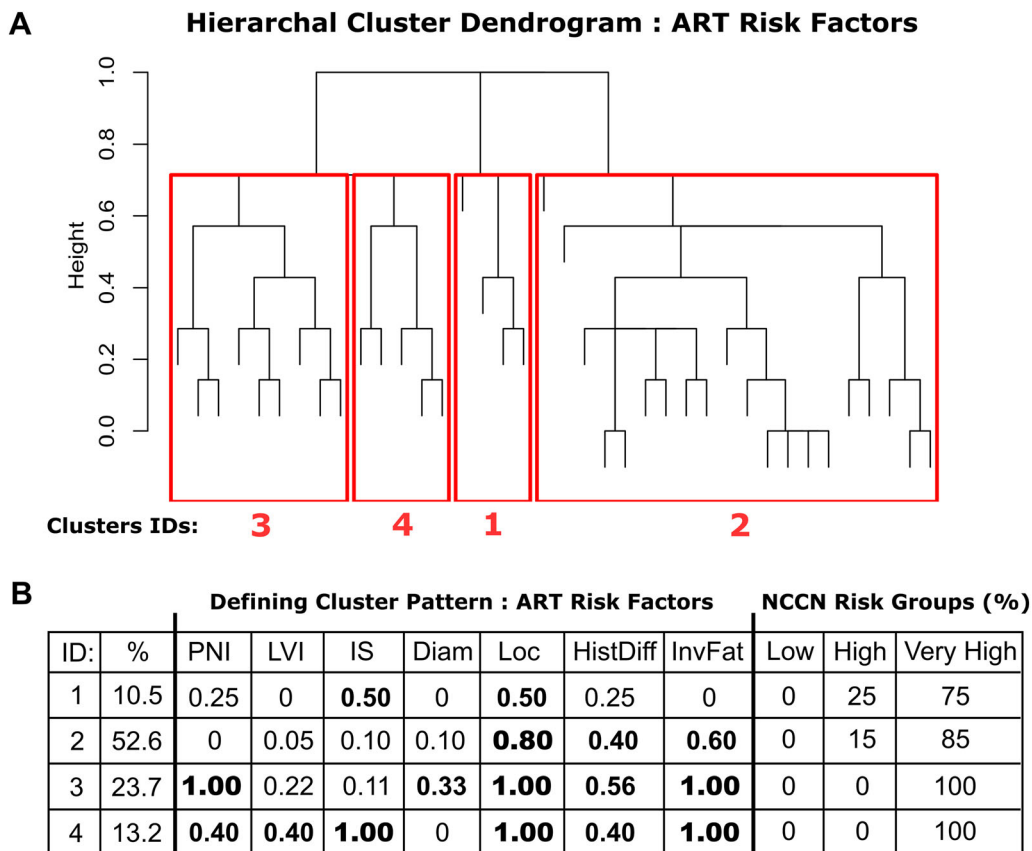


Fig. 2 Dendrogram showing clustering of risk factor profiles across ART-treated patients, and the proportion of patients presenting with the different risk factors used to define the attributes of each patient cluster. **a** Unsupervised hierarchal clustering was used to determine the primary patterns of risk factors that define ART-treated patients in this cohort. Red boxes highlight identified clusters of patients denoted by cluster identification; note that the numbers within a cluster are less important than the strength of an identified pattern. **b** The relative incidence of the highest risk factor levels observed based on cluster assignment was used to determine the specific pattern defining each cluster. Note a patient could have more than one highest level risk factor so proportions do not sum to 1. Heavy bold text indicates all or nearly all patients reported that factor, bold indicates patients must

have one of those additional factors, and regular text indicates patient does not have highest factor level. For example, cluster 2 is defined by tumor location on head or neck (80% of patients) AND either poor histological differentiation (40%) or invasion of fat (60%). Note that clusters conform to increasingly greater proportions designated as NCCN very high risk, in accordance with clinical risk. *ID* cluster identification, % percentage of ART patients in each cluster, *PNI* perineural invasion (> 0.1 mm involved nerve caliber), *LVI* lymphovascular invasion (present), *IS* immunosuppressed patient (yes), *Diam* tumor diameter > 6 cm, *Loc* tumor location on head or neck, *HistDiff* poor histological differentiation, *InvFat* invasion beyond subcutaneous fat (yes), percentage of cluster patients falling into NCCN high or very high risk groups

(median 1 risk factor, IQR 1–2), which included perineural invasion, lymphovascular invasion, immunosuppression, tumor diameter > 6 cm, tumor location on head or neck, poor histological differentiation, and invasion beyond subcutaneous fat (Table 1). Of these untreated

patients, 98% were categorized as High or Very High Risk and 70% were not treated despite being eligible for ART. This discordance between eligibility and treatment identifies the clinical need for improved risk stratification to consistently guide decisions regarding ART.

Of the ART-treated patients, all were categorized as High Risk or Very High Risk cSCC. Sixty-eight percent (38/56) of ART-treated patients had no residual tumor reported, of which only 32% (12/38) reported PNI of a nerve caliber ≥ 0.1 mm. The remaining ART-treated patients with no residual tumor had between 0 and 5 of the highest level of risk factors present (median 2 factors, IQR 1–3), showing a similar range of risk factor numbers as the untreated patients described above and reporting just one additional factor on average. Of these, seven patients identified only one of the highest level risk factors, [including one patient with invasion beyond subcutaneous fat, one immunosuppressed patient with a tumor at a special site (anogenital area, acral, pretibial), five patients with a tumor located on the head or neck with diameters between 2 and < 6 cm ($n = 3$) or ≤ 2 cm ($n = 2$)], one patient had none of the highest level risk factors but a tumor at a special site that was 2–4 cm in diameter, and 18 patients had two or more of the highest level risk factors.

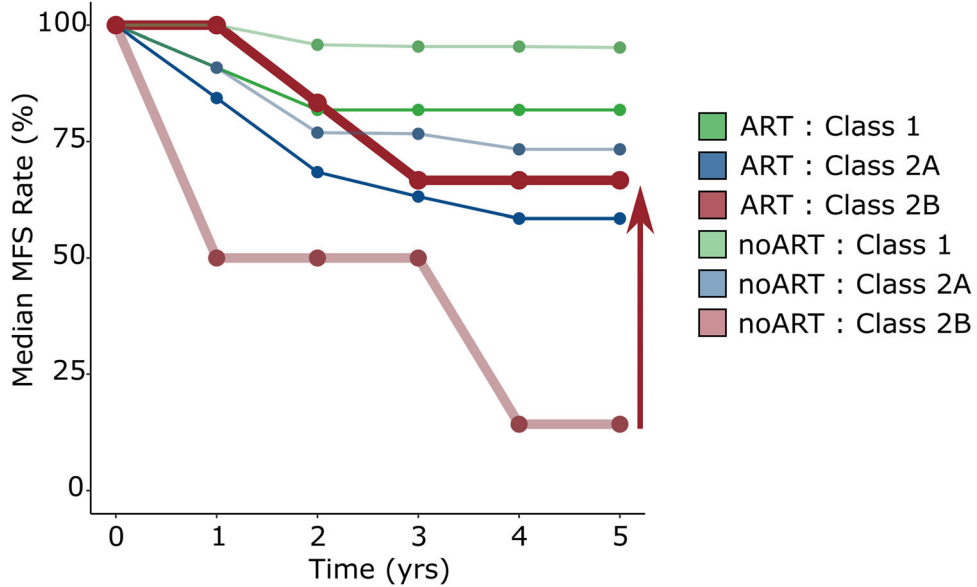
Clustering of Clinicopathologic Factors in ART-Treated Patients and Impact of 40-GEP Informed Decision-Making

To better determine the patterns of clinicopathologic factors associated with the ART-treated patients, unsupervised hierarchical clustering was performed and the distribution of features was used to assess level of risk for each cluster of factors. Four clusters were identified (Fig. 2a). The proportion of clustered patients that reported the highest level risk factors was used to determine the specific pattern of clinical presentation defining each cluster (Fig. 2b). Cluster 1 is marked by immunosuppressed patients or tumor location on the head or neck. Cluster 2 comprised patients with tumors on the head or neck with either poor histological differentiation or invasion of subcutaneous fat. Patients in cluster 3 all have PNI, tumors on the head or neck, and invasion of subcutaneous fat in addition to either poor histological differentiation or tumor diameter > 6 cm. Patients in cluster 4 are all

immunosuppressed and reporting tumors of the head or neck with invasion of subcutaneous fat in addition to either PNI, LVI, or poor histological differentiation. Clusters 3 and 4 represent the most risky patterns of clinicopathologic factors. The distribution of NCCN Low Risk, High Risk, and Very High Risk categories within each cluster support the level of risk suggested by the clustered pattern of risk factors (Fig. 2b).

A comparison of MFS between ART-treated and untreated patients with 40-GEP test results demonstrates the clinical utility of the 40-GEP test in guiding ART recommendations. As a result of the clinicopathologic discordance in determining who received and did not receive ART, as demonstrated in the previous section, there was a need to control patient selection bias. This was achieved by matching ART-treated and untreated patients with cSCC on a number of clinicopathologic factors, including type of definitive surgery received. Matched patient pairs were randomly resampled into balanced cohorts and MFS analysis was repeated 10,000 times to simulate population metrics. The results showed that patients predicted by the 40-GEP test to have the highest likelihood of metastasis, a Class 2B test result, saw an associated absolute improvement in MFS of approximately 50% increase on average at 5 years (Fig. 3a). In contrast and as expected on the basis of the risk-stratification performance of the 40-GEP, patients with a low-risk Class 1 test result were shown to have a low risk of metastatic events overall ($< 10\%$) and, as expected because of the low risk of events, patients with a Class 1 test results did not see an associated improvement in MFS. This lack of ART benefit is clinically and economically significant as Class 1 results were observed in 55% of patients [16]. Even within the ART-treated group presented here, Class 1 results were observed across all clustered risk factor groups, showing the independence of 40-GEP results from clinicopathologic factors (Fig. 3b, top).

A Matched Cohorts : Metastasis-Free Survival



B

ART Treated Patient Clusters NCCN Risk Group (%)

ID:	%	Class 1	Class 2A	Class 2B	Low	High	Very High
1	10.5	1	2	1	0	25	75
2	52.6	2	14	4	0	15	85
3	23.7	2	5	2	0	0	100
4	13.2	1	4	0	0	0	100

Untreated Patient Cluster Assignment NCCN Risk Group (%)

ID:	%	Class 1	Class 2A	Class 2B	Low	High	Very High
1	83.8	289	193	19	0	82.2	17.8
2	14.5	35	46	6	0	0	100
3	1.0	1	4	1	0	0	100
4	0.7	3	2	0	0	0	100

◀ **Fig. 3** Kaplan–Meier plot of 5-year metastasis-free survival (MFS) shows a median improvement of approximately 50% for class 2B patients treated with ART versus class 2B patients not treated with ART (red arrow), and identification of patients within the ART-treated group that could have reconsidered ART treatment and within the untreated cohort who may have benefitted from ART. **a** Recent findings in which patients diagnosed with cSCC having at least one high-risk feature were matched on clinical risk between ART-treated and untreated patients. Matched patients were randomly resampled into balanced cohorts and analyzed for MFS, with the median differences observed for each GEP class shown here. **b** Defined clusters representing ART patient risk factor patterns were applied to untreated patients. Top table shows ART-treated patient clusters (cluster 1 as lowest risk to clusters 3 and 4 as highest risk, see text), 40-GEP test result distributions and percentage of patients within NCCN risk groups. Bottom table shows cluster risk pattern definitions applied to untreated patients that matched one of the patterns and 40-GEP results. class 2B identifies patients that could have benefitted from ART and class 1 identifies patients that could defer ART to watchful waiting. Note that both results were observed across all cluster risk levels, highlighting the utility of the 40-GEP test in refining treatment decisions for ART within the space of NCCN guidelines. Note the reverse distribution of NCCN high versus very high patients between ART-treated and untreated for cluster 1 likely reflects patients that are either immunosuppressed or tumor located on head or neck with larger tumors (but ≤ 4 cm) or risky subtype in the ART-treated versus untreated patients for that lower risk cluster

APPLICATION OF CLUSTER PATTERNS OF CLINICOPATHOLOGIC FACTORS TO PATIENTS THAT DID NOT RECEIVE ART AND IMPACT OF 40-GEP TESTING

We then applied the clinicopathologic risk cluster groups associated with ART treatment to the untreated patients to identify patients that fell within the same cluster of risk factors but did not receive ART. In total, 598 patients fell within one of the four ART-treated cluster groupings but did not receive ART (70% of the untreated cohort). Of these, 83.8% were in

cluster 1, 15% in cluster 2 ($n = 87$), and less than 2% in clusters 3 or 4 (Fig. 3b, bottom). A Class 2B test result was reported in 26 patients falling within one of the four ART risk-factor cluster, identifying patients who could have benefitted from ART but did not receive treatment. Only one of those patients was in cluster 3 or 4, with the remaining falling in cluster 1 or 2, the less risky clusters based on clinicopathologic factors alone. However, the Class 2B result for these patients indicates tumor biology with a true risk of metastasis significantly greater than assumed by clinicopathologic factors alone. On the basis of study data described above, these patients are most likely to have significant clinical benefit from ART.

In contrast to clusters 1 and 2, clusters 3 and 4 are associated with more risk as assessed by clinicopathologic factors. However, one-third of those patients received a Class 1 result (Fig. 3b, top). Together with a low risk of disease progression and no clear benefit of ART demonstrated, a clinician can have confidence in deferring ART for these patients. The identification of clustered patterns of risk factors in ART-treated patients allowed for the assignment of untreated patients to the same clusters of risk factors. Similarly, 324 untreated patients that matched Cluster 1 or 2 received a Class 1 result (Fig. 3b, bottom).

The distribution of 40-GEP results across untreated patients reveals opportunities to further focus ART treatment on patients most likely to benefit and identify patients for whom a clinician can confidently defer ART. Use of the 40-GEP test for the untreated cohort qualifying for ART according to NCCN guidelines would have recommended 53% of patients to defer ART on the basis of tumor biology and low risk of metastasis, 42% would be guided to consider ART in the context of other clinicopathologic risk factors, and 5% would be recommended for ART on the basis of highest risk of metastasis and highest likelihood to receive a benefit (Fig. 4).

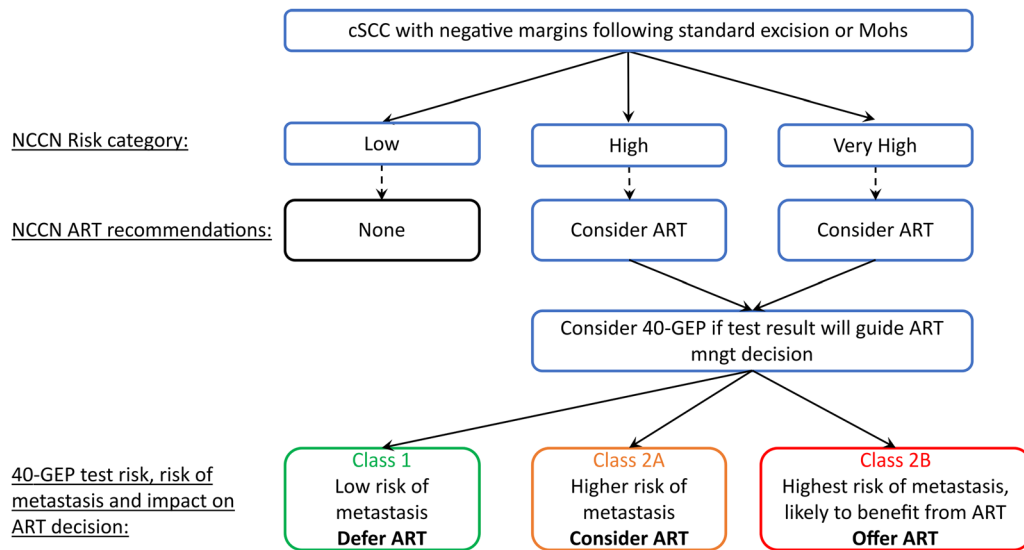


Fig. 4 Workflow for patients with cSCC with negative margins following excision or Mohs surgery and NCCN-defined history and physical and ART guidance according

to NCCN risk category. Subsequent 40-GEP use further refines prognostic risk and expected benefit from ART

DISCUSSION

Determining which patient is likely to benefit from ART is clinically challenging, and can result in unnecessary treatments and complications for patients as well as substantial costs to the healthcare system [16]. Patients with clear surgical margins undergoing ART by definition have no evidence of disease (NED) at the time of ART initiation. As such, patients with NED must carefully weigh the known cost and side effects of ART versus a theoretical benefit, as any individual patient may not relapse or progress in the absence of ART and thus receive no benefit. Identifying which patients are most likely to benefit from ART is an unmet clinical need. The NCCN guidance that ART should be considered in patients with NED “at high risk of regional or distant metastasis” is supported by all relevant societies and guidelines, including AAD, ACR, and ASTRO. These recommendations are based on published evidence that ART improves outcomes when patients with high metastatic risk are treated, including a large cohort study from Brigham and Women’s Hospital (BWH) and Cleveland Clinic [2]. Despite these consistent recommendations for the use of ART in patients at high risk of

regional or distant metastasis, there is still a significant clinical conundrum of which patients to treat, as the majority of patients will not experience a metastatic event. This study indicates that risk factors alone are inconsistently assessed for guidance of ART treatment, likely a result of the broad criteria in NCCN guidelines that qualify a patient for eligibility. Published data on the independent prognostic value of the 40-GEP test [9, 10, 17] and demonstrated utility to inform risk aligned management decisions [12, 13], including prediction of benefit from ART [15], demonstrate that a refinement of NCCN guidelines to include consideration of gene expression profile testing would result in more consistent decision-making regarding ART within the field.

Ruiz and colleagues demonstrated that within risk groups defined by the BWH staging system, ART is associated with a roughly 50% reduction of poor outcomes in patients with the highest risk of regional and distant metastasis identified by this staging system [2]. However, in patients with a moderate-to-high risk of metastasis identified by this staging system, a modest reduction in poor outcomes was noted, indicating a need to improve upon risk assessment to better identify which patients receive

benefit from ART [2]. This represents an opportunity to prevent overtreatment of patients with low biological risk of metastasis while identifying patients that have the highest likelihood to respond to ART treatment. The 40-GEP test has been validated to address this clinical hurdle by identifying patients with a high risk of regional or distant metastasis independent from established clinicopathologic factors.

Previous studies have proven that the biologically based risk-stratification performance of the 40-GEP test is independent from individual clinicopathologic factors and the clinicopathologic based risk systems used in clinical practice today—NCCN, BWH, and AJCCv8 [9, 10]. These studies also demonstrated, using multivariate analyses, that the 40-GEP Class 2B identifies the highest level of metastatic risk and has the highest independent positive predictive value compared to other risk stratification factors and systems. It is noteworthy that the 40-GEP results are distributed across the spectrum of risk identified by clinical and pathological risk factors; each result can be identified in each clinicopathologic risk factor-based category. As such, the test can be used alongside staging information, regardless of which risk assessment method is used, to improve the accuracy of metastatic risk assessment.

In addition to the risk-stratification performance of the 40-GEP in NCCN High Risk and very high risk patients with cSCC that has been previously demonstrated, this study highlights that ART-treated patients with a 40-GEP Class 2B test result experienced an approximately 50% absolute treatment benefit compared to untreated patients. In addition, in patients with a Class 1 test result and a low overall risk of metastasis, there was a non-significant benefit from ART treatment. As such, the 40-GEP is able to guide treatment recommendations for ART by informing overall risk stratification of the tumor, and in demonstrated improvement in outcomes where ART is focused on a specific population, those with Class 2B results. Importantly, because more than 55% of patients receive a Class 1 result, a substantial proportion of ART-eligible patients can safely defer ART treatment from which they

are unlikely to benefit. Recent studies have demonstrated this use of 40-GEP is associated with substantial healthcare savings [16].

Note that specific risk factors identified for each cluster for ART-treated patients reported here may vary in different set of ART-treated patients. Nevertheless, because the identified clusters were applied to untreated patients within the same cohort, the use of these specific risk factor cluster profiles is valid.

In summary, these data demonstrate that many untreated patients with clear indications for considering or recommending ART did not receive treatment, and the patterns of ART use confirm that clinicians currently rely on more ad hoc evaluation of “other poor prognostic factors” and assessment whether a tumor has a “high risk for regional or distant metastasis” as defined by guidelines to identify cSCC tumors that received or deferred ART. The clinical use of the 40-GEP test to guide treatment plan decisions, including ART recommendations, has been previously documented in both real-world clinician studies as well as a prospective, multicenter study [12, 13]. Including tumor biology-based prognostic information from the 40-GEP refines risk and identifies patients who are most appropriate and likely to benefit from ART, and those that can consider deferring ART. Prior clinical validation and clinical use studies combined with data from this current study support the improved decision-making that the 40-GEP test provides within NCCN High Risk and Very High Risk cSCC patient populations.

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article, have contributed important intellectual content for critically revising the manuscript, take responsibility for integrity of the data and approve the manuscript in its final form.

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Data Availability. The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of Interest. Ally-Khan Somani and Brent Moody are speakers and principal investigators for CBI. Aaron Farberg is an investigator and advisor for CBI. We thank the participants of the study. Aaron Farberg is an Editorial Board member of *Dermatology and Therapy*. Aaron Farberg was not involved in the selection of peer reviewers for the manuscript, nor any of the subsequent editorial decisions.

Ethical Approval. The source data used for the current work had obtained appropriate institutional review board (IRB) ethical approval via Western IRB (approval number 20162697) and a waiver of consent was obtained. The study adhered to the Declaration of Helsinki and its subsequent amendments.

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