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REVIEW

Sentinel lymph node biopsy in melanoma: beyond histologic factors

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Abstract

Sentinel lymph node (SLN) biopsy should be performed with the technical expertise required to correctly identify the sentinel node, in the context of understanding both the likelihood of positivity in a given patient and the prognostic significance of a positive or negative result. National Comprehensive Cancer Network guidelines recommend SLN biopsy for all cutaneous melanoma patients with primary tumor thickness greater than 1 mm and in select patients with thickness between 0.8 and 1 mm, yet admit a lack of consistent clarity in its utility for prognosis and therapeutic value in tumors < 1 mm and leave the decision for undergoing the procedure up to the patient and treating physician. Recent studies have evaluated specific patient populations, tumor histopathologic characteristics, and gene expression profiling and their use in predicting SLN positivity. These data have given insight into improving the physician's ability to potentially predict SLN positivity, shedding light on if and when omission of SLN biopsy in specific patients based on clinicopathological characteristics might be appropriate. This review provides discussion and insight into these recent advancements.

Keywords Melanoma · Gene expression profiling · DecisionDx-Melanoma · Sentinel lymph node biopsy · Metastatic melanoma

Abbreviations

SLN	Sentinel lymph node
MSS	Melanoma specific survival
CLND	Completion lymph node dissection
DFS	Disease free survival
OS	Overall survival
31-GEP	31 Gene expression profile test
DMFS	Distant metastasis free survival
RFS	Recurrence free survival
HR	Hazard ratio

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Introduction

Prognosis and sentinel lymph node status

Sentinel lymph node (SLN) biopsy is a procedure whereby preoperative and intraoperative lymphatic mapping is performed followed by selective lymphadenectomy of the first lymph node found in the lymphatic drainage pathway from the primary tumor to the regional nodal basin. This procedure identifies the lymph node which is most likely to contain any cellular metastasis from the primary tumor, diagnosing clinically occult disease [1, 2]. The frequency of SLN positivity increases with increasing primary tumor thickness and ulceration, and the presence of a pathologically positive sentinel node is the best morphologic prognostic factor for recurrence and survival [3, 4]. More specifically, SLN positivity differentiates intermediate and high-risk primary melanomas into subgroups with a better or worse overall prognosis, facilitating the identification of patients who would benefit from adjuvant therapies and rarely additional surgery such as regional completion lymph node dissection [5, 6].

Evidence from the Multicenter Selective Lymphadenectomy I (MSLT-I) trial showed that compared to nodal observation, performing SLN biopsy does not confer a

benefit in melanoma specific survival (MSS) among intermediate-thickness (defined in MSLT-I as > 1.2–3.5 mm) and thick (defined in MSLT-I as > 3.5 mm) melanoma. However, survival was improved when comparing those patients with a positive SLN vs. those observed who recurred in the nodal basin clinically [4]. The Multicenter Selective Lymphadenectomy II (MSLT-II) and German Dermatologic Oncology Cooperative Group (DeCOG-SLT) trials evaluated active surveillance with nodal observation versus completion lymph node dissection (CLND) in SLN positive patients. Both trials independently concluded there was no difference between groups in MSS, although it is important to note that patients with high-risk disease (extracapsular extension, microsatellitosis, > 3 nodes involved) were excluded from the MSLT-II analysis [7, 8]. A study looking at post MSLT-II practices recently evaluated these patients who were excluded from MSLT-II through a propensity-score matched comparison of nodal surveillance versus CLND. The authors found similar recurrence free survival (RFS) and MSS between the two groups, suggesting that the use of ultrasound surveillance in place of CLND is also appropriate in these patients who would have been excluded from MSLT-II. (Broman et al. Active Surveillance of Melanoma Patients with Sentinel Node Metastasis: An International Multi-Institution Evaluation of Post-MSLT-2 Adoption and Early Outcomes. *Cancer*. 2020. *In press*.)

Omission of sentinel lymph node biopsy

Recommendation for SLN biopsy is made for patients with no clinical evidence of lymph node metastasis and who demonstrate certain primary tumor factors (i.e. thickness greater than 1.0 mm and in some reports thickness greater than 0.8 mm or thinner with ulceration) [9]. Prior studies note increasing SLN positivity with increasing primary tumor Breslow thickness and ulceration [9]. (Table 1) Based off these studies, and common practice at most institutions, SLN biopsy is routinely offered where the risk of sentinel node positivity is deemed to be greater than or equal to 5% [9].

Although the utility of SLN biopsy has been long debated for thin melanomas < 1 mm in thickness, Han et al. demonstrated SLN metastases in 8.4% of thin melanomas ≥ 0.76 mm, including 5% of T1a melanomas ≥ 0.76 mm [10]. The authors later confirmed these findings in a larger study of 1250 patients, reporting SLN positivity in multivariate analysis more likely in primary tumors with ulceration and thickness ≥ 0.76 mm compared to those < 0.76 mm regardless of ulceration status as well as worse MSS with positive SLN biopsy [11]. A systematic review including this study and 59 others regarding thin melanoma found significantly increased likelihood of SLN positivity with thickness ≥ 0.75 mm, presence of microsatellites, and ≥ 1 mitoses/mm^2 [12]. More recently, further evaluation of MSS in melanomas < 1 mm by the Sentinel Lymph Node Study Group

Table 1 Recent single and multi-institutional studies looking at predictors of sentinel lymph node positivity

Study	Patient number (N)	Tumor thickness (mm)	Independent covariates	SLN positivity rate (%)
Han 2013 [11]	1250	≤ 1.0	Thickness ≥ 0.75 mm	6.3
			Ulceration	11.6
Cordeiro 2016 [12]	Median 75 per study	≤ 1.0	Thickness ≥ 0.75 mm	8.8
			MR ≥ 1/mm^2	8.8
Tejera-Vaquerizo 2019 [13]	3018	< 0.8	MR ≥ 1/mm^2	4.0
	1231	0.8–1.0	MR ≥ 1/mm^2	9.9
Egger 2019 [15]	6894	0.8–1.0	MR = 0/mm^2	3.0
			MR ≥ 1/mm^2, age < 56	8.0
		0.8–0.9	MR ≥ 1/mm^2, age > 56	3.7
		1.0	MR ≥ 1/mm^2, age > 56	5.4
Egger 2019 [16]	12,918	1.0–2.0	Age ≤ 56	14.5
			Age > 56, + LVI	26
			Age > 56, no LVI	7.8
Hanna 2019 [17]	23,440	1.0–4.0	+LVI	39.2
			No LVI, ≥ 1.7 mm thickness	18.3
			No LVI, < 1.7 mm thickness, Age < 56	15.2
			No LVI, < 1.7 mm thickness, Age > 56	4.9

MR mitotic rate, *LVI* lymphovascular invasion

in Melanoma (SENTIMEL) reported SLN positivity as the most important prognostic factor in those who underwent SLN biopsy, and a SEER database study associated significantly improved overall survival (OS) and MSS in patients who underwent SLN biopsy compared to those who did not have a SLN biopsy [13, 14]. Because of these studies and others, it is recommended that SLN biopsy be considered in those melanomas including thin melanomas where the positivity rate is greater than or equal to 5%.

Age and melanoma sentinel lymph node positivity

The question remains: Are there any scenarios where we could identify a patient population with less than 5% risk of SLN metastases (other than thin primary tumors)? For example, is there a certain age group along with certain tumor depth in which we can safely avoid SLN biopsy? Egger et al. reported on 6894 patients with primary tumor thickness 0.8–1.0 mm, noting that patients who were 56 years or younger were at significantly higher risk for SLN positivity. In that study, two groups were identified which had risk of SLNB positivity < 5% and made up 55% of patients with T1b non-ulcerated melanoma who underwent SLN biopsy: patients with mitotic rate of 0/mm^2 and patients with mitotic rate ≥ 1/mm^2, age > 56 years, and thickness 0.8–0.9 mm [15]. Egger et al. went on to report on AJCC 8 T2 melanomas using data from the National Cancer Database, finding an increased risk of SLN positivity in patients < 40 years old versus 40–65 years and > 65 years (Hazard ratios: reference, 0.6, 0.39, respectively; $p < 0.001$) [16].

Additionally, patients > 56 years who also demonstrated primary tumor lymphovascular invasion were at higher risk of SLN positivity compared to those without lymphovascular invasion. Among elderly patients (defined as > 75 years old) with primary tumor thickness < 1.2 mm the risk of SLN positivity was 4.9% (95% confidence interval 3.3–7.1%) [16]. In a separate National Cancer Database study, Hanna et al. reviewed the relationship between age and lymph node metastasis in intermediate thickness melanoma among 23,440 patients. SLN positivity was more common with lymphovascular invasion, thickness ≥ 1.7 mm, and age < 56 years. When relating age to tumor thickness, the authors developed a model for patients with non-ulcerated primary tumors, demonstrating that every 10 years above 50 years corresponded to an additional 0.5 mm depth beyond 1 mm depth where the estimated risk of SLN positivity stayed below < 5% [thickness = (age × 0.05) − 1.5] [17].

Based on these studies, it appears that it is possible to potentially identify a population of patients who may be eligible for safe omission of SLN biopsy, in which the positivity would be less than 5% based on primary tumor depth (particularly T1 and T2 melanomas), age, mitotic rate, lymphovascular invasion and ulceration.

Guidance of sentinel lymph node biopsy using gene expression profiling

SLN biopsy and melanoma management

Historically, melanoma treatment is driven by risk of SLN positivity as judged by tumor thickness and ulceration, and risk of recurrence as judged by tumor thickness, ulceration, and SLN status. MSLT-I and subsequent studies have observed a SLN biopsy false negative rate of 5–15%, therefore the current practice is to recommend SLN biopsy if the risk of metastases is ≥ 5% [4, 18]. Current guidelines recommend SLN biopsy for patients with melanomas stage T1b and above, and patients with stage T1a melanomas can be considered for SLN biopsy when the primary tumor exhibits high-risk features such as high mitotic rate or ulceration [9, 19].

Of patients undergoing SLN biopsy, 88% will have a negative result [20–22] and an average pooled rate of 11% will experience a complication, most commonly post-operative seroma (5.1%) and infection (2.9%) which are able to be managed expectantly [23]. This emphasizes the need for physicians to provide the right treatment for the right patient at the right time. Low-risk patients, typically those with stage I-IIA disease, are given less frequent follow-up and, after 2 years, recommended to follow-up primarily with their dermatologist only [19]. High-risk patients, typically those with stage IIB disease or higher, are given higher intensity surveillance plans through frequent clinical follow-up with their oncologist for 5 years, advanced imaging, and consideration of adjuvant therapy and/or enrollment in a clinical trial [19].

DecisionDx-Melanoma

The DecisionDx-Melanoma test was developed to assess the risk of recurrence independent from traditional clinical and histological factors in patients with stage I–III melanoma. Residual primary tumor tissue from the standard formalin-fixed paraffin-embedded block of the initial biopsy or wide local excision is assessed for expression of 31 genes using reverse transcriptase polymerase chain reaction (RT-PCR) (Fig. 1).

Gene expression levels are determined using a proprietary gene expression profile test (31-GEP), which involves a radial basis predictive modeling algorithm that determines whether the genetic profile of a particular tumor is more strongly associated with low-risk (Class 1, with subclasses 1A and 1B) cases or high-risk (Class 2, with subclasses 2A

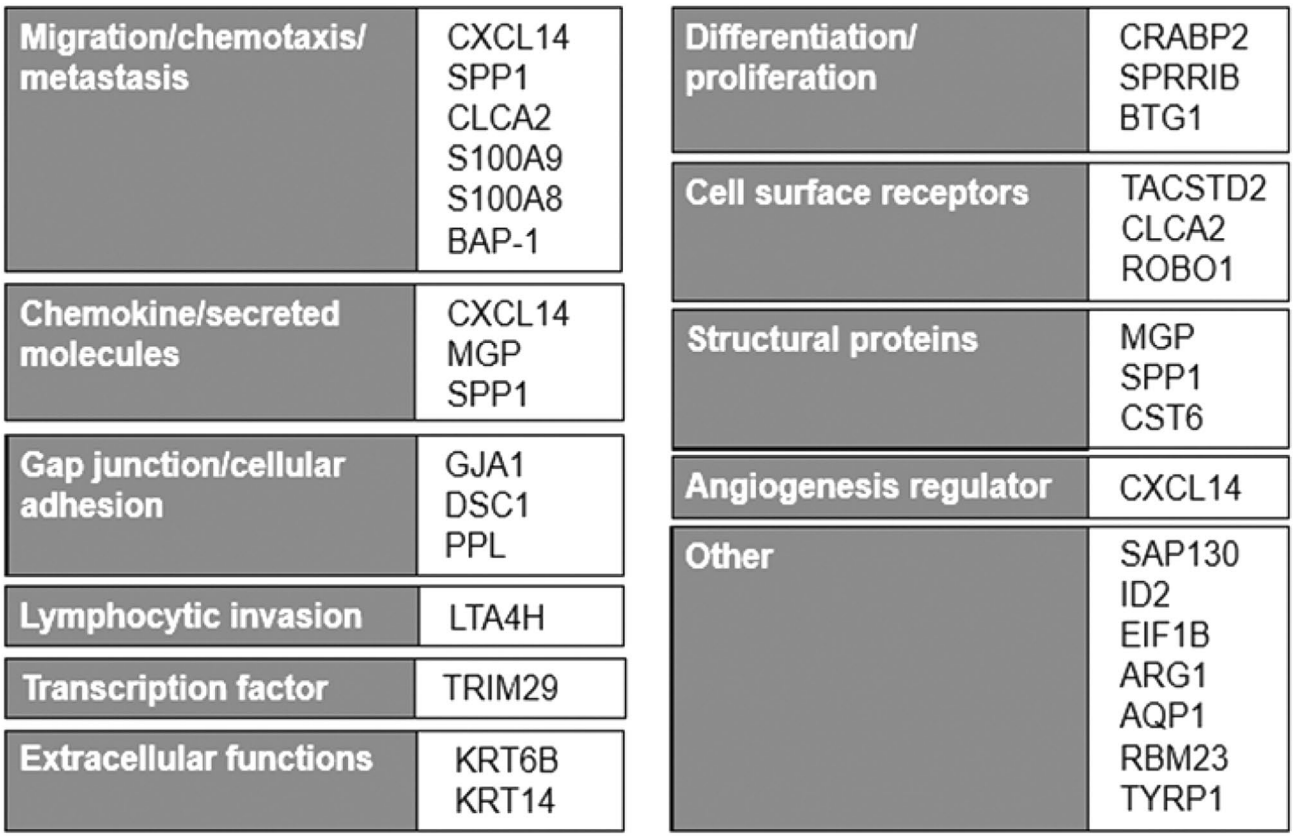

Fig. 1 Cellular functions represented in the DecisionDx-Melanoma gene signature [23]

and 2B) cases, as previously reported [24]. Approximately 85% of tests fall within class 1A or 2B.

Validation of gene expression profiling

The 31-GEP test has been studied with the goal to identify a melanoma patient population that is below the threshold of 5% likelihood of SLN positivity, stratifying patients into low-versus high-risk groups with the aim to improve patient selection for SLN biopsy. Twenty-one manuscripts regarding > 3100 patients have been published in peer-reviewed journals, validating the development of DecisionDx-Melanoma (Fig. 2). These include three multi-institutional studies with expanded performance cohorts, where approximately 70% of patients in the clinical validation program had stage I or II melanoma. The total validation cohort consisted of 690 patients staged I–III, followed over median 7 years [24–29]. Additionally, four prospective, independent studies and a prospective registry study involving a total of 1887 patients were used to determine the best model to identify patients at low risk for a positive SLN [30–33]. Recently published, a prospective, multi-institutional study of 1421 patients showed that DecisionDx-Melanoma may provide some further prognostic information to be used along with the above-mentioned primary tumor and patient-related factors to aid in predicting SLN positivity [34]. Throughout the published studies, Class 1 patients have consistently shown lower rates of SLN positivity, with Class 2 patients demonstrating approximately 3× the rate of SLN positivity compared to Class 1 (Table 3).

Development of a model to identify a patient population with < 5% risk of SLN positivity utilized clinical and gene expression data from retrospective review of 946 patients with stage I–IV melanoma [35]. The Radial Basis Machine predictive algorithm coupled with Breslow depth was the best performing modeling method among regression models, neural networks, and others, and identified a low-risk patient population defined by Class 1 patients in combination with tumor thickness ≤ 2 mm, which met the < 5% threshold. Validation of the algorithm was then performed across two prospective, multi-institutional cohorts of 584 (Castle Biosciences study [33, 36]) and 837 (independent study [34]) patients (Table 2).

Of the 1421 patients evaluated in the validation study, 79% had a SLN biopsy performed, including 34% of T1a patients. Overall, 1065 patients had T1-2 tumors, and of those with T1-2 tumors and Class 1A designation, the SLN positivity rate was 4.6%. For T1-2 tumors with Class 2B

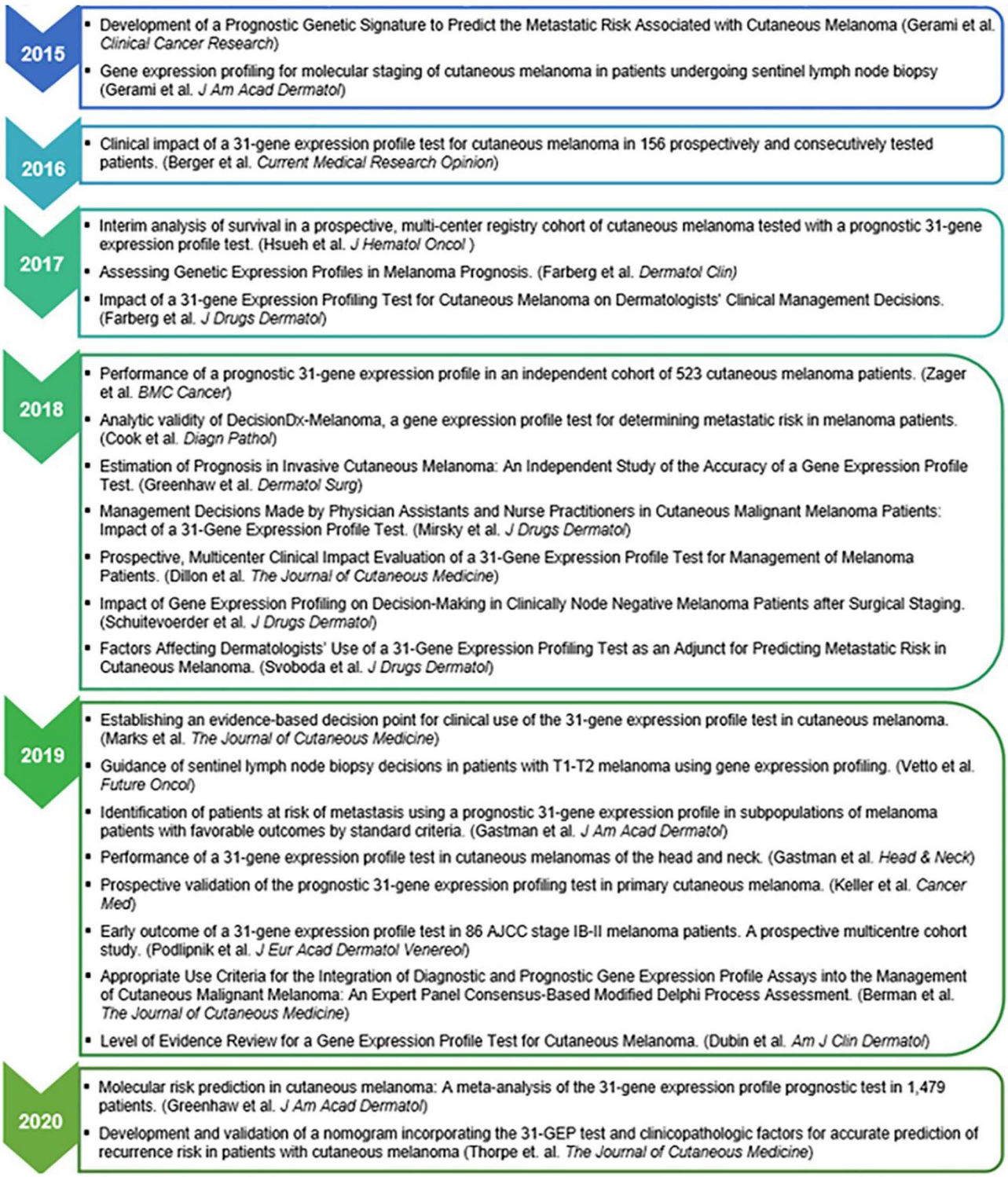

Fig. 2 Flowchart of manuscripts evaluating DecisionDx-Melanoma gene expression profiling

designation, the SLN positivity rate was 18.8% [34]. As the risk of SLN positivity decreases with increasing age [37–40], patients were stratified by age into three groups of <55, 55–64, and ≥ 65 years old, based off model inflection points. When looking at age and tumor depth, patients with Class 1A, T1–2 tumors, and ≥ 65 years demonstrated SLN

Table 2 Clinicopathologic characteristics of prospective validation cohorts [33, 34, 36]

Variable	Cohort 1 (n = 584) Castle studies [33, 36]	Cohort 2 (n = 837) Independent study [34]	p value
Median age, years (range)	61 (18–100)	63 (12–101)	0.05
Median Breslow depth, mm (range)	1.2 (0–18)	1.16 (0–60)	0.21
Ulceration present	19%	25%	0.006
Mitotic rate ≥ 1/mm^2	59%	64%	0.043
SLN biopsy positive	14%	12%	0.33
Tumor stage			
T1	44%	42%	0.67
T2	31%	32%	
T3	17%	17%	
T4	7%	9%	

SLN sentinel lymph node

positivity of 1.6%, significantly less than the same cohort of Class 2B patients with SLN positivity rate of 11.9% ($p < 0.02$) [34]. (Table 3) Long-term follow-up estimation of survival outcomes for the target population of Class 1A patients with T1–2 melanomas (regardless of SLN biopsy outcome) showed an improved MSS, OS, distant metastasis free survival (DMFS), and RFS at 5 years compared to Classes 1B-2B [34].

In expansion upon the validation studies, further evaluation of the 31-GEP clinical effectiveness in identifying patients with low risk of SLN positivity is currently being performed in a prospective expansion cohort at centers who routinely use DecisionDx-Melanoma. A total of 2578 patients with known SLN status have been evaluated, with 1905 cases having a T1–2 melanoma and 866 cases being ≥ 65 years in age. In Class 1A patients ≥ 65 years with T1–2 melanoma (n = 367), there was a significantly lower rate of SLN positivity of 2.7% compared to 8.8% in Class 1B/2A (n = 170) and 18.5% in Class 2B (n = 92) ($p < 0.01$). In patients of all ages with T1–2 melanoma, Class 1A patients (n = 1317) demonstrated a SLN positivity rate of 4.9%, compared to 11.1% and 13.4% in Class 1B/2A (n = 398) and Class 2B (n = 588), respectively ($p < 0.01$). A manuscript that presents the final analysis of this second study is currently in preparation. It will be interesting to see the SLN positivity rates when further broken down by T substage (T1a, T1b, T2a and T2b) and age.

Table 3 Probability of sentinel lymph node positivity in T1/T2 tumors by DecisionDx-Melanoma [34]

Result	All (n = 1065)	≥ 65 years (n = 448)	55–64 years (n = 247)	< 55 years (n = 370)
Class 1A	4.6%	1.6%	4.9%	7.6%
Class 1B/2A	10.8%	6.9%	7.7%	19.6%
Class 2B	18.8%	11.9%	30.8%	24%
p value	< 0.001	0.007	0.01	0.009

DecisionDx-Melanoma in clinical practice

Incorporating DecisionDx-Melanoma, along with looking at primary tumor and patient-related factors in thinner melanomas, into patient discussions and treatment recommendation could aid decisions regarding the appropriateness of SLN biopsy in certain populations, especially those greater than 65 years old with T1–2 tumors. With this in mind, the DecisionDx-Melanoma Impact on Sentinel Lymph Node Biopsy Decisions and Clinical Outcomes (DECIDE) multi-institutional registry trial is currently enrolling patients with the primary outcome of studying the association of 31-GEP test results with surgical decision making in patients with SLN biopsy eligible T1–2 melanoma. Enrolled patients will undergo DecisionDx-Melanoma testing within 2 months of diagnosis and are recommended SLN biopsy based off Class designation and patient-physician choice. The secondary outcome of the study aims to track and evaluate post-operative and 5-year outcomes among patients with T1–T4 melanoma, stratified by each 31-GEP subclass, including patients who did and did not undergo SLN biopsy.

Following resection of a primary melanoma and decision to undergo SLN biopsy, the next clinical discussion revolves around a patient's risk for recurrence. Historically, the risk for melanoma recurrence has been based off the clinicopathologic factors of Breslow thickness, ulceration status, and SLN positivity. Variance in these three variables places each patient into a staging category detailed in the American Joint Commission on Cancer 8th edition staging system for melanoma (AJCC8) [41]. As patients exhibit higher stage, their risk for recurrence and overall prognosis worsens, leading physicians to recommend further treatment. Patients with stages I-IIA melanoma are generally considered low risk and are recommended for somewhat lower frequency follow-up.

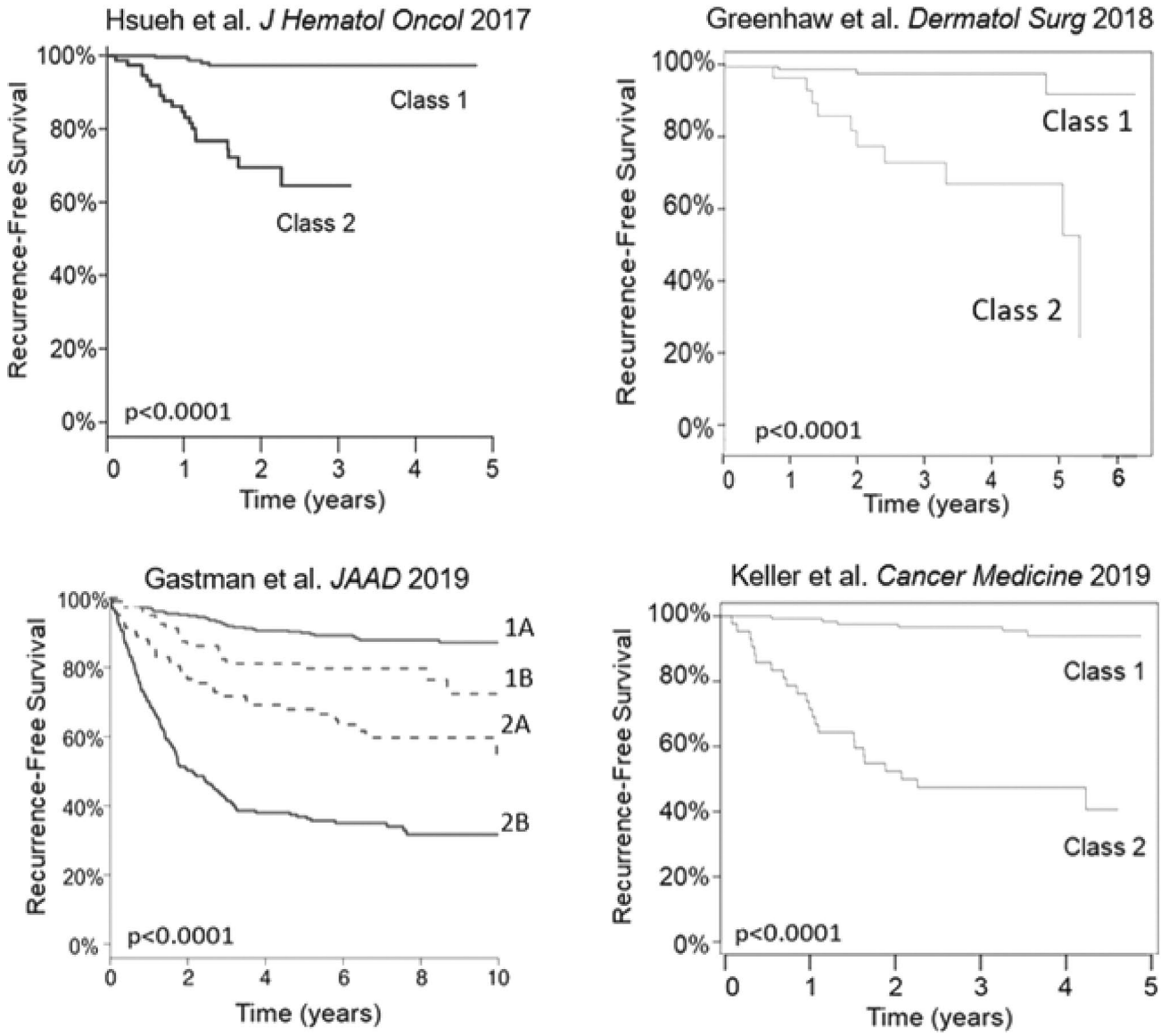

Fig. 3 DecisionDx-Melanoma results for recurrence risk [25, 29, 32, 34]

Patients with stage IIB or higher melanoma are at higher risk and may be recommended higher frequency follow-up, full-body surveillance imaging, initiation of adjuvant therapy, or clinical trial enrollment. Evaluating the 31-GEP test for utility in discussion of a patient's risk of recurrence, one retrospective study [35], and three prospective studies [30, 32, 33] determined significant differences in recurrence rates between Class 1 and Class 2 designations (Fig. 3).

In assessment of the 31-GEP test for ability to provide accurate prognostic information on survival, a pooled cohort of 901 melanoma cases from 22 centers were analyzed for 5-year MSS. MSS of Class 1A and Class 2B patients within each T-stage was compared with the 5-year MSS of the AJCC8. The 31-GEP test cohort exhibited similar rates of 5-year MSS across stages I-III melanoma relative to that of the AJCC8 cohort, with distinct RFS and DMFS between each 31-GEP Class. Additionally, 31-GEP was found to provide additional prognostic stratification within each T-stage for rate of MSS [42] (Table 4).

In the stage I patients, DecisionDx-Melanoma identifies Class 2B designation with a worse prognosis, closer to that of stage IIIA patients. Stage II patients with Class 2B designation have a prognosis similar to stage IIIB patients, and stage III patients with Class 2B designation have a much lower rate of MSS and worse prognosis than stage IIIC patients. Conversely, the Class 1A patients within each substage identified patients with a high rate of MSS, similar to that seen in stages IA–IIA patients. A PRISMA

Table 4 Stratification of patients into low- and high-risk groups within AJCC8 stages by 5-year melanoma specific survival

Cohort	Stage I	Stage II	Stage III	Risk
Class 1A	99.7%	97%	94.5%	Low (stage I–IIA)
AJCC8	98%	90%	–	
Class 2B	92.8%	87.4%	60.9%	High (stage IIB–III)
AJCC8	–	–	77%	

AJCC8: American Joint Commission on Cancer, 8th edition

based systematic review and meta-analysis was performed to further evaluate the prognostic ability across four study cohorts totaling 1479 patients with stages I–III melanoma, finding that independent of other clinical factors such as tumor thickness, ulceration, and regional lymph node status, DecisionDx-Melanoma provides a risk assessment for melanoma recurrence and metastasis. Multivariate analysis showed Class 2B patients are almost three times as likely to recur than Class 1A patients (HR 2.9, $p < 0.001$) and almost three times as likely to experience distant metastasis as well (HR 2.75, $p < 0.001$) [43]. A second PRISMA based systematic review conducted a meta-analysis of gene expression profiling for melanoma, only including 6 studies which all reported on DecisionDx-Melanoma. When compared to Class 1 designation, Class 2 designation was associated with SLN positivity (odds ratio = 2.99), worse RFS (pooled HR 7.22), DMFS (pooled HR 6.62), and OS (pooled HR 7.06) (all $p < 0.001$) [44].

Recommendations for DecisionDx-Melanoma in management of melanoma

Upon biopsy-proven diagnosis of cutaneous melanoma, wide local excision with or without SLN biopsy, based on Breslow depth and other clinicopathological factors aforementioned in this review, is needed to accurately stage the patient according to National Comprehensive Cancer Network (NCCN) guidelines. For patients with tumor depth ≥ 0.3 mm, DecisionDx-Melanoma offers further prognostic information to help guide surveillance planning of clinical follow-up and imaging [35].

Conclusion

The use of molecular expression signatures to guide treatment pathways in the direction of using healthcare resources for the patients at higher risk is routine for patients with thyroid, prostate, and lung cancers. For patients with cutaneous melanoma, there are notable subgroups who may not require invasive therapy, high frequency follow-up, or surveillance imaging depending on a patient's age and primary tumor mitotic rate, Breslow depth, lymphovascular invasion, or ulceration. DecisionDx-Melanoma may provide an additional variable to aid clinicians in potentially identifying a patient population with < 5% likelihood of SLN positivity and provide valuable prognostic information on recurrence and disease survival, leading to decreased cost and optimization of resources.

Author contributions All authors made substantial contributions to the conception or design, interpretation of data, and drafting/revision of the manuscript. All authors approved the final version for publication and agree to be accountable for all aspect of the work in ensuring that questions related to the accuracy or integrity of the work are appropriately investigated and resolved.

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Declarations

Conflict of interest M.J.C. has no conflicts of interest to disclose. F.A.M. is a former employee and current stock option holder at Castle Biosciences, Inc. J.S.Z. has advisory board relationships with Novartis, Sanofi/Regeneron, Merck; receives research funding from Amgen, Delcath Systems, Philogen, Provectus; consults for Castle Biosciences and Philogen, speaker's bureau for Castle Biosciences, Pfizer and SunPharma. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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