Application to the Danish Health Technology Council regarding the Merlin Assay to predict nodal metastasis in patients with pT1-pT2 primary cutaneous melanoma

Merlin

A GENE EXPRESSION-BASED TEST

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1. List of abbreviations

Abbreviation	Meaning
AJCC	American Joint Committee on Cancer
BT	Breslow Thickness
CM	Cutaneous melanoma
CP-GEP	Clinicopathological-gene expression profile
DMFS	Distant metastasis-free survival
DNA	Deoxyribonucleic acid
EADO	European Association of Dermato-Oncology
EFP	Extended follow-up
FFPE	Formalin-fixed paraffin-embedded
FP	False positive
FN	False negative
GDPR	General Data Protection Regulation
HR	Hazard ratio
MERLIN_001	MElanoma Research Lymph Node Prediction Implementation National_001
MSS	Melanoma-specific survival
NCCN	National Comprehensive Cancer Network
NPV	Negative predictive value
OS	Overall survival
SPECT-CT	single-photon emission computerized tomography computed tomography
RFS	Recurrence-free survival
RNA	Ribonucleic acid
RT-qPCR	Quantitative reverse transcriptase poly chain reaction
SLN	Sentinel lymph node
SLNB (RR)	Sentinel lymph node biopsy (reduction rate)
SMR	Society of Melanoma Research
TAT	Turnaround Time
TNM	TNM Classification of Malignant Tumors
US	United States
UV	Ultraviolet
WCM	World Congress of Melanoma

2. Summary

Melanoma is among the most common and rapidly rising cancers in Denmark, with approximately 3,000 new cases diagnosed each year. The country currently ranks third globally in melanoma incidence, with a rate of 31.1 per 100,000, the second highest in the world (1). This growing trend poses a significant burden on both individuals and the national healthcare system, making melanoma a critical public health concern.

Sentinel lymph node biopsy (SLNB) surgery is a widely used procedure important for staging and management of melanoma patients by assessing whether tumor cells have spread into the sentinel lymph nodes (SLN). If tumor spread to SLNs has been identified this will guide whether a patient is eligible for further immunotherapy. However, there are several unmet needs associated with SLNB:

- Low Diagnostic Yield: Approximately 80–85% of SLNBs return negative for nodal metastasis. In these cases, the procedure offers no therapeutic benefit but still exposes patients to its risks (2).
- Invasiveness: The procedure requires surgical intervention, often under general anesthesia, with associated risks of scarring and postoperative discomfort.
- Complications: Up to 17.4% of patients experience adverse effects such as seroma, hematoma, infection, wound dehiscence, and lymphedema (3–5).
- Resource-Intensive: SLNB demands multiple hospital visits and coordination across a multidisciplinary team, placing a strain on both patients and healthcare resources (6).
- Psychosocial Impact: Beyond physical side effects, SLNB might potentially contribute to long-term psychological distress, particularly due to the anxiety associated with waiting for biopsy results.

The Merlin Assay (“Merlin”) is a non-invasive in vitro diagnostic test (CE-IVD marked) that allows for a personalized risk assessment based on clinicopathological features and gene expression profiling (CP-GEP) of 8 genes. By assessing the tumor's aggressiveness and metastatic propensity, Merlin predicts a newly diagnosed melanoma patient’s risk of nodal metastasis to guide SLNB referral decision-making. Merlin uses primary biopsy material; therefore, no additional interventions are needed to perform the test and reports a binary result: either Low-Risk or High-Risk for nodal metastases. The CP-GEP algorithm was first published in 2020 and has been extensively validated in both analytical and clinical context, in Merlin’s intended use population: SLNB eligible patients. Tumors are pathologically staged according to their thickness, with the assumption that thicker tumors are more likely to metastasize to SLNs. Although Merlin's performance has been studied across all primary tumor classifications (pT1-pT4), the test has the most clinical impact in the pT1-pT2 group; therefore, the dossier focuses on this group of pT1-pT2 melanomas, accounting for roughly 68% of the newly diagnosed patients undergoing SLNB in 2023 (7).

The main value of Merlin in Danish clinical practice is considered:

- Reducing SLNB surgeries for primary cutaneous melanoma patients who are SLNB-eligible
- Forgoing SLNB surgery can additionally reduce associated SLNB-related complications and resources
- Providing extra guidance in melanoma patient groups of specific interest, such as the elderly, head and neck patients, and patients for whom the SLNB is contraindicated
- Giving long-term survival outcome insights
- Providing prognostic information in patients who are contraindicated or cannot be operated on

2.1.1 Nodal metastasis prediction

This dossier focuses on the research question: “Should Merlin be used to triage patients with primary cutaneous malignant melanoma (T1-T2) to determine whether patients should be further investigated with

SLNB?". The clinically acceptable baseline risk of nodal metastasis before recommending SLNB surgery varies by practice. In Denmark, this baseline is set at 10% (8–10). Therefore, to be clinically relevant, Merlin must identify as many patients as possible who can safely forgo SLNB surgery, while maintaining a negative predictive value (NPV) of approximately 90% to align with the 10% acceptable baseline risk. Studies generally report a high NPV (11–18), see **Table 1**, underscoring Merlin's strong potential to exclude patients below the threshold commonly used in clinical practice for SLNB referral. This was further supported by a meta-analysis conducted by Wong et al., which reported NPVs of 94% and 96% for pooled analysis of pT1 and pT2 patients, respectively (19).

The large body of clinical evidence that is available shows the consistent performance of Merlin across different countries, continents, and healthcare settings in risk-stratifying patients who can forgo SLNB due to their low risk of nodal metastasis:

- The Merlin test reliably stratifies patients with pT1–pT2 melanoma by their risk of nodal metastasis to inform SLNB decision-making (11–18)
- Merlin has a consistently high NPV across multiple international studies (11–18), exceeding the 90% risk threshold used in Danish guidelines (8–10)
- Merlin can safely identify a large proportion of patients who may forgo SLNB without compromising clinical outcomes (11–18)
- Pooled data from independent cohorts in a meta-analysis confirm Merlin's robust predictive performance across diverse populations and healthcare systems (19)
- Forgoing SLNB would lead to fewer adverse events associated with SLNB (4)

Table 1. Overview of main study outcomes for Merlin in pT1-pT2 primary cutaneous melanoma patients

Reference: author (year) country	Cohorts & substages	Sample size	SLNB RR, % [95% CI]	Negative predictive value, % [95% CI]
Bellomo (2020)	T1-T2	549	56.1	97.1
(11)	pT1b	184	80 [74-86]	98 (95-100)
United States	pT2a	290	48 [42-54]	95 (91-98)
	pT2b	66	24 [14-34]	96 (91-100)
Hieken (2025)*	pT1a	23	82.6 [61.2-95]	100 [82.4-100]
(12)	pT1b	448	68.5 [64-72.8]	95.4 [92.5-97.5]
United States	pT2a	683	36.2 [32.6-39.9]	92.3 [88.2-95.3]
	pT2b	163	17.2 [11.7-23.9]	82.1 [63.1-93.9]
Stassen (2023)	T1-T2	162	53	96.0
(13)	pT1	62	83.9 [72.3-92.0]	94.2 [84.1-98.8]
Netherlands	pT2	100	33.0 [23.9-43.1]	100 [89.4-100]

Mulder (2020)	pT1	11	90.9	100 [72.2-100]
(14)	pT2	94	29.8	89.3 [72.8-96.3]
Netherlands				
Johansson (2021)	T1-T2	240	35.4 [29.4-41.8]	96.5 [90.0-90.3]
(15)	pT1	30	53.3 [34.3-71.7]	93.8 [69.8-99.8]
Sweden	pT2	210	32.9 [26.5-39.7]	97.1 [89.9-99.6]
Yousaf (2021)	T1-T2	153	41.8 [33.9-50.1]	93.8 [84.8-98.3]
(16)	pT1	74	60.8 [48.8-72.0]	93.3 [81.7-98.6]
United States	pT2	79	24.1 [15.1-35.0]	94.7 [74.0-99.9]
Weitemeyer	pT1	176	72.7%	94.5 [89.1-97.8]
(2025) (17)	pT2	251	35.5%	87.6 [79.0-93.7]
Denmark				
Pazhava (2025)	T1-T2	167	56.3 [48.4-63.9	98.9 [94.2-100]
(18)	pT1	52	73.1 [59-84.4]	100 [90.7-100]
United States	pT2	115	48.7 [39.3-58.2]	98.2 [90.4-100]
Wong (2025) (19)	pT1	177	72 [55-85]	94 [89-97]
	pT2	483	31 [27-35]	96 [91-98]

*Hieken et al., 2025 updated data after WLE.

The Merlin test is optimized to reduce as many SLNBs as possible (reduction rate) while maintaining a high negative predictive value (NPV), minimizing the number of false negative (FN) tests. While studies do report sensitivity and specificity on a subgroup level, Merlin's performance metrics are generally focused on SLNB reduction rates in combination with NPVs to ensure that patients can be ruled out effectively and safely. Information on sensitivity and specificity is provided in **Table 38**.

2.1.2 Safety

Excluding patients from clinical interventions is a decision that should not be made lightly. If a patient is classified as low risk for nodal metastases by Merlin but does, in fact, have metastatic spread (false negative), they may receive systemic therapy at a later stage, potentially reducing their survival rate. Ensuring a high NPV minimizes the number of false negatives, thereby enhancing patient safety. Moreover, there is additional evidence supporting the safety of Merlin beyond its high NPV:

- All patients included in studies are followed for a minimum period of 5 years to assess their long-term survival. Generally, the longitudinal data shows that Merlin Low Risk patients have a good prognosis and better long-term survival compared to High-Risk patients (18,20–24). See **Figure 1** below.
- Conduct further investigations to determine the underlying causes of false negatives. Weitemeyer et al. (2025) reported that most false-negative tests in the Danish cohort would not have had a clinical impact, as they primarily involved clinical stage IIIA with low SLN tumor burden, ineligible for adjuvant therapy (17)
- Include pure prognostic cohorts where the Merlin test is used to assess the patient's recurrence-free survival (RFS), distant-metastasis-free survival (DMFS), melanoma-specific survival (MSS), and overall survival (OS) in patients with negative SLNBs (21–23) and patients who did not undergo SLNB (24). These studies show good and better long-term survival for patients receiving a Merlin Low Risk label, compared to the High Risk label, beyond SLNB status.

Considering the clinically acceptable baseline risk for nodal metastasis of 10% (8–10) before recommending SLNB and considering Merlin's high NPV of 90% and above, combined with robust favorable long-term survival data for Merlin Low-Risk patients, Merlin test can be used to deselect patients for SLNB surgery reliably and safely.

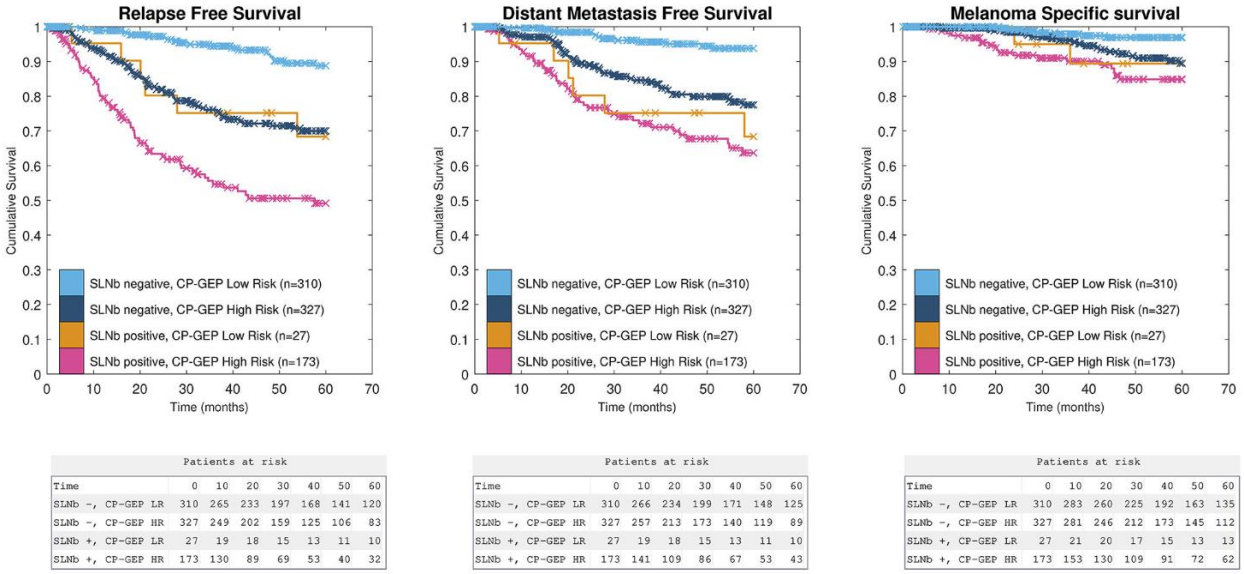


Figure 1. Kaplan-Meier curves for relapse-free survival, distant metastasis-free survival, and melanoma-specific survival stratified by SLNB status and Merlin (CP-GEP) classification (n=837) (20)**.**

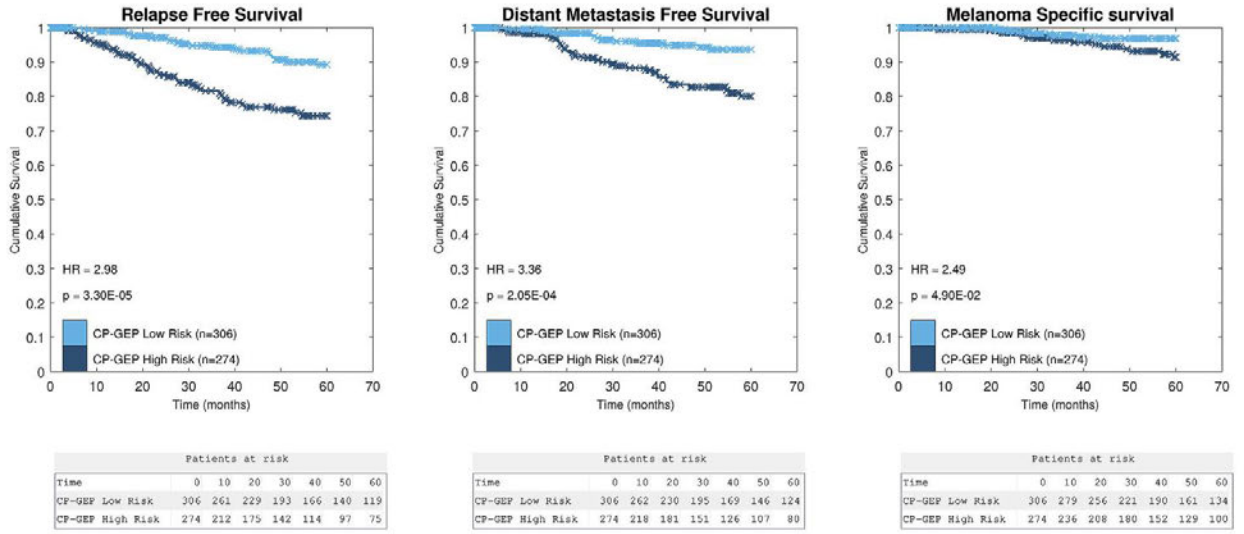


Figure 2. Kaplan-Meier curves for relapse-free survival, distant metastasis-free survival, and melanoma-specific survival stratified by Merlin (CP-GEP) classification in SLNB negative patients (n=580) in a US cohort (20)**.**

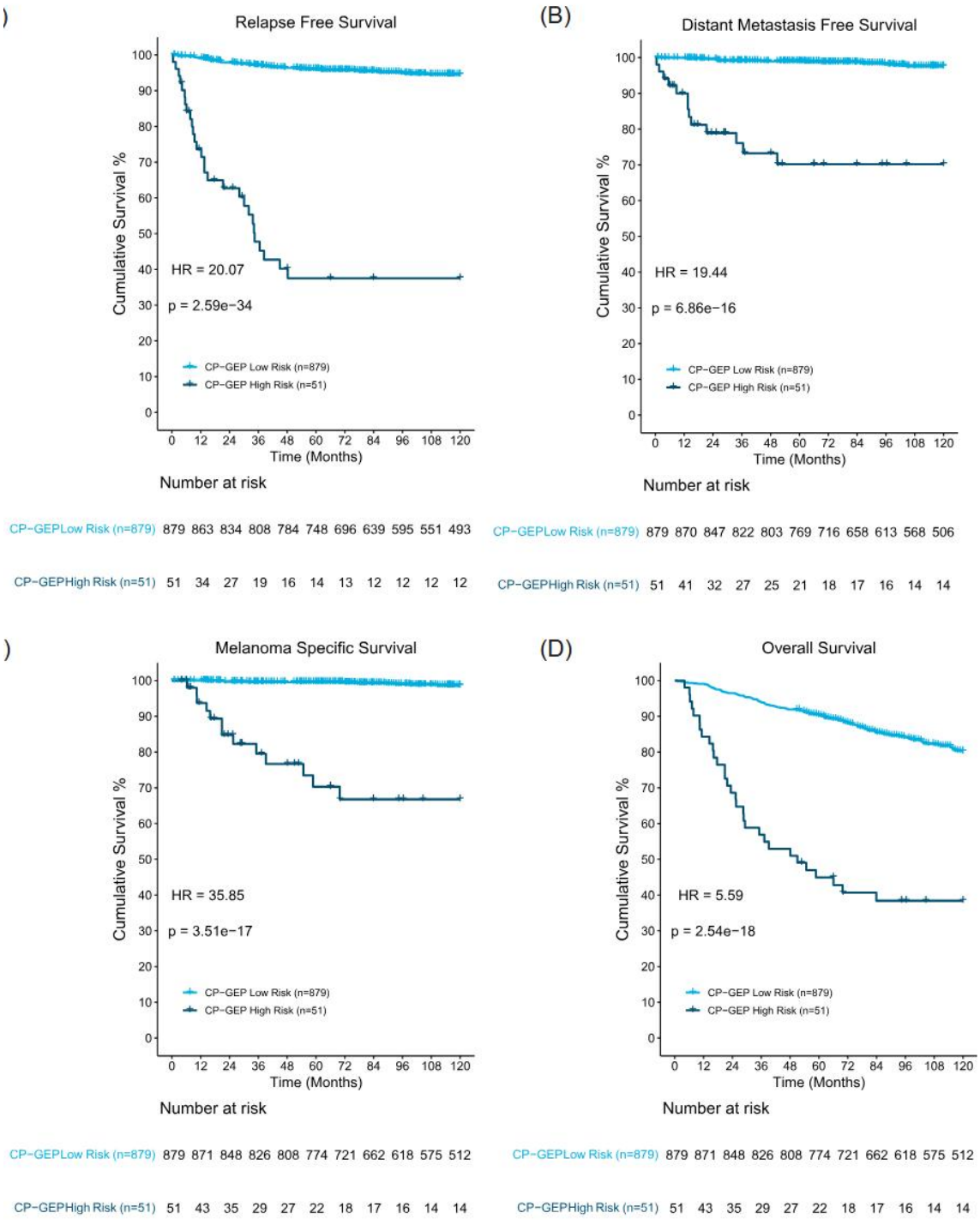


Figure 3. Kaplan-Meier curves for relapse-free survival (A), distant metastasis-free survival (B), melanoma-specific survival (C), and overall survival (D) stratified by Merlin (CP-GEP) classification in patients who did not undergo SLNB (n=930) in a German cohort (24)**.**

A patient is a false positive (FP) when Merlin indicates the patient as high-risk for nodal metastases, whereas in fact, there are no nodal metastases that can be detected in the nodes. Due to Merlin's outcome, this patient is unnecessarily referred to surgery. Although the preferred scenario would be a Merlin Low Risk

result, referral for SLNB is currently also the standard of care; therefore, the patient does not run into additional (safety) risks with Merlin.

2.1.3 Patient perspective

From a patient perspective, forgoing SLNB can lead to fewer incurred resources from the healthcare system. (6), eventually resulting in fewer visits to the hospital and fewer traveling, but also fewer adverse events associated with this procedure (4). Additionally, patients won't be confronted with adverse effects caused by this procedure (3,4). Adverse effects consist of seroma, infection, hematoma, dehiscence, and lymphedema. Especially the latter can persist and cause long-term issues, decreasing the quality of life of patients (25,26). Introducing Merlin would lead to:

- Reducing SLNB procedures and decreasing the risk of adverse effects from this procedure such as infection, seroma, and long-term lymphedema (4).
- Leads to fewer incurred resources from the healthcare system, eventually resulting in fewer visits to the hospital and fewer traveling (6).

2.1.4 Organizational implications

From an organizational perspective, the implementation of this intervention could lead to improvements in clinical workflows and resource utilization. By reducing the number of unnecessary biopsies and complications related to SLNB, healthcare institutions may experience a reduction in hospital admissions, postoperative care, and overall treatment costs. Moreover, the intervention could streamline patient pathways, making melanoma management more efficient and reducing waiting times for critical diagnostic results. A logistical process has been established to facilitate the shipment of samples to the laboratory in Rotterdam, following the specified instructions. This process includes the completion and submission of the required test requisition form and is designed to ensure full compliance with General Data Protection Regulation (GDPR). This logistical framework has been successfully piloted using retrospective samples from Herlev and Gentofte Hospital, confirming the feasibility of this approach. Looking ahead, and pending access to the necessary equipment and infrastructure, the establishment of a centralized testing hub within Denmark may be possible, further enhancing operational efficiency and turnaround times for test results.

2.1.5 Health economics

In terms of health economics, the data indicates that the innovation is dominant in almost all scenarios, with a Merlin testing strategy emerging as the preferred approach. A Merlin testing strategy leads to cost savings and provides a modest health benefit. Sensitivity analysis confirm the robustness of the base-case results. For the budget impact analysis, despite a relatively low increase in diagnostic cost per patient, implementing a Merlin testing strategy is cost-efficient over time, as less budget impact is seen in terms of therapy cost, complication cost, and out-of-pocket costs. In addition, the budget impact model explores resources that are freed up for healthcare practitioners to reinvest in patients who require their support on other fronts. Overall, the cost-effectiveness model and budget impact model show that a Merlin testing strategy enables a more sustainable and effective allocation of healthcare resources. These findings support the potential for wider implementation of the Merlin test within the Danish healthcare setting.

2.1.6 Overall conclusion

The Merlin test under consideration represents a significant step forward in the management of melanoma, addressing both clinical and economic needs by offering a less invasive and less costly alternative to SLNB. Merlin is an effective triaging tool for guiding SLNB decisions in patients with T1–T2 melanoma. It helps avoid unnecessary surgeries without compromising diagnostic accuracy or patient safety, and supports more personalized, resource-efficient, and cost-effective care.

3. Introduction

3.1 Patient/target population

Malignant cutaneous melanoma is a serious form of skin cancer that begins in cells known as melanocytes (pigment-producing cells, which give skin its color). Ultraviolet rays (UV) are the major cause of melanoma and come from sunlight or tanning lamps, and sunbeds. UV rays can damage DNA, which in turn may result in uncontrolled cellular growth. While melanoma accounts for approximately 1 out of 5 annual skin cancer diagnoses, and is the most lethal form of all skin cancers (27).

Melanoma is typically diagnosed by taking a biopsy of the suspicious skin area (lesion). This lesion is analyzed for malignancy by a pathologist who can confirm whether it is melanoma. Multiple pathology characteristics of the primary tumor are reported, of which the thickness of the tumor, Breslow thickness (BT) carries the most weight, as staging follows the widely adopted TNM system: (tumor) Thickness, Nodal involvement, Metastasis (beyond lymph nodes). The thicker the tumor, the higher the risk of having nodal metastasis. Nodal involvement can be assessed by means of an SLNB. Nodal metastasis is an indicator to refer patients for systemic adjuvant immunotherapy.

A simple representation of the melanoma patient journey in Denmark is summarized in **Figure 4**. Current Danish clinical guidelines recommend referring the patient for SLNB when their risk of nodal metastasis is ~10% or higher (8–10). If patients are SLNB-eligible, an appointment is scheduled to perform the SLNB procedure. The SLNB procedure is normally carried out in a 1–2-day procedure in which the SLN is detected by performing pre-operative lymphoscintigraphy by injecting blue V dye to determine and localize the SLNs. Specialists can identify the SLN in 95% of the cases. Then, either immediately after or on the second day, the patient is brought under general anesthesia and the SLN is removed to pathologically examine whether any tumor cells have spread to the sentinel node. During the SLNB procedure, a wide local excision (WLE) is performed to surgically remove any possible remaining abnormal or cancerous tissue, along with a surrounding margin of healthy tissue. If tumor cells are present in the SLN, patients can be classified under stage III (or IV in case of metastasis beyond SLNs to distant parts of the body) and would be eligible for systemic adjuvant immunotherapy (8). Approved adjuvant therapies in Denmark are pembrolizumab monotherapy and nivolumab monotherapy.

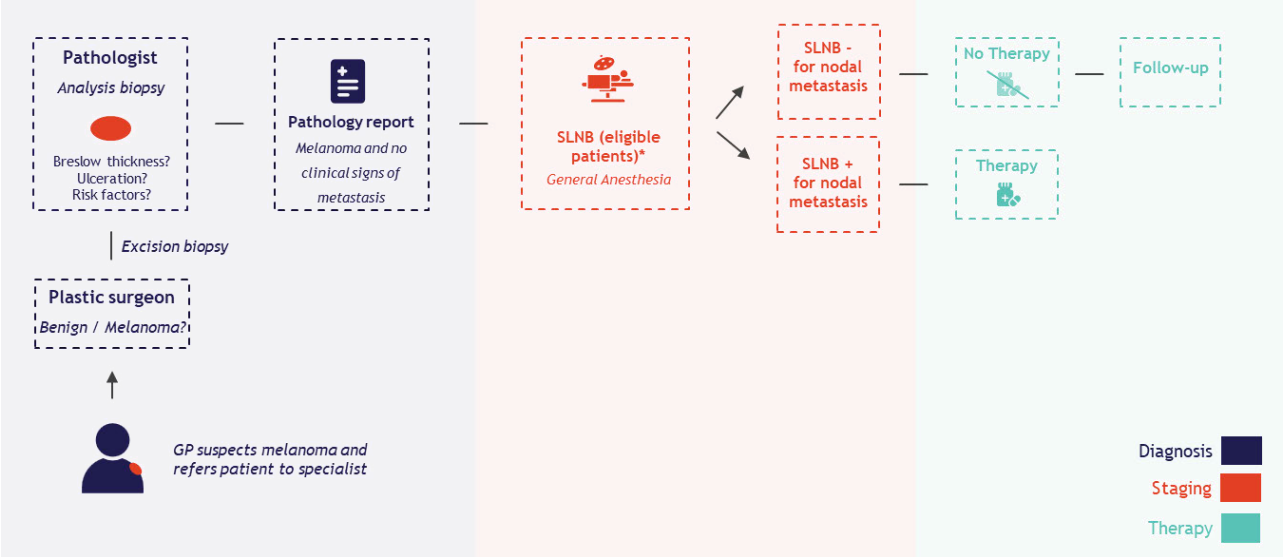


Figure 4. Patient pathway from diagnosis to treatment

In 2023, a total of 2,700 Danish patients were diagnosed with T1-T3 primary cutaneous melanoma, of which 1,043 were deemed SLNB-eligible. **Table 2** below shows the distribution of patients per pathological subgroup according to their tumor thickness (7). In the 2023 Danish Melanoma Database is described that approximately 50% of patients that underwent SLNB were between 61-80 years old, 20% was below 50 years old, and another 20% was between 51-60 years old. In terms of gender, no clear indication is provided on the distribution in the patients that underwent SLNB, however in the total group of melanoma patients (2980) exactly 50% were men and 50% were women (7).

Table 2. Incidence of Danish melanoma patients per pathological stage T1-T3, SLNB eligibility, and percentage of detected positive nodes in 2023 from the Danish Melanoma Database

Pathological stage	Patients with invasive melanoma	Patients with SLNB	Patients with a positive node
T1a	1,374	116 (8.4%)	# (#)
T1b	462	171 (37.0%)	9 (5.3%)
T2a	511	465 (91.0%)	46 (9.9%)
T2b	52	41 (78.8%)	9 (22.0%)
T3a	183	159 (86.9%)	38 (23.9%)
T3b	118	91 (77.1%)	26 (28.6%)
Total	2,700	1043 (38.6%)	128 (12.3%)

Adapted from Dansk Melanom Database·National årsrapport 2023 Regionernes Kliniske Kvalitetsudviklingsprogram · www.rkkp.dk. Data for T4 patients is available in the report but not shown here. (7)

Table 2 shows that eventually 39% of patients were referred to SLNB surgery. Fewer surgeries are performed in thinner melanomas and more in thicker melanomas (T1 versus T2-T3). In most SLNs, no metastasis was found; SLNB has no discernible therapeutic effect if the node is negative. SLNB under general anesthesia can be costly (28), complex, time-consuming endeavor (6) and is an invasive procedure that carries a 10-17% risk of potentially lifelong complications (4).

3.2 Comparator

Clinical staging of primary cutaneous melanoma patients determines the patient's care path. Assessing metastatic spread to the SLN is a crucial step, as large population studies show that patient survival decreases with nodal involvement. In the figures below, this is reflected in melanoma-specific survival curves that are less favorable when patients are upstaged from clinical stage I and II to III, in which nodal metastasis is detected (29), see **Figure 5** and **Figure 6**.

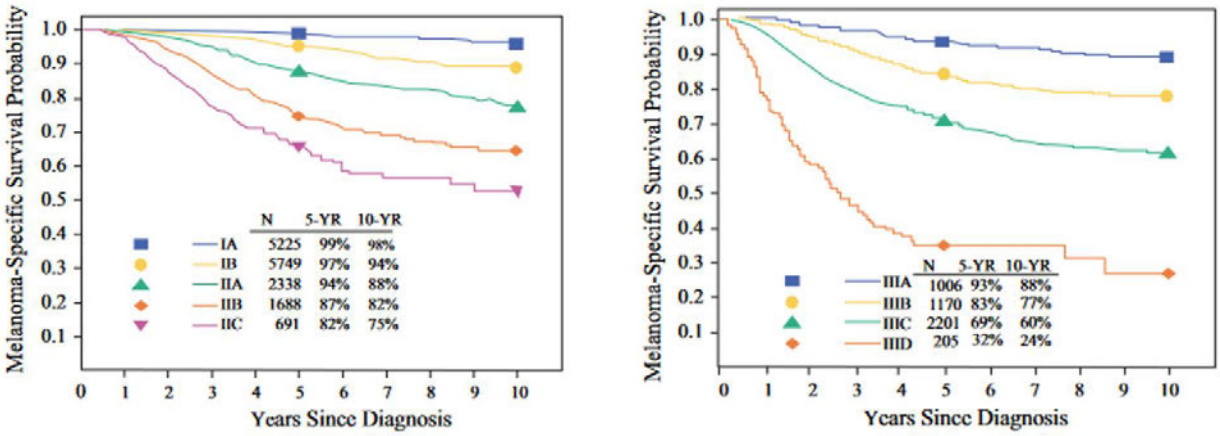


Figure 5. Adapted from Gershenwald et al., 8th edition melanoma melanoma-specific survival in stages I and II stage groups (left) and stage III stage groups (right) (29)

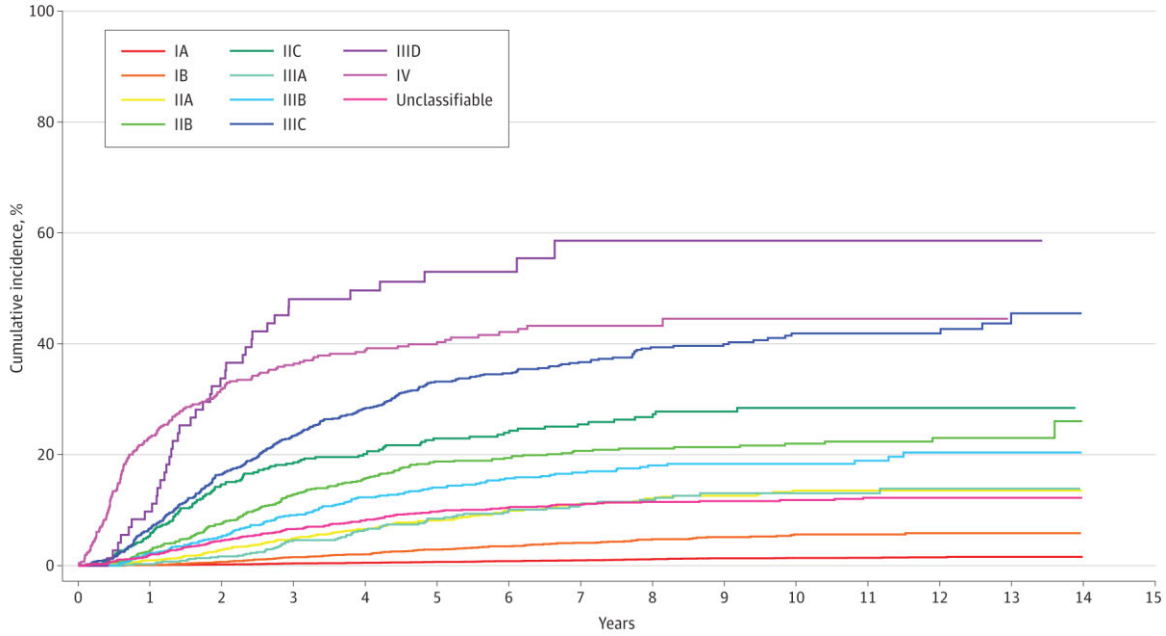


Figure 6. Adapted from Helvind et al., 8th edition melanoma cumulative incidence of death from melanoma in stage I-III Danish patients. (30)

Current Danish clinical guidelines recommend referring the patient for SLNB when their risk of nodal metastasis is ~10% or higher (8–10). **Table 2** shows that the referral pattern translates to almost 40% of newly diagnosed T1-T3 melanoma patients receiving SLNB surgery. In this group, 12.3% have nodal involvement and are eligible for adjuvant immunotherapy. For the other 87.7%, this invasive surgical intervention holds no discernible therapeutic effect. The results are even more striking in the T1b and T2a subgroups. While T1b patients are less often referred (37% of all newly diagnosed), there was no lymph node metastasis detected in 94.7% of all biopsies. **Table 2** also shows that almost all newly diagnosed T2a patients (91%) received SLNB surgery, whilst 90.1% of the SLNB cases were negative. Patients undergoing SLNB surgery are exposed to surgery-related complications in 10-17% of the cases, regardless of the SLNB outcome. These complications include seroma, lymphedema, infection/cellulitis, hematoma, and dehiscence, and might require post-

procedural emergency room visits and readmission. Especially, lymphedema may persist for an extended period.

In addition, **Table 2** underlines that although patients might be eligible for the procedure (T2), not every patient is referred to SLNB (9.0% for T2a and 21.2% for T2b). Considerations why these T2 patients did not undergo could include complex locations such as the iliac or head and neck region, (significant) comorbidities, frailty, or patients rejecting surgery. Currently, there is no alternative for patients who have a high risk for nodal involvement and should be referred for SLNB (according to clinical guidelines), but have not undertaken one, due to aforementioned reasons.

Despite its widespread use, SLNB presents several clinical challenges and unmet needs. SLNB surgery has been the standard tool to assess nodal involvement with the goal to accurately stage the patient. However, one could argue that this method does not capture all information, as many biopsies are negative, but these patients still relapse. Actually, a large group of SLNB negative patients relapse or die of melanoma (31). In addition, it is an invasive procedure often under general anesthesia, with the risk of scarring and discomfort. The procedure can lead to potential complications, including seroma, hematoma, infection, wound issues, and lymphedema. It has a high resource demand as it involves multiple hospital visits and coordination with a multidisciplinary team, with a low diagnostic yield, as 80–85% of biopsies are negative, offering no therapeutic benefit in these patients. Furthermore, it may cause psychological Burden as it may cause anxiety and emotional stress while awaiting results.

Hence, there is a need for non-invasive, personalized risk assessment, beyond the tumor thickness, that includes the tumor's metastatic potential for a more accurate risk of metastasis to guide SLNB referral decision-making.

3.3 Intervention

The Merlin Assay ("Merlin") is a non-invasive in vitro diagnostic test (CE-IVD marked; risk class C) that allows for a personalized risk assessment. By assessing the tumor's aggressiveness and metastatic propensity, Merlin predicts a newly diagnosed melanoma patient's risk of nodal metastasis to guide SLNB referral decision-making. It provides a binary outcome: the patient is at Low-Risk for nodal metastasis and could consider forgoing surgery, or the patient is at High Risk for nodal metastasis and should be referred for surgery. (11).

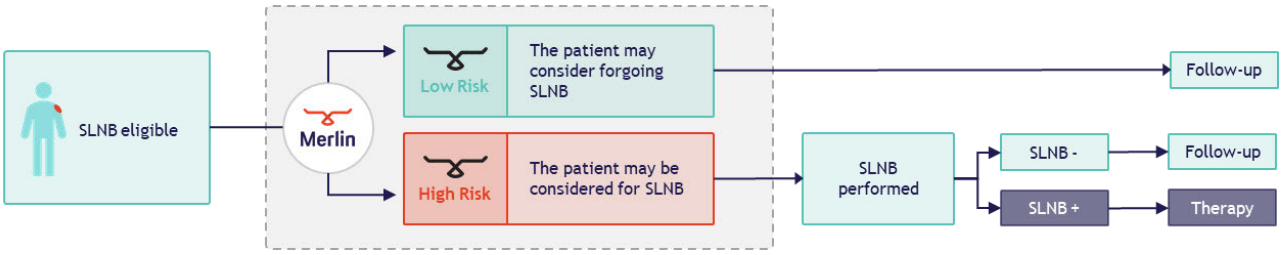


Figure 7. Patient flow after introducing Merlin

The test was co-developed by SkylineDx B.V., Netherlands, and the Mayo Clinic in the United States and utilizes the CP-GEP algorithm. CP-GEP is an integration of the clinical variables *age at diagnosis*, and pathological variable *Breslow Thickness,* and the gene expression data from 8 genes related to melanoma-specific biological pathways: *MLANA, GDF15, PLAT, ITGB3, LOXL4, SERPINE2, TGFBR1*, and *CXCL8* and two housekeeping genes. Merlin is performed on primary excision tumor biopsy material and therefore does not require any additional interventional steps.

The clinicopathologic information is routinely available. The GEP component requires formalin-fixed paraffin-embedded (FFPE) tissue from the primary tumor obtained via a shave or punch biopsy or full excision during diagnosis; no microdissection is needed. Five sections of 10 microns (50 µm in total) are used for RNA extraction and subsequently cDNA synthesis (reverse transcription). Quantitative polymerase chain reaction (qPCR) obtains expression levels of the 8 target genes. Proprietary software normalizes the gene expression levels against two housekeeping genes and integrates clinicopathologic information to calculate the patient's risk for nodal metastasis. A clinical report with the patient's outcome label and guidance on interpretation is provided to the requesting physician, showing a Merlin Low Risk or Merlin High Risk test result.

As Merlin's clinical applicability is guiding physicians in referral to SLNB, the test's intended use population is SLNB-eligible patients:

- Newly diagnosed invasive malignant melanoma of the skin (based on the AJCC 8th edition staging guidelines).
- Without clinical evidence of having SLN metastases or distant metastasis (staging code cN0M0).
- T1b-T3 melanomas (Breslow thickness ≥0.8mm to 4mm or <0.8mm with ulceration).
- T1a (Breslow thickness < 0.8 mm) and one or more of the following risk features:
    - Mitotic rate ≥ 2/mm2.
    - Patient age at time of primary biopsy: <40 years old.
    - Presence of lymphovascular invasion.

The main utility of the Merlin test lies in:

- Reducing SLNB surgeries for primary cutaneous melanoma patients who are SLNB-eligible.
- Forgoing SLNB surgery can additionally reduce associated SLNB-related complications.
- Providing extra guidance in melanoma patient groups of specific interest, such as the elderly, head and neck patients, and patients for whom the SLNB is contraindicated.
- Give insights into the long-term survival outcome of Low Risk and High Risk patients.
- Providing prognostic information in patients who are contraindicated or cannot be operated on.

Under the EU In Vitro Diagnostic Regulation (IVDR 2017/746), CE-IVD medical devices are classified into four risk classes: Class A, B, C, and D. Class A is the lowest risk, and Class D is the highest. This classification determines the level of regulatory scrutiny and conformity assessment required for CE marking. Merlin is a class C device.

4. Evidence base

The CP-GEP model in Merlin was first published in 2020 (11) and has been substantially validated in both analytical and clinical context. Analytical validation evidence is well documented in SkylineDx's Quality Management System (externally audited annually following the ISO13485 standard) and ensures the quality, reliability, and consistency of test results. Throughout several peer-reviewed manuscripts, SkylineDx has prepared and executed Merlin's clinical validation studies diligently to allow for consistent study designs and data reporting:

- In all studies, multiple sites participated, reflecting both national and regional populations. These sites included larger academic institutes and smaller community hospitals. Furthermore, all patients within the study period were included (patient samples from retrospective cohorts were prospectively collected) to reflect real real-world setting, which allowed for generalizability of the results.
- In all studies, the cohort characteristics are clearly detailed.
- Between validation studies, the study design and used performance metrics are comparable, whereas study results from national and regional populations globally show similar results. Furthermore, studies analyzing prognostic data have at least 5 years of follow-up to study long-term outcomes.
- The use of older retrospective cohorts might create a slight differentiation from what is seen in current clinical practice. Therefore, large prospective trials have been initiated to confirm Merlin's performance seen in the retrospective cohorts.
- In 2023, a peer-reviewed manuscript was published describing the prospective results from a Dutch multicenter trial, confirming Merlin's retrospective performance (13). SkylineDx also concluded a large multicenter prospective study in the United States (MERLIN_001) that included 1,761 patients. (32).
- All studies are independently executed by independent investigators to avoid conflict of interest as much as possible. Study sites have full control over the data and perform the related analysis independently as well.
- All studies adhere, at the minimum, to Category C Level of evidence II, according to Simon et al 2009. Both prospective validation studies adhere to the highest level of evidence (Category A, Level of Evidence I) and are satisfactory for the determination of medical utility (33).

All studies investigating Merlin's ability to predict SLNB metastasis are observational studies. An interventional study will not add any significance as the SLNB procedure itself is not therapeutic. A randomized controlled trial (RCT) would not be ethically defensible (Scientific ad-board, minutes & testimonials per request). The Merlin test Low Risk patients have a good long-term outcome for recurrence-free survival (RFS), distant-metastasis-free survival (DMFS), and melanoma-specific survival (MSS), see section 5.2.2.2. If observational evidence shows that low-risk patients could forgo SLNB and have a good MSS, it would not be defensible to perform surgery in an interventional setting to prove the SLNB outcome. Additionally, it would not be ethically defensible to withhold SLNB surgery in Merlin test High Risk patients as a positive node in these higher risk patients would make them eligible for systemic treatment.

In line with the Danish Health Technology Council's evaluation design, a systematic literature search was carried out to identify existing published literature as part of the four topics on which the DHTC bases its assessment:

- Clinical effectiveness and safety
- Patient perspective
- Organizational implications
- Health economics.

Additionally, a search was carried out with the aim of identifying reviews.

4.1 Selection of relevant studies

A systematic literature review was carried out using Covidence software with publications originating from the following databases: PubMed, Embase, Web of Science, and Scopus. Herein, two reviewers independently conducted screening during the title and abstract stage using the inclusion and exclusion criteria defined in **Table 3**. In case of discrepancies between reviewers' inclusion and exclusion of specific scientific literature, a discussion between reviewers took place to reach consensus.

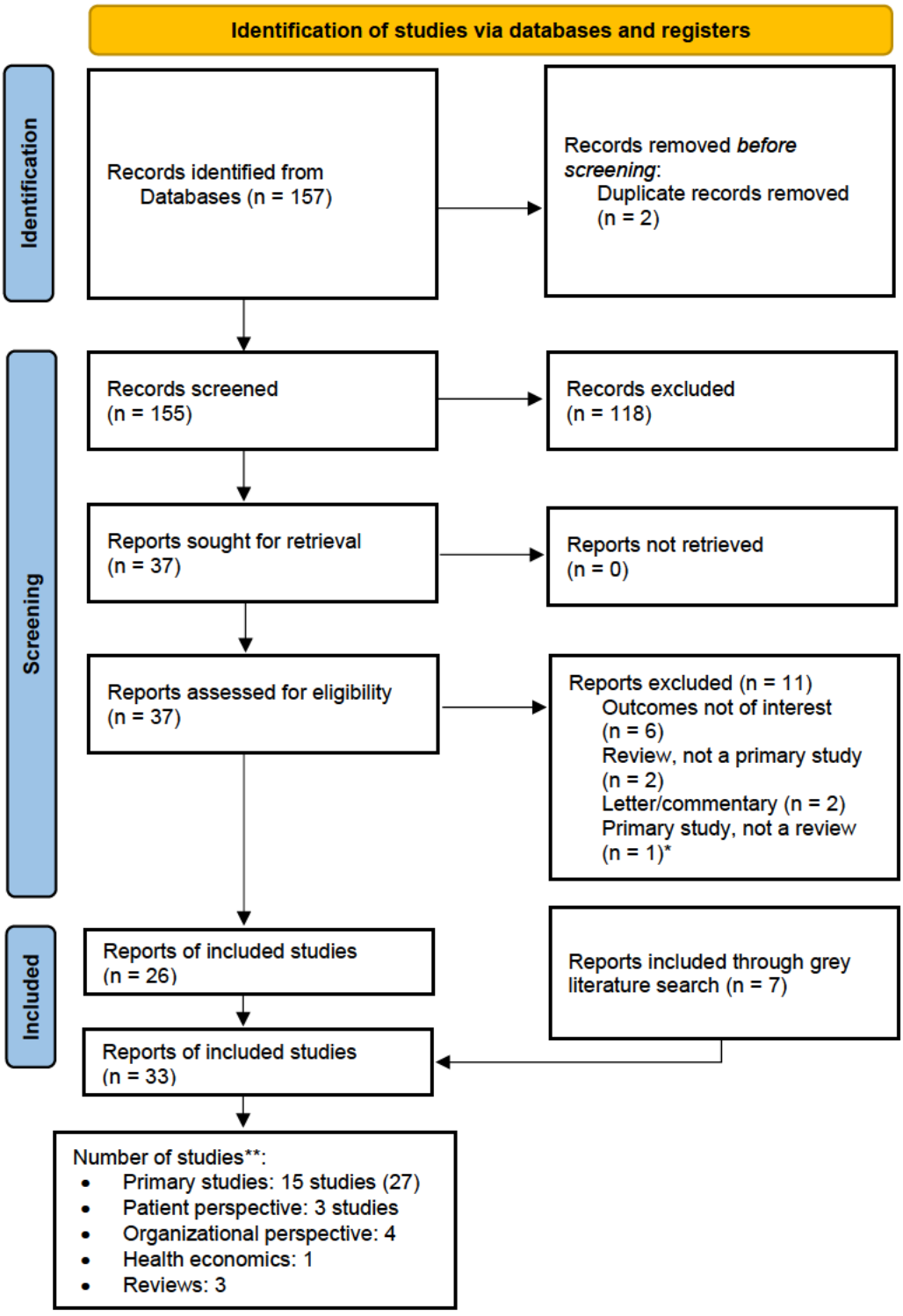
Table 3. Inclusion criteria: systematic literature search

	Inclusion criteria
Population	Patients over 18 years of age with newly diagnosed T1-T2 primary cutaneous malignant melanoma who are candidates for SLNB conform to current clinical guidelines. Subgroups divided by tumor thickness (T): • T1a < 0.8 mm and < 65 years • T1b • T2a • T2b
Intervention	Merlin Assay (CP-GEP)
Comparator	SLNB
Outcomes	Outcomes related to: • Clinical effectiveness and safety • Patient perspective • Organizational implications • Health economics

CP-GEP, clinicopathological-gene expression profiling; SLNB, sentinel lymph node biopsy.

A PRISMA flowchart of the systematic literature review (**Figure 8**), summarizing the identification of studies via systematic searches in the aforementioned databases. Searches were carried out on 5 June 2024 and complemented with desk research to retrieve the most recent publications (annotated with[§]). Overall, 155 references were screened for eligibility during title and abstract phase, of which 118 were excluded. Subsequently, 37 references were screened in the full-text selection. During full-text selection, 11 references were excluded, leading to a total of 26 eligible reports, pertaining to 14 different studies. Additionally, desk research was carried out to select the most recent publications, which led to another 7 publications found through desk research or that were published after the date of searching for a total of 33 reports (18 separate studies) shown in **Table 4**.

- 15 studies report clinical effectiveness and safety data
- 3 studies report patient perspectives
- 3 studies report organizational implications
- 1 study reports health economics3 reviews



**Literature search aimed at searching for reviews in the 4 topics of interest, hence primary studies were not of interest: reports [studies]. **Literature search identified publications that pertain to multiple topics, as such the count is higher than 33 reports of included studies*

Figure 8. PRISMA flow chart of systematic literature review for reviews, clinical effectiveness and safety, patient perspective, organizational implications, and health economics. Please find an overview of the reasons for exclusion during full-text selection in the appendix section 11.1, Table 37

Table 4. List of publications on each evaluation topic

Studies	Clinical effectiveness and safety	Patient perspective	Organizational implications	Health economics
1a	Stassen, 2023 (13)	Hieken, 2022b (4)**	Hieken, 2022b (4)**	Thao 2023 (28)
1b	Stassen, 2023 (34)	Meves 2020a (6)**	Meves 2020a (6)**	
2a	Hieken, 2022a (35)	Stassen (2024)§ (36)	Stassen, 2023 (13)	
2b	Hieken, 2023 (32)			
2c	Hieken, 2024§* (12)			
3a	Bellomo, 2020 (11)			
3b	Meves, 2020 (37)			
4	Arias-Meijas (37)			
5a	Mulder, 2019 (38)			
5b	Mulder, 2021 (14)			
6a	Yousaf, 2021 (16)			
6b	Meves, 2020b (39)			
7	Johansson, 2022 (15)			
8a	Weitemeyer 2024§(40)			
8b	Weitemeyer 2025§ (17)			
9	Hieken, 2022b (4)			
10a	Pittelkow, 2024 (41)			
10b	Pazhava, 2025§ (18)			
11	Wong 2025§ (19)			
12a	Eggermont 2020 (20)			
12b	Eggermont, 2020 (42)			
12c	Wever 2020 (23)			
13a	Amaral, 2022 (43)			
13b	Amaral, 2023 (21)			
14a	Mulder, 2022 (22)			
14b	Mulder, 2023 (44)			
15	Amaral, 2025§ (24)			

*§Manuscripts and abstracts that have not been identified through the search but are relevant to the research questions of this dossier. ** Meves and Hieken, were not obtained in the systematic search for patient perspective but contained data that is relevant for that topic.*

In addition to Table 4, 3 separate reviews were identified:

- Sadurni 2023: Discusses the benefits of gene expression profiling in clinical practice (45)
- Quattrocchi 2023: Discusses intended use population and potential patient pathways for implementing CP-GEP in clinical practice (46)
- Kött 2024: Provides an overview of different available gene expression profiles and discusses their use in forgoing the SLNB procedure (47)

5. Clinical effectiveness and safety

Merlin's intended use population is SLNB eligible patients. Tumors are pathologically staged according to their respective thickness. The assumption is that thicker tumors are more likely to have metastasized. Therefore, in current clinical practice, T3 and T4 patients' risk of having nodal metastases is so significant that these patients are generally referred to SLNB. Merlin validation studies support these findings, as most T3-T4 patients receive a Merlin High Risk label. Although Merlin's performance has been studied across all primary tumor classifications (pT1-pT4), the test has the most clinical impact in the pT1-pT2 group; therefore, the dossier focuses on this group. pT1-pT2 melanomas account for roughly 68% of the newly diagnosed patients undergoing SLNB in 2023. (see **Table 2**).

As described previously, a total of 15 different studies have been identified reporting on the clinical effectiveness and safety of Merlin. Studies are subdivided into studies that focus on nodal metastasis prediction (see **Table 5**), and studies focusing on safety (see **Table 6**).

Table 5. Overview of clinical effectiveness and safety studies: Nodal metastasis prediction

STUDIES FOCUSING ON NODAL METASTASIS PREDICTION			
Reference (author, year, country)	**Identification no. / short description**	**Intervention**	**Comparator**
Stassen (2023) (13,34) **Netherlands**	Prospective clinical validation study	Merlin	SLNB
Hieken (2025) (12,32,35) **United States**	Prospective clinical trial (MERLIN-001, NCT04759781)	Merlin	SLNB
Bellomo (2020) (11,37) **United States**	Signature discovery paper: prospectively collected data from archived patient samples.	Merlin	SLNB
Mulder (2021) (14,38) **Netherlands**	Clinical validation study; prospectively collected data from archived patient samples and medical records.	Merlin	SLNB
Yousaf (2021) (16,39) **United States**	Clinical validation study; prospectively collected data from archived patient samples and medical records.	Merlin	SLNB
Johansson (2021) (15) **Sweden**	Clinical validation study; prospectively collected data from archived patient samples.	Merlin	SLNB
Weitemeyer (2025) (17,40) **Denmark**	Clinical validation study; prospectively collected data from archived patient samples.	Merlin	SLNB
Hieken (2022) (4) **United States**	Complication study; prospectively collected data from archived patient samples and medical records.	Merlin	SLNB
Pazhava (2025) (18,41) **United States**	Clinical validation study in Head and Neck patients; prospectively collected data from archived patient samples.	Merlin	SLNB
Wong (2025) (19) **Netherlands, Sweden, United States**	Meta-analysis of 4 main cohorts	Merlin	SLNB

MERLIN_001, MElanoma Research Lymph Node Prediction Implementation National_001

Table 6. Overview of clinical effectiveness and safety studies: safety

STUDIES FOCUSING ON SAFETY

Reference (author, year), country)	Identification no. / short description	Intervention	Comparator
Eggermont (2020) (20,23,42) **United States**	Prognostic utility study & Sub analysis of IIA patients; Prospectively collected data from archived patient samples and medical records	Merlin	SLNB
Amaral (2023) (21,43) **Germany**	Prognostic utility study in SLNB negative patients	Merlin	
Mulder (2022) (22,44) **Netherlands**	Prognostic utility study in SLNB negative patients, prospectively collected data from archived patient samples and medical records	Merlin	
Pazhava (2025) (18,41) **United States**	Prognostic utility study in Head and Neck patients, prospectively collected data from archived patient samples and medical records	Merlin	
Amaral (2025) (24) **Germany**	Prognostic utility study in patients not undergoing SLNB, prospectively collected data from archived patient samples and medical records	Merlin	

5.1 Study and population characteristics.

Merlin's performance was assessed in a clinical setting, whereas multiple sites participated, reflecting both national and regional populations. These sites include larger academic institutes and smaller community hospitals. Furthermore, all patients within a certain period are included (patient samples from retrospective cohorts are prospectively collected) to reflect real-world setting and allow for generalizability of the results. In addition, the cohort characteristics are well described in each publication. Detailed study and population characteristics for these studies can be found in the appendix (section 11.2), whereas a short summary of the study design per study can be found in the following sections.

5.2 Should Merlin be used to triage patients with primary cutaneous malignant melanoma (T1-T2) to determine whether patients should be further investigated with SLNB?

Initially the DHTC requested data on pT1-pT3. However, in current clinical practice, pT3 and pT4 patients' risk of having nodal metastases is so significant that these patients are generally referred to for SLNB. Although Merlin's performance has been studied across all primary tumor classifications (pT1-pT4), the test has the most clinical impact in the pT1-pT2 group; therefore, the dossier focuses on this group of pT1-pT2 melanomas accounting for roughly 68% of the newly diagnosed patients undergoing SLNB in 2023 (7).

5.2.1 Studies used

In total, 17 studies were identified. These studies were conducted in collaboration with several renowned hospitals, covering two continents, and 5 countries (United States, Germany, Denmark, Sweden, Netherlands). Over 4,700 unique samples have been processed and over 6,400 including the MERLIN_001 (NCT04759781) prospective trial for which initial results were presented during the Society for Melanoma Research (SMR) conference in October 2024 by Vernon Sondak, at the society of surgical oncology (SSO) annual meeting in March 2025 by Tina Hieken and combined European Association of Dermato-Oncology (EADO) and World Congress of Melanoma (WCM) in April 2025 by Mike Egger, meanwhile the data has been published online as of October 22nd 2025 (12). To ensure scientific rigor and robustness in the conducted studies, a representative and broad cohort of primary cutaneous melanoma patients was considered,

including patients with and without ulceration, different Breslow thicknesses (pT1a-pT4), and melanoma on different parts of the body (e.g., head and neck, trunk, extremities and acral). To be eligible for inclusion, all patients either underwent (retrospective) or were scheduled for SLNB (prospective), allowing a direct comparison between the Merlin and SLNB status (metastasis yes/no). The signature described in the discovery paper was clinically validated in multiple retrospective and prospective studies. Additionally, to allow for independent reviews of the test performance, the samples were processed anonymously at SkylineDx's laboratory. The results were shared in a blinded manner with the clinical collaborators enabling independent data analysis and objective validation of performance. For an overview of study design and characteristics, see:

- Merlin study design & characteristics: Nodal metastasis prediction **Table 7**
- Merlin study design & characteristics safety **Table 8**

The main purpose of the evidence base is to understand how accurate Merlin can identify and predict which patients may forgo SLNB. As described in Bellomo, the CP-GEP model is optimized to reduce a clinically meaningful number of SLNBs at a clinically acceptable level of FN results (11). Therefore, all studies report at a minimum the SLNB Reduction Rate (RR) and the NPV. Additionally, to understand the prognosis of patients that forgo SLNB and whether it is safe to forgo SLNB in the long-term, survival data was gathered for a minimum of 5 years. For an overview of the outcome data on nodal metastasis prediction and long-term safety, see:

- Nodal metastasis prediction Section 5.2.2.1
- Safety Section 5.2.2.2

Multiple cohorts from different countries and study sites were investigated, reflecting different care pathways and referral patterns per geography or even institutions. For a detailed overview per study, see **Table 7** and **Table 8**. A more detailed description per study can be found in the appendix in section 11.2.

Table 7. Merlin study design & characteristics Nodal metastasis prediction

STUDIES FOCUSING ON NODAL METASTASIS PREDICTION

Reference	Patient population	Age	SLNB positivity rate	Biopsy location	Breslow Thickness	Ulceration	Outcomes of interest	Study sites
Stassen (2023) (13,34) **Netherlands**	Patients with pT1b-T4 primary CM (n=260)	Median: 63 (IQR: 50–71)	35/222	Head and neck: 60 Trunk: 89 Arm: 51 Leg: 56 Missing: 4	Median: 1.4 mm (IQR: 1.0–2.2)	38 (14.6%)	- Sensitivity - Specificity - NPV - SLNB RR - Turnaround time	Erasmus medical center, ISALA hospital, Leiden University Medical Center, Maastricht University Medical Center
Hieken (2025) (12,32,35) **United States**	Patients with T1-T3 primary CM (n=1,761)	Median 64	17.6% (297/1,686)	Head and neck: 273 Trunk: 542 Extremities: 871	Median 1.3 mm	370 (21.9%)	- Sensitivity - Specificity - NPV - SLNB RR	9 well renown university hospitals in the US
Bellomo (2020) (11,37) **United States**	Patients with T1b-T3b primary cutaneous melanoma (CM) (n=754)	Node- 61.0 (SD: 15.87) Node+ 53.66 (SD: 17.11)	16.9% (128/754)	Head and neck: 148 Trunk: 251 Upper ext.:179 Lower ext.: 114 Acral: 62	0.50-1: 192 1.01-2: 359 2.01-4: 203	160 of 750	- Sensitivity - Specificity - NPV - SLNB RR	Mayo Clinic tertiary care centers between 2004 and 2018
Mulder (2022) (14,38) **Netherlands**	Patients with T1-T4 primary CM eligible for SLNB (n=210)	Median 55 (IQR: 45–65)	27% (56/210)	Head and neck: 1 Trunk: 110 Arm: 33 Leg: 66	Median 2.05 mm (1·40–3·30)	55 of 210	- Sensitivity - Specificity - NPV - SLNB RR	Erasmus Medical Centre Cancer Institute between 2007 and 2017
Johansson (2021) (15) **Sweden**	Patients with T1-T4 primary CM (n=489)	Median: 60 (IQR: 49-71)	13% (54/421)	Trunk: 87 Arm: 202 Leg: 132	Median: 1.8 mm (IQR: 1.3-3.2)	133 (32%)	- Sensitivity - Specificity - NPV - SLNB RR	Sahlgrenska University Hospital, Gothenburg, Sweden between 2006 and 2014

Yousaf (2021) (16,39) **United Sates**	Patients with T1-T4 primary CM (n=208)	59 (45, 70)	44/208	Head and neck: 31 Trunk: 78 Upper ext.:40 Lower ext.: 44 Acral: 15	Median: 1.30 mm (IQR: 0.90-2.10)	49 (23.5%)	- Sensitivity - Specificity - NPV - SLNB RR	Mayo Clinic centers between 2004 and 2019 and West Virginia University between 2007 and 2014
Weitemeyer (2025) (17,40) **Denmark**	Patients with T1-T3 primary CM (n=543)	58 (44-70)	18.1% (97/536)	Head and neck: 33 Trunk: 270 Extremities: 230 Genitalia: 3	1.3 (1.0-1.8)	83 (15.5%)	- Sensitivity - Specificity - NPV - SLNB RR	Two Danish university hospitals between 2010 and 2015
Hieken (2023) (4) **United States**	Patients with T1-T4 primary CM (n=558)	Median: 64.2 (IQR: 53.0–73.8) Median: 64.2 (IQR: 54.8–75.3)	Not applicable*	Head and neck: 144 Trunk: 179 Upper ext.: 147 Lower ext.: 88	1.6 mm (SD: 1.0) 1.5 mm (SD: 0.8)	Not available	- Complication rate	Mayo Clinic tertiary care centers between 2004 and 2018
Pazhava (2025) (18,41) **United States**	Patients with head and neck CM (n=250)	median 68 (IQR: 56–76)	14% (35/250)	H&N= 250	median 1.60 mm (IQR: 1.10–2.50)	49 (19.6)	- SLNB RR - NPV - 5-year RFS - 5-year DMFS - 5-year MSS	Two US centers between 2004 and 2021

**SLNB-positive patients routinely underwent immediate complete lymph node dissection, and as such patients that were SLNB positive were excluded to prevent dilution of the results with events that could potentially be caused by CLND instead as there are high complication rates associated with this procedure. CM, cutaneous melanoma; DMFS, distant-metastatic-free survival; MSS, melanoma-specific survival; NPV, negative predictive value; RFS, relapse-free survival; SLNB RR, sentinel lymph node biopsy reduction rate.*

Table 8. Merlin study design & characteristics: safety

STUDIES FOCUSING ON SAFETY

Reference	Patient population	Age	SLNB positivity rate	Biopsy location	Breslow Thickness	Ulceration	Outcomes of interest	Study sites
Eggermont (2020) (20,23,42) **United States**	Patients with T1b-T3b primary CM (n=837) Patients with IIA primary CM (n=131)	Mean 58.9 (SD: 16.4)	23.9% (200/837)	Not available/ see Bellomo	Not available/ see Bellomo	192 (22.9%)	- 5-year RFS - 5-year DMFS - 5-year MSS	Mayo Clinic tertiary care centers between 2004 and 2018
Amaral (2023) (21,43) **Germany**	Patients with stage I/II primary CM (n=543)	66 (54, 74)	Not applicable*	Head and neck: 101 Trunk: 197 Upper ext.:58 Lower ext.: 109 Acral: 78	Median 1.70 mm (IQR: 1.20, 2.80)	135 (24.9%)	- 5-year RFS - 5-year DMFS - 5-year MSS	University hospital Tübingen between 2000 and 2017
Mulder (2022) (22,44) **Sweden & Netherlands**	Patients with stage I/II primary CM (n=535)	60 (48–70)	Not applicable*	Head and neck: 1 Trunk: 256 Upper ext.: 110 Lower ext.: 167	Median: 1.80 mm (IQR: 1.30-3.00)	145 (27.1%)	- 10-year RFS - 10-year DMFS - 10-year OS	Sahlgrenska University Hospital between October 2006 and 2014 and Erasmus MC Cancer Institute between 2007 and 2017
Amaral (2025) (24) **Germany**	Patients who did not undergo SLNB (n=930)	64 (52, 75)	Not available***	Head and neck: 206 Trunk: 407 Upper ext.: 136 Lower ext.: 180 Other: 1	Median: 0.50 (IQR: 0.40, 0.70)	44 (4.7%)	- 10-year RFS - 10-year DMFS - 10-year MSS - 10-year OS	University hospital Tübingen between 2000 and 2020
Pazhava (2025) (18) **United States**	Patients with head and neck CM (n=250)	median 68 (IQR: 56–76)	14% (35/250)	Head and neck: 250	median 1.60 mm (IQR: 1.10–2.50)	49 (19.6)	- SLNB RR - NPV - 5-year RFS - 5-year DMFS - 5-year MSS	Two US centers between 2004 and 2021

**This particular cohort consisted of stage I/II primary cutaneous melanoma patients. ***This cohort consisted of patients that did not undergo SLNB. CM, cutaneous melanoma; DMFS, distant-metastatic-free survival; MSS, melanoma-specific survival; NPV, negative predictive value; RFS, relapse-free survival; SLNB RR, sentinel lymph node biopsy reduction rate.*

In addition to the above studies, an independent systematic review and meta-analysis was carried out by the worldwide-renowned Melanoma Institute of Australia (19). Following systematic searches in Embase and Medline, 4 studies assessing the CP-GEP ("Merlin") model were included in the review (13–16). Estimates of sensitivity, specificity, NPV, PPV, and SLNB reduction rate were pooled using a fixed-effects model. See **Table 7** For details on study and patient characteristics of these studies.

5.2.2 Results at study level

5.2.2.1 Nodal metastasis prediction

Often, molecular tests are designed to identify whether a patient is positive for a specific disease or mutation. Merlin was developed to understand whether a patient has a high risk or low risk of nodal metastasis based on the aggressiveness of the primary tumor. By combining the expression of 8 genes in combination with clinical pathological variables, age and BT, the Merlin assay provides biological insight into metastatic propensity of the primary tumor. The clinical unmet need that Merlin addresses is based on the following: 80-85% of the patients that undergo an invasive SLNB surgery would have a negative lymph node, SkylineDx together with the Mayo Clinic collaborated on an innovative solution to risk stratify patients in either low risk or high risk, in which low risk patients may forgo the SLNB surgery based on a low risk of nodal metastasis.

For Merlin to be clinically impactful, it should identify as many patients with a low risk of nodal metastasis as possible who could forgo SLNB surgery. For Merlin to be safe, it should maintain a high NPV to minimize FN results that are considered clinically acceptable and in line with current clinical guidelines. Hence, Merlin has been optimized to achieve a high SLNB RR at a high NPV. Herein, sensitivity and specificity are measures of test performance and its concordance with SLNB positivity, while PPV and NPV indicate the likelihood that Merlin can successfully identify whether patients do or do not have a positive node.

Merlin was initially developed to follow recommendations regarding SLNB. In international guidelines, a patient is offered SLNB if the probability of a positive node is above 10%, and if the probability is between 5-10%, SLNB is discussed and considered (48). The clinically acceptable baseline risk of nodal metastasis before recommending SLNB surgery varies by country and practice. In Denmark, this baseline is set at 10% (8–10). Therefore, to be clinically relevant, Merlin must identify as many patients as possible who can safely forgo SLNB surgery, while maintaining a negative predictive value (NPV) of approximately 90% to align with the 10% acceptable baseline risk. Studies generally report a high NPV (11–18), underscoring Merlin's strong potential to exclude patients below the threshold commonly used in clinical practice for SLNB referral.

Another observation on the performance data is that the number of patients with a Merlin Low Risk decreases in thicker melanomas. Especially in thinner melanoma, the Merlin test significantly enhances patient management by minimizing the number of unnecessary SLNB procedures. In the Merlin discovery paper, Merlin was able to reduce, on average, 42.4% of SLNB procedures at 96.9% NPV in the T1-T4 patient population. Even higher SLNB RR were observed in T1-T2 primary cutaneous melanoma patients (SLNRR: 56.1%; NPV: 97.1%). NPV for these specific substages stays well within the range of <10%. Please find an overview of the SLNB RR and NPV in **Table 9**. This clinical development cohort confirmed the CP-GEP model's robustness, demonstrating high NPV and SLNB RR in predicting SLN involvement (11). These findings underscore the potential of combining molecular and clinical data to guide decision-making in melanoma management, ultimately reducing unnecessary biopsies and optimizing treatment pathways.

Table 9. Merlin discovery Cohort

Reference: author (year) country	Cohorts & substages	Sample size	SLNB RR, % [95% CI]	Negative predictive value, % [95% CI]
Bellomo (2020)	T1-T2	549*	56.1	97.1
United States	T1b	184	80 [74-86]	98 (95-100)
	T2a	290	48 [42-54]	95 (91-98)
	T2b	66	24 [14-34]	96 (91-100)

*SLNB RR, sentinel lymph node biopsy reduction rate. *1 T2 patient and 8 T1a patients*

Recently the prospective study Merlin MERLIN_001 study (12) was published, a prospective trial in which 1,761 T1-T3 primary cutaneous melanoma patients were enrolled and available at JAMA surgery (12). In this multicenter study, nine renowned institutes collaborated to evaluate the predictive utility and performance of Merlin to correctly stratify patients in low risk or high risk of nodal metastasis. Patients were enrolled in the study after which a sample was sent in for testing, test results were not disclosed, and SLNB was carried out according to standard clinical practice. Results from the MERLIN_001 prospective trial underscore and reinforce the previously collected retrospectively data, matching metrics such as NPV within the ranges of the NCCN guidelines (48) and Denmark (<10%) (8–10). Results from MERLIN_001 show that SLNB can be forgone in 68.2%, and 32.9% of the T1, and T2 patients, respectively (12). Looking closer at Merlin_001 data there is one difference to consider when comparing USA data with Danish and European data. That is the method of biopsy. In Danmark, the entire suspicious mole is completely excised in 90% of the cases (7). The biopsy methods used in Denmark and Europe ensure the correct BT from the first pathological assessment. In contrast, in the USA suspected melanomas are "shaved" biopsied and sent to pathology for diagnosis (49). In about 10% of the case not the entire melanoma is removed via this shave method, while complete depth of the melanoma is revealed at wide excision surgery (with or without SLNB), thus it can happen that patients turns out to have higher BT than originally estimated (shown in eFigure 2 in Supplement 2), as a result these patients may be upstaged for example from a T1b → T2a. Since BT is part of the Merlin assay the risk label may also change, which happened in 32 cases in Merlin_001 study in the USA (12). Based on these observations, we decided to use the Merlin_001 metrics after updated wide local excision data (shown in eTable 2 in Supplement 2) to mimic the Danish situation in which the correct BT is known from the initial diagnosis. With this consideration the data becomes as follows: 1,701 T1-T3 patients, with 69.2% of T1 patients having a Low Risk Merlin result and 32.5% of T2 patients having a low risk result. The breakdown per T-category) is shown in **Table 10** and **Table 38**.

Table 10. Prospective clinical Validation Cohorts

Reference: author (year) country	Cohorts & substages	Sample size	SLNB RR	Negative predictive value
Hieken (2025)* (12)	pT1a	23	82.6	N/A
United States	pT1b	448	68.5	95.4
	pT2a	683	36.2	92.3
	pT2b	163	17.2	82.1
Stassen (2023) (13)	T1-T2	162	53	96.0
Netherlands	pT1	62	83.9	94.2
	pT2	100	33.0	100

*Hieken et al., 2025 updated data after WLE.

In another prospective study, primary cutaneous melanoma patient samples (n=260) from 4 different hospitals in the Netherlands were assessed in a non-interventional manner. Analysis confirmed Merlin's ability to significantly reduce the number of SLNB surgeries in particular in T1 with 83.9% reduction rate at

an NPV of 94.2% and in T2 a 33% reduction rate a NPV of 100%. In three cases, the CP-GEP model classified patients as low risk while the post-operative histopathological examination revealed nodal metastasis. Two of these patients had a micrometastasis in the SLN (13). Patients with a micrometastasis would classify as stage IIIA and would not have been eligible for immunotherapy treatment according to the Danish guidelines (50).In total, five clinical validation studies have been published so far (for details please see **Table 11**). These studies analyzed cohorts from the Netherlands, Denmark, Sweden, and the United States and included T1-T4 primary cutaneous melanoma patient samples (13–16). In a publication by Mulder and colleagues evaluated the accuracy of Merlin using prospectively collected archived patient samples with T1-T4 primary cutaneous melanoma in the Netherlands (n=210). This validation cohort confirmed the ability of Merlin in predicting SLN involvement with SLNB RR of 90.9% in T1 melanoma patients, and 29.8% in T2 patients. In one of the four misclassified SLN, only a small number of tumor cells was identified in the lymph node (micrometastasis) (14). As such, the tumor burden is deemed low and the patient would be classified as stage IIIA not receiving any further treatment in the Danish setting (50).

Secondly, the accuracy of Merlin was further evaluated by Johansson and colleagues in a cohort of Swedish T1-T3 primary cutaneous melanoma patients (n=421). Results from the validation study demonstrated that the algorithm reliably identified patients for whom SLNB can be safely omitted with a high NPV for the total cohort and individual substages, reducing the need for invasive SLNB without compromising patient outcomes. SLNB RR in this cohort was 53.3%, and 32.9% in T1, and T2 primary cutaneous melanoma patients, respectively (15).

A third publication by Yousaf et al., describes the predictive utility of Merlin using patient samples from a cohort of T1-T3 primary cutaneous melanoma patients (n=208) in the US. Merlin showed promise in reducing unnecessary SLNB while ensuring High Risk patients receive appropriate surgical staging with a SLNB RR of 60.8%, 24.1% in T1, and T2 primary cutaneous melanoma patients, respectively. This particular study also included patients of 65 years and older in which a similar trend was observed where findings showed higher SLNB RR in thinner melanomas (16). Data on these elderly patients resulted in obtaining Medicare reimbursement in the United States for patients 65 years and older. These patients are less likely to be SLN-positive, and physicians are more reluctant to refer them for SLNB surgery, also due to the higher likelihood of suffering from surgery-related complications and comorbidities, emphasizing the clinical relevance of Merlin in this specific group of patients. In the US, SLNB is often dismissed in older patients (51). Merlin might provide a good alternative or triaging method in older patients.

As part of the ongoing clinical validation of Merlin, the Herlev and Gentofte Rigshospitalet in Denmark independently evaluated the predictive utility of Merlin in forgoing the SLNB. In total, 543 archived samples were prospectively collected and tested with Merlin. In cutaneous melanoma patients with pT1 a NPV of 95 with a SLNB RR of 73% was achieved. The NPV in patients with pT2 was 88 with a SLNB RR of 35.5%. However, compared to previous studies the number of FN identified in the Danish cohort was higher than average. However, of the 19 patients with SLN metastases classified as CP-GEP Low Risk (FN), 17 (89.5%) were considered micrometastases (metastasis with low tumor volume) and were stage IIIA (17). In Denmark as part of the Danish guidelines, patients with a micrometastasis are staged under IIIA and would not have been eligible for immunotherapy (50). Authors concluded that Merlin was a promising and well-validated non-invasive tool that can deselect patients for SLNB for patients having a low risk of nodal metastasis.

In a fifth publication in patients with patients with primary cutaneous melanoma of the head and neck published by Pazhava and colleagues, similar results were observed. SLNB RR was 56.3% in T1-T2, and 73.1% and 48.7% in T1 and T2 patients, respectively with high NPV. This shows that Merlin might provide a good alternative or triaging method in patients with head and neck melanoma (18).

The Merlin test is optimized to reduce as many SLNBs as possible (reduction rate) while maintaining a high negative predictive value (NPV), minimizing the number of false negative (FN) tests. While studies do report sensitivity and specificity, Merlin's performance metrics are generally focused on SLNB reduction rates in combination NPVs to ensure direct clinically relevant patient outcomes measures. In 2025, a meta-analysis was performed to better understand the pooled predictive utility of Merlin. The authors conducted an SLRin Embase and MEDLINE to identify external validation studies assessing the CP-GEP model ("Merlin") publications from 2020 until2024. In total 4 relevant studies were included, namely the retrospective publications by Mulder, Johannson, Yousaf and the prospective study by Stassen. TP, TN, FN, FP were extracted to analyze the predictive utility of Merlin and calculate sensitivity, specificity, PPVNPV, and SLNB RR. Subsequently, pooled estimates were obtained using a fixed effects model. Overall, Merlin shows consistent performance across different countries and their respective patient cohorts. [redacted] Summarizing, Merlin aims to substantially reduce SLNB surgeries with no negative impact on the healthcare outcome parameters, therefore NPV and SLNB RR are predominantly used as real-world outcome measures.

Table 11. Clinical Validation Cohorts

Reference: author (year) country	Cohorts & substages	Sample size	SLNB RR, % [95% CI]	Negative predictive value, % [95% CI]
Mulder (2020) (14)	pT1	11	90.9	100 [72.2-100]
Netherlands	pT2	94	29.8	89.3 [72.8-96.3]
Johansson (2021)	T1-T2	240	35.4 [29.4-41.8]	96.5 [90.0-90.3]
(15)	pT1	30	53.3 [34.3-71.7]	93.8 [69.8-99.8]
Sweden	pT2	210	32.9 [26.5-39.7]	97.1 [89.9-99.6]
Yousaf (2021) (16)	T1-T2	153	41.8 [33.9-50.1]	93.8 [84.8-98.3]
United States	pT1	74	60.8 [48.8-72.0]	93.3 [81.7-98.6]
	pT2	79	24.1 [15.1-35.0]	94.7 [74.0-99.9]
Weitemeyer (2025)	pT1	176	72.7%	94.5 [89.1-97.8]
(17)	pT2	251	35.5%	87.6 [79.0-93.7]
Denmark				
Pazhava (2025) (18)	T1-T2	167	56.3 [48.4-63.9	98.9 [94.2-100]
United States	pT1	52	73.1 [59-84.4]	100 [90.7-100]
	pT2	115	48.7 [39.3-58.2]	98.2 [90.4-100]
Wong (2025) (19)	pT1	177	72 [55-85]	94 [89-97]
Multiple countries	pT2	483	31 [27-35]	96 [91-98]

CI, confidence interval; SLNB RR, sentinel lymph node biopsy reduction rate

Forgoing SLNB surgery leads to fewer adverse events associated with this procedure. In a study by Hieken et al., results showed a reduced number of adverse events, but also emergency department visits and readmissions if decision based on Merlin was applied (See **Table 12**). In a cohort of 558 patients with pT1-T4 primary cutaneous melanoma undergoing SLNB, with SLNB complication rates defined as the overall 90-day complication rate specific to SLNB was taken for procedures performed between 2004 and 2018 at the Mayo Clinic. Excluding patients with a positive SLNB as these patients at the time underwent complete lymph node

dissection, which has a high complication rate (4). In another paper, SLNB status was proven not to affect SLNB complication rate (52). The overall observed complication rate was 17.4% and of which most common complications were seroma, infection, cellulitis and lymphedema. Omission of SLNB surgery in these 279 patients would have decreased complications proportionally by 59.2% (4). Additionally, Merlin's ability to decrease postoperative morbidity and hospital readmission in the Merlin Low Risk group, could directly impact a patient's quality of life. Forgoing SLNB by deselecting patients with Merlin would lead to less complications in these patients, thereby requiring less resources from hospitals.

Table 12. Complications in patients undergoing SLNB

Reference: author (year) country	SLNB complications	Reference; n (%)	With Merlin decision applied; n (%)	% reduction
Hieken (2022) (4) **United States**	Undergoing SLNB	558 (100)	268 (49.0)	51%
	Any complications	97 (17.4)	39 (7.1)	59.2%
	Seroma	52 (9.3)	16 (2.9)	68.8%
	Lymphedema	24 (4.3)	10 (1.8)	58.1%
	Infection/cellulitis	27 (4.8)	14 (2.6)	45.8%
	Hematoma	18 (3.3)	6 (1.1)	66.7%
	Dehiscence	14 (2.5)	7 (1.3)	50.0%
	Emergency department visits	6 (1.1)	0	100%
	Readmission to the hospital	9 (1.6)	5 (0.9)	43.8%

SLNB, sentinel lymph node biopsy. ED visits and readmission were post-procedure

In conclusion, the large body of clinical evidence that is available shows the consistent performance of Merlin across different countries, continents and healthcare settings in risk-stratifying patients who can forgo SLNB due to their low risk of nodal metastasis:

- Reliably stratifying patients with pT1–pT2 melanoma by their risk of nodal metastasis to inform SLNB decision-making (11–18)
- Merlin has a consistently high NPV across multiple international studies (11–18), exceeding the 90% risk threshold used in Danish guidelines (8–10)
- Merlin can safely identify a large proportion of patients who may forgo SLNB without compromising clinical outcomes (11–18)
- Pooled data from independent cohorts from a meta-analysis confirm Merlin's robust predictive performance across diverse populations and healthcare systems (19)
- Forgoing SLNB would lead to fewer adverse events associated with SLNB (4)

The Merlin test is optimized to reduce as many SLNBs as possible (reduction rate) while maintaining a high negative predictive value (NPV), minimizing the number of false negative (FN) tests. While studies do report sensitivity and specificity, Merlin's performance metrics are generally focused on SLNB reduction rates in combination NPVs to ensure that patients can be ruled out effectively and safely. Information on sensitivity and specificity is provided in **Table 38**.

5.2.2.2 Safety

Excluding patients from clinical interventions is a decision that should not be made lightly. If a patient is classified as low risk for nodal metastases by Merlin but does, in fact, have metastatic spread (FN), they may receive systemic therapy at a later stage, potentially reducing their survival rate. Ensuring a high NPV minimizes the number of FNs, thereby enhancing patient safety. Moreover, there is additional evidence supporting the safety of Merlin beyond its high NPV:

- All patients included in studies are followed for a minimum period of 5 years to assess their long-term survival. Generally, the longitudinal data shows that Merlin Low Risk patients have a good prognosis and better long-term survival compared to High Risk patients (18,20–24).
- Conduct further investigations to determine the underlying causes of false negatives. Weitemeyer et al. (2025) reported that most false-negative tests in the Danish cohort would not have had a clinical impact, as they primarily involved clinical stage IIIA with low SLN tumor burden, ineligible for adjuvant therapy (12).
- Including pure prognostic cohorts where the Merlin test is used to assess the patient's RFS, DMFS, MSS, and OS in patients with negative SLNBs (21–23) and patients who did not undergo SLNB (24). These studies show good and better long-term survival for patients receiving a Merlin Low Risk label, compared to the High Risk label, beyond SLNB status.

Considering the clinically acceptable baseline risk for nodal metastasis of 10% (8–10) before recommending SLNB and considering Merlin's high NPV of 90% and above, combined with robust favorable long-term survival data for Merlin Low-Risk patients, Merlin test can be used to deselect patients for SLNB surgery reliably and safely.

To better understand the long-term safety of Merlin and the capability of Merlin to prognosticate patients, long-term survival data were analyzed in patients with stage I-III or I/II (SLNB-negative) primary cutaneous melanoma. Three studies investigated the long-term survival of Merlin Low versus High Risk patients in a US, Swedish/Dutch, and German cohort (Eggermont et al., 2020 (20), Mulder et al., in 2022 (22) and Amaral et al., in 2023 (21), respectively) and reported relapse-free survival (RFS), distant metastasis-free survival (DMFS), and melanoma-specific survival (MSS) or overall survival (OS) (20–22).

In a paper published by Eggermont et al. (20), the survival of patients with a Merlin Low Risk and High Risk was assessed in SLNB positive and negative patients (stage I-III), aiming to understand whether Merlin could identify patients who are at high risk for relapse. In negative SLNB patients, CP-GEP was shown to be able to identify patients at high risk of relapse who are not identified by conventional staging and therefore are currently ineligible for clinical trials or other clinical interventions.

For RFS, in patients that were classified as SLNB negative (n=637), 51% of patients were categorized as Merlin High Risk and had a significantly lower 5-year RFS (70% [95% CI: 63% – 76%]) versus Low Risk patients (89% [95% CI: 83% – 93%]) with a hazard ratio (HR) of 3.61 (95% CI: 2.23 – 5.84); $P < 0.0001$. Interestingly, in patients that were SLNB positive (n=200), Merlin was also able to risk-stratify patients in two groups: SLNB positives with Merlin Low Risk had a much better survival outlook than Merlin High Risk patients. Of the patients categorized as high risk, 5-year RFS was substantially lower (49% [95% CI: 40% – 58%]) compared to CP-GEP Low Risk patients (68% [95% CI: 42% – 85%]) with a HR of 2.06 (95% CI: 0.89 – 4.74); $P < 0.1$. In SLNB-positive patients, 27 out of 200 were classified as Low Risk, in this group it is worth noting that many of these cases (44%) had an ambiguous SLN tumor burden, for example individual tumor cells were detected or consisted of cell clusters less than 0.1 mm diameter. A FN patient is when Merlin indicates the patient as Low Risk for nodal metastases, whereas in fact, there are nodal metastases that can be detected by SLNB surgery. Not detecting metastases in time and consequently delaying start of systemic therapy may impact the patient’s survival outlooks. Although it would be the most desirable to avoid any FN, Merlin’s long-term follow-up data shows that these Merlin test Low Risk patients also have a low risk for recurrence. Similar trends were observed for DMFS and MSS. Please see **Figure 9** and Table 13 for a visual representation of the survival data and the full study result details, respectively (20).

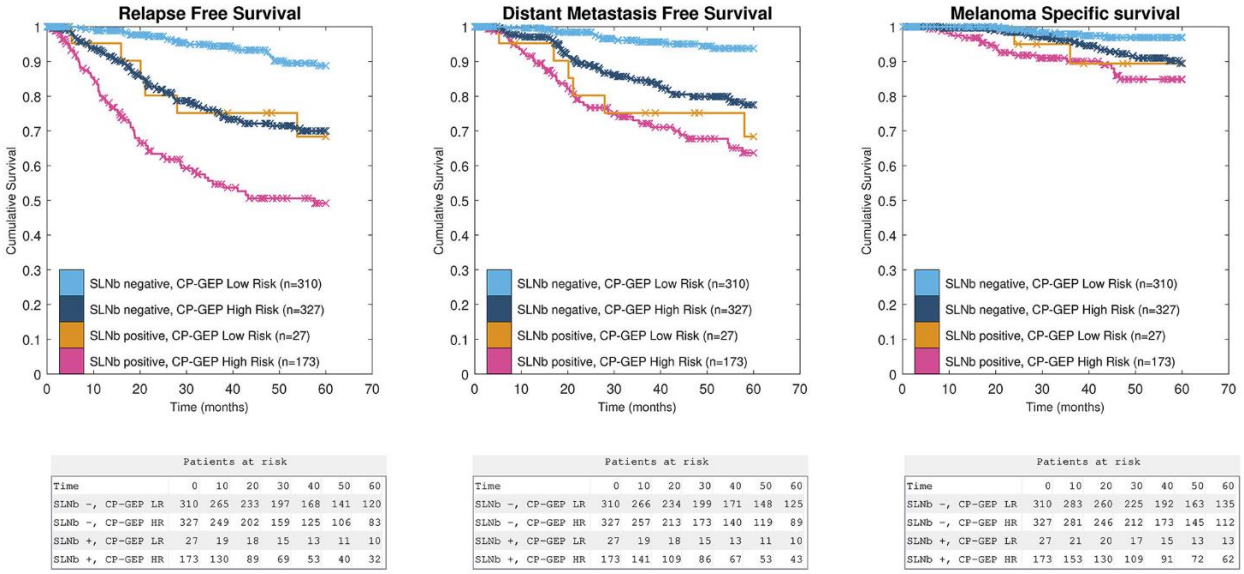


Figure 9. Kaplan-Meier curves for relapse-free survival, distant metastasis-free survival, and melanoma-specific survival stratified by SLNB status and Merlin (CP-GEP) classification (n=837) (20)**.**

Additionally, subgroup analysis was performed in patients with stage I/IIA primary cutaneous melanoma (n=580). Herein, 47% of the patients were classified as High Risk and 53% as Low Risk. The 5-year RFS of patients with a High Risk test result was 74% (95% CI: 67% – 80%) versus 89% (95% CI: 84% – 93%) in patients with Low Risk Merlin with an HR of 2.98 (95% CI: 1.78 – 4.98); P < 0.0001. Similar trends were observed in DMFS, MSS in which SLNB negative Low Risk patients had significantly better DMFS and MSS compared to High Risk patients (DMFS: HR, 3.36 [95% CI: 1.77 – 6.36]; P < 0.001; MSS HR, 2.49 [95% CI: 1.00 – 6.16); P < 0.0]). Please see **Figure 10** and Table 13 for a visual representation of the survival data and the full study result details, respectively (20).

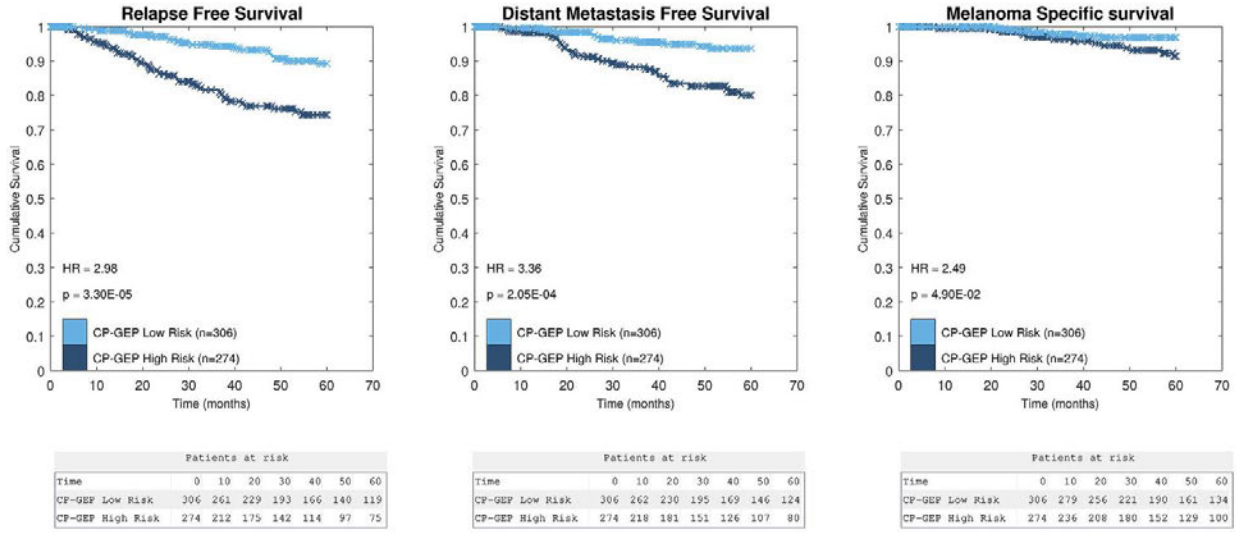


Figure 10. Kaplan-Meier curves for relapse-free survival, distant metastasis-free survival, and melanoma-specific survival stratified by Merlin (CP-GEP) classification in SLNB negative patients (n=580) in a US cohort (20)**.**

In a paper published by Pazhava et al. (18), the prognostic utility of Merlin was investigated in a cohort of 250 stage I, II, and III patients from the US with head and neck melanoma. In patients with a Low Risk, the 5-year RFS was 85% (95% CI, 75.0–91.3%). In comparison, in patients with High Risk, the 5-year RFS was 61.4% (95% CI, 51.2–70.2%). Similar trends were observed in DMFS and MSS, where Merlin was able to risk-stratify patients with an HR of 3.31 p<0.05 and 3.06 p<0.05 in DMFS and MSS, respectively. This indicates that Merlin is able to enhance risk stratification in head and neck melanoma patients.

In the paper published by Mulder et al. (44), similar to the paper from Eggermont, the study aimed to investigate whether Merlin was capable of identifying patients with a high risk of recurrence. Among all patients, the 5-year RFS was 83.5% (95% CI: 79.9–86.5). The 5-year DMFS and OS rates were 88.8% (95% CI: 85.6–91.3) and 86.7% (95% CI: 83.4–89.4), respectively. To evaluate the prognostic significance of CP-GEP, stratification based on CP-GEP risk groups revealed a 5-year RFS of 92.9% (95% CI: 86.4–96.4) for Low Risk patients compared to 80.7% (95% CI: 76.3–84.3) for High Risk patients (HR 2.93, 95% CI: 1.41–6.09, $p < 0.004$). At 10 years, Low Risk patients had an RFS of 90.6% (95% CI: 83.1–94.9) versus 74.9% (95% CI: 69.8–79.2) for High Risk patients (HR 2.84, 95% CI: 1.47–5.45, $p < 0.002$). Similar patterns were observed in DMFS and OS. Please see **Figure 10** and Table 13 for a visual representation of the survival data and the full study result details, respectively (22).

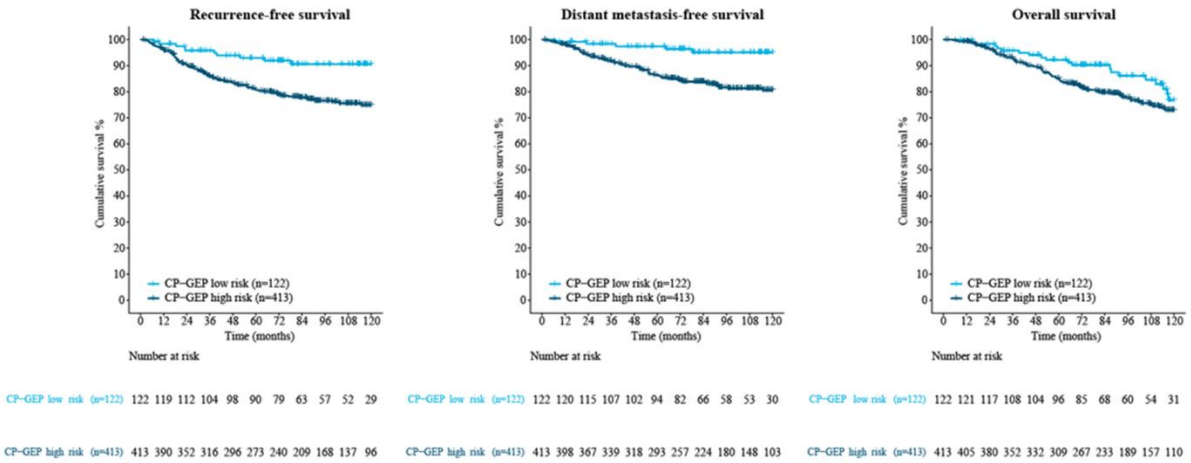


Figure 11. Kaplan-Meier curves for relapse-free survival, distant metastasis-free survival, and melanoma-specific survival stratified by Merlin (CP-GEP) classification in SLNB negative patients (n=535) in a Swedish/Dutch cohort (22)**.**

In a paper published by Amaral et al., the prognostic utility of Merlin was investigated in a German cohort of 543 stage I/II primary cutaneous melanoma patients with negative SLNB. In patients with a Low Risk, the 5-year RFS was 92.9% (95% CI: 88.0-95.8) for stage I patients and 90.6% (95% CI: 67.3-97.6) for stage II patients. In comparison, in patients with High Risk, the 5-year RFS was 86.0% (95% CI: 76.6-91.8) in stage I patients and 63.4% (95% CI: 56.1-69.9) in stage II patients. For stage I/II, CP-GEP classified 311 patients as high risk for disease recurrence, with an HR of 4.73 (p < 0.001). Overall, Merlin Low Risk captured 83 of the 98 reported relapses in the complete cohort. Similar trends were observed in DMFS (HR: 4.6 [p<0.001]) and OS (HR: 3 [p<0.001]). For more details on specific cohorts and the total cohort, see **Figure 12** and **Table 13** (21).

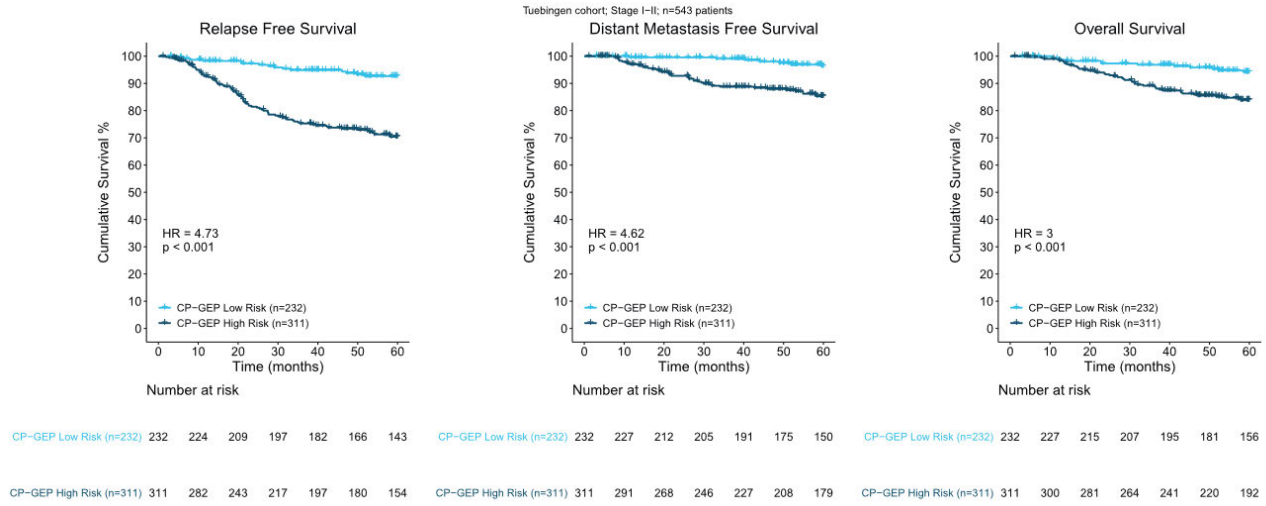


Figure 12. Kaplan-Meier curves for relapse-free survival, distant metastasis-free survival, and melanoma-specific survival stratified by Merlin (CP-GEP) classification in SLNB negative (stage I/II) primary cutaneous melanoma patients (n=543) in a German cohort (21)**.**

Next to patients with stage I/II primary cutaneous melanoma, Amaral and colleagues investigated patients who did not undergo SLNB. A similar trend was observed in stage I/II patients with primary cutaneous melanoma in which Merlin was able to distinguish between patients with a low and a high chance of progressing (HR: 20.07 [p<0.0001]), albeit in a small patient sample. Similar trends were observed in DMFS, MSS, and OS (see **Table 13** and **Figure 13**).

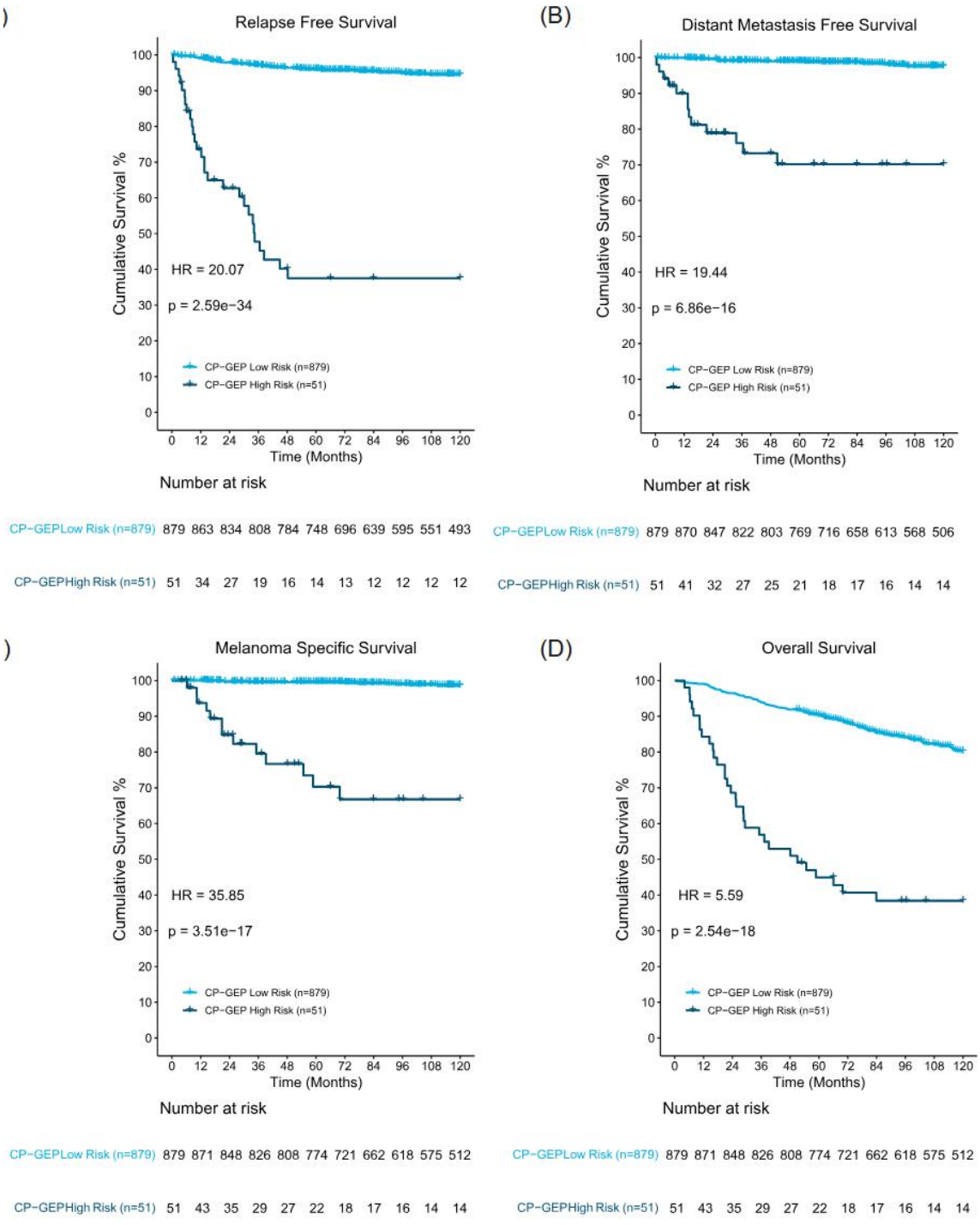


Figure 13. Kaplan-Meier curves for relapse-free survival (A), distant metastasis-free survival (B), melanoma-specific survival (C), and overall survival (D) stratified by Merlin (CP-GEP) classification in patients who did not undergo SLNB (n=930) in a German cohort (24)**.**

Peer-reviewed publications by Eggermont et al., Amaral et al., and Mulder et al. highlight that patients with a Low Risk test result have a good prognosis, whereas the study by Pazhava et al. establishes this principle in a specific cohort of head and neck melanoma patients in which Merlin was able to separate patients who have a high risk of relapse, distant metastasis, and death. For an overview of the study results per study,

please see **Table 13**. Other research has been presented and confirms and reinforces the results from earlier publications. Abstracts from Yu et al., 2023, and Hill et al., 2024 (not included in the main search), underscore that Merlin can identify patients with higher chances of a relapse, distant metastasis, or death currently not detected by SLNB (results are presented in the annex, **Table 39**).

Beyond guiding decisions around SLNB, the Merlin Low Risk result is strongly associated with favorable long-term outcomes. Five-year follow-up data show that patients classified as Low Risk experience significantly improved survival rates and fewer recurrences. Among SLNB-negative patients, the Merlin assay provides additional prognostic insight, those with a Low Risk result demonstrate markedly better survival outcomes compared to their High Risk counterparts. This suggests that for node-negative primary cutaneous melanoma patients, a Low Risk Merlin result not only identifies individuals with a good prognosis but may also serve as a safe alternative to SLNB in the long term.

Table 13. Relapse-free survival, distant metastasis-free survival, and melanoma-specific survival/overall survival in Merlin Low- and High Risk primary cutaneous melanoma patients

Reference: author (year) country	Cohort & Sample size	Recurrence-free survival, % (5-year)	Distant metastatic free survival, % (5-year)	Overall survival/ Melanoma-specific survival, % (5-year)	Hazard Ratio (95% CI, p-value)
Eggermont (2020) (20) **United States**	All patients (837)	73 [69-76]	81 [77-84]	91 [94-99]	Stage I-III
	SLNB negative (637)	79 [75-83]	86 [82-89]	93 [90-95]	Low risk versus High Risk node node-
	SLNB positive (200)	52 [43-60]	64 [55-72]	85 [78-90]	RFS: 3.61 [2.23-5.84]; P < 0.0001
	Stage I-III (837)				Low risk versus High Risk node node+
	Low risk & node- (310)	89 [83-93]	94 [89-96]	96 [94-99]	RFS: 2.06 [0.89-4.74]; P < 0.1
	High risk & node- (327)	70 [63-76]	78 [71-83]	89 [84-93]	Stage I-IIA
	Low risk & node+ (27)	68 [42-85]	68 [42-85]	89 [64-97]	RFS: 2.98 [1.78-4.98]; P <0.001
	High risk & node+ (173)	49 [40-58]	64 [54-72]	84 [77-90]	DMFS: 3.36 [1.77-6.36]; P <0.001
	Stage I-IIA (580)				MSS: 2.49 [1.00-6.16]; P <0.05
(MSS)	CP-GEP Low Risk (306)	89 [84-93]	94 [89-96]	97 [93-98]	
	CP-GEP High Risk (274)	74 [67-80]	80 [73-85]	91 [86-95]	
Wever (2020) (23) **Sub analysis United States (MSS)**	Stage IIA				Stage IIA
	CP-GEP Low Risk (78)	78%	85%	89	RFS: 2.23; P <0.05
	CP-GEP High Risk (63)	56%	58%	90	
Mulder (2022) (22) **Sweden & Netherlands (OS)**	Stage I-II (535)	83.5 [79.9-86.5]	88.5 [85.6-91.3]	86.7 [83.4-89.4]	5-year RFS:
	CP-GEP Low Risk (122)	92.9 [86.4-96.4]	97.4 [92.1-99.2]	92.1 [85.4-95.8]	2.93 [1.41-6.09]; P <0.004
	CP-GEP High Risk (413)	80.7 [76.3-84.3]	86.2 [82.3-89.3]	85.1 [81.1-88.3]	10-year RFS:
	Stage I-II: 10-year survival (535)	78.4 [74.2-82.1]	83.8 [79.9-87.0]	73.9 [69.1-78.1]	2.84 [1.47-5.45]; p<0.002
	CP-GEP Low Risk (122)	90.6 [83.1-94.9]	95 [88.3-97.9]	76.9 [64.9-85.1]	
	CP-GEP High Risk (413)	74.9 [69.8-79.2]	80.6 [75.8-84.5]	73 [67.6-77.6]	
Amaral (2023) (21) **Germany**	Stage I/II (543)	79.9 [76.0-83.2]	90.0 [86.9-92.4]	88.3 [85.1-90.8]	Stage I/II
	CP-GEP Low Risk (232)	92.7 [88.1-95.5]	96.3 [92.3-98.2]	94.2 [89.9-96.7]	RFS: 4.73; P <0.001
	CP-GEP High Risk (311)	70.4 [64.6-75.4]	85.3 [80.4-89.0]	83.9 [79.1-87.7]	DMFS: 4.62; P <0.001
	Stage I/IIA (424)	86.0 [82.0-89.1]	94.4 [91.5-96.4]	92.9 [89.8-95.1]	OS: 3; P <0.001
	CP-GEP Low Risk (229)	93.0 [88.5-95.8]	96.7 [92.7-98.5]	94.6 [90.3-97.0]	
	CP-GEP High Risk (195)	77.8 [70.9-83.3]	91.8 [86.6-95.1]	91.0 [85.7-94.4]	Stage I/IIA
	AJCC Low Risk stage I/IIA (424)	86.0 [82.0-89.1]	94.4 [91.5-96.4]	92.9 [89.8-95.1]	RFS: 3.53; P <0.001
	Stage I (301)	90.7 [86.6-93.7]	96.0 [92.7-97.8]	95.6 [92.4-97.5]	DMFS: 2.79; P <0.05
	CP-GEP Low Risk (208)	92.9 [88.0-95.8]	96.9 [92.7-98.7]	95.8 [91.7-97.9]	OS: 1.72; P =0.17
	CP-GEP High Risk (93)	86.0 [76.6-91.8]	94.0 [86.2-97.5]	95.4 [88.1-98.2]	

	Stage II (242)	66.1 [59.2-72.0]	82.2 [76.2-86.9]	79.0 [73.0-83.9]	
	CP-GEP Low Risk (24)	90.6 [67.3-97.6]	90.6 [67.3-97.6]	79.3 [53.1-91.8]	
	CP-GEP High Risk (218)	63.4 [56.1-69.9]	81.4 [74.9-86.3]	79.0 [72.6-84.0]	
	Patients who did not undergo SLNB	7 events	4 events	7 events	
(OS)	CP-GEP Low Risk (69)	1 event	0 events	3 events	
	CP-GEP High Risk (11)	6 events	4 events	4 events	
Amaral (2025)					Patients who did not undergo SLNB
(24) **Germany**	Complete cohort (930): 5 years	93.1 [91.2-94.6]	97.6 [96.3-98.4]	98.2 [97.1-98.9]	RFS: 20.07; P<0.0001
	CP-GEP Low Risk (879)	96.0 [94.4-97.1]	98.9 [98.0-99.4]	99.5 [98.8-99.8]	DMFS: 19.44; P<0.0001
	CP-GEP High Risk (51)	37.5 [23.2-51.8]	70.2 [53.8-81.7]	70.3 [53.1-82.2]	MSS: 35.85; P<0.0001
	Complete cohort (930): 10-years	91.7 [89.6-93.3]	96.2 [94.6-97.3]	97.2 [95.8-98.1]	OS: 5.59; P<0.0001
	CP-GEP Low Risk (879)	94.5 [92.7-95.9]	97.6 [96.1-98.5]	98.6 [97.4-99.3]	
(MSS)	CP-GEP High Risk (51)	37.5 [23.2-51.8]	70.2 [53.8-81.7]	66.8 [49.0-79.6]	
Pazhava (2024)	Stage I-III:				SLNB negative versus positive
(18)	Head & Neck (250)	71.3 [64.2-77.3]	80.8 [74.2-85.8]	88.4 [82.1-92.7]	RFS: 4.12; P<0.001
United States	SLNB negative (215)	76.9 [69.4-82.8]	84.3 [77.4-89.2]	90.6 [83.8-94.6]	DMFS: 3.43; P<0.001
	SLNB positive (35)	36.8 [17.0-57.0]	61.4 [41.6-76.2]	72.5 [45.5-87.7]	OS: 3.53; P<0.05
	CP-GEP Low Risk (103)	85.0 [75.0-91.3]	91.6 [83.1-95.9]	95.4 [88.2-98.3]	CP-GEP Low Risk versus High Risk
	CP-GEP High Risk (147)	61.4 [51.2-70.2]	72.6 [62.4-80.4]	82.6 [71.8-89.5]	RFS: 3.04; P<0.001
(MSS)					DMFS: 3.31; P<0.01
					OS: 3.06; P<0.05

CI, confidence interval; CP-GEP, clinicopathologic gene expression profiling; DMFS, distant metastatic-free survival; MSS, melanoma-specific survival; OS, overall survival; RFS, recurrence-free survival/relapse-free survival

5.2.2.3 Turnaround time test

It is imperative to understand how Merlin will affect the overall testing strategy in Danish patients. As such, understanding whether Merlin will disrupt the current diagnostic work-up and scheduling of an SLNB procedure would help assess whether it can become part of the standard of care. The turnaround time (TAT) of Merlin has been analyzed in two different studies of which one in a prospective multicenter study in the Netherlands. The aim of the study was clinical validation and implementation of the CP-GEP model in a multicenter setting (13).

Although prognostic accuracy is of the utmost importance, equally important is the duration it takes to discuss the results of Merlin for implementation in routine daily clinical practice. Test results should be readily available before scheduling an SLNB procedure. In the Netherlands, with a median of 16 (14-20) days between ordering the test, and reporting the final results of the test, the TAT was sufficient to discuss the results of Merlin between physician and patient and to consider forgoing the SLNB surgery in patients with a Low Risk test result. A prolonged IC-to-report time was found in 16 of the 260 patients (median value exceeding 30 days). The main contributors that caused this delay were incorrect tissue shipment (n = 4) and extended shipment duration (exceeding 25 days) (13). Additionally, in 2016, Oude Ophuis et al. published data that showed that there is no effect of the time interval between diagnosis and SLNB on 5-year survival or SN-positivity rate up to three months after diagnosis of primary cutaneous melanoma (53). Given that the TAT is well within the 3-month timeframe and was deemed well accepted within the Dutch study it is anticipated that this would cause no harm and could be implemented in current routine daily clinical practice in Denmark.

Table 14. Organizational utility of Merlin

Reference: author (year) country	Organizational utility	Days, median (IQR)
Stassen (2023) (13) **Netherlands**	• Order to revision	5 (3-6)
	• Revision to shipment	4 (2-6)
	• Shipment to report	7 (6-9)
	• Processing time at SkylineDx	4 (3-5)
	• Order to report	16 (14-20)

IQR, interquartile range.

5.2.3 Methodologies for comparison analysis (intervention versus comparator)

To determine the accuracy of Merlin in all substages, all clinical validation cohorts [five retrospective studies (14–18) and two prospective studies (13)] described in **Table 10** and **11** were combined in a whole cohort analysis (unpublished, see **Table 38**) and predictive performance was assessed against SLNB outcome. To conduct the whole cohort analysis (meta-analysis), the statistical programming language R was used with the "metaprop" function from the "meta" package (v.8.1). To join the proportions from the individual studies we made use of a random intercept logistic regression model as described in Stijnen et al., 2010 (54). We decided in favor of the random intercept model because each study is based on a different cohort, from different hospitals and countries and the random intercept can account for cohort specific variations.

All seven studies are peer-reviewed and publicly available, however detailed subgroup analysis was not always reported. Combined analysis resulted in a T1-T2 cohort of 2,577 patients with a SLNB positivity rate of [redacted]. In this group, [redacted] of patients were risk stratified as Merlin Low Risk (SLNB RR), the sensitivity was [redacted] specificity was [redacted], and the NPV was [redacted] A subgroup breakdown

is shown in **Table 38**. To assess the impact Merlin on Health outcomes (and costs) a simulation model was used. In this model a strategy with the Merlin assay (in which Merlin Low Risk patients forgo SLNB surgery) was compared to a strategy in which all SLNB elected patients would undergo SLNB. The methodology of this simulation (Health Economic) model is described in Section 8.

5.2.4 Results of the comparative analysis (intervention versus comparator)

The results of the simulation model are shown in section 8.

6. Patient perspective

This section presents data for Merlin in terms of clinical effectiveness and safety in patients with Breslow thickness T1a to T3b with primary cutaneous melanoma. Overall, 11 references were screened for eligibility during the title and abstract phase, of which 8 were excluded. Subsequently, 3 references were screened in the full-text selection. During full-text selection, 2 references were excluded, leading to a total of 1 eligible report through screening. Another 2 studies were added as part of the full search, as these studies could provide more information on patient perspective but weren't retrieved through its original search. For an overview of the different studies, see **Table 15**.

Table 15. Overview of patient perspective studies

Reference (author, year, country)	Objectives	Method	Population	Intervention	Comparator
Hieken (2022) (4) **United States**	Evaluate the number of potentially avoidable SLNB-associated complications by applying the Merlin test results to the Merlin.	Complications evaluated in this study were lymphedema, seroma, infection/cellulitis, hematoma, and wound dehiscence.	Merlin development cohort, n=754 patients (Bellomo)	CP-GEP (Merlin)	SLNB
Meves & Eggermont (2020) (6) **United States**	Evaluate whether the Merlin Assay can be used as a triage tool during pandemic times to optimize healthcare resources.	Compare utilization of hospital resources in current clinical care settings versus a clinical care setting with the implementation of Merlin testing.	1 patient case from the Merlin development cohort (Bellomo 2020)	CP-GEP (Merlin)	SLNB
Stassen (2024) (36) **Netherlands**	To validate the impact of CP-GEP on treatment decisions in clinical practice	Patients were consulted on the test result, and a treatment decision was made following shared decision making to forgo SLNB or not.	45 patients	CP-GEP (Merlin)	SLNB

CP-GEP, clinicopathological-gene expression profiling; SLNB, sentinel lymph node biopsy

6.1 Summary of the findings concerning the patient's perspective

From a patient's perspective studies suggest that if patients can forgo the SLNB based on a Low Risk result, it would require fewer visits and traveling to the hospital as the SLNB does not have to be carried out and

fewer incurred resources from the healthcare system (6), eventually resulting in fewer visits to the hospital and fewer traveling, but also less adverse events associated with this procedure (4)

In 10% to 17.4% of the SLNB cases, patients will be confronted with adverse effects caused by this procedure (3,4). Adverse effects consist of seroma, infection, hematoma, dehiscence, and lymphedema. Especially the latter can persist and can cause long-term issues and decrease quality of life of patients (25,26). From 2017 to 2021, lymphedema was observed in 3.5% to 6.8% in Denmark during a 10–26-month follow-up (2017-2022) (7).

In a study by Hieken et al.(4), results showed a likely reduced number of adverse events, but also emergency department visits and readmissions if the Merlin decision had been applied. In a cohort of 558 patients with pT1-T4 primary cutaneous melanoma undergoing SLNB, with SLNB complication rates was defined as the overall 90-day complication rate specific to SLNB was taken for procedures performed between 2004 and 2018 at the Mayo Clinic. Excluding patients with a positive SLNB, as these patients at the time underwent complete lymph node dissection, which has a high complication rate (4). In another paper, SLNB status was proven not to affect SLNB complication rate (52). The overall observed complication rate was 17.4% and of which the most common complications were seroma, infection, cellulitis and lymphedema. Omission of SLNB surgery in these 279 patients would have decreased complications proportionally by 59.2%. Additionally, Merlin's ability to decrease postoperative morbidity and hospital readmission in the Merlin Low Risk group could directly impact a patient's quality of life. Forgoing SLNB by deselecting patients with Merlin would lead to less complications in these patients, thereby requiring fewer resources from hospitals (See **Table 12** in section 5.2.2.1).

To diagnose patients and understand their nodal involvement, multiple steps and hospital resources need to be considered. In 2020, a case study was published by Meves & Eggermont in which a patient's journey was described. This study was carried out by following a patient who was enrolled at the Mayo Clinic. After the diagnosis of primary cutaneous melanoma, the patient underwent preoperative evaluation with customized testing to ensure a safe recovery. To understand where the SLNs reside. Lymphoscintigraphy was carried out using technetium-99m (single-photon emission computerized tomography [SPECT]) in nuclear medicine combined with computed tomography (CT) to produce SPECT-CT scans. Subsequently, the patient had SLNB and a WLE of the primary tumor under general anesthesia. Tissue was paraffin-embedded, sectioned, and stained with hematoxylin and eosin, and melanocyte lineage-specific immunohistochemistry was determined and interpreted by anatomic pathologists. For this patient, from melanoma diagnosis to finalized SLN biopsy result took 3 weeks. After surgery, the patient received postoperative care. Eventually, the biopsy turned out to be negative for this specific patient (6).

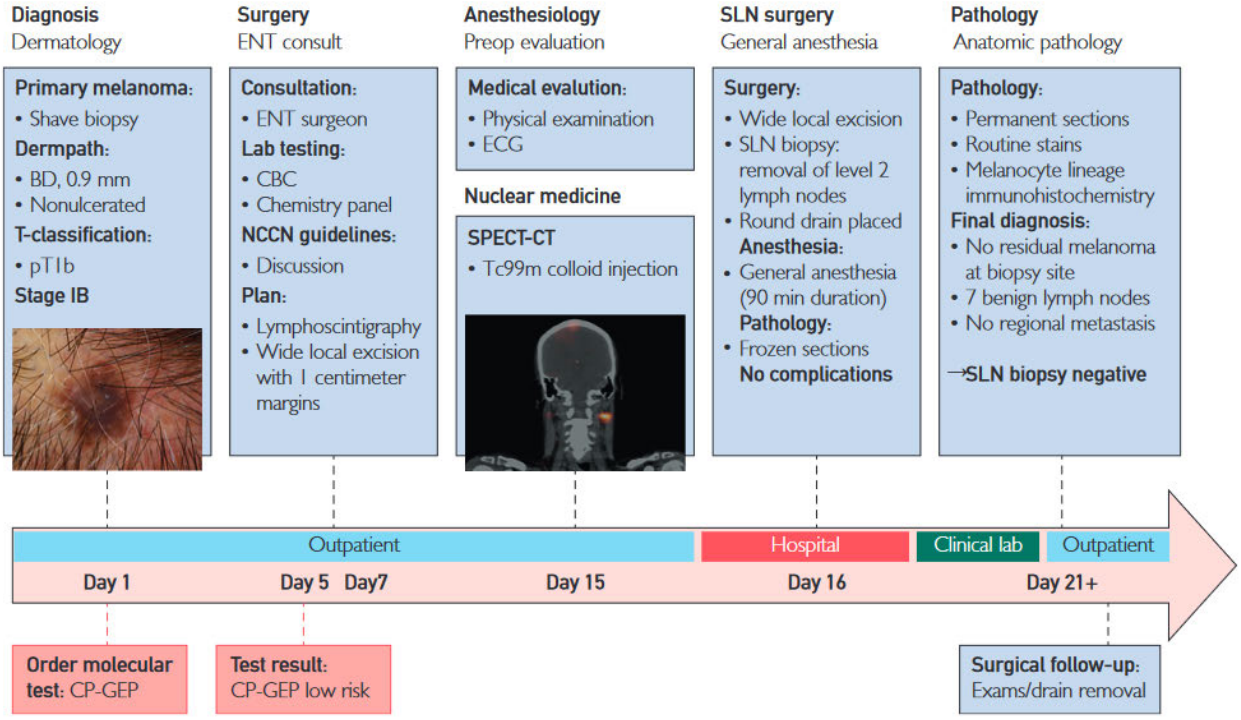


Figure 14. Summary of a case enrolled in a pilot study for clinicopathologic variables and gene expression profile (CP-GEP) based molecular testing at Mayo Clinic who underwent SLNB in late 2019. Adapted from Meves & Eggermont (6)**.**

Note: the extensive use of hospital infrastructure, medical specialists, laboratory tests, and time to arrive at a negative SLN biopsy test result. In contrast, a simple molecular test performed on diagnostic biopsy tissue correctly identified the low-risk nature of the patient's primary melanoma with a turnaround time of just a few days. BD = Breslow depth; CBC = complete blood cell count; ECG = electrocardiogram; ENT = ear, nose and throat science (otorhinolaryngology); NCCN = National Comprehensive Cancer Network; SPECT-CT = single photon-emission computed tomography.

Results from a study carried out in a Dutch setting in two specialized melanoma centers, Erasmus Medical Centre and ISALA described how CP-GEP can guide further decisions in the clinical care pathway. This study enrolled patients of 18 years or older with primary cutaneous melanoma (pT1b-pT4), who were eligible for SLNB. Patients were consulted regarding SLNB and the possibility of forgoing SLNB following a "low risk" CP-GEP result. Most patients were T1b-T2a, whereas the Merlin yielded a low risk for 29 (64%) of 45 patients. After discussion between the physician and patient, 23 of the 29 patients opted for follow-up instead of SLNB (36). Recently, a German case report study has been submitted to journal Die Hausartz in which 5 patient cases are described for which Tuebingen Universitäts-Hautklinik decided, in consultation with the patient, to order Merlin to help guide patient pathway. The case report will describe guidance on SLNB surgery and importantly, the patient perspective. These case reports were first presented at German skin cancer conference ADO in September 2025. Currently, no further qualitative evidence about the patient's experience and perspective is available.

6.2 Considerations regarding user requirements and accessibility

No additional user requirements are needed. In agreement with the physician, the tissue from the first excision will be processed. It is imperative that the tissue that is processed contains tumor cells from the primary tumor.

7. Organizational implications

This section presents data for Merlin in terms of organizational implications. Overall, 16 references were screened for eligibility during title and abstract phase, of which 14 were excluded. Subsequently, 2 references were screened in the full-test selection. During full-text selection, 1 reference was excluded, leading to a total of 1 eligible report through screening. Two additional studies were added through the full search. For an overview of the studies, see **Table 16**. The study by Stassen (13) investigated the predictive utility and the clinical implementation of Merlin in 4 different well-renowned hospitals in the Netherlands. Another study by Meves & Eggermont (6) describes a clinical case study of a patient and the patient's journey.

Table 16. Overview of studies on organizational implications

Reference (author, year, country)	Type of study/ type of data	Purpose of the study/ data collection	Context (Year, location, who)	Respondents (number, characteristics)	Results
Meves & Eggermont (2020) (6) **United States**	Case study of the patient journey	To describe the clinical patient journey and the extensive use of hospital infrastructure and medical specialists	Mayo Clinic tertiary care centers in Minnesota, Arizona, or Florida between 2004 and 2018	Case study of 1 patient	An overview of the patient journey, hospital infrastructure, and multidisciplinary team
Hieken (2022) (4) **United States**	Complication study	Evaluate the number of potentially avoidable SLNB-associated complications by applying the Merlin test results to the Merlin. development patient cohort.	Procedures were performed between 2004 and 2018 at the Mayo Clinic.	Merlin development cohort, n=754 patients (Bellomo)	List of complications per risk-stratified patient group
Stassen (2023) (13) **Netherlands**	Prospective clinical validation study.	To evaluate clinical validation and implementation of the CP-GEP model in a multicenter prospective Dutch study.	July 2020 until August 2022 in: Erasmus Medical Center, Leiden University Medical Center, Isala Hospital, Maastricht University Medical Center	This study enrolled 260 patients, of which 260 were tested with Merlin and 222 underwent SLNB.	Results between Merlin and SLNB were compared.

CP-GEP, clinicopathological-gene expression profiling; SLNB, sentinel lymph node biopsy

Currently, all collaborating sites either ship samples to our CAP/CLIA-certified laboratory in the United States (San Diego) or send samples to our accredited laboratory site in the Netherlands (Rotterdam). For establishing testing with Merlin, different options can be considered: 1) sending patient samples to our accredited laboratory at the SkylineDx facility in Rotterdam, the Netherlands, or 2) conducting the analysis in a centralized pathology hub in one or more main hospitals in Denmark.

7.1 Summary of findings regarding the organizational perspective

Forgoing SLNB can lead to:

- Fewer incurred resources from the healthcare system (6)
- Fewer adverse events are associated with this procedure (4)

From a hospital's perspective, this suggests that if patients can forgo the SLNB based on a Low Risk result, it would require fewer consultations, fewer resources from a multidisciplinary team, and less pressure on the operating room as the SLNB does not have to be carried out (6).

In 10% to 17.4% (3,4) of the SLNB cases, patients will be confronted with adverse effects caused by this procedure, which may lead to additional hospital resources being needed. Hence, the implementation of Merlin may lead to less organizational impact and more resources becoming available.

Additionally, data were collected prospectively from primary cutaneous melanoma patients (n=260) in 4 different hospitals in the Netherlands. Although the main goal of this study was to understand the prognostic accuracy of Merlin, the time required to complete the test is also crucial for its practical use in daily clinical settings. Efficient logistics and timely reporting of results are essential before a potentially scheduled SLNB. Study results showed that the time frame is adequate for discussing test results with patients and considering the option to avoid surgery for those with a Low Risk of SLN positivity. In 2016, Oude Ophuis et al. demonstrated that the time interval between diagnosis and SLNB does not impact the 5-year survival rate or the rate of sentinel node positivity, even when it occurs up to three months after the primary melanoma diagnosis (53).

7.2 Organizational description

7.2.1 Compatibility of Merlin

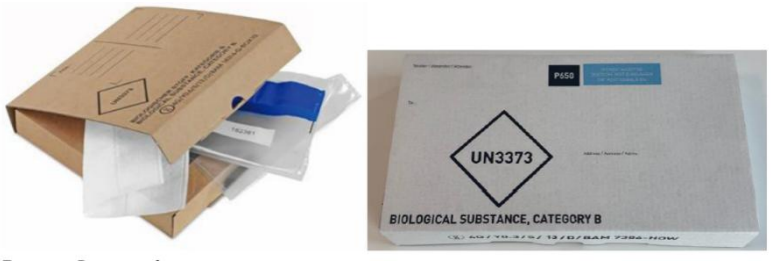
For establishing testing with Merlin, different options can be considered: 1) sending patient samples to our accredited laboratory at the SkylineDx facility in Rotterdam, the Netherlands, or 2) conducting Merlin in a centralized pathology hub in one/or more main hospitals in Denmark.

7.2.1.1 Samples shipped to Rotterdam (diagnostic service)

For shipment and Merlin as a diagnostic service, samples must consist of Formalin-Fixed and Paraffin-Embedded (FFPE) primary cutaneous melanoma biopsy tissue containing tumor cells. These can be submitted as either freshly cut curls in a microcentrifuge tube, which is the preferred method, or mounted on charged, unstained, unbaked glass slides placed in a slide box. The required specimen volume is 50 µm of tissue, consisting of 5 to 10 recuts in a single standard 1.5 mL microcentrifuge tube. Each specimen must be clearly labelled with the Patient ID. Test requests must be authorized by a medical doctor, and the latest version of the test requisition form (TRF, see Appendix section 11.12.1) must be signed by the ordering physician. If the form is unsigned, it must be accompanied by a signed document or authorization letter. Samples must be stored and transported at room temperature (25°C) or under cool conditions (4°C) but freezing or refrigeration below 4°C is prohibited.

For shipping, the completed test requisition form, any required physician authorization letter, and the specimen(s) should be placed in a bubble envelope or box. Samples may be shipped using the hospital's preferred courier or postal service, or through SkylineDx's DHL account, following the guidelines provided in the attached customer instructions (See appendix, section 11.12.2). It is important to note that SkylineDx assumes responsibility for the samples only once they have entered its laboratory. Potential risks and issues

that may arise from international shipment are listed below. In our experience the packaging is important to start with, the best results have been with the following boxes:



The advantage of these packages compared to bubble envelopes; it helps going through customs easier in our experience and a box may have a lower risk of getting lost. Furthermore, it is sturdy and provides several layers of protection to the FFPE patient material (box, bubble envelope, microcentrifuge tube).

Transit or Customs Hold-ups can also cause shipment delays: we have experienced shipping delays due to logistical issues, customs inspections, or incorrect documentation all contributing to potential extended transit time. We provide clear documentation to describe the shipment process step-by-step.

Loss or Misplacement: Samples may be lost or misplaced in transit due to labelling errors, inadequate tracking systems, or human error, leading to loss of valuable samples.

Regulatory or Compliance Issues: Failure to meet shipping regulations for hazardous, biological, or restricted materials could result in shipment rejection. This risk is minimized by providing boxes, stickers and step-by-step guidance.

Very Low chances:
Temperature fluctuations: the samples should be held at room temperature (RT, 25 ^{o}C) or cooled (4°C) conditions during storage and transport. The samples should not be exposed to heat.

Physical Damage: Due to improper packaging, rough handling, or stacking during transport can cause breakage if they are fragile or stored in glass or plastic containers. With over 6,000 samples handled in our laboratory in Rotterdam, this rarely happened. Since glass slides should be in a glass holder and 1.5 ml tubes don't break easily especially when transported in carton box.

Contamination or Cross-Contamination: Inadequate sealing, improper separation, or breaches in packaging could allow contamination from external sources or between samples. In the guide it is emphasized to make sure that if sending multiple samples in 1 shipment they are separated from each other by foil or extra external packaging such as small containers.

All the above scenarios may delay the diagnostic processing and affect waiting times. We have adequate experience of dealing with these situations where it happens. Currently we receive clinical samples from German institutions and manage to report outcomes in a timely manner.

Deliveries using services such as DHL must arrive by 5:00 PM CET (preferably 4:00 PM) from Monday to Friday to ensure receipt by lab personnel. Samples should be sent to SkylineDx. Once the shipment is arranged, the hospital must notify SkylineDx via email at merlinsample@skylinedx.com, providing the tracking number to inform lab personnel of the incoming sample. Shipments should not be sent on dry ice, though a cold pack may be used during extreme heat conditions when packed inside the transport kit.

The sectioned tissues must contain tumor cells, whereas the sample should be stored refrigerated (cannot be frozen) or kept at room temperature to ensure the highest success rate. After receiving the FFPE sample at the SkylineDx laboratory, RNA extraction is conducted with the Qiagen RNeasy FFPE Kit, using xylene for deparaffinization and a standard elution volume of 20 µL. The RNA should be stored at -80°C. For cDNA synthesis, an aliquot of RNA (500 ng) is used as input for Merlin. cDNA synthesis should be conducted using SuperScript VILO Master Mix from ThermoFisher. Subsequently, quantitative poly chain reaction (qPCR) is performed in the QuantStudio 5 Dx (ThermoFisher) on 10 times diluted cDNA using ThermoFisher's PowerUp SYBR Green Master Mix and Merlin qPCR Template. In certain cases samples can fail, the technical succes rate in the Merlin_001 study with almost 2,000 samples was 97.7%, thus a failure rate of 2.3% (12). For failing samples SkylineDx has a QC-parameter report in place. Causes of an invalid test result will always be explained directly with the reporting. If other questions arise, the SkylineDx lab can have direct contact with the requesting physician/pathology department to discuss that specific sample. Examples of sample failures: Not sufficient material was available in the FFPE material resulting in very low RNA yield of bad quality, action would be to request a new sample, which will be rerun. Sample can fail on qPCR, cause can be degraded RNA if possible rerun cDNA material or request new sample. Human errors, certain steps in the protocol (RNA extraction, qPCR preparation) went wrong, if possible rerun remainder of existing sample or request a new sample.

After the diagnostic work-up of the sample, data analysis is performed using the installed Merlin Assay Analysis Software (MAAS) on a standalone local computer, ideally the computer that is used in conjunction with the QuantStudio5Dx. The software is part of the workflow and will be performed where the Merlin Assay is run (either in SkylineDx lab when assay is performed there, or at customer's site if they run the Merlin Assay in-house). The generated output file (.eds format) from the QuantStudio 5 Dx is used as input for the MAAS. From this file, several quality control (QC) parameters are derived and checked against their acceptance criteria. If the sample is successfully analyzed and complies with all the acceptance criteria, the QuantStudio 5 Dx output is combined with manually entered clinicopathologic (CP) data, age, and Breslow depth, whereafter the Merlin result is calculated. A report is generated with High Risk or Low Risk. The QuantStudio 5 Dx data, the entered clinical data, and the results are stored on a local computer in encrypted and password-protected databases. A flow chart of the full process can be found in **Figure 15**, whereas more information about the full procedure can be found in the latest version of the instructions for use (IFU). This process will either be carried out as part of outsourcing this diagnostic service to the laboratory in the Netherlands but can also be established on site.

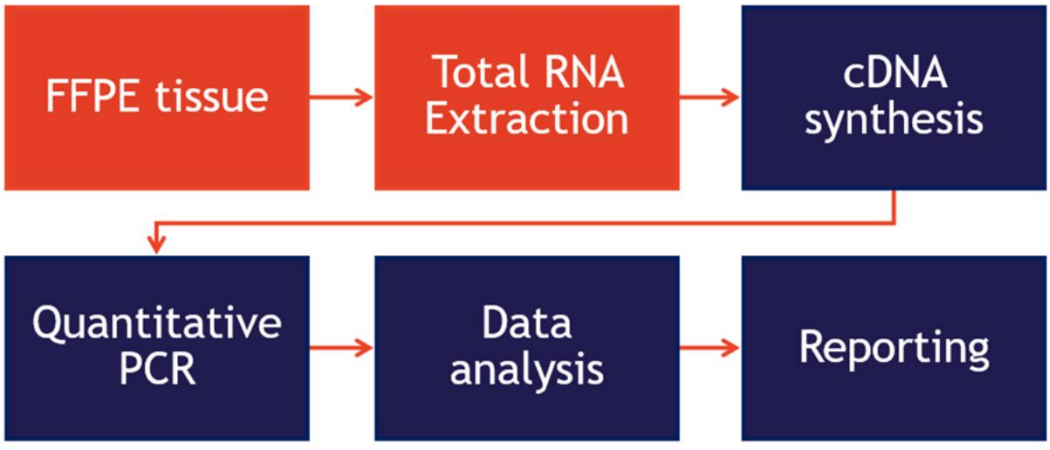


Figure 15. Summary of the Merlin analytical process and sample preprocessing

SkylineDx generates a test report within 5 working days after receiving the sample. The report will be securely shared with the requesting physician or designated contact person via a secure portal (Egress portal, see Appendix section 11.12.3). SkylineDx adheres strictly to GDPR, ensuring that samples and data are not stored, shared, or used for any purpose other than diagnostic testing. Access to data is limited to those who require it, and data storage duration depends on contractual requirements. Generally, data is retained for up to six months and deleted thereafter, unless otherwise instructed by the Danish Health Authorities. This comprehensive approach ensures the integrity and confidentiality of the testing process from start to finish.

The sample must be FFPE, as the performance characteristics of the signature have not been established for tissue in other primary cutaneous melanocytic lesion biopsies. In addition, the signature has not been validated on re-excised lesions, metastatic lesions (i.e., non-primary melanomas), non-melanocytic neoplasms, samples not fixed in neutral buffered formalin, or lesions subjected to prior treatment or biopsy. Therefore, these samples are not suitable for testing and although results could be achieved, these would be invalid. Furthermore, the performance characteristics have not been validated for RNA extraction methods other than the RNAeasy FFPE kit QIAGEN™, therefore it is strongly recommended for processing of the sample.

7.2.1.2 Centralized pathology hub

SkylineDx will collaborate with an identified laboratory to serve as the centralized hub for testing. Preference will be given to laboratories that already have a QuantStudio5Dx machine on-site. If a suitable laboratory lacks the required equipment, SkylineDx will explore the possibility of providing and installing a QuantStudio5Dx to facilitate the testing process. To ensure adherence to proper procedures, SkylineDx will conduct comprehensive training sessions for the laboratory staff, covering all aspects of the Merlin test workflow to guarantee consistent and reliable results. The training will be held onsite, at the centralized laboratory, and performed by our highly skilled and qualified trainers. First, we will share and discuss Merlin Assay – Checklist; listing materials included as part of the kit, further materials required for testing, consumables required, and equipment requirements. Also, the MAAS software is provided in advance. We want to make sure the centralized lab confirms the availability of all the necessary items before the Merlin Assay Proficiency Training can start. The Proficiency Training procedure for the Merlin Assay with Merlin Assay Proficiency Training Instructions will be conducted by our trainer onsite and will take one or two working days. Ideally all lab personnel that will be involved in performing the Merlin Assay will take part in the training including training on MAAS software and proper result reporting. Upon completion, personnel will receive a Merlin Assay Proficiency Certificate. After the training the expectation is that the staff is able to confidently process samples with Merlin Assay. We advise running a couple of test samples. Our laboratory experts are available to answer any questions that may arise or provide further technical or client support in a timely manner. So far more than 10 sites have been successfully trained on the Merlin Assay.

The TRF will be customized by SkylineDx to meet the specific requirements of the chosen laboratory, ensuring compliance with local regulations and operational practices. Samples must be stored and transported at room temperature (25°C) or under cooled conditions (4°C), with freezing or refrigeration below 4°C strictly prohibited to preserve sample integrity. The centralized hub will establish and manage its own shipping processes, tailoring logistical arrangements to its operational needs while maintaining the required standards for sample handling. Additionally, the centralized hub will determine and implement the turnaround time for the Merlin test, ensuring it aligns with local clinical needs and operational capacity.

The hub will also take responsibility for the reporting of test results, establishing a secure, accurate, and timely process to communicate findings to the ordering physician or designated contact. Therefore, the MAAS software is installed locally to allow timely reporting by the centralized pathology hub, thus there is no delay in reporting the sample result. Furthermore, the centralized hub will be responsible for managing data rights, storage, and access in compliance with GDPR and other applicable data protection regulations.

The diagnostic services with shipment melanoma samples to Rotterdam come with additional costs. Additional costs are related to the: i) shipment cost of the samples from any location in Denmark to the service location in Rotterdam and ii) the processing costs of the sample on top of the Merlin Kit that SkylineDx uses in its laboratory as well. When the Merlin Kits are purchased by the pathology hub from SkylineDx and used in the centralized pathology hub in Denmark itself, the centralized pathology hub only pays for the Merlin Kits and the related shipment costs (with less frequency and more kits at once) of the kits, and has to account for expenses related to own laboratory personal, reagents, materials etc. Since we are unaware of the costs of the central lab related expenses we cannot make this comparison.

7.2.2 General data protection regulation (GDPR)

Under the centralized pathology hub, SkylineDx's main priority is to deliver Merlin kits to the center performing the diagnostic service, and as such, no GDPR is required as this exchange does not include any patient confidential information or sensitive data. With regards to the diagnostic service in which the Merlin Assay is carried out in the laboratory in Rotterdam, appropriate technical and organizational measures have been put in place so that personal data processing will meet the requirements of the national and European Union laws and will ensure the protection of the patient's rights. In this process, the requesting site will share a TRF per patient and ship a tissue sample. The TRF will communicate a unique patient identification number and information on age and Breslow thickness to SkylineDx to allow for running the algorithm in relation to the test result. The final report will be shared via a secure portal (Egress) with the requesting physician or designated contact person. The provided patient identification number with the date of specimen collection will be communicated by SkylineDx on the test report. The patient will remain anonymous to SkylineDx. SkylineDx will not store, share, or use the samples and data for any other purposes, according to the GDPR directives. In addition, SkylineDx has a dedicated data protection officer (DPO). For any complaints, the DPO can be directly contacted via the following email address: dpo@skylinedx.com. The sample (50 µm of tissue, consisting of 5 to 10 recuts) that has been shared with SkylineDx will be destroyed.

7.2.3 Required training and skills

The procedure follows basic principles of science and diagnostic work-up, and it is not required to have additional accreditation or certifications to conduct the procedures described in the IFU, no special training or competences are required. Training of lab personnel can be carried out by SkylineDx and includes running the procedures with a dedicated lab personnel trainer and getting familiar with the procedures in the IFU.

7.2.4 Changes in workflow with Merlin

The implementation of Merlin in clinical practice would risk-stratify patients based on their Low or High Risk for nodal metastasis, in which patients identified as Low Risk may forgo SLNB surgery and Merlin High Risk patients should be considered for SLNB. An overview of the patient flow for standard of care and with the introduction of Merlin can be found in **Figure 16**.

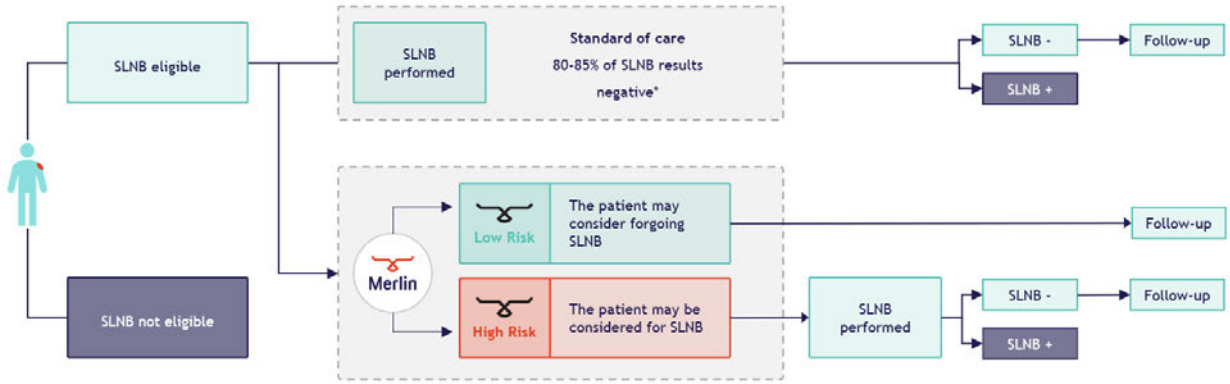


Figure 16. Patient flow after introducing Merlin compared to current clinical practice

To diagnose patients and understand their nodal involvement, multiple steps and hospital resources need to be considered. In 2020, a case study was published by Meves & Eggermont (6) in which a patient's journey was described. This study was carried out by following a patient who was enrolled at the Mayo Clinic. The study is fully described in section 7.1. The patient journey, as per the publication by Meves & Eggermont, was discussed with a plastic surgeon from a Danish perspective and resulted in the overview as described in **Table 17**. The procedure for Danish patients would follow the activities as described in the case study by Meves & Eggermont (6) in which a multidisciplinary team would be required using multiple resources of the hospital infrastructure. In addition, substantial time is associated with each patient case. In the case of Merlin this would be replaced with the pathway described in **Table 18**

Table 17. Overview of primary cutaneous melanoma patient journey in Denmark

Steps	Activity	Type of specialist(s), or other health care personnel	Time in hours*	Associated DRG codes
Visit 1	Melanoma diagnosis (Referred to department of plastic surgery from general practitioner or private Dermatologist + Plastic surgeon performs excision biopsy)	Plastic surgeon	30 min	09PR04
	Pathology of the excised biopsied tissue	Pathologist	Not available	-
Visit 2	Consultation (patient informed about the diagnosis and SLNB + planning of the SLNB)	Plastic surgeon	45-60 minutes	09MA98
Visit 3	Pre-operative anesthesiologic visit	Anesthesiologist	10-15 minutes	36PR06 (same DRG code for dynamic and static lymphoscintigraphy)
	Pre-operative plastic surgical visit	Plastic surgeon (trainee)	10-15 minutes	
	Lymphoscintigraphy (0-1 days before SLNB)	Nuclear medicine	45-60 minutes	

Visit 3-4	Pre-operative tracer injection (on the day of SLNB)	Nuclear medicine	15 minutes	05PR01
	Surgery (SLNB + wide local excision [WLE])	Plastic surgeon, anesthesiologist, nurse anesthesiologist, surgical assistant	1.5 hours*	
	Pathology (SLN+WLE)	Pathologist	Not available	-
	WLE, simple (e.g. primary excision biopsy with free margins)			-
	WLE, complex (e.g. primary excision biopsy without free margins)			-
	1 sentinel lymph node (SLN)			-
	Additional SLNs, per SLN			-
Visit 4-5	Consultation (patient informed about the SLNB result and further follow up)	Plastic surgeon	30 min	09MA98

**This was gathered as part of conversations with Herlev and Gentofte and is specific to that center and might not be reflective of the time associated per patient in other centers. DRG, diagnosis-related group; SLN, sentinel lymph node; SLNB, sentinel lymph node biopsy; WLE, wide local excision. ** if 2+ nodes need to be removed, surgery takes longer.*

Table 18. Overview of primary cutaneous melanoma patient journey in Denmark with Merlin Low Risk test result

Steps	Specific steps in clinical pathway	Type of specialist(s), or other health care personnel	Time in hours*	Associated DRG codes
1	Melanoma diagnosis (Referred to department of plastic surgery from general practitioner or private Dermatologist + Plastic surgeon performs excision biopsy)	Plastic surgeon	30 min	09PR04
2	Consultation (patient informed about Merlin result and further follow up) + WLE under local anesthesia	Plastic surgeon	45-60 min	09PR04
3	Biopsy results by letter	Plastic surgeon	15	65TE01
	Total		~1.5 hour	

*This was gathered as part of conversations with Herlev and Gentofte and is specific to that center and might not be reflective of the time associated per patient in other centers. DRG, diagnosis-related group; WLE, wide local excision.

7.3 Applicant's summary of the transferability of the research setting

The clinical evidence supporting Merlin has been generated through a robust body of research conducted across diverse geographic and organizational contexts, including both retrospective and prospective studies in Europe and the United States. These studies span academic centers, high-throughput dermatology practices, and multidisciplinary melanoma units, with many incorporating multicenter cohorts. This breadth of study design strengthens external validity and enhances the generalizability of findings across healthcare systems.

The Danish healthcare system, characterized by its publicly funded structure, centralized care coordination, and emphasis on standardized, evidence-based clinical pathways, shares several operational and structural similarities with healthcare systems in which the Merlin Assay has already been validated, most notably, the Netherlands in the context of a recent prospective trial. This parallel supports the generalizability of study outcomes to the Danish context.

Crucially, Denmark-specific evidence is also emerging. A recent validation study (Weitemeyer et al., 2025) (17) conducted within the Danish healthcare setting has confirmed the predictive performance of the Merlin Assay in Danish patients. This adds a layer of internal validity and supports the feasibility of integration into existing diagnostic and treatment pathways. Furthermore, logistical and organizational feasibility has already been demonstrated in Denmark. A collaboration with Herlev and Gentofte Hospital piloted the retrospective testing process for CP-GEP, including the shipment of biopsy samples to our laboratory in Rotterdam. The infrastructure established includes a standardized test requisition form and full compliance with GDPR for handling sensitive health data.

In summary, the Merlin Assay and the associated CP-GEP model demonstrate high potential for transferability to the Danish healthcare system. The consistency of performance across international studies, alignment with Danish clinical guidelines, and the presence of early Danish implementation efforts provide a strong foundation for further adoption. With the necessary adaptations in infrastructure, training, and policy alignment, the model could be effectively integrated into national melanoma care to support more efficient, patient-centered care.

8. Health economics

This section describes the health economic value of Merlin. It comprises literature obtained via the literature review in accordance with the Danish Health technology council as well as two health economic models to support this application to the Danish health Technology Council. The first is an existing published cost analysis. The second is a cost-effectiveness model which is specifically developed to assess the cost-effectiveness of Merlin in different contexts of use.

8.1 Existing (health) economic analysis

Overall, 20 references were screened for eligibility during title- and abstract phase, of which 17 were excluded. Subsequently, 3 references were screened in the full-test selection. During full-text selection, 2 references were excluded, leading to a total of 1 eligible publication. This publication describes a health economic analysis of Merlin and was published by Thao et al., in 2022 (28). This study was conducted in collaboration with the Department of dermatology of the Mayo Clinic Rochester (MCR) in the US. This cost evaluation comprised a decision tree comparing a no-testing strategy and Merlin testing strategy. A decision tree that was used for the model can be found in the annex, section 11.6. Primary cutaneous melanoma patients with T1 and T2 who underwent WLE and SLNB and controls with T1 only undergoing WLE at the MCR between 2007 and 2018 were included. Costs were inflated to 2021 U.S. dollars (USD). To understand the cost per patient, a scenario of patients undergoing SLNB and WLE was compared to a setting in which patients only underwent WLE. Costing data was retrieved from within MCR, converting internal costs to standardized costs (55). Other parameters that were used as input for this cost evaluation were: sensitivity, specificity, the rate of a positive SLNB, and the SLNB complication rate. For the T1b group, the Merlin strategy breaks even at a $7,350 price point. In the T1-T2 group breakeven is reached at a $4,600 price point. The higher breakeven price for the T1b melanoma group is explained by the higher SLNB reduction rate. Across all studies, payments from private insurers are much higher than Medicare payments for both hospital and physician services. Therefore, where Merlin's breakeven price point is calculated at $4,600 for the T1b-T2 melanoma patient group based on Medicare rates, the breakeven price point might be significantly higher in the private setting (28).

Table 19. Published health economic analysis on Merlin Assay (CP-GEP)

Author, Year	Country & setting	Analysis type	Patient population	Intervention/ comparator	Time horizon	Final outcome
Thao (2022) (28)	United States Healthcare perspective	Decision tree (See annex, section 11.6)	T1b + T2 primary cutaneous melanoma patients	Usual care versus Merlin testing	Up to 1 year due to definition Lymphedema complication	Break-even: T1: $7,350 T1-T2: $4,600

8.2 Health economic analysis

To assess the cost-effectiveness (more specifically the cost-utility) of Merlin testing compared to SLNB, a cost-effectiveness model was developed. The model has been established to evaluate the incremental cost-utility ratio of using Merlin testing as a rule-in test for SLNB versus the current standard of care in which all patients undergo SLNB. As clinical data shows, Merlin allows patients to forgo SLNB on the basis of a Low- Risk test result. The health economic analysis shows that Merlin can be cost-effective, with cost-savings and no or

minimal changes in QALYs. Implementing Merlin results in less financial resources to be used by the Danish healthcare system but will also lead to time saved as the process of SLNB requires multiple disciplines to work together (e.g., plastic surgeon, nuclear medicine, pathology).

The health economic analysis was performed using an individual level patient simulation model combining a decision tree and a health state model. The model is accessible online via a portal and has been coded as part of a backend and frontend. Model elements have been coded with R. Portal can be accessed via a link on: https://www.medipanalytics.com/. Below the specifications of the model are described. Please find an overview of the model specifications in **Table 20**.

Table 20. Model specifications

Item	Analysis element	Expert committee specifications	Element applied in the analysis	Elaborated in section
1	**Time horizon**	Must be long enough to include relevant effects and costs. For relapse: after the longest possible follow-up time For intermediate effect measure: equivalent to an investigation course incl. management of complications after SNB	Lifetime horizon (50 years). At this point in time, most patients have been deceased	8.2.3
2	**Intervention**	Intervention: Merlin to qualify potential SLNB	Merlin result followed by SLNB if the test result is "High Risk"	8.2.2
3	**Comparator(s)**	Comparator: Current standard practice in the form of SLNB cf. current clinical guidelines	SLNB	8.2.2
4	**Analysis method**	Cost Effectiveness Analysis results must be presented separately for each subgroup as per the PIROT	A base-case analysis has been carried out for patient with primary cutaneous melanoma pT1a-pT2b Separate analysis have been carried out for: • pT1a • pT1b • pT2a • pT2b	8.2.6
5	**Outcome measure**	Primary analysis with patient-relevant efficacy measures: Relapse section 4.2.1 Secondary analysis with intermediate efficacy measure: Overlooked metastatic case (false negative findings)	The current model includes relapse-free survival, distant-metastatic free survival and overall survival, including patients that have a false negative finding	8.2.6
6	**Method of data extrapolation, if relevant**	Carried out to the relevant extent of the Treatment Council's technical appendix and the Medical Council's guidance on the use of course data in health economic analysis	It was assumed that a plateau in the mortality risk would occur after a period of 15 years as this was shown in the data	8.2.5.6

7	**Analysis perspective**	Limited societal perspective	Although this might substantially impact on cost considerations, societal costs have not been considered as they did not fit the current framework of the model. Societal costs have been considered in the budget impact model	8.2.7.3
8	**Minimum cost components to be estimated**	Costs should include but are not limited to: • Technology costs (Merlin and any software, updates, licenses, etc.) • Costs associated with the use of the Merlin Assay, including analysis and dissemination of the test findings • Costs associated with training in the use of the Merlin Assay • Costs associated with the SLNB procedure • Costs associated with re-excision (wide local excision • Costs associated with treatment of complications after the SNB procedure, including seroma, infection, lymphoedema etc. • Transport and time for patients associated with treatment.	The following costs have been considered: • Merlin acquisition cost / cost diagnostic service • SLNB costs • WLE costs • Cost of complications • Cost of immunotherapy following SLNB outcome	8.2.7.3
9	**Sensitivity analysis that should be carried out as a minimum***	One-way analysis where the minimum and maximum values of all input estimates are used to test their impact on the base case result Scenario analysis where Merlin 's diagnostic goals are changed to reflect best/worst case scenarios for the test's diagnostic accuracy on all included subgroups Scenario analysis where the complication rate for the SLNB procedure is changed to reflect the best/worst case Scenario analysis(s) which reflect different assumptions about future treatment effects if the decision model includes patient-relevant effects of subsequent treatment	One-way sensitivity analysis was carried out, changing all (non-zero) values by ±20% to test their impact on the base-case results and per substage. Results have been presented in tornado diagram format	8.2.8.2

Analysis elements included in the health economic analysis as stated in the expert committee's evaluation design and the analysis elements used in the health economic analysis completed in this application.

8.2.1 Patient population

The patient population for this analysis consists of individuals diagnosed with primary cutaneous melanoma, with T1a-T2b melanoma, who meet the following inclusion criteria: eligible for SLNB according to the Danish guidelines. The model assumes that patients have no prior SLNB. The patient population reflects a representative sample of the population likely to receive the Merlin within Denmark. As per the request of the DHTC, the analysis is subdivided into an analysis for the t1a-t2b patients and sub-analysis per substage.

Hence, SkylineDx has evaluated T1a-T2b patients with primary cutaneous melanoma and the following substages:

- T1a
- T1b
- T2b
- T2a

In addition, there are patients who are not eligible for SLNB due to frailty, comorbidities, pregnancy or regions that are difficult to operate such as the iliac region or head and neck cutaneous melanoma. These are currently not considered in the model. However, Merlin would offer a robust alternative for these patients that for any specific reason are not able or cannot undergo SLNB but would be eligible.

8.2.2 Intervention and comparator(s)

The intervention under consideration is Merlin (CP-GEP) which is intended to provide more insight into the tumor's aggressiveness and provides a binary outcome, either a Low Risk or High Risk for nodal metastasis. In the current setting, SkylineDx anticipates that when patients have a Low-Risk Merlin classification, they forgo the SLNB, whereas if a High-Risk classification is retrieved patients would undergo SLNB for further assessment of the lymph nodes. Subsequently, a positive lymph node is an indication for immunotherapy. The comparator is the current standard of care, consisting of carrying out SLNB in all patients. This corresponds with the Danish guideline which states that patients who are suspected of nodal metastasis should undergo SLNB. In addition, a WLE is performed to remove the melanoma using specific margins from the primary site. In the case of forgoing SLNB, a WLE would still need to be carried out.

8.2.3 Time horizon

The time horizon for this analysis is 50 years, chosen to capture both short-term and long-term effects of Merlin on patient outcomes, as well as the associated costs. A time horizon of 50 years is considered sufficient to assess the full impact of Merlin as most patients have deceased after that period. The cycle length of the model is 1 year. Costs and benefits realized are discounted at an annual rate of 3.5%.

8.2.4 Analysis structure

Using the model, the change in costs (Δ costs), the change in quality-adjusted life years (Δ QALY), and the incremental cost-effectiveness ratio (ICER) of the strategy with Merlin against the standard of care is calculated. Data for Merlin, serving as input for the model, is based on data from the MERLIN_001 clinical trial, the largest Merlin trial to date.

8.2.4.1 Data from seven studies including a clinical trial

Input data used in the health economic model came from a whole cohort analysis based on seven validation studies as described in **section 5.2.3** and shown in **Table 38**. The rationale for using the whole cohort analysis including the Danish retrospective study (17) is that it provides the largest samples set with least bias since it combines retrospective and prospective data from various European countries and the USA. Included in the data set is the largest prospective clinical trial data set for gene expression profiling in melanoma to date with 1,701 enrolled patients. furthermore, given that the Danish Health Technology Council requested analysis per substage and Merlin's added value is considered most optimal in T1-T2 melanomas, it is preferred to use all data available to obtain the largest sample size. In all clinical validation studies, Merlin shows consistent performance across different countries and their respective patient pathways. Additionally, clinical trials that prospectively enroll, and conduct specimen collection, processing in real time are considered highest level of evidence for tumor marker studies (33).

8.2.4.2 Health economic model

The Health Economic model was developed by MedipAnalytics and accessible using the "CEA-melanoma" application. This application allows the user to define the Standard of care and the strategy in which Merlin is used. Furthermore, model input like patient characteristics or costs can be adjusted in the software to match multiple settings in which Merlin is used. These model inputs are configured as a JSON file and sent to the cost-effectiveness model. The underlying model is constructed in R (version 4.4.2; R Foundation for Statistical Computing) which simulates individual patients in a microsimulation health-state model. The model is structured in three parts, including a decision tree (part 1&2) and a health state model (part 3):

1) In the First part, the “true” disease status is simulated for each individual patient (see **Figure 17**).
2) Based on the true disease status (from part 1) and the sensitivity and specificity of SLNB and Merlin (± SLNB) diagnostic outcomes are simulated in the second part of the model. Patients who have positive nodes (Stage III) can either be detected and correctly classified as Stage III or can be missed and therewith under classified as Stage IA-IIb/c (the clinical stage). Patients who do not harbor positive nodes undergo an unnecessary SLNB or no SLNB (see **Figure 18**)
3) In the third part of the model, the long-term consequences of the diagnostic outcomes (from part 2) are simulated. Patients without positive nodes start in their respective stage (IA – IIb/c) and can stay in this health state or can progress to regional metastases or distant metastases disease over time. Patients with positive nodes start in either the treated stage III or missed stage III, which defines their risk of developing distant metastases over time. Treatment for stage III occurs with immunotherapy (monotherapy). Some patients will potentially receive more extensive follow-up instead of monotherapy. To be conservative regarding the value of Merlin we assumed that these patients will have the same oncological outcomes as patients treated with monotherapy. Patients with metastatic disease can die from the disease (metastasis-related death). Patients can also die due to other reasons from every health state in the model. (see **Figure 19**)

For the results described in this report, 500,000 individual patients were sampled for each analysis. For each patient, associated costs, life years, and utility values are calculated. After running the 500,000 samples, the results are averaged and compared to a scenario where only SLNB is performed.

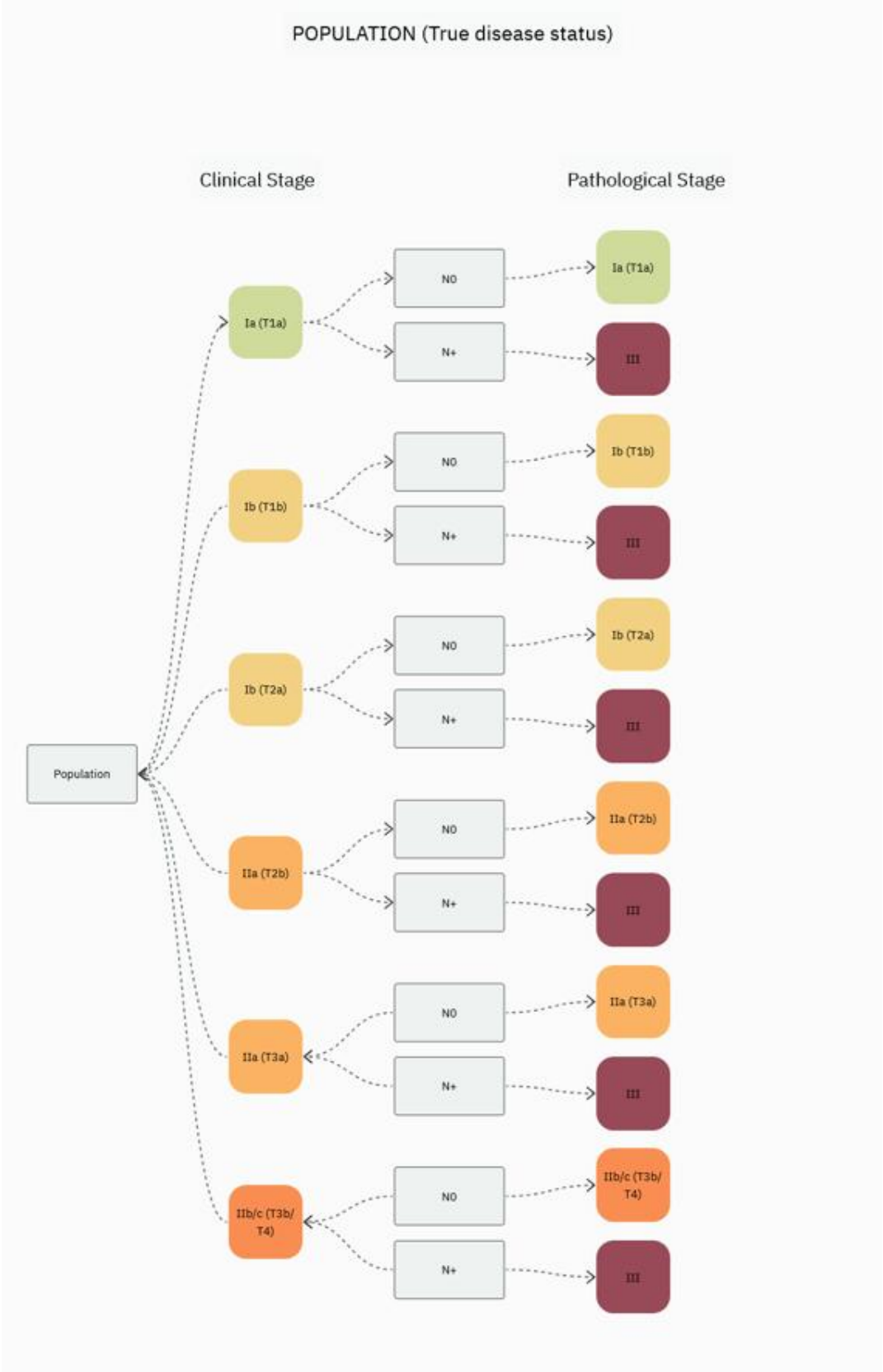
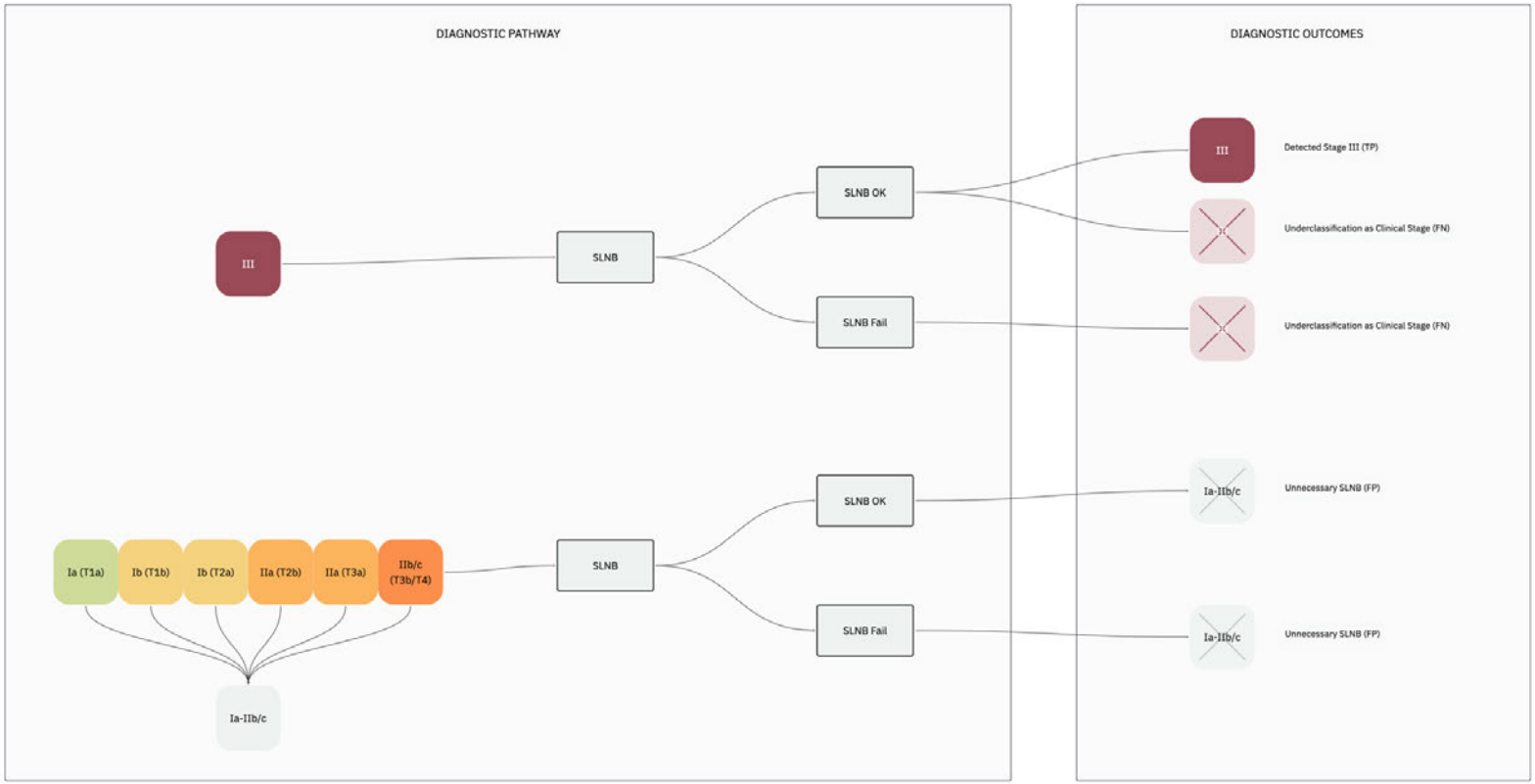


Figure 17. Calculation of the true disease status per primary cutaneous melanoma patient


DIAGNOSTIC PATHWAY
III
SLNB
SLNB OK
SLNB Fail
Ia (T1a)
Ib (T1b)
Ib (T2a)
IIa (T2b)
IIa (T3a)
IIb/c (T3b/T4)
SLNB
SLNB OK
SLNB Fail
Ia-IIb/c
DIAGNOSTIC OUTCOMES
III
Detected Stage III (TP)
Underclassification as Clinical Stage (FN)
Underclassification as Clinical Stage (FN)
Ia-IIb/c
Unnecessary SLNB (FP)
Ia-IIb/c
Unnecessary SLNB (FP)

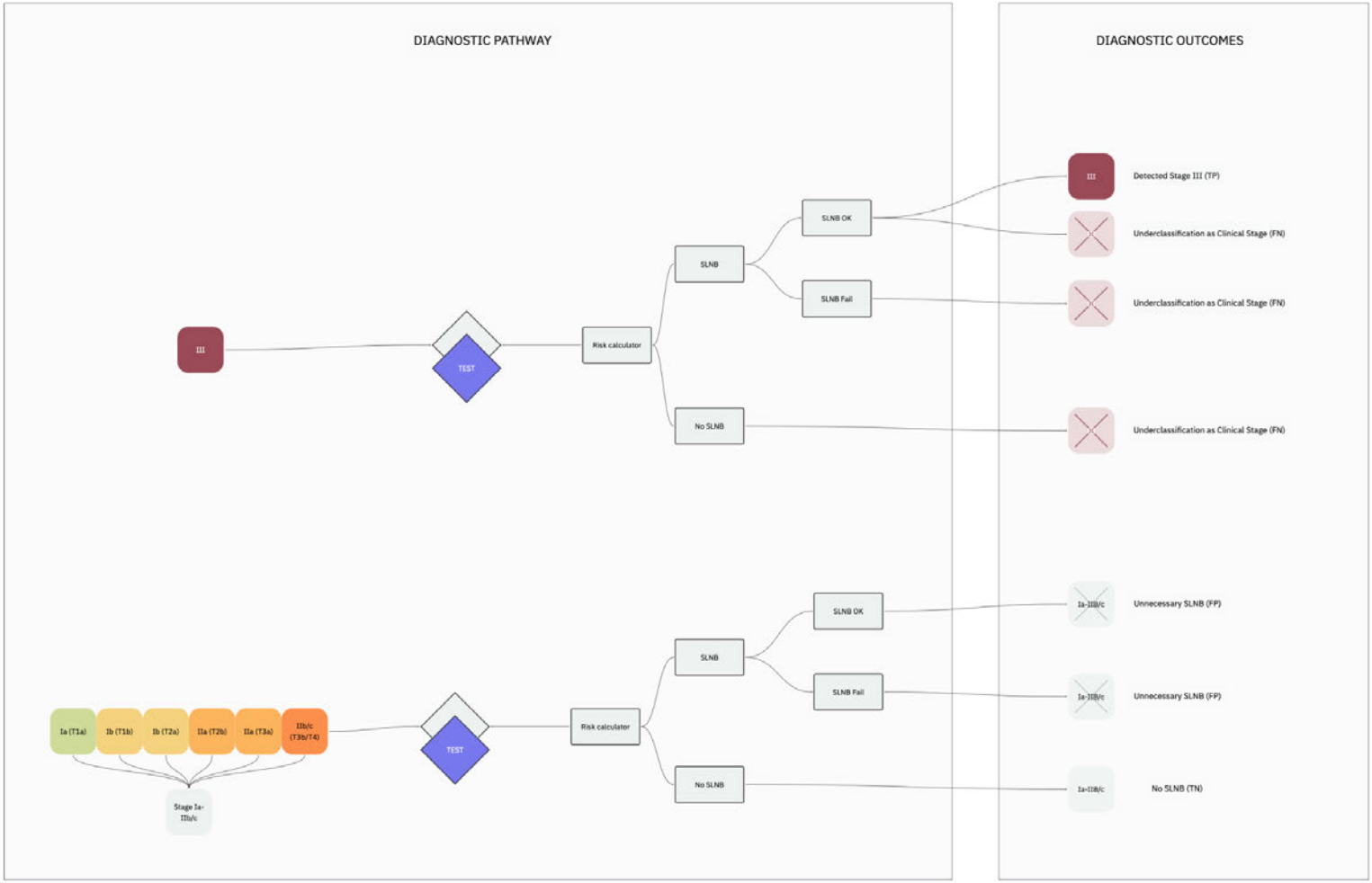


Figure 18. Diagnostic pathway and associated diagnostic outcomes for standard of care and pathway when using the Merlin Assay.

*SLNB fail is defined as the unsuccessful mapping of the sentinel node, meaning no sentinel node was detected and therefore no sentinel node could be removed and assessed.

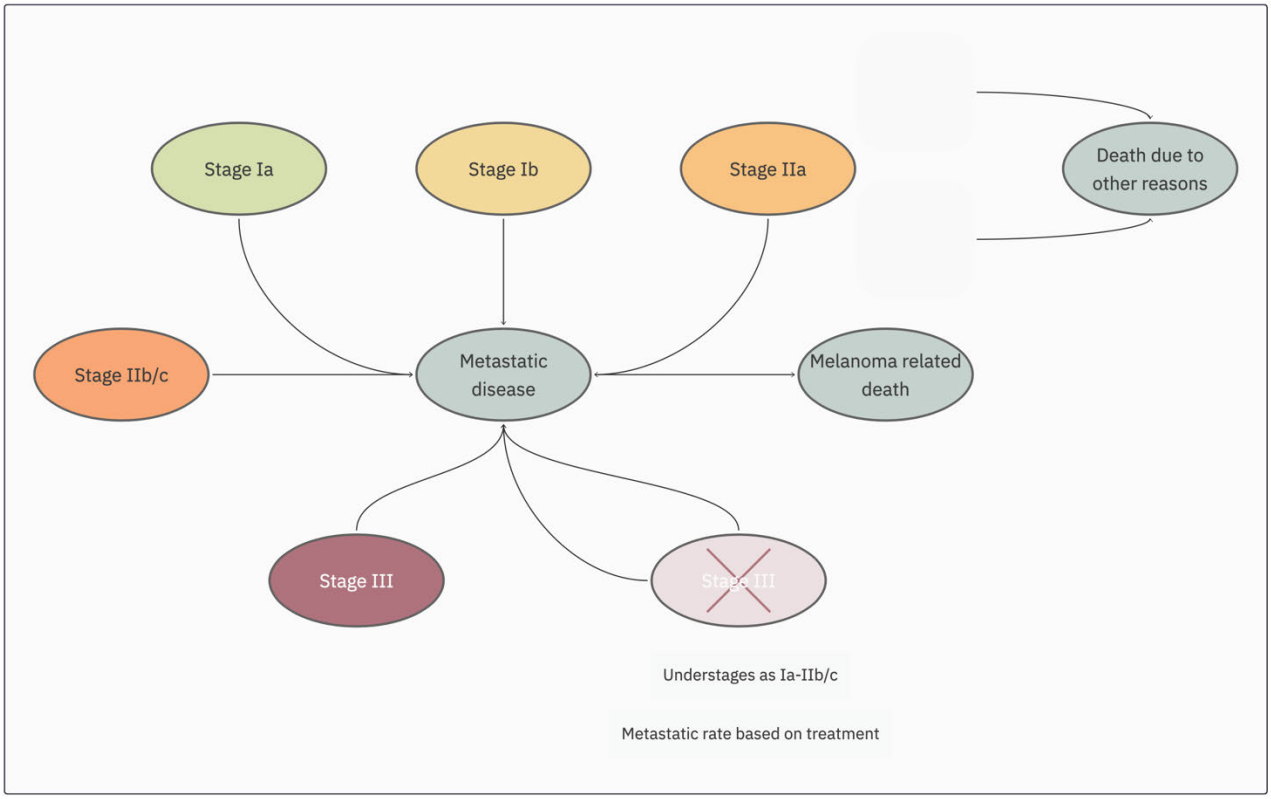


Figure 19. Health status used in the cost-utility model

8.2.4.2.1 Model validation

The clinical patient pathway and corresponding codes have been externally validated by a clinician experienced with these codes. Additionally, pathology codes have undergone further validation by a pathology expert using a point system. Assumptions related to the treatment regimen, WLE, and the interpretation of changes in billing have been reviewed and discussed with an expert. Internal validation of the model was performed based on scenario testing, including extreme value testing.

8.2.5 Probability data

Data regarding probabilities in the model include the probability of node involvement (per stage), sensitivity and specificity of Merlin, adverse event rates after SLNB, Distant metastases rate per stage with and without additional therapy, as well as the risk of mortality after obtaining metastasis. These probabilities were obtained from different sources, five retrospective studies and two prospective studies including the MERLIN_001 clinical trial, a complication paper by Hieken, and data from major clinical trials for progression and mortality. Distribution per substage was retrieved, as well as the identified number of positive nodes in Denmark.

8.2.5.1 Use of epidemiological data

To calculate a patient's disease status, distribution across different substages is needed. Additionally, lymph nodes in thinner melanomas tend to show less involvement compared to thicker melanomas. To assess lymph node status for these patients, data on node involvement from Danish primary cutaneous melanoma

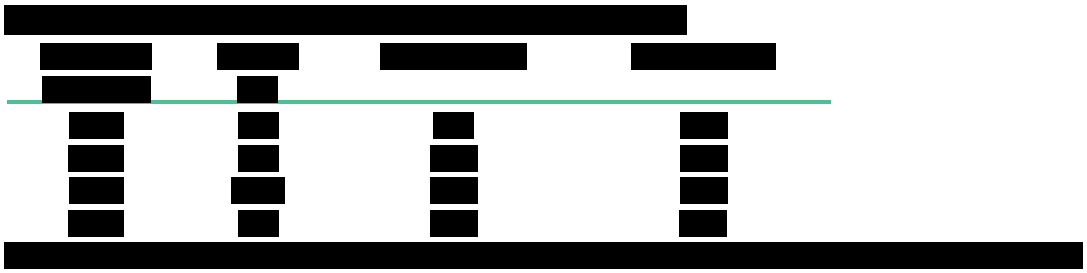
patients were utilized. When analyzing a specific substage (e.g., IA), the clinical stage distribution was set to 100%. The data was derived from the annual report of the Danish Melanoma Database. (7). During each cycle in the model, patients have a probability of dying due to causes other than Melanoma. This background mortality is based on the age dependent mortality of the Danish population (Danmarks Statistik).

Table 21. Clinical stage distribution and node involvement per substage

Substage	Clinical stage distribution	Node involvement	Source
IA (T1a)	15% (115)	2%	(7)
IB (T1b)	22% (167)	5%	(7)
IB (T2a)	58% (469)	10%	(7)
IIA (T2b)	5% (42)	22%	(7)

8.2.5.2 Use of data from clinical trials

Input data for the accuracy of Merlin came from a combined whole cohort analysis (unpublished, see **Table 38**) described in **section 5.2.3.** This whole cohort analysis included five retrospective studies (14–18) and two prospective studies were combined (12, 13) comprising of 2,577 T1-T2 patients. This data included the Danish retrospective patient cohort publication with 427 T1-T2 patients. The rationale to combine all available retrospective and prospective validation cohort was to obtain the most representative cohort with the least bias. In short, R was used with metaprop function for the whole cohort analysis (meta-analysis). To join the proportions from the individual studies we made use of a random intercept logistic regression model as previously described (54). We decided in favor of the random intercept model because each study is based on a different cohort, from different hospitals and countries and the random intercept can account for cohort specific variations. Data from the combined analysis is used to set the model's sensitivity and specificity per substage (see **Table 22**).



Rates for most complications following SLNB were retrieved from a publication by Hieken et al., published as part of a study to understand the complication rate in Merlin Low Risk and High Risk patients (4). Alternatively, other papers were identified highlighting the complication rate after SLNB (5,7,56,57). These factors will be considered in the sensitivity analysis. Other side effects that have not been considered but were identified through literature are postoperative pain, suture granuloma, and myocardial infarction. Removal of an increased number of lymph nodes (2 or more), increases the chances of complications. Additionally, this study showed that especially patients who undergo parotid SLNB have a higher chance of complications (5). See **Table 23** for an overview of identified SLNB complication rates.

Table 23. Adverse event rates after SLNB

Parameter	Adverse effect, %	Source
First-year side effect:		
Nerve injury	1%	(58)

Seroma	9%	(4)
Hematoma	3%	(4)
Infection	5%	(4)
Lymphedema	4%	(4)
Long-term side effects		
Lymphedema	2%	(59)

SLNB, sentinel lymph node biopsy

8.2.5.3 Use of proxy outcome measures

See the section on cost statement.

8.2.5.4 Transformation of data

To model background mortality, transforming these into survival estimates, conversion to probabilities is required. The formula that was used is 1 – $EXP^{(-rate)}$. Herein, the rate represents the mortality rate per unit of time (e.g., per year) to estimate the probability of surviving each subsequent year. As such, as per the formula, mortality rates are transformed into probabilities for survival for the provided time horizon.

8.2.5.5 Changes in probabilities over time

Not applicable

8.2.5.6 Extrapolation of data

For the data regarding the risk of regional metastases and distant metastasis, survival data from peer-reviewed publications of large melanoma trials were selected (60–62). Data was split for stage I/IIA, stage IIB/C, and stage III. Data regarding regional and distant metastases were available for 5 years. An overview of the identified risks is provided below (see **Table 24**).

Table 24. Survival rates per stage and with or without therapy

Parameter & substages	1-year	2-year	5-year	Source
Risk of regional metastasis				
Stage IA/B	0%	1%	3%	(60)
Stage IIA	3%	6%	11%	(60)
Stage IIB/C				
No treatment	4%	8%	19%	(61)
Monotherapy	3%	6%	16%	(61)
Risk of distant metastasis				
Stage IA/B	0%	1%	2%	(60)
Stage IIA	4%	7%	13%	(60)
Stage IIB/C				
No treatment	5%	10%	26%	(61)
Monotherapy	3%	6%	9%	(61)
Stage III				
No treatment	25%	34%	43%	(62)
Monotherapy	14%	20%	28%	(62)

Additionally, the presence of regional or distant metastasis significantly reduces a patient's survival. To model this effect, the cumulative mortality risk associated with metastasis was considered (63). Mortality data from Wolchock et al. provided follow-up data for the first 10 years. The 15-year mortality was extrapolated using a generalized gamma distribution, see supplementary document 1 on extrapolation of metastasis-related mortality. It was assumed that a plateau in the mortality risk would occur after a period of 15 years, as was shown in the data, see

Table 25.

Table 25. Cumulative mortality risk after metastasis

Parameter & substages	1-year	2-year	5-year	10-year	15-year	Source
Cumulative mortality risk with metastasis	25%	35%	48%	57%	62%	(63)

8.2.6 Measurement of outcomes

This model evaluates several key outcomes, including the average lifetime cost and quality-adjusted life years (QALYs) per patient. When applicable, incremental cost-effectiveness ratios (ICERs) are calculated between the strategy including Merlin and the current practice. QALYs are used as a measure of offering a comprehensive evaluation of overall health. This measure not only accounts for the quantity or duration of life but also the quality of life during that period, factoring in the potential negative side effects of complications, treatments, and misdiagnosis. The main goal of the analysis is to determine the QALY gain or loss, while also considering the economic impact of combining Merlin ± SLNB compared to SLNB alone.

8.2.6.1 Cost-consequence and cost-effectiveness analysis

Not applicable

8.2.6.2 Cost-utility analysis

A cost-utility analysis (CUA) was conducted regarding Merlin ± SLNB compared to SLNB alone for patients with primary cutaneous melanoma in T1-T2. The analysis was performed from the perspective of the Danish healthcare system, incorporating costs and health outcomes that are relevant to the Danish context (if available).

8.2.6.2.1 Health-related quality of life for complications and different health states

The model uses a base utility for patients from which disutility values are subtracted based on the stage of melanoma and potential side effects of SLNB. The base utility in the model was based on the age dependent EQ-5D utility reported in Hvidberg et al. (64). A search of the literature was conducted to identify relevant disutility values for patients with primary cutaneous melanoma. Four studies were identified: Morton et al., 2017 (59), Gjorup et al, 2017 (65), Tran et al., 2018 (66), and Bensimon et al., 2019 (67). Disutility for complications was taken from a paper published by Morton et al. and describes mean weighted EQ-5D values for patients with stage I/II melanoma and postoperative complications. Given that this study was a cross-sectional study, no baseline EQ-5D data were available. To calculate disutility, the highest utility value for complications was taken and considered the baseline for calculating disutility for infection, seroma, and nerve injury (limb trauma). Alternatively, another approach could exist in using baseline EQ-5D data generated from another publication and using that to calculate the disutility of complications. However, this approach would lead to larger differences compared to the first approach, and as such, the first approach would be more conservative and was used for this cost-utility model.

8.2.6.2.2 Data basis for the impact on health-related quality of life

Please find an overview of the different health states and events and associated (decremental) utility in **Table 26**.

Table 26. List of (decremented) utilities associated with health states and events

Health state/event	(Dis)utility	Instrument and value set	Mapping used? yes/no	Comment	Ref.
Age 16 - 24	0.902	EQ-5D	No	Danish population-based estimates of EQ-5D (64)	
Age 25 - 34	0.893	EQ-5D	No		
Age 35 - 44	0.871	EQ-5D	No		

Age 45 - 54	0.839	EQ-5D	No		
Age 55 - 64	0.833	EQ-5D	No		
Age 65 - 74	0.832	EQ-5D	No		
Age 75+	0.749	EQ-5D	No		
Stage 1	-0.03	Mixed	No	Systematic review &	(66)
Stage 2	-0.03	Mixed	No	meta-analysis of	(66)
Stage 2b	-0.03	Mixed	No	studies in melanoma	(66)
Stage 3	-0.23	Mixed	No		(66)
Metastases	-0.24	Mixed	No		(66)
Lymphedema	-0.04	EORTC-QLQ-C30	Yes (68)	Difference in EORTC-QLQ-C30 scores in patients with and without lymphedema	(65)
Infection	-0.04	EQ-5D	No	Mapping used in the	(59)
Seroma	-0.03	EQ-5D	No	study itself (Cheung	(59)
Nerve Injury	-0.05	EQ-5D	No	2009)	(59)
Hematoma	-0	EQ-5D	No	Assumption, baseline	(59)
No additional treatment	-0			Assumption	
Monotherapy	-0.074	EQ-5D	no	KEYNOTE-054	(67)

EORTC-QLQ-C30, EORTC (European Organization for Research and Treatment of Cancer) Core Quality of Life questionnaire; TTO, time-to-Trade Off.

8.2.7 Cost statement

The cost statement includes all relevant direct costs associated with the intervention and comparator, including diagnostic costs (WLE and SLNB), Merlin acquisition costs, cost of immunotherapy, cost of (cancer) death, and cost of complications due to SLNB. The costs for the intervention and comparator were derived and composed of a combination of interactive diagnosis-related groups (DRG) as listed on the following website:

https://sundhedsdatastyrelsen.dk/data-og-registre/sundhedsoekonomi/drg-gruppering/interaktiv-drg

8.2.7.1 Costs of using health technology and comparator(s)

To understand the costs associated with WLE and SLNB during the full trajectory of the patient, associated DRGs have been identified and summarized. Costs from the first melanoma diagnosis up until consultation about further follow-up or treatment plans would incur 26,539 kr. or 80.980 kr. depending on the length of stay in the hospital, with a higher price for patients staying longer than 12 hours. As per expert opinion, we assumed that approximately 10% of the patients would stay longer than 12 hours within the hospital. In contrast, if WLE is to be carried out without further pathological research, the cost it would incur would amount to 11,845 kr. For a full breakdown of the associated DRG codes, types of specialists involved, and time associated with specific activities for SLNB and WLE, see **Table 27** and **Table 28**, respectively. [redacted] As this would lead to separate discussions with external parties, such as ThermoFisher with variable prices, separate discussions would need to be held to agree on a price per kit. The laboratory hub would agree to purchase the kit directly from SkylineDx.

Table 27. Breakdown of the costs and corresponding to DRG codes and pathology for SLNB

Steps	Specific steps in clinical pathway	Type of specialist(s), or other health care personnel	Time in hours	Associated DRG codes	2025 cost (DKK)
1	Melanoma diagnosis (Referred to department of plastic surgery from GP or private Dermatologist OBS MM Plastic surgeon performs excision biopsy)	Plastic surgeon	30 min	09PR04	5,879 kr.
2	Consultation (patient informed about the diagnosis and SLNB + planning of the SLNB)	Plastic surgeon	45-60 minutes	09MA98	1,578 kr.
3	Pre-operative anesthesiologic visit	Anesthesiologist	10-15 minutes	36PR06 (same DRG	4,565 kr.
4	Pre-operative plastic surgical visit	Plastic surgeon (trainee)	10-15 minutes	code for dynamic and	
5	Lymphoscintigraphy (0-1 days before SLNB)	Nuclear medicine	45-60 minutes	static lympho-scintigraphy)	
6	Pre-operative tracer injection (on the day of SLNB)	Nuclear medicine	15 minutes	Included in SLNB codes	
7	Surgery (SLNB + wide local excision [WLE])	Plastic surgeon, anesthesiologist, nurse anesthesiologist, surgical assistant	1.5 hours*	36PR06 05PR01	8,374 kr. OR 62,815 kr.*
8	Consultation (patient informed about the SLNB result and further follow up)	Plastic surgeon	30 min	09MA98	1,578 kr.
	Total		HCP's ~9.5 hours	90% of the cases	<12 hours hospital stay after SLNB = 21,974
			Patient ~4.5 hours + 5 visits	10% of the cases	>=12 hours= 76.415**
					Weighted average = 27,418 kr.

*HCP, healthcare practitioner; SLN, sentinel lymph node; SLNB, sentinel lymph node biopsy; WLE, wide local excision; *Four different specialists would be required for this activity, and time is associated with removal of one lymph node, generally 1-2 lymph nodes are removed; **depends on length of hospital stay (<12 hours versus ≥12 hours).*

Table 28. Breakdown of the costs and corresponding to DRG codes and pathology for WLE

Steps	Specific steps in clinical pathway	Type of specialist(s), or other health care personnel	Time in hours*	Associated DRG codes	2025 cost (DKK)
1	Melanoma diagnosis (Referred to department of plastic surgery from general practitioner or private Dermatologist + Plastic surgeon performs excision biopsy)	Plastic surgeon	30 min	09PR04	5,879 kr.
2	Consultation (patient informed about Merlin result and further follow up) + WLE under local anesthesia	Plastic surgeon	45-60 min	09PR04	5,879 kr.
3	Biopsy results by letter	Plastic surgeon	15	65TE01	87 kr.
	Total		~1.5 hour		11,845 kr.

WLE, wide local excision

Generally, if a positive node is encountered after SLNB, patients would be eligible for immunotherapy. In Denmark, patients with stage IIIA would not be eligible for therapy. To understand the cost impact of Merlin on further immunotherapy in patients with primary cutaneous melanoma, the cost of immunotherapy was calculated using information from: https://medicinpriser.dk/. To estimate the full price, a patient's body weight of 80 kg was assumed using the following schedules for 1) Nivolumab; 240 mg every 2 weeks over 30 minutes or 480 mg every 4 weeks over 60 minutes or over 30 minutes (3mg/kg, EPAR) continued as long as clinical benefit is observed or until treatment is no longer tolerated by the patient (and up to maximum duration of therapy if specified for an indication). For adjuvant therapy, the maximum treatment duration with OPDIVO is 12 months. 2) Pembrolizumab; 160 mg/kg bodyweight (up to a maximum of 200 mg), every 3 weeks administered as an intravenous infusion over 30 minutes (2 mg/kg, EPAR) and should be administered until disease recurrence, unacceptable toxicity, or for a duration of up to one year. For a breakdown of the costs for Nivolumab and Pembrolizumab, see **Table 29**. For the model, we assumed that patients were treated with pembrolizumab based on expert opinion amounting for: 749,953 kr. As not all patients would receive immunotherapy due to frailty or comorbidities or are categorized as stage IIIA, we assumed that 25% would not receive immunotherapy as per discussions with experts.

Table 29. breakdown of the costs of immunotherapy & administration

Immunotherapy	Price per vial	Per dose	Dose per patient	Total dose per year	Total price	Source
Nivolumab	20,457.13 kr.	240 mg	480 mg per 4 weeks	6,240 mg	531,885 kr.	Link
Pembrolizumab	21,573.58 kr.	100 mg	400 mg per 6 weeks	3,467 mg	747,884 kr.	

Immunotherapy	Price per administration	Number of administrations per year	Yearly price of admin	Total price	Source
Nivolumab	477.43 kr.	13	6,207 kr.	538,092 kr.	EPAR
Pembrolizumab	238.72 kr.	8.67	2,070 kr.	749,953 kr.	EPAR

Position	Effective hourly salary	Source
Ikke-ledende sundhedsplejersker (non-executive nurse)	**477.43**	**krl**

8.2.7.2 Costs associated with health conditions and events

Costs for adverse effects were estimated for complications following SLNB and costs of adverse events following immunotherapy. Find an overview of the costs for specific adverse events in **Table 30**. Specific Danish costs for complications were not identified. As such, complication costs from the UK for infection and lymphedema were considered. To ensure that costs were not driving model's model outcomes we kept complication costs relatively low.

In addition, costs associated with cancer death and with death were retrieved from a recently published paper by Ten Ham including Danish cost data, authors estimated that cost associated with death was €1577, converted to 12,918.97 kr. adjusting for inflation. In addition, we assumed that if a patient was identified that required immunotherapy due to regional or distant metastasis additional costs would be incurred for immunotherapy of 749,953 kr.

Table 30. Complication costs

Adverse effect	Cost year	Country of origin & currency	Original cost	Converted cost	Source
Infection	2019	United Kingdom, £	£333.76	4298.58 kr.	(69)
Seroma	2019	United Kingdom, £	£333.76	4298.58 kr.	Proxy (69)
Hematoma	2019	United Kingdom, £	£333.76	4298.58 kr.	Proxy (69)
Lymphedema	2021	United Kingdom, £	£723.00	8788.68 kr.	(70)

Costs were converted using the following tool: CCEMG – EPPI-Centre Cost Converter. Accessed at: https://eppi.ioe.ac.uk/costconversion/default.aspx

8.2.7.3 Limitations of the cost statement

Complication costs were not obtained using Danish sources, instead these were converted to Danish Krone. The model does not allow for a breakdown in societal costs; these costs have been considered in the budget impact model instead. Societal costs are not deemed a major driver for this model but might favor implementation of Merlin, as foregoing SLNB based on a Merlin Low Risk test result could lead to less hospital

visits, less appointments required and less complications that occur. These events could impact productivity of patients. The budget impact model considers some of these costs from a patient perspective. Other costs that have not been considered but might affect the model are costs for adverse effects due to immunotherapy.

8.2.8 Result of the health economic analysis

8.2.8.1 Base case results

Base case results are presented for T1a-T2b primary cutaneous melanoma patients. In addition, sub-analysis have been performed on the following substages:

- pT1a
- pT1b
- pT2a
- pT2b

Merlin was found to be dominant, with an incremental QALY gain of [redacted] and cost savings of [redacted] per patient compared to the comparator. This indicates that the innovation provides small health benefits at a lower cost in t1a-t2b primary cutaneous melanoma patients.

Table 31. Cost-effectiveness base-case results Merlin testing strategy

Population	Intervention	Total cost, DKK	QALY	ΔC, DKK	ΔE	ICER	Statement of dominance
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]

Please note that there might be minor discrepancies due to rounding

Merlin was found to be dominant in specific substages of t1a, t1b and t2a, with an incremental QALY gain ranging from [redacted] and cost savings from [redacted] per patient compared to standard of care. This indicates that the innovation provides small health benefits at a lower cost in t1a-t2a primary cutaneous melanoma patients. See **Table 32** for an overview of the aggregated and disaggregated costs and QALY's.

In patients with t2b primary cutaneous melanoma, the intervention was associated with [redacted] Of additional costs, compared to the comparator along with a slight decrease in effectiveness, with an incremental QALY of [redacted] See **Table 32** for an overview of the aggregated and disaggregated costs and QALY's.

[redacted]. These marginal variations may reflect model uncertainty or minor differences in patient outcomes that do not translate into meaningful health gains. Despite this, the intervention was associated with substantial cost savings per patient, suggesting potential economic value. From a health system perspective, such cost reductions may support the adoption of the intervention, particularly in a resource-constrained setting. [redacted]

[redacted]

Please note that there might be minor discrepancies due to rounding

8.2.8.2 Sensitivity analysis

A sensitivity analysis was conducted to assess the robustness of the base-case cost-effectiveness results by varying key model parameters within plausible ranges by ±20%. The results are presented in the form of a tornado diagram, which illustrates the impact of each parameter on the incremental cost impact and the incremental QALY impact. Please find an overview of the sensitivity analysis per group and substages in the following sections:

- Sensitivity analysis T1a-T2b — Section 8.2.8.2.1
- Sensitivity analysis T1a — Section 0
- Sensitivity analysis T1b — Section 8.2.8.2.3
- Sensitivity analysis T2a — Section 8.2.8.2.4
- Sensitivity analysis T2b — Section 8.2.8.2.5

8.2.8.2.1 Sensitivity analysis T1a-T2b

The tornado diagram ranks parameters in descending order of influence in terms of incremental costs and incremental QALY's, with the most influential variables appearing at the top. Each bar represents the change in that specific parameter, while holding all other inputs constant.

The three main drivers in this model are the sensitivity of Merlin in T2a patients, the cost of SLNB and the cost of Merlin: Varying this parameter within its plausible range had the largest impact on costs (**Figure 20**). In terms of impact on QALY, the main driver in the model is metastases: stage 3 WE value (year 5) and sensitivity of Merlin in T2a patients (**Figure 21**). In contrast, parameters such as disutility metastases (yearly) had a minimal impact on QALY suggesting that uncertainty in these inputs does not materially alter the conclusions of the base-case analysis. Across all tested scenarios, Merlin continued to generate net cost savings. In addition, a small but consistent health benefit was observed, measured in QALYs, even when input values were set to be unfavorable. Based on these findings, Merlin may be considered cost-effective for these scenarios in patients with T1a-T2b primary cutaneous melanoma within the context of the Danish healthcare system.

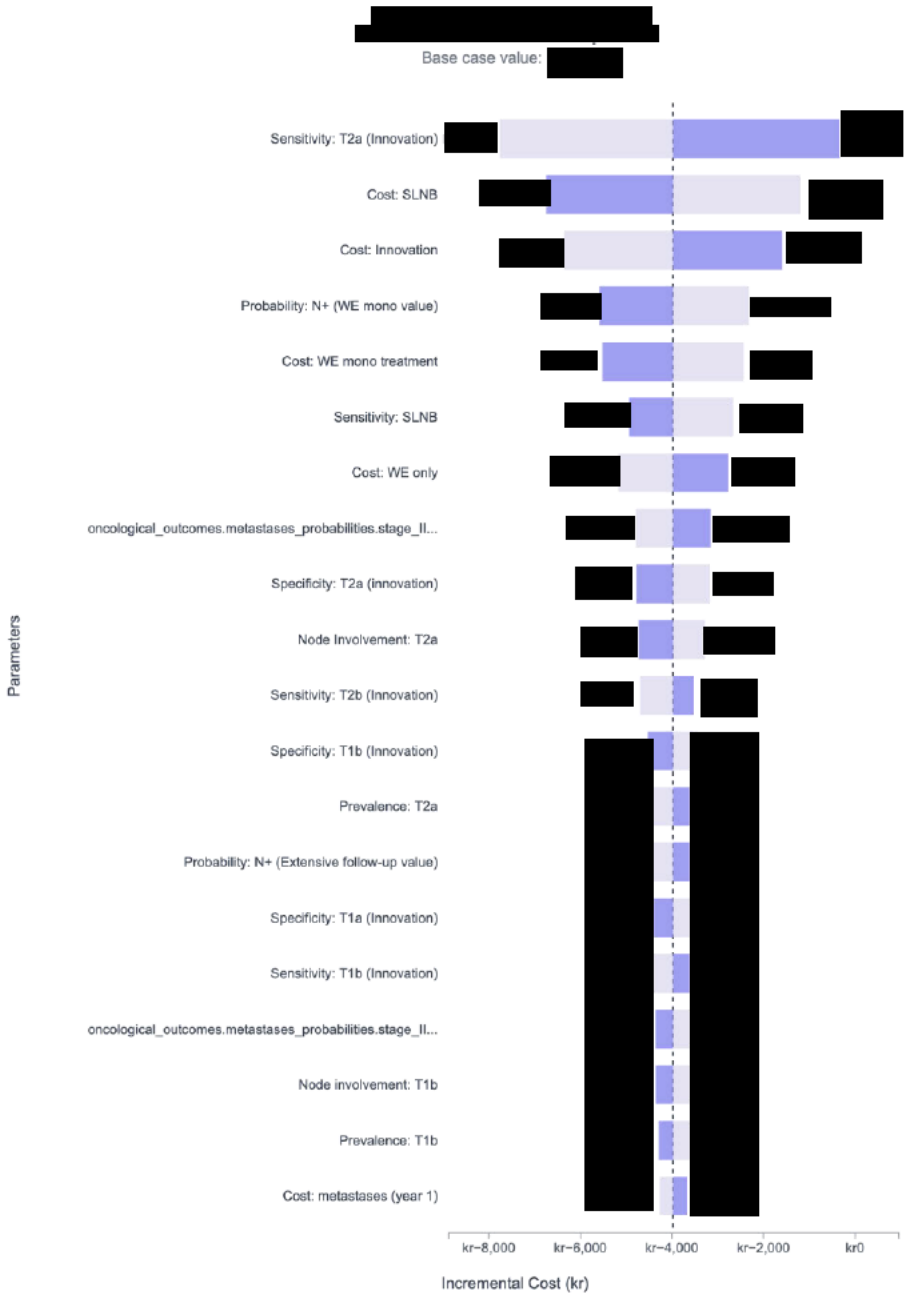


Figure 20. Incremental costs following sensitivity analysis in t1a-t2b primary cutaneous melanoma patients. Increase of parameter (light colour), decrease of parameter (dark colour).

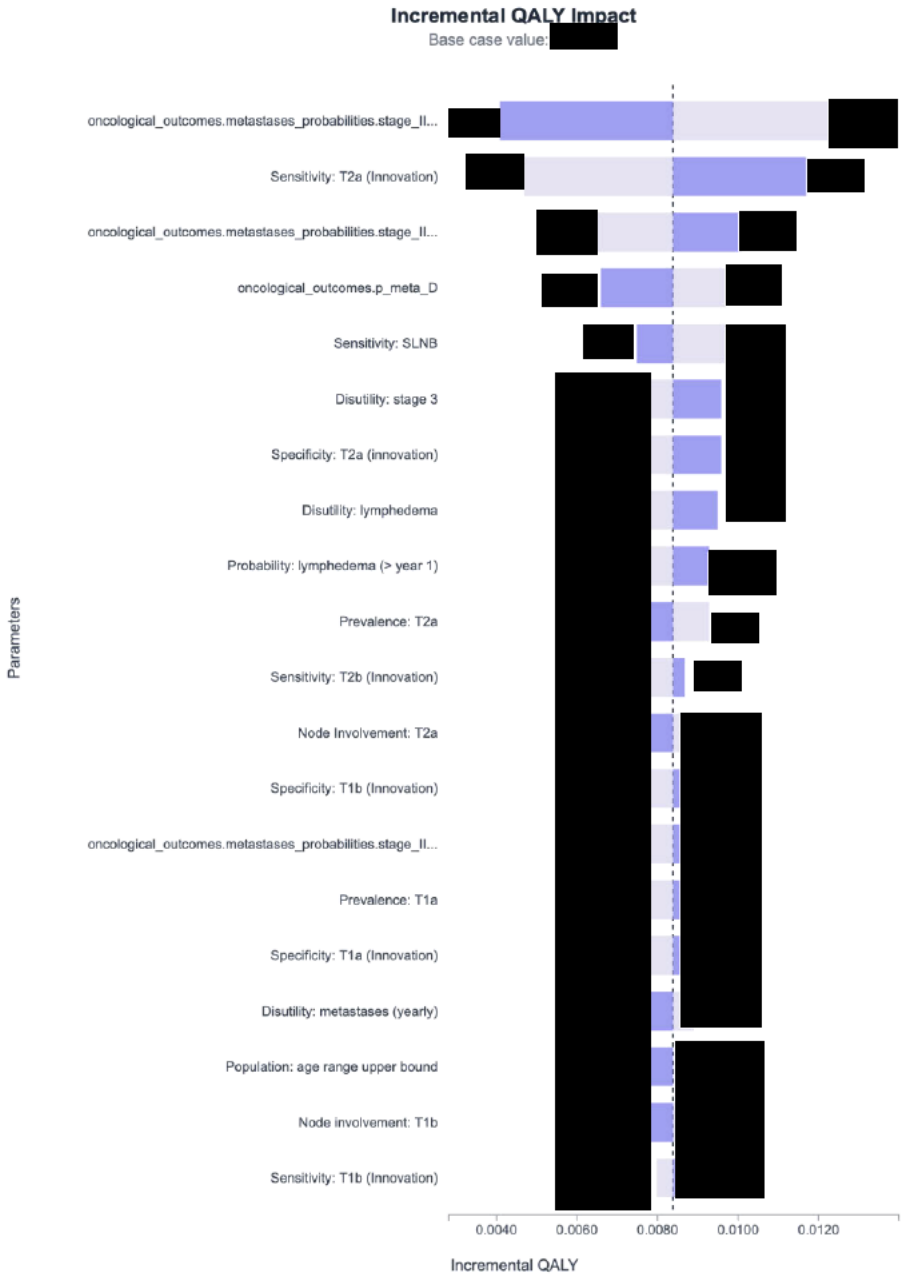


Figure 21. Incremental effectiveness following sensitivity analysis in t1a-t2b primary cutaneous melanoma patients. Increase of parameter (light colour), decrease of parameter (dark colour).

8.2.8.2.2 Sensitivity analysis T1a

The tornado diagram ranks parameters in descending order of influence in terms of incremental costs and incremental QALY's, with the most influential variables appearing at the top. Each bar represents the change in that specific parameter, while holding all other inputs constant.

The main drivers in this model are the cost of SLNB, the specificity of Merlin in T1a patients, the cost of Merlin and the cost of WLE: varying this parameter within its range had the largest impact on costs (**Figure 22**). In terms of impact on QALY, the main drivers in the model are the specificity of Merlin in T1a patients, and the population age range upper and lower bound as well as the disutility of lymphedema and probability after 1 year (**Figure 23**). In contrast, parameters changing the probability or disutility of several complications had a minimal impact on QALY suggesting that uncertainty in these inputs does not substantially modify the conclusions of the base-case analysis. Across all tested scenarios, Merlin continued to generate net cost savings, except in a scenario where the cost of SLNB was decreased by 20%. In addition, a small but consistent health benefit was observed, measured in QALYs, even when input values were set to be unfavorable. Based on these findings, Merlin may be considered cost-effective for these scenarios in patients with T1a primary cutaneous melanoma within the context of the Danish healthcare system.

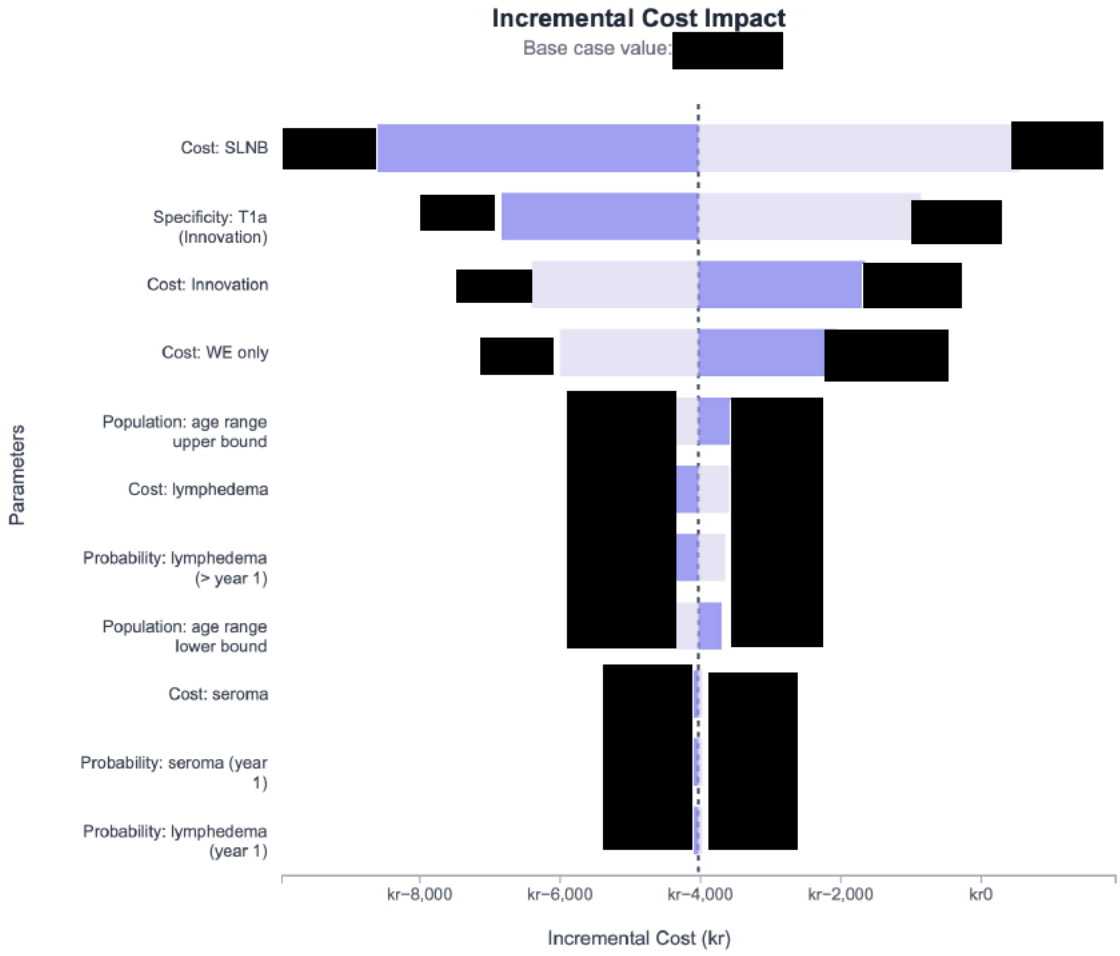


Figure 22. Incremental cost following sensitivity analysis in T1a primary cutaneous melanoma patients. Increase of parameter (light colour), decrease of parameter (dark colour).

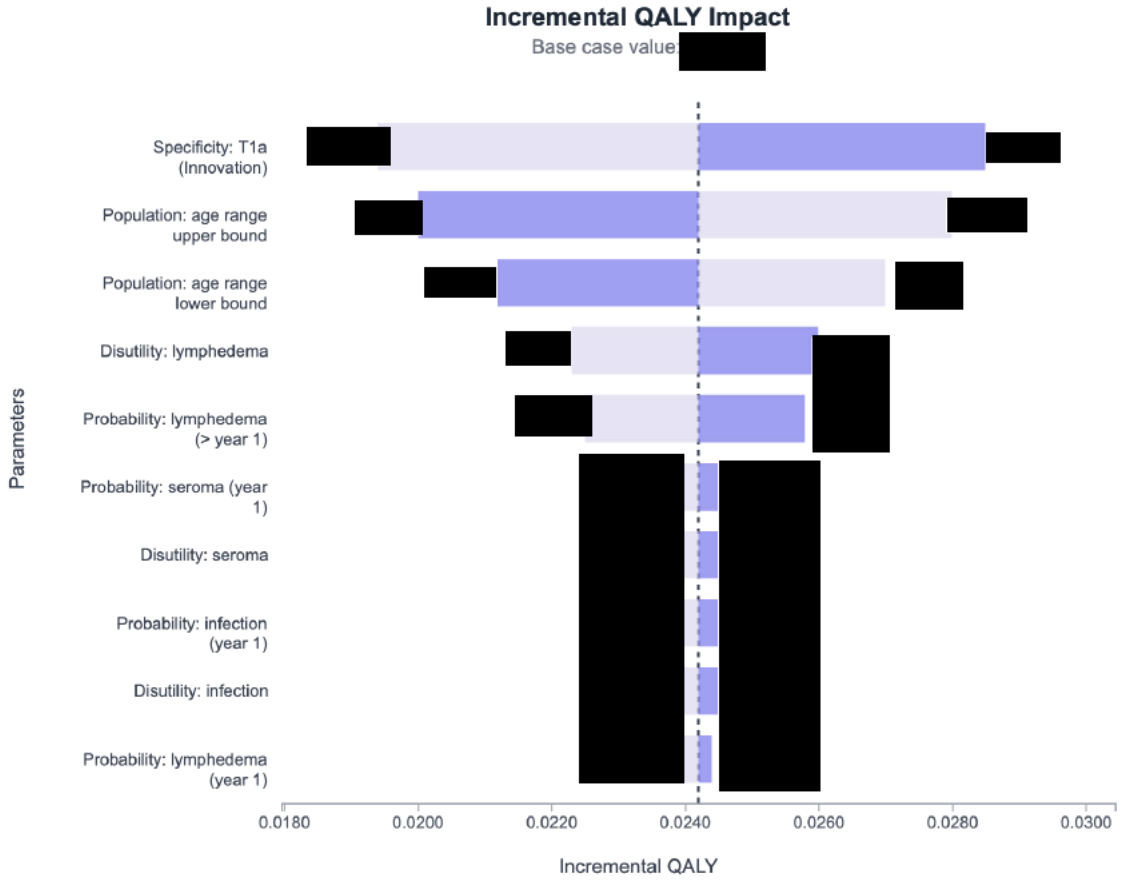


Figure 23. Incremental effectiveness following sensitivity analysis in t1a primary cutaneous melanoma patients. Increase of parameter (light colour), decrease of parameter (dark colour).

8.2.8.2.3 Sensitivity analysis T1b

The tornado diagram ranks parameters in descending order of influence in terms of incremental costs and incremental QALY's, with the most influential variables appearing at the top. Each bar represents the change in that specific parameter, while holding all other inputs constant.

The three main drivers in this model are the specificity of Merlin in T1b patients, the cost of SLNB and the cost of Merlin, varying these parameters within these ranges (±20%) had the largest impact on costs (**Figure 24**). In terms of impact on QALY, the main drivers in the model are metastases, the specificity and sensitivity of Merlin in T1b (**Figure 25**). Across all tested scenarios, Merlin continued to generate net cost savings. In addition, a small but consistent health benefit was observed, measured in QALYs, even when input values were set to be unfavorable. Based on these findings, Merlin may be considered cost-effective for these scenarios in patients with T1b primary cutaneous melanoma within the context of the Danish healthcare system.

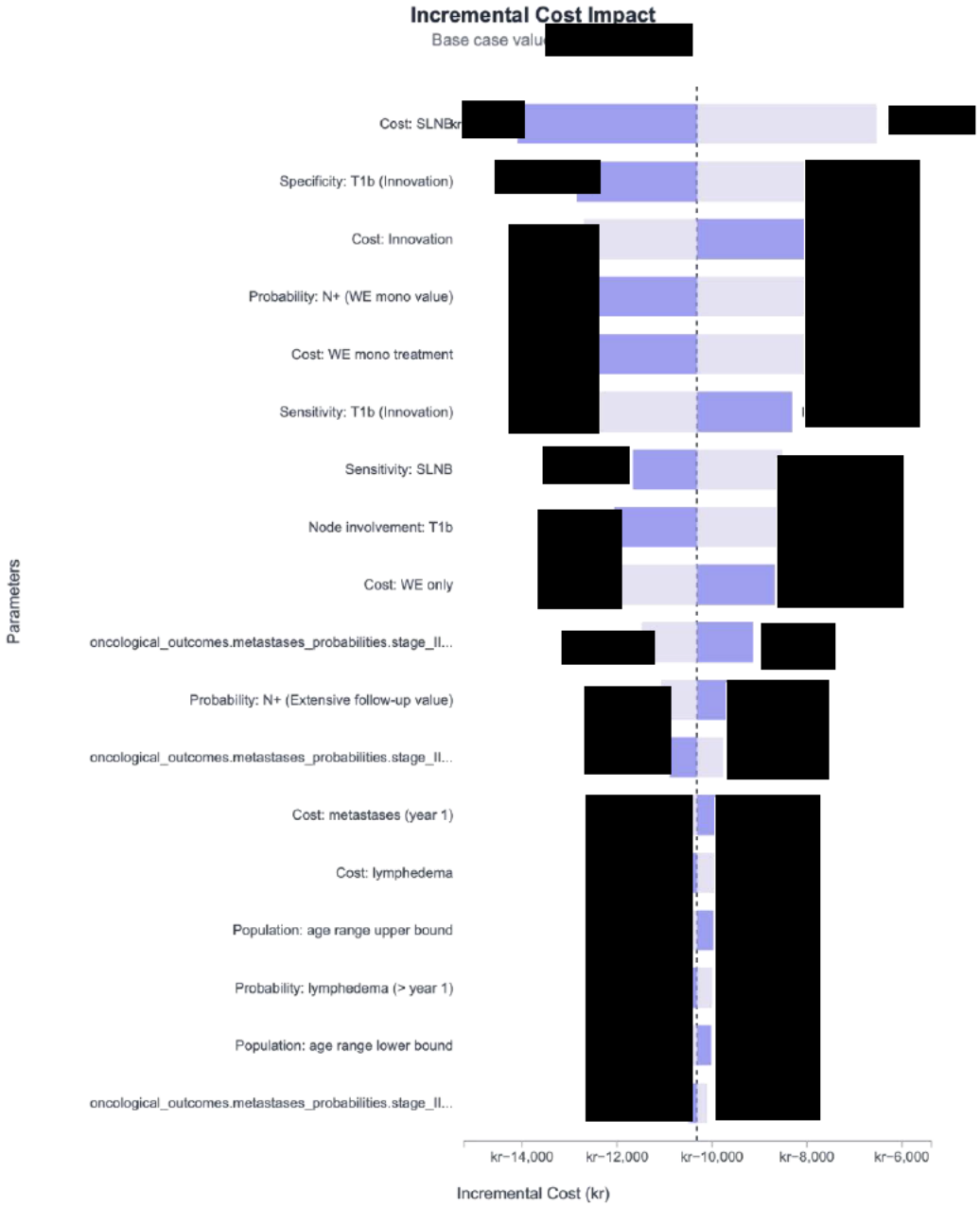


Figure 24. Incremental cost following sensitivity analysis in t1b-primary cutaneous melanoma patients. Increase of parameter (light colour), decrease of parameter (dark colour). EFP, extensive follow-up

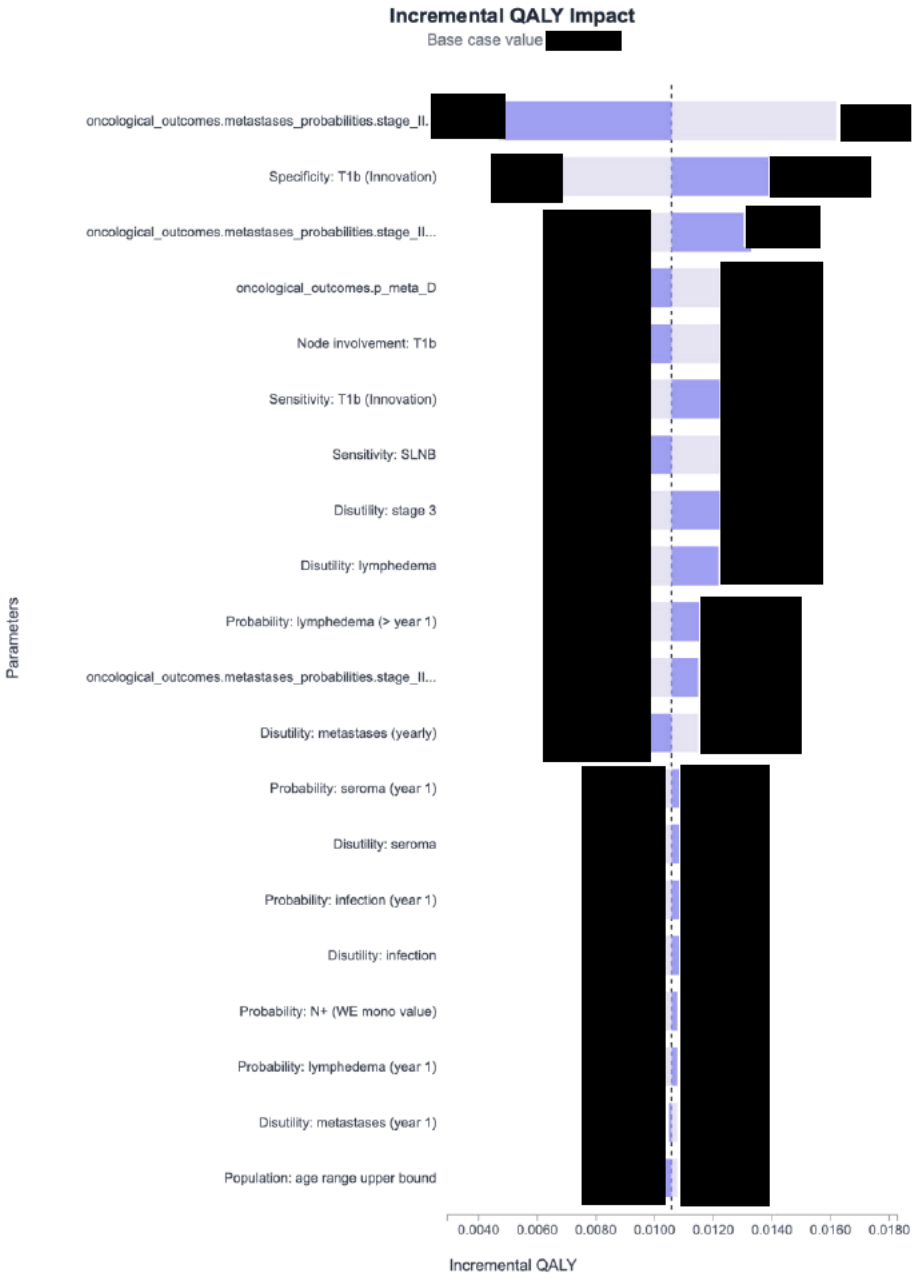


Figure 25. Incremental effectiveness following sensitivity analysis in t1b primary cutaneous melanoma patients. Increase of parameter (light colour), decrease of parameter (dark colour).

8.2.8.2.4 Sensitivity analysis T2a

The tornado diagram ranks parameters in descending order of influence in terms of incremental costs and incremental QALY's, with the most influential variables appearing at the top. Each bar represents the change in that specific parameter, while holding all other inputs constant.

The main drivers in this model are the sensitivity of Merlin in T2a patients, the cost of SLNB, Merlin and monotherapy, varying these parameters had the largest impact on costs (**Figure 26**). In terms of impact on QALY, the main driver in the model is metastases: stage 3 WE value (year 5) and sensitivity of Merlin in T2a patients (**Figure 27**). Other parameters have less impact suggesting that uncertainty in these inputs does not substantially alter the conclusions of the base-case analysis. Across all tested scenarios, Merlin continued to

generate net cost savings, except in a scenario where the sensitivity of Merlin is higher. In addition, a small but consistent health benefit was observed, measured in QALYs, even when input values were set to be unfavorable, except for a lower sensitivity of Merlin. Based on these findings, Merlin may be considered cost-effective for these scenarios in patients with T2a primary cutaneous melanoma within the context of the Danish healthcare system.

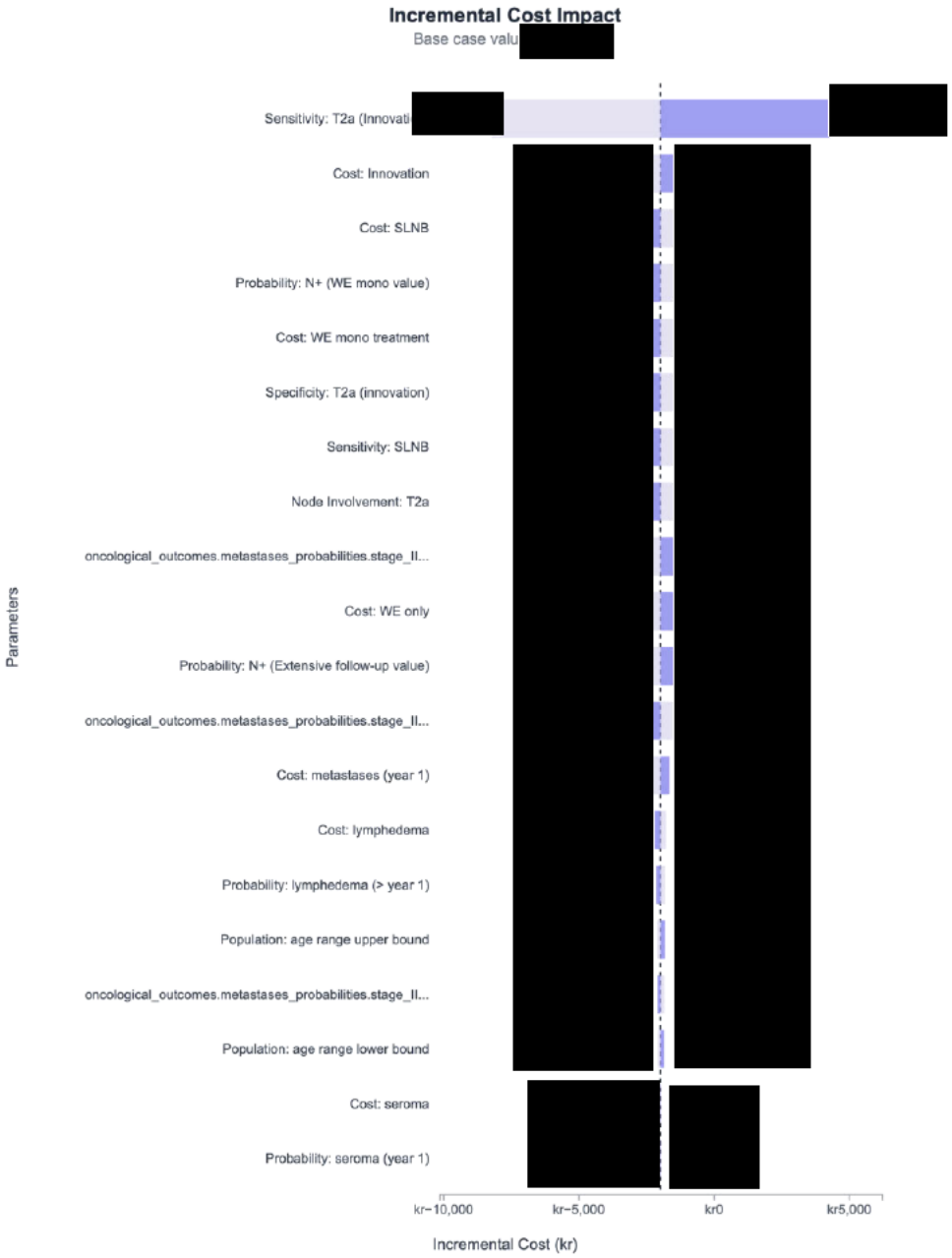


Figure 26. Incremental cost following sensitivity analysis in T2a primary cutaneous melanoma patients. Increase of parameter (light colour), decrease of parameter (dark colour). EFP, extensive follow-up



Figure 27. Incremental effectiveness following sensitivity analysis in T2a primary cutaneous melanoma patients. Increase of parameter (light colour), decrease of parameter (dark colour). EFP, extensive follow-up

8.2.8.2.5 Sensitivity analysis T2b

The tornado diagram ranks parameters in descending order of influence in terms of incremental costs and incremental QALY's, with the most influential variables appearing at the top. Each bar represents the change in that specific parameter, while holding all other inputs constant.

The main driver in this model is the sensitivity of Merlin in T2b patients, varying this parameter had the largest impact on costs (**Figure 28**). In terms of impact on QALY, the main driver in the model is again the sensitivity of Merlin in T2b patients (**Figure 29**). Other parameters have less impact suggesting that uncertainty in these inputs does not substantially alter the conclusions of the base-case analysis. Across all

tested scenarios, Merlin continued to generate additional cost savings. In addition, a small but consistent health detriment was observed. Based on these findings, Merlin may not be considered cost-saving for almost all these scenarios in patients with T2b primary cutaneous melanoma within the context of the Danish healthcare system, with very low net decrease in QALY.

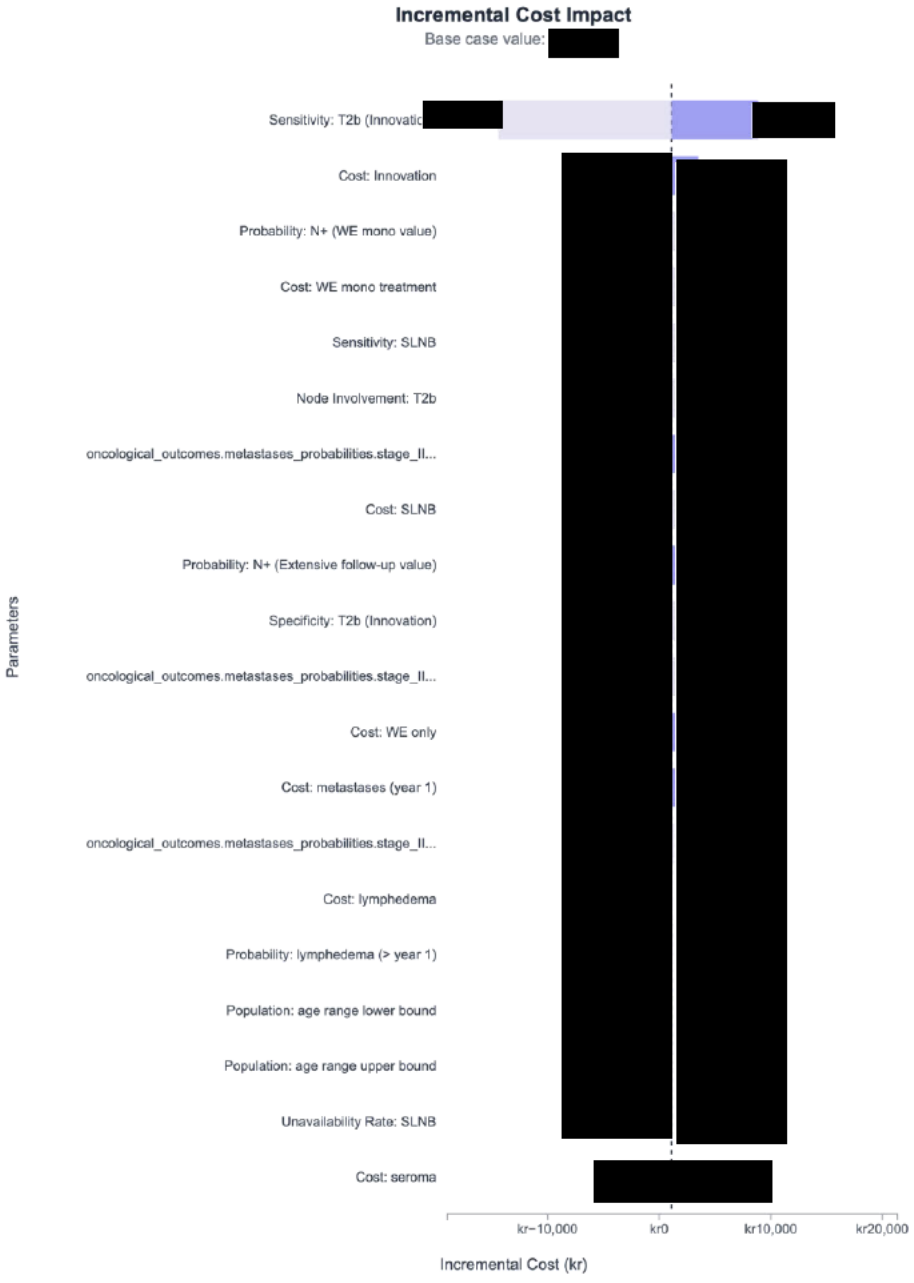


Figure 28. Incremental cost following sensitivity analysis in T2b primary cutaneous melanoma patients. Increase of parameter (light colour), decrease of parameter (dark colour). EFP, extensive follow-up.

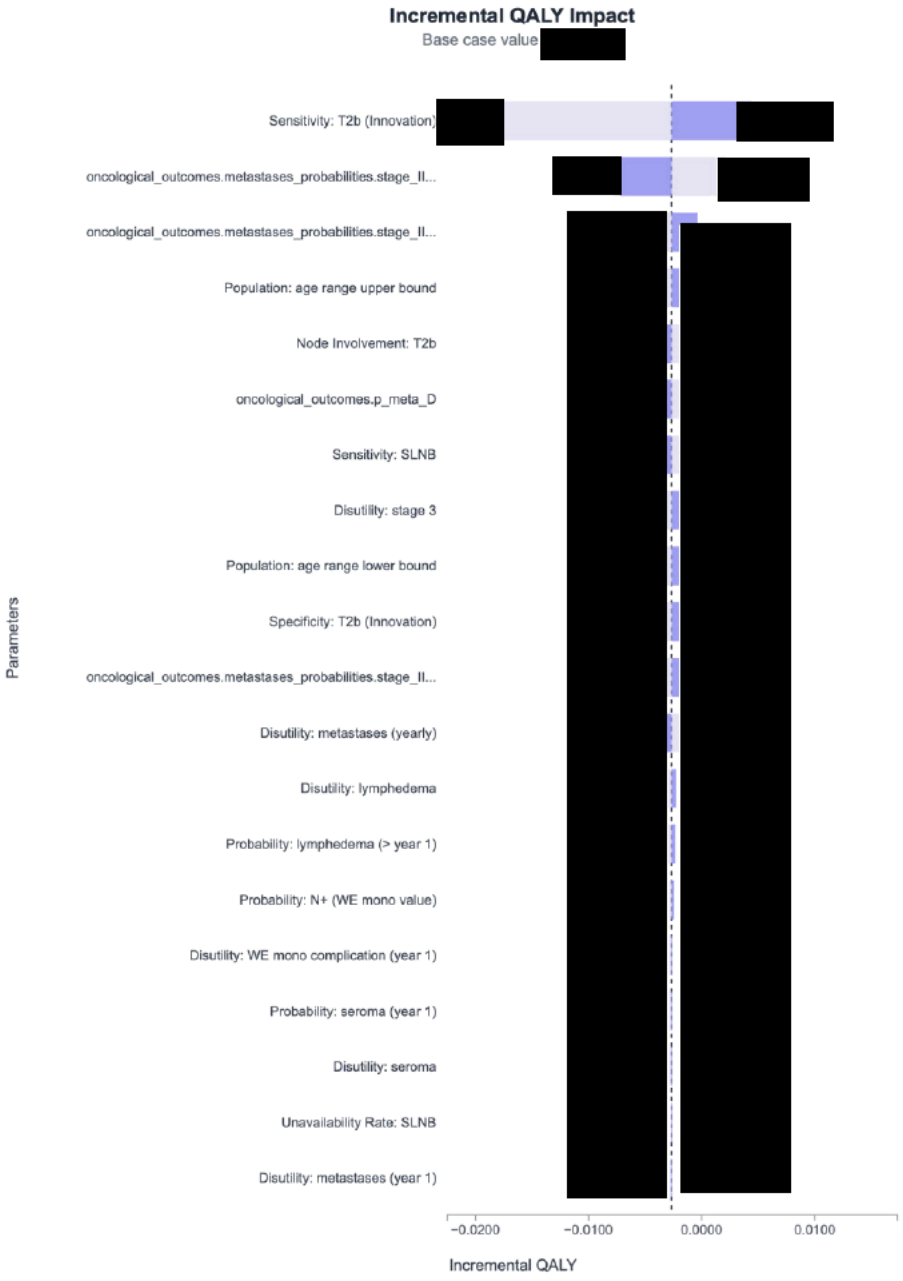


Figure 29. Incremental effectiveness following sensitivity analysis in T2b primary cutaneous melanoma patients. Increase of parameter (light colour), decrease of parameter (dark colour). EFP, extensive follow-up

8.2.9 Applicant's interpretation of the health economic analysis

The health economic analysis conducted for the Danish context indicates overall cost savings associated with Merlin, accompanied by a small gain in QALYs. This trend is consistently observed across substages T1a, T1b and T2a. [redacted]

Sensitivity analysis confirm the robustness of the base-case results. Also, the sensitivity analysis highlight a few key parameters that have the highest impact on the outcomes. The sensitivity of Merlin (especially for the T2a patients), the costs associated with SLNB and the innovation-related expenditures had the highest

potential impact on costs. With respect to QALYs, the rate of metastases for Stage III cancers that receive no treatment (besides WE) and the sensitivity of Merlin (especially T2a) have the highest impact on outcomes.

With regards to the sensitivity of Merlin in T2a patients, a meta-analysis recently published by Wong et al. (19), calculated the weighted sensitivity of the main studies that were available at the time and concluded that for pT2 melanomas, the pooled sensitivity was [redacted] which is slightly higher compared to the results used in this analysis (whole cohort analysis) and suggests that sensitivity in pT2 patients is robust. Consequently, it is reasonable to assume that the sensitivity of Merlin is stable and reliable.

Additionally, the cost for SLNB is one of the other main drivers in the model. At the moment, the cost calculation considers that only 10% of the patients will reside in the hospital for longer than 12 hours, which seems a conservative estimate. Staying in the hospital for longer would substantially increase the costs of SLNB. The rate of metastases of stage III cancers was based on the KEYNOTE-054 study, a large RCT with 1019 patients. Therefore, we believe that uncertainty regarding this parameter is very limited.

In addition to cost savings and minor health benefit, a merlin strategy reduces resources since less time is required from multidisciplinary teams (e.g. plastic surgeon, anesthesiologist, nuclear medicine, nurse) and alleviates pressure on the operating room as SLNB is performed under general anesthesia, whereas WLE can be performed under local anesthesia. In addition, a number of SLNB-related complications can be prevented. Besides cost savings, this also reduces resources required for the treatment of complications. Also, for the patient there could be a reduction in time as the patient must travel less. The impact on resources and time of the patient is currently not reflected in the model but does have a beneficial impact on the Danish Healthcare system. Furthermore, costs associated with adverse effects associated with immunotherapy have not been considered. Additionally, there have been rare cases reported in literature where patients have deceased due to immune-related adverse effects from adjuvant immunotherapy (71) but have not been considered for this cost model.

At the moment there are no other health economic papers that have a similar model design in which results can be compared. The model has a few strengths and weaknesses. As this model uses microsimulation, it allows for patient-level heterogeneity, making it well-suited for modeling individualized risk profiles, such as those generated by a diagnostic test (Merlin). By combining a decision tree (for short-term diagnostic and treatment decisions) with a Health state model (for long-term disease progression and survival) it captures both immediate consequences (e.g., SLNB complications) and downstream health outcomes (e.g., recurrence, survival, immunotherapy use) into one cost-effectiveness model. At the other end, the combination of decision trees and Health state models increase complexity, which can hinder transparency and make validation more difficult. A wide range of cost components and inputs have been considered, such as test acquisition, immunotherapy, SLNB procedure and complications—as well as quality-of-life adjustments (disutilities). Especially for the quality-of-life impact of complications, there was limited data available. We believe we used conservative estimates and showed the impact of different values in the sensitivity analysis.

Overall, the data indicates that the innovation is dominant in most scenarios, with Merlin testing strategy emerging as the preferred approach. It delivers cost savings and provides a modest health benefit. Furthermore, it can contribute to more efficient use of hospital resources.

8.3 Budget impact analysis

In addition to the cost-effectiveness model, a budget impact model was developed to estimate the budgetary consequences of introducing Merlin to the Danish clinical practice in patients with primary cutaneous

melanoma T1a-T2b for 5 years. The budget impact model consists of different types of costs, namely, 1) acquisition & diagnostic costs for the diagnostic service, including costs for WLE, and SLNB, 2) costs of follow-up, 3) opportunity costs, 4) therapy costs including costs for those patients that relapse, 5) complications costs, and 6) out of pocket costs and time spent going to the hospital. Results have been specified per type of cost and per diagnostic test separately. Costs were not discounted.

Average costs were calculated per subtype of cost and applied to the market share per year and population sample size per year. This led to an overall budget impact for a scenario with the current standard of care in which all eligible patients would undergo SLNB compared to a scenario in which patients would first undergo Merlin testing and subsequently underwent SLNB if patients received a Merlin High Risk test result. These two scenarios were then subtracted to calculate the budget impact per year. The budget impact model can be adapted to calculate costs for specific substages, costs are presented in Danish Krone and were converted to reflect 2024-2025 prices unless otherwise stated.

8.3.1 Patient population

The model determines the cost impact from 2025 to 2029, focusing on patients with primary cutaneous melanoma with pathological stage T1a-T2b eligible for SLNB. Incidence of different substages and patients eligible for SLNB were retrieved from the Danish melanoma database year report of 2023 in which in total from T1a-T2b, 828 patients would be eligible for SLNB. The average increase or decrease in incidence of melanoma was calculated based on incidence from 2017 to 2023 averaging to a 2.2% yearly increase in melanoma incidence (7).

A report from the Danish Melanoma database (7) reported the number of patients per pathological substage that had an SLNB performed. If applied to pathological substages T1a-T2b, 793 patients would be eligible for SLNB in 2023. Applying the 2.2% yearly increase in incidence, 828 primary cutaneous melanoma patients should be eligible for SLNB in 2025. See **Table 33** and **Table 34** for an overview of the market shares per strategy.

Table 33. The size of the patient population without recommendation of Merlin

	Year 1	Year 2	Year 3	Year 4	Year 5
Merlin ± SLNB	0	0	0	0	0
SLNB	828	846	865	884	904
Total patient population	828	846	865	884	904

Table 34. The size of the patient population with recommendation of Merlin

	Year 1	Year 2	Year 3	Year 4	Year 5
Merlin ± SLNB	83	169	303	486	633
SLNB	745	677	562	398	271
Total patient population	828	8466	865	884	904

8.3.2 Results of the budget impact analysis

The budget impact analysis suggests that the introduction of the new strategy is associated with net savings for the Danish healthcare system. While diagnostic costs increase, these are offset by substantial reductions in therapy costs, complication-related expenses, patient out-of-pocket payments, and opportunity costs as hospital resources are freed up.

Over the 5-year time horizon, increasing uptake of the strategy results in greater cost savings, although diagnostic expenditures rise in parallel. Therapy costs remain the primary cost driver, representing a significant proportion of the hospital budget per year. Due to some patients being missed by the diagnostic

strategy, there are fewer upfront therapy costs, and while some positive nodes may go undetected, resulting in a slightly higher relapse rate at later stages, overall cost savings are still realized.

Follow-up costs are assumed to remain unchanged in the current model, as no budget shifts are expected in this area. However, this could be explored in future analysis, particularly given that patients with a Merlin Low Risk result typically have a favorable prognosis, potentially enabling de-escalated follow-up regimens.

Regarding complication costs, a conservative approach was taken. These costs were assumed to be low but still contributed to savings due to fewer complications observed with the Merlin testing strategy.

Although reimbursed DRG tariffs were used to estimate costs for SLNB and WLE, and typically include physician time, opportunity costs were calculated separately. These estimates were based on the average time saved per patient when SLNB is avoided with those with a Merlin Low Risk result. Time and personnel data were collected from Herlev and Gentofte Hospital and used to calculate total hours saved, which were then multiplied by the hourly tariffs of relevant healthcare staff. Given the scarcity of clinical time, these saved hours represent a valuable resource and were incorporated into the model accordingly.

Out-of-pocket costs included patient travel to and from the hospital, and time spent receiving treatment (used as a proxy per Danish costing guidelines). While this represents a smaller share of the total budget, it remains a meaningful burden to patients and contributes to the overall benefit of the strategy.

In summary, despite increased diagnostic costs, the strategy is cost-efficient over time and enables a more sustainable and effective allocation of healthcare resources. The findings support the potential for wider implementation within the Danish healthcare setting. See **Table 35** and **Table 36** for a breakdown in costs per cost category or diagnostic, respectively.

Table 35. [redacted]

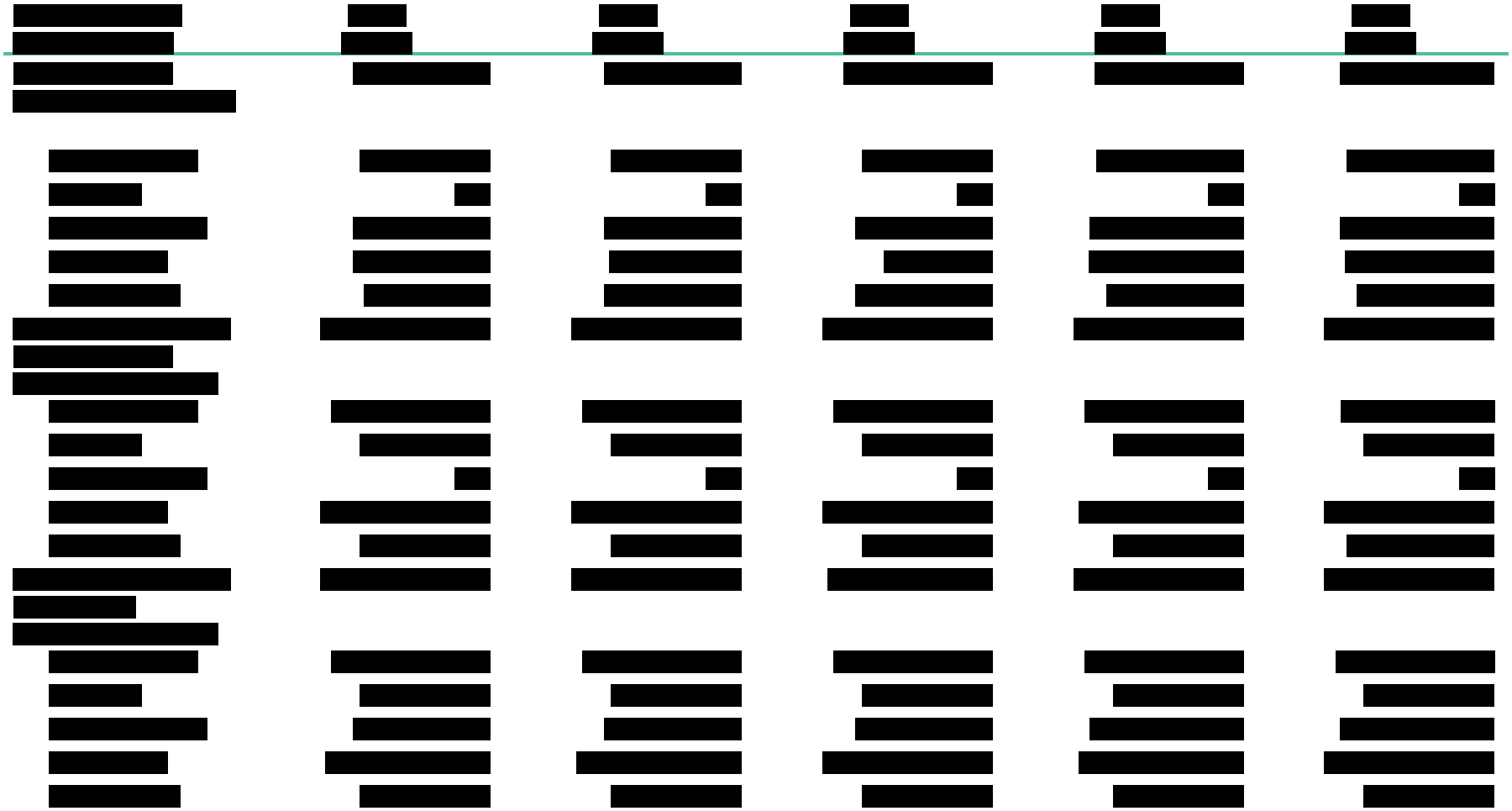
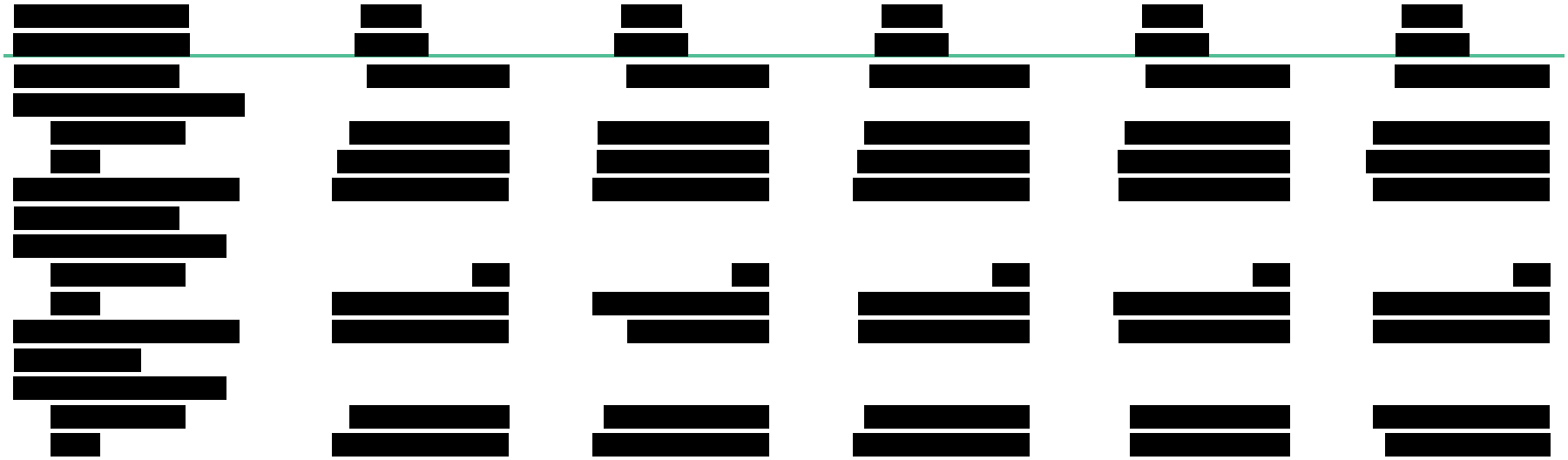


Table 36. [redacted]



9. Discussion on the documentation submitted

9.1.1 Nodal metastasis prediction and safety

For the Merlin test to be clinically meaningful, it must safely identify a substantial proportion of patients who can forgo SLNB without compromising oncological outcomes. The Merlin test's strength lies in its high NPV, which is critical in minimizing FN results. In the context of current Danish clinical guidelines, SLNB is not recommended if the predicted risk of nodal metastasis is 10% or lower (8–10). Merlin's large body of clinical evidence shows the consistent performance of Merlin across different countries, continents and healthcare settings in risk stratifying patients that can forgo SLNB due to their low risk of nodal metastasis. Merlin has a consistently high NPV across multiple international studies (11–18), exceeding the 90% risk threshold used in Danish guidelines (8–10). Importantly, this risk is lower than that of missing nodal metastases under current standard practice. Although ideally the aim is to avoid FN entirely, long-term follow-up data offers further reassurance as patients classified as Low Risk by Merlin have demonstrated low recurrence rates and favorable survival outcomes (18,20–24).

Conversely, patients may be falsely classified as high risk (false positives) and undergo SLNB despite not having nodal metastases. However, since SLNB remains the standard of care in this group regardless of Merlin's result, such patients are not exposed to any additional procedural risks beyond current practice.

Merlin also provides a practical advantage in patient populations for whom SLNB is contraindicated or not feasible. For example, patients with significant comorbidities, head and neck melanoma, or older age (16,18).

Taken together, the evidence supports the clinical utility and prognostic value of the Merlin test, particularly for Low Risk patients. It enables safer de-escalation of SLNB and helps guide individualized care, aligning well with modern principles of precision oncology.

9.1.2 Patient perspective

While SLNB remains a standard staging procedure, its invasive nature and potential consequences highlight the need for more tailored approaches. The use of Merlin to triage patients allows those at low risk of nodal metastasis to avoid unnecessary surgery, aligning with a more personalized, less burdensome care model that prioritizes both clinical safety and patient quality of life.

From the patient's perspective, the ability to forgo SLNB based on a Low Risk result from the Merlin test offers meaningful benefits. Avoiding SLNB not only reduces exposure to surgical risks (4) but also minimizes the number of required hospital visits, travel time, (6) and the psychological stress associated with awaiting biopsy results. This can be particularly impactful for patients living in rural areas or those with limited mobility or support systems.

In addition to logistical and potential emotional burdens, SLNB carries a well-documented risk of adverse events, including seroma, infection, hematoma, wound dehiscence, and lymphedema (4). Of these, lymphedema is especially concerning due to its potential for long-term morbidity and quality-of-life impact (25,26).

9.1.3 Organizational implications

From an organizational standpoint, the implementation of the Merlin test presents an opportunity to enhance clinical workflows and optimize the use of healthcare resources. By reducing the number of unnecessary SLNBs and the associated complication rates, healthcare providers may see a downstream

impact in terms of fewer hospital admissions, reduced postoperative care needs, and overall cost containment.

Importantly, the test also has the potential to streamline patient pathways. Avoiding unnecessary surgical procedures not only eases pressure on surgical departments but may also reduce diagnostic delays, improving access and efficiency in melanoma care. This is particularly relevant in settings where healthcare capacity is constrained, but also if patients live in remote areas or need to travel from afar.

Operationally, a logistical process has already been established to support the shipment of biopsy samples to the central testing laboratory in Rotterdam. This framework includes clearly defined handling protocols, the use of a standardized test requisition form, and full compliance with GDPR requirements for the management of personal health data. The feasibility of this process has been demonstrated through prior successful collaboration with Herlev and Gentofte Hospital using retrospective samples as well as other sites such as the Erasmus Medical Center during the prospective trial.

Looking ahead, there is a clear opportunity to further strengthen the integration of Merlin testing into Danish clinical practice. With appropriate investment in infrastructure and equipment, it may be feasible to establish a centralized testing hub within Denmark. Such a move could further reduce turnaround times and enhance the long-term sustainability and scalability of the testing program.

9.1.4 Health economics

The health economic evaluation of the Merlin testing strategy suggests a favorable profile across multiple modeled scenarios. In most cases, the test is shown to be dominant, meaning it leads to both cost savings and improved health outcomes compared to standard care. While the diagnostic cost per patient increases modestly with the addition of Merlin, these costs are offset over time by reductions in downstream expenditures, particularly concerning SLNB procedures, treatment of associated complications, and patient out-of-pocket expenses.

The sensitivity analyses reinforce the robustness of these findings, demonstrating that the cost-effectiveness of Merlin remains consistent even when key parameters are varied. Moreover, the budget impact analysis highlights the potential for resource reallocation: by avoiding unnecessary surgical interventions, healthcare providers may free up clinical capacity and funding, which can then be redirected to patients with greater clinical need.

Taken together, these results indicate that integrating Merlin into the melanoma care pathway is not only clinically beneficial but also economically sustainable. The evidence supports its broader implementation within the Danish healthcare system, aligning with national priorities around efficient resource use and value-based care delivery.

9.1.5 Overall conclusion

The Merlin test represents a meaningful advancement in the clinical management of melanoma, with the potential to address both patient-centered and economic priorities. By providing a less invasive, risk-stratified alternative to SLNB, it offers measurable value in terms of reducing unnecessary procedures, minimizing patient burden, and optimizing resource use within the healthcare system.

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11. List of annexes

11.1 Selection of the literature

Literature has been excluded as per the pre-defined inclusion criteria in section 4.1 from the literature search during full-text selection. Please see the overview in **Table 37**.

Table 37: Excluded literature during full-text selection and reason for exclusion

Title	Category	Reason exclusion
MERLIN-001: A prospective registry study of a primary melanoma gene-signature to predict sentinel node (SN) status and determine its prognostic value for more accurate staging of patients with SN-negative melanoma https://ascopubs.org/doi/10.1200/JCO.2022.40.16_suppl.TPS9606	Reviews	Wrong publication type (not a review)
From diagnostic labs to rapid near-patient testing: Bridging across RT-qPCR platforms of a clinicopathological and gene expression model for cutaneous melanoma patients https://aacrjournals.org/cancerres/article/84/6_Supplement/3780/738448/Abstract-3780-From-diagnostic-labs-to-rapid-near	CE Primary	Wrong outcomes
Prognostic significance of sentinel lymph node status in thin melanoma: a retrospective analysis https://pmc.ncbi.nlm.nih.gov/articles/PMC10957307/	CE Primary	Not a primary study
Eggermont, Alexander M.M. et al. The letter responds to comment on Identification of stage I/IIA melanoma patients at high risk of disease relapse using a clinicopathologic and gene expression model. European Journal of Cancer, Volume 157, 518 - 519	CE Primary	Letter/Commentary
Domenico Bellomo et al. Reply to E. K. Bartlett et al and A. H. R. Varey et al. JCO Precis Oncol 4, 992-994(2020). DOI:10.1200/PO.20.00289	CE Primary	Letter/Commentary
Thao V, Dholakia R, Moriarty JP, Borah BJ, Dwarkasing J, Meves A. Cost evaluation of the Merlin assay for predicting melanoma sentinel lymph node biopsy metastasis. Int J Dermatol. 2023 Jan;62(1):56-61. doi: 10.1111/ijd.16515. Epub 2022 Nov 28. PMID: 36440797; PMCID: PMC10098626.	Patient perspective	Wrong outcomes
Quattrocchi E, Meves ES, Meves A. CP-GEP (Merlin) gene expression profiling: can my melanoma patient forgo sentinel lymph node biopsy? Ital J Dermatol Venerol. 2023 Aug;158(4):292-301. doi: 10.23736/S2784-8671.23.07621-1. PMID: 37539500.	Patient perspective	Not a primary study
Stassen RC, Mulder EEAP, Mooyaart AL, Francken AB, van der Hage J, Aarts MJB, van der Veldt AAM, Verhoef C, Grünhagen DJ. Clinical evaluation of the	Patient perspective	Wrong outcomes

clinicopathologic and gene expression profile (CP-GEP) in patients with melanoma eligible for sentinel lymph node biopsy: A multicenter prospective Dutch study. Eur J Surg Oncol. 2023 Dec;49(12):107249. doi: 10.1016/j.ejso.2023.107249. Epub 2023 Oct 26. PMID: 37907016.		
Pozza, L., Zumrutcu, S., Bosman, L., van der Spek, A., Moreau, M., Tempel, D., Dwarkasing, J.T., & Bellomo, D. (2024). Abstract 3780: From diagnostic labs to rapid near-patient testing: Bridging across RT-qPCR platforms of a clinicopathological and gene expression model for cutaneous melanoma patients. *Cancer Research*.	Organizational implications	Wrong outcomes
Tina J. Hieken et al. **MERLIN_001: A prospective registry study of a primary melanoma gene-signature to predict sentinel node (SN) status and determine its prognostic value for more accurate staging of patients with SN-negative melanoma. *JCO* 40, TPS9606-TPS9606(2022). DOI:**10.1200/JCO.2022.40.16_suppl.TPS9606	Health economics	Wrong outcomes
Tina J. Hieken, Michael E. Egger, Christina V. Angeles, Erin E. Burke, Michael C. Lowe, John R. Hyngstrom, Georgia M. Beasley, Edmund K. Bartlett, Vernon K. Sondak; Abstract CT053: Merlin_001: a prospective registry study of a primary melanoma gene-signature to predict sentinel node (SN) status and determine its prognostic value for more accurate staging of SN-negative melanoma patients. *Cancer Res* 15 April 2023; 83 (8_Supplement): CT053. https://doi.org/10.1158/1538-7445.AM2023-CT053	Health economics	Wrong outcomes

11.2 Tables for description of the characteristics of the studies and populations included

Title	**Model Combining Tumor Molecular and Clinicopathologic Risk Factors Predicts Sentinel Lymph Node Metastasis in Primary Cutaneous Melanoma**
Link to abstract	Link
Reference (first author, year)	Bellomo, 2020
Overall objective of the study	Development of a clinicopathologic and gene expression- based model for the prediction of SLNB status in primary cutaneous melanoma patients
Study type and design	Retrospective study with data from archived patient samples. Logistic regression was used for algorithm development and double-loop cross validation. The average performance of the model was evaluated by repeated cross-validation or bootstrapping. Here, we opted for a repeated cross validation scheme (ie, double-loop cross-validation). The key idea of DLCV is to get a reliable estimate of the out of-sample performance of a classifier by averaging the performance of multiple classifiers trained in cross-validation a number of times.

Follow-up period	Not applicable, explanation: Predicting SLNB status outcome is the endpoint, as the model under development is intended as a prediction tool. No follow-up period in these studies is required, as SLNB status outcome is already known during the study and is used as reference against the output label of the model.
Inclusion and exclusion criteria	Inclusion: Patients were eligible for this study if they met one of three conditions, which included Breslow thickness of 1.0-4.0 mm; Breslow thickness of 0.75-0.99 mm and presence of ulceration, mitoses, and/or age < 40 years; or Breslow thickness of 0.50-0.74 mm and presence of at least two of the following: ulceration, mitoses, and age < 40 years. Exclusion: Lesions with a Breslow thickness of > 4 mm were excluded because they were considered a priori high-risk lesions with a rate of nodal involvement > 40%. During following validation studies T4 patients can be included as part of the SLNB-eligible population. Other exclusion criteria were M1 disease within 90 days of primary diagnosis; insufficient primary tumor diagnostic biopsy tissue; inadequate RNA harvested; and, for Minnesota, denial of access to medical records for research purposes.
Intervention	CP-GEP model ("Merlin"), a model that combines clinicopathologic features patient's age and Breslow thickness, with the expression of eight genes from the primary tumour. Genes included are: *ITGB3, PLAT, SERPINE2, GDF15, TGFBR1, LOXL4, CXCL8 and MLANA.*
Comparator	Sentinel Lymph Node Biopsy (SLNB)
Baseline characteristics	Baseline characteristics of the 754 primary cutaneous melanoma patients • Gender distribution (n, %): SLNB negative patients: male sex n= 472 (62.6 %) SLNB positive patients: male sex n= 79 (10.5 %) • Age (mean, ±SD) SLNB negative patients: 61 years (15.87 years) SLNB positive patients: 54 years (17.11 years) • Previously treated (n, %): not applicable, only newly diagnosed patients included for whom the primary biopsy was tested. This biopsy was treatment naïve. • Weighting (ave., SD): data not collected in this study • Severity of disease: the prevalence of SLNB nodal metastases in this study is 17% (128 patients had an SLNB positive outcome) We included two more clinically relevant variables as baseline characteristics for the patient populations, which are Breslow Thickness and ulceration: • Breslow thickness: SLNB negative patients: 0.5-1 mm: n = 186 1.01-2 mm: n = 309 2.01-4 mm: n = 131 SLNB positive patients: 0.5-1 mm: n = 6 1.01-2 mm: n = 50 2.01-4 mm: n = 72

Primary and secondary outcome measures **Analysis method** **Relevant sub-group analysisanalysis** **Supplementary articles based on the same study**	• Ulceration (presence, %): SLNB negative patients: present in 114 out of 622 patients (18.3%) SLNB positive patients: present in 46 out of 128 patients (35.9%) Primary outcome measures: SLNB reduction rate (proportion of Low Risk assigned patients by the model, that theoretically could have forgone SLNB surgery) Negative Predictive Value (NPV) Secondary outcome measures: Positive Predictive Value (PPV) Sensitivity Specificity • Samples with a probability of metastasis greater than the cutoff were classified as positive, whereas samples with a probability lower than the cutoff were classified as negative. • SLNB RR = (TN +FN)/ (TP + TN + FN + FP) • NPV = TN/ (TN + FN) • PPV = TP/ (TP +FP) • Sensitivity = TP/ (TP + FN) • Specificity = TN/ (TN +FP) No subgroup analysisanalysis have been conducted in this study Not applicable

Study identification no.	**Validation of a clinicopathological and gene expression profile model for sentinel lymph node metastasis in primary cutaneous melanoma**
Link to abstract	Link
Reference (first author, year)	Mulder, 2020
Overall objective of the study	Clinically validate the CP-GEP model in an independent Dutch cohort
Study type and design	Retrospective study with data from archived patient samples.
Follow-up period	Not applicable, explanation: Predicting SLNB status outcome is the endpoint, as the model under development is intended as a prediction tool. No follow-up period in these studies is required, as SLNB status outcome is already known during the study and is used as reference against the output label of the model.
Inclusion and exclusion criteria	Inclusion: The study included patients aged 18 years or older who were diagnosed with primary cutaneous melanoma and underwent SLNB at the Erasmus Medical Centre (MC) Cancer Institute, between 2007 and 2017. Exclusion: Patients who underwent SLNB more than 90 days after the primary melanoma diagnosis were excluded as well as patients with multiple primary melanomas, or missing data on Breslow thickness or age (at the time of primary diagnosis).

Intervention	CP-GEP model ("Merlin"), a model that combines clinicopathologic features patient's age and Breslow thickness, with the expression of eight genes from the primary tumour. Genes included are: *ITGB3, PLAT, SERPINE2, GDF15, TGFBR1, LOXL4, CXCL8 and MLANA*
Comparator	Sentinel Lymph Node Biopsy (SLNB)
Baseline characteristics	Baseline characteristics of the 210 primary cutaneous melanoma patients • Gender distribution (n, %): All patients: male sex n= 110 (52 %) • Age (median, interval) All patients: 55 years (45-65 years) • Previously treated (n, %): not applicable, only newly diagnosed patients included for whom the primary biopsy was tested. This biopsy was treatment naïve. • Weighting (ave., SD): data not collected in this study • Severity of disease: the prevalence of SLNB nodal metastases in this study is 27% (56 patients had an SLNB positive outcome) We included two more clinically relevant variables as baseline characteristics for the patient populations, which are Breslow Thickness and ulceration: • Breslow thickness (median, interval): All patients: 2.05 mm (1.40-3.30 mm) • Ulceration (Presence, %): All patients: present in 55 out of 210 patients (26%)
Primary and secondary outcome measures	Primary outcome measures: SLNB reduction rate (proportion of Low Risk assigned patients by the model, that theoretically could have forgone SLNB surgery) Negative Predictive Value (NPV) Secondary outcome measures: Positive Predictive Value (PPV) Sensitivity Specificity
Analysis method	• Samples with a probability of metastasis greater than the cutoff were classified as positive, whereas samples with a probability lower than the cutoff were classified as negative. • SLNB RR = (TN +FN)/ (TP + TN + FN + FP) • NPV = TN/ (TN + FN) • PPV = TP/ (TP +FP) • Sensitivity = TP/ (TP + FN) • Specificity = TN/ (TN +FP)
Relevant sub-group analysis	No subgroup analysis have been conducted in this study
Supplementary articles based on the same study	Not applicable

Study identification no.	**Deselecting melanoma patients for sentinel lymph node biopsy during COVID-19: clinical utility of tumor molecular profiling.**
Link to abstract	Link
Reference (first author, year)	Meves & Eggermont, 2020
Overall objective of the study	Evaluate use of CP-GEP as triage tool for deselecting primary cutaneous melanoma patients for SLNB during pandemic times.
Study type and design	Retrospective patient case study, representative case.
Follow-up period	Not applicable, explanation: Predicting SLNB status outcome is the endpoint, as the model under development is intended as a prediction tool. No follow-up period in these studies is required, as SLNB status outcome is already known during the study and is used as reference against the output label of the model.
Inclusion and exclusion criteria	Inclusion: A representative case who enrolled in a feasibility study of a molecular method aimed to identify melanoma patients who are so low risk for metastasis that they can forgo SLN biopsy.
Intervention	CP-GEP model ("Merlin"), a model that combines clinicopathologic features patient's age and Breslow thickness, with the expression of eight genes from the primary tumour. Genes included are: *ITGB3, PLAT, SERPINE2, GDF15, TGFBR1, LOXL4, CXCL8 and MLANA*
Comparator	Sentinel Lymph Node Biopsy (SLNB)
Baseline characteristics	One primary cutaneous melanoma patient • Gender distribution: male • Age: not reported • Previously treated (n, %): not applicable, newly diagnosed patient included for whom the primary biopsy was tested. This biopsy was treatment naïve. • Weighting (ave., SD): data not collected in this study • Severity of disease: the prevalence of SLNB nodal metastases in this study is 0% We included two more clinically relevant variables as baseline characteristics for the patient populations, which are Breslow Thickness and ulceration: • Breslow thickness: 0.9 mm • Ulceration: not ulcerated
Primary and secondary outcome measures	Primary outcome measures: Change in clinical care path with the implementation of CP-GEP
Analysis method	• Evaluate hospital resources that are used upon SLNB referral versus CP-GEP testing
Relevant sub-group analysis	No subgroup analysis have been conducted in this study
Supplementary articles based on the same study	Bellomo, 2020

Study identification no.	**Validation of a clinicopathological and gene expression profile model to identify patient with cutaneous melanoma where sentinel lymph node biopsy is unnecessary.**
Link to abstract	Link
Reference (first author, year)	Johansson, 2022
Overall objective of the study	Validate the CP-GEP test in a population-based single-center consecutive cohort of melanoma patients treated with SLNB and to compare the results with two clinicopathological models in clinical use for SLNB assessment, the Memorial Sloan Kettering Cancer Center (MSKCC) and the Melanoma Institute Australia (MIA) nomograms, respectively.
Study type and design	Retrospective study with data from archived patient samples.
Follow-up period	Not applicable, explanation: Predicting SLNB status outcome is the endpoint, as the model under development is intended as a prediction tool. No follow-up period in these studies is required, as SLNB status outcome is already known during the study and is used as reference against the output label of the model.
Inclusion and exclusion criteria	Inclusion: All SLNB-eligible patients with primary cutaneous melanoma undergoing SLNB at Sahlgrenska University Hospital, Gothenburg, Sweden. All types of cutaneous melanoma from all body regions, except melanoma of the head and neck region. Exclusion: age <18 years (n = 0), missing consent to use of archival tissue for research purposes (n = 1), history of Jacob Creutzfeldt disease (n = 1), primary tumor paraffin blocks unavailable from remote laboratories (n = 42), insufficient amount of archival primary tumor tissue in the paraffin blocks (n = 12), erroneous histological diagnosis of primary invasive cutaneous melanoma (n = 4), distant metastatic disease (M1a,b,c) present at primary diagnosis or documented within 90 days of primary diagnosis (n = 0), documented clinically positive nodes at primary diagnosis (n = 0), documented history of another primary invasive melanoma (cutaneous or extra-cutaneous) at the time of the primary melanoma biopsy (n = 2), full SN pathology report unavailable (n = 0) or SLNB procedure failed (n = 2).
Intervention	CP-GEP model ("Merlin"), a model that combines clinicopathologic features patient's age and Breslow thickness, with the expression of eight genes from the primary tumor. Genes included are: *ITGB3, PLAT, SERPINE2, GDF15, TGFBR1, LOXL4, CXCL8 and MLANA*
Comparator	Sentinel Lymph Node Biopsy (SLNB)
Baseline characteristics	Baseline characteristics of the 421 primary cutaneous melanoma patients • Gender distribution (n, %): All patients: male sex n= 214 (51%) • Age (median, interval) All patients: 60 years (49-71 years) • Previously treated (n, %): not applicable, only newly diagnosed patients included for whom the primary biopsy was tested. This biopsy was treatment naïve. • Weighting (ave., SD): data not collected in this study

	• Severity of disease: the prevalence of SLNB nodal metastases in this study is 13% (54 patients had an SLNB positive outcome) We included two more clinically relevant variables as baseline characteristics for the patient populations, which are Breslow Thickness and ulceration: • Breslow thickness (median, interval): All patients: 1.80 mm (1.30-3.20 mm) • Ulceration (Presence, %): All patients: present in 133 out of 421 patients (32%)
Primary and secondary outcome measures	Primary outcome measures: SLNB reduction rate (proportion of Low Risk assigned patients by the model, that theoretically could have forgone SLNB surgery) Negative Predictive Value (NPV) Secondary outcome measures: Positive Predictive Value (PPV) Sensitivity Specificity
Analysis method	• Samples with a probability of metastasis greater than the cutoff were classified as positive, whereas samples with a probability lower than the cutoff were classified as negative. • SLNB RR = (TN +FN)/ (TP + TN + FN + FP) • NPV = TN/ (TN + FN) • PPV = TP/ (TP +FP) • Sensitivity = TP/ (TP + FN) • Specificity = TN/ (TN +FP) Subgroup analysis have been conducted for comparison with MIA and MSKCC nomograms, T1-T3 subgroup (n = 303) and T1-T2 subgroup (n = 202)
Relevant sub-group analysis	• SLNB RR = (TN +FN)/ (TP + TN + FN + FP) • NPV = TN/ (TN + FN) • PPV = TP/ (TP +FP) • Sensitivity = TP/ (TP + FN) • Specificity = TN/ (TN +FP)
Supplementary articles based on the same study	Not applicable

Study identification no.	**Validation of CP-GEP (Merlin Assay) for predicting sentinel lymph node metastasis in primary cutaneous melanoma patients: a U.S. cohort study.**
Link to abstract	Link
Reference (first author, year)	Yousaf, 2021
Overall objective of the study	First validation of CP-GEP ("Merlin") in a U.S. cohort with a subgroup analysis of patients 65 years or older.
Study type and design	Retrospective study with data from archived patient samples.
Follow-up period	Not applicable, explanation: Predicting SLNB status outcome is the endpoint, as the model under development is intended as a prediction tool. No follow-up period in

Inclusion and exclusion criteria	these studies is required, as SLNB status outcome is already known during the study and is used as reference against the output label of the model. Inclusion: Primary cutaneous melanoma patients (age≥18 years) who underwent an SLNB within 90 days of their primary diagnosis at the Mayo Clinic in Minnesota, Arizona, or Florida between 2004 and 2019 or the West Virginia University between 2007 and 2014. Exclusion: No SLNB performed; prior melanoma diagnosis; SLNB after 90 days of primary diagnosis; M1 disease within 90 days of primary diagnosis; insufficient primary tumor diagnostic biopsy tissue; missing data on Breslow thickness or patient age; inadequate RNA harvested; duplicate samples, and, for Minnesota, denial of access to medical records for research purposes (per Minnesota State law).
Intervention	CP-GEP model ("Merlin"), a model that combines clinicopathologic features patient's age and Breslow thickness, with the expression of eight genes from the primary tumour. Genes included are: *ITGB3, PLAT, SERPINE2, GDF15, TGFBR1, LOXL4, CXCL8 and MLANA*
Comparator	Sentinel Lymph Node Biopsy (SLNB)
Baseline characteristics	Baseline characteristics of the 208 primary cutaneous melanoma patients • Gender distribution (n, %): All patients: male sex n= 113 (54.3%) • Age (median, interval) All patients: 59 years (45-70 years) • Previously treated (n, %): not applicable, only newly diagnosed patients included for whom the primary biopsy was tested. This biopsy was treatment naïve. • Weighting (ave., SD): data not collected in this study • Severity of disease: the prevalence of SLNB nodal metastases in this study is 21% (44 patients had an SLNB positive outcome) We included two more clinically relevant variables as baseline characteristics for the patient populations, which are Breslow Thickness and ulceration: • Breslow thickness (median, interval): All patients: 1.30 mm (0.90-2.10 mm) • Ulceration (Presence, %): All patients: present in 49 out of 208 patients (23.5%)
Primary and secondary outcome measures	Primary outcome measures: SLNB reduction rate (proportion of Low Risk assigned patients by the model, that theoretically could have forgone SLNB surgery) Negative Predictive Value (NPV) Secondary outcome measures: Positive Predictive Value (PPV) Sensitivity Specificity

Analysis method	• Samples with a probability of metastasis greater than the cutoff were classified as positive, whereas samples with a probability lower than the cutoff were classified as negative. • SLNB RR = (TN +FN)/ (TP + TN + FN + FP) • NPV = TN/ (TN + FN) • PPV = TP/ (TP +FP) • Sensitivity = TP/ (TP + FN) • Specificity = TN/ (TN +FP) Subgroup analysis have been conducted for 77 patients who were 65 years or older (Medicare population)
Relevant sub-group analysis	• Samples with a probability of metastasis greater than the cutoff were classified as positive, whereas samples with a probability lower than the cutoff were classified as negative. • SLNB RR = (TN +FN)/ (TP + TN + FN + FP) • NPV = TN/ (TN + FN) • PPV = TP/ (TP +FP) • Sensitivity = TP/ (TP + FN) • Specificity = TN/ (TN +FP)
Supplementary articles based on the same study	Not applicable

Study identification no.	**Clinical evaluation of the clinicopathologic and gene expression profile (CP-GEP) in patients with melanoma eligible for sentinel lymph node biopsy: A multicenter prospective Dutch study.**
Link to abstract	Link
Reference (first author, year)	Stassen, 2023
Overall objective of the study	Evaluate clinical validation and implementation of the CP-GEP model in a multicenter prospective Dutch study.
Study type and design	Prospective study with data from four dedicated melanoma centers (Erasmus Medical Center (MC), Isala Hospital, Leiden University Medical Center and Maastricht University Medical Center) in the Netherlands from July 2020 until August 2022.
Follow-up period	Not applicable, explanation: Predicting SLNB status outcome is the endpoint, as the model under development is intended as a prediction tool. No follow-up period in these studies is required, as SLNB status outcome is already known during the study and is used as reference against the output label of the model.
Inclusion and exclusion criteria	Inclusion: Patients ≥18 years, with newly diagnosed primary cutaneous melanoma, eligible for SLNB (pT1b – pT4 according to American joint committee on cancer (AJCC) 8th edition) were assessed for study eligibility Exclusion: Exclusion was based on documented clinical positive nodes at diagnosis, satellite or in-transit metastases, or multiple primary melanomas with lymphatic drainage to the same lymph node basin. Other exclusion criteria included distant metastatic disease present at primary diagnosis, prior history of a primary invasive melanoma (>pT1b)

	within the last 5 years, non-cutaneous melanoma, and missing pathology report or paraffin embedded tissue of the primary melanoma.
Intervention	CP-GEP model ("Merlin"), a model that combines clinicopathologic features patient's age and Breslow thickness, with the expression of eight genes from the primary tumour. Genes included are: *ITGB3, PLAT, SERPINE2, GDF15, TGFBR1, LOXL4, CXCL8 and MLANA*
Comparator	Sentinel Lymph Node Biopsy (SLNB)
Baseline characteristics	Baseline characteristics of the 260 primary cutaneous melanoma patients • Gender distribution (n, %): All patients: male sex n= 147 (56.5%) • Age (median, interval) All patients: 63 years (50-71 years) • Previously treated (n, %): not applicable, only newly diagnosed patients included for whom the primary biopsy was tested. This biopsy was treatment naïve. • Weighting (ave., SD): data not collected in this study • Severity of disease: the prevalence of SLNB nodal metastases in this study is 13.5% (35 patients had an SLNB positive outcome) We included two more clinically relevant variables as baseline characteristics for the patient populations, which are Breslow Thickness and ulceration: • Breslow thickness (median, interval): All patients: 1.40 mm (1.0-2.2 mm) • Ulceration (Presence, %): All patients: present in 38 out of 260 patients (14.6%)
Primary and secondary outcome measures	Primary outcome measures: SLNB reduction rate (proportion of Low Risk assigned patients by the model, that theoretically could have forgone SLNB surgery) Negative Predictive Value (NPV) Secondary outcome measures: Positive Predictive Value (PPV) Sensitivity Specificity
Analysis method	• Samples with a probability of metastasis greater than the cutoff were classified as positive, whereas samples with a probability lower than the cutoff were classified as negative. • SLNB RR = (TN +FN)/ (TP + TN + FN + FP) • NPV = TN/ (TN + FN) • PPV = TP/ (TP +FP) • Sensitivity = TP/ (TP + FN) • Specificity = TN/ (TN +FP)
Relevant sub-group analysis	No subgroup analysis have been conducted in this study
Supplementary articles based on the same study	Not applicable

Study identification no.	**Identification of stage I/IIA melanoma patients at high risk for disease relapse using a clinicopathologic and gene expression model**
Link to abstract	Link
Reference (first author, year)	Eggermont, 2020
Overall objective of the study	Investigate the potential of CP-GEP to identify stage I/IIA patients at high risk for relapse who might benefit from adjuvant therapy or intensive surveillance.
Study type and design	Retrospective study with data from archived patient samples. The prognostic value of the CP-GEP output labels was assessed with respect to three survival endpoints: relapse free survival (RFS); distant metastasis free survival (DMFS) and melanoma-specific survival (MSS).
Follow-up period	Five years after melanoma diagnosis
Inclusion and exclusion criteria	Inclusion: Breslow thickness greater than 1.0 mm; Breslow thickness of 0.75-0.99 mm and presence of ulceration, mitoses, and/or patient age less than 40 years; or Breslow thickness of 0.50-0.74 mm and presence of at least two of the following: ulceration, mitoses and patient age less than 40 years. Exclusion: M1 disease within 90 days of primary diagnosis; insufficient primary tumour diagnostic biopsy tissue; inadequate RNA harvested and, for Minnesota, denial of access to medical records for research purposes (per Minnesota State law).
Intervention	CP-GEP model ("Merlin"), a model that combines clinicopathologic features patient's age and Breslow thickness, with the expression of eight genes from the primary tumour. Genes included are: *ITGB3, PLAT, SERPINE2, GDF15, TGFBR1, LOXL4, CXCL8 and MLANA*
Comparator	Sentinel Lymph Node Biopsy (SLNB)
Baseline characteristics	Baseline characteristics of the 837 primary cutaneous melanoma patients • Gender distribution (n, %): All patients: male sex n= 526 (62.8%) • Age (median, interval) All patients: 60 years (15-89 years) • Previously treated (n, %): not applicable, only newly diagnosed patients included for whom the primary biopsy was tested. This biopsy was treatment naïve. • Weighting (ave., SD): data not collected in this study • Severity of disease: the prevalence of SLNB nodal metastases in this study is 23.9% (200 patients had an SLNB positive outcome) We included two more clinically relevant variables as baseline characteristics for the patient populations, which are Breslow Thickness and ulceration: • Breslow thickness (median, interval): Not reported • Ulceration (Presence, %): All patients: present in 192 out of 837 patients (22.9%)
Primary and secondary outcome measures	Primary outcome measures: Relapse free survival (RFS) Secondary outcome measures:

Analysis method	Distant metastasis free survival (DMFS) Melanoma-specific survival (MSS) Survival was assessed by Kaplan-Meier curves and Cox proportional hazard analysis The Wald test was used to assess the statistical significance of the difference in survival between groups. The median follow-up was calculated based on reverse Kaplan-Meier estimator
Relevant sub-group analysis	No subgroup analysis have been conducted in this study
Supplementary articles based on the same study	Not applicable

Study identification no.	**Using a Clinicopathologic and Gene Expression (CP-GEP) Model to Identify Stage I–II Melanoma Patients at Risk of Disease Relapse**
Link to abstract	Link
Reference (first author, year)	Mulder, 2022
Overall objective of the study	investigate whether the CP-GEP model could identify patients with stage I–II melanoma and a high risk of recurrence based on two independent European patient cohorts.
Study type and design	Retrospective study with data from archived patient samples. The prognostic value of the CP-GEP output labels was assessed with respect to three survival endpoints: relapse free survival (RFS); distant metastasis free survival (DMFS) and overall survival (OS).
Follow-up period	Five and ten years after melanoma diagnosis
Inclusion and exclusion criteria	Inclusion: Patients with cutaneous melanoma aged ≥18 years and with a negative SLNB at the Sahlgrenska University Hospital (between October 2006 and December 2014) and Erasmus MC Cancer Institute (between January 2007 and December 2017). Exclusion: Patients with multiple primary melanomas prior to SLNB, missing data on age or Breslow thickness, missing formalin-fixed paraffin-embedded (FFPE) tissue from the primary tumor (excised for diagnostic purpose), or an invalid quantitative polymerase chain reaction (qPCR) test.
Intervention	CP-GEP model (“Merlin”), a model that combines clinicopathologic features patient's age and Breslow thickness, with the expression of eight genes from the primary tumour. Genes included are: *ITGB3, PLAT, SERPINE2, GDF15, TGFBR1, LOXL4, CXCL8 and MLANA*
Comparator	Sentinel Lymph Node Biopsy (SLNB)
Baseline characteristics	Baseline characteristics of the 535 primary cutaneous melanoma patients • Gender distribution (n, %): All patients: male sex n= 273 (51.0%) • Age (median, interval) All patients: 60 years (48-70 years) • Previously treated (n, %): not applicable, only newly diagnosed patients included for whom the primary biopsy was tested. This biopsy was treatment naïve.

	• Weighting (ave., SD): data not collected in this study • Severity of disease: only SLNB negative patients included in this study We included two more clinically relevant variables as baseline characteristics for the patient populations, which are Breslow Thickness and ulceration: • Breslow thickness (median, interval): 1.80 mm (1.30- 3.00 mm) • Ulceration (Presence, %): All patients: present in 145 out of 535 patients (27.1%)
Primary and secondary outcome measures	Primary outcome measures: Relapse free survival (RFS) Secondary outcome measures: Distant metastasis free survival (DMFS) Melanoma-specific survival (MSS)
Analysis method	• Survival was assessed by Kaplan-Meier curves and Cox proportional hazard analysis • The Wald test was used to assess the statistical significance of the difference in survival between groups. • The median follow-up was calculated based on reverse Kaplan-Meier estimator
Relevant sub-group analysis	No subgroup analysis have been conducted in this study
Supplementary articles based on the same study	Not applicable

Study identification no.	**Identification of stage I/II melanoma patients at high risk for recurrence using a model combining clinicopathologic factors with gene expression profiling (CP-GEP)**
Link to abstract	Link
Reference (first author, year)	Amaral, 2023
Overall objective of the study	Validate CP-GEP for the identification of stage I/II patients at high risk for disease recurrence.
Study type and design	Retrospective study with data from archived patient samples. The prognostic value of the CP-GEP output labels was assessed with respect to three survival endpoints: relapse free survival (RFS); distant metastasis free survival (DMFS) and overall survival (OS).
Follow-up period	Five years after melanoma diagnosis
Inclusion and exclusion criteria	Inclusion: Patients aged ≥18 years, diagnosed with melanoma stage I/II between 2000 and 2017, and with a negative SLNB outcome e SLNB performed within 90 days of their diagnosis between 2000 and 2017.
Intervention	CP-GEP model (“Merlin”), a model that combines clinicopathologic features patient's age and Breslow thickness, with the expression of eight genes from the primary tumour. Genes included are: *ITGB3, PLAT, SERPINE2, GDF15, TGFBR1, LOXL4, CXCL8 and MLANA*
Comparator	Sentinel Lymph Node Biopsy (SLNB)
Baseline characteristics	Baseline characteristics of the 543 primary cutaneous melanoma patients • Gender distribution (n, %): All patients: male sex n= 309 (56.9%)

	• Age (median, interval) All patients: 66 years (54-74 years) • Previously treated (n, %): not applicable, only newly diagnosed patients included for whom the primary biopsy was tested. This biopsy was treatment naïve. • Weighting (ave., SD): data not collected in this study • Severity of disease: only SLNB negative patients included in this study We included two more clinically relevant variables as baseline characteristics for the patient populations, which are Breslow Thickness and ulceration: • Breslow thickness (median, interval): 1.70 mm (1.20- 2.80 mm) • Ulceration (Presence, %): All patients: present in 135 out of 543 patients (24.9%)
Primary and secondary outcome measures	Primary outcome measures: Relapse free survival (RFS) Secondary outcome measures: Distant metastasis free survival (DMFS) Melanoma-specific survival (MSS)
Analysis method	• Survival was assessed by Kaplan-Meier curves and Cox proportional hazard analysis • The Wald test was used to assess the statistical significance of the difference in survival between groups. • The median follow-up was calculated based on reverse Kaplan-Meier estimator Subgroup analysis was performed for 80 patients who did not undergo SLNB surgery to investigate the prognostic ability of CP-GEP in patients with unknown SLNB.
Relevant sub-group analysis	Outcome measures: RFS, DMFS, MSS Survival was assessed by Kaplan-Meier curves and Cox proportional hazard analysis The Wald test was used to assess the statistical significance of the difference in survival between groups. The median follow-up was calculated based on reverse Kaplan-Meier estimator
Supplementary articles based on the same study	Not applicable

Study identification no.	NCT04759781: MElanoma Research Lymph Node Prediction Implementation National_001 (MERLIN_001)
Link to abstract	Submitted / under review
Reference (first author, year)	Hieken, 2025
Overall objective of the study	Prospectively validate the CP-GEP model in an independent multicenter cohort of primary cutaneous melanoma patients, who undergo lymphatic mapping and SLNB per current clinical guidelines.
Study type and design	Prospective study with data from an observational registry clinical trial, nine participating institutions across the US. Planned enrolment of 2,340 patients.

Follow-up period	Five years after melanoma diagnosis.
Inclusion and exclusion criteria	Patients with clinically node-negative cutaneous melanoma and planned SLNB using current guideline indications are eligible for the study and will be followed for five years. Both patients and investigators are blinded to the results.
Intervention	CP-GEP model ("Merlin"), a model that combines clinicopathologic features patient's age and Breslow thickness, with the expression of eight genes from the primary tumor. The genes included are: *ITGB3, PLAT, SERPINE2, GDF15, TGFBR1, LOXL4, CXCL8 and MLANA*
Comparator	Sentinel Lymph Node Biopsy (SLNB)
Baseline characteristics	Baseline characteristics not reported in abstract
Primary and secondary outcome measures	Primary outcome measures: SLNB reduction rate (proportion of Low Risk assigned patients by the model, that theoretically could have forgone SLNB surgery) Negative Predictive Value (NPV) Risk of recurrence (RFS, DMFS, OS) Secondary outcome measures: Positive Predictive Value (PPV) Sensitivity Specificity
Analysis method	• Samples with a probability of metastasis greater than the cutoff were classified as positive, whereas samples with a probability lower than the cutoff were classified as negative. • SLNB RR = (TN +FN)/ (TP + TN + FN + FP) • NPV = TN/ (TN + FN) • PPV = TP/ (TP +FP) • Sensitivity = TP/ (TP + FN) • Specificity = TN/ (TN +FP) • Survival was assessed by Kaplan-Meier curves and Cox proportional hazard analysis • The Wald test was used to assess the statistical significance of the difference in survival between groups. • The median follow-up was calculated based on reverse Kaplan-Meier estimator
Relevant sub-group analysis	No subgroup analysis have been conducted in this study
Supplementary articles based on the same study	Not applicable

Study identification no.	**Using a clinicopathologic and gene expression model to predict sentinel lymph node metastasis in primary cutaneous melanoma could potentially reduce the rate of sentinel lymph node biopsies with >40%: a multicenter Danish cohort study**
Link to abstract	Submitted / accepted
Reference (first author, year)	Weitemeyer, 2025
Overall objective of the study	Validate the prognostic utility of the CP-GEP in an independent multicenter Danish cohort of stage I-II melanoma patients.
Study type and design	The study included 438 patients with T1-T3 cutaneous melanoma and negative sentinel node biopsies (stage I-II) performed between 2010 and 2015 at two university clinics in Denmark. Archived formalin-fixed paraffin-embedded primary

	melanoma tissue was collected, and CP-GEP was applied to each case. Data regarding recurrence was obtained from the Danish Melanoma Database.
Follow-up period	Five years after melanoma diagnosis
Inclusion and exclusion criteria	Patients eligible for SLNB surgery based on national clinical guidelines.
Intervention	CP-GEP model ("Merlin"), a model that combines clinicopathologic features patient's age and Breslow thickness, with the expression of eight genes from the primary tumor. Genes included are: *ITGB3, PLAT, SERPINE2, GDF15, TGFBR1, LOXL4, CXCL8 and MLANA*
Comparator	Sentinel Lymph Node Biopsy (SLNB)
Baseline characteristics	Baseline characteristics not reported in abstract
Primary and secondary outcome measures	Primary outcome measures: Risk of recurrence (RFS, OS)
Analysis method	• Survival was assessed by Kaplan-Meier curves and Cox proportional hazard analysis • The Wald test was used to assess the statistical significance of the difference in survival between groups. • The median follow-up was calculated based on reverse Kaplan-Meier estimator
Relevant sub-group analysis	No subgroup analysis have been conducted in this study
Supplementary articles based on the same study	Not applicable

Study identification no.	**Risk stratification using the Merlin Assay (CP-GEP) in an independent cohort of 930 patients with clinical stage I/II melanoma who did not undergo sentinel lymph node biopsy**
Link to abstract	Link
Reference (first author, year)	Amaral, 2025
Overall objective of the study	Investigate CP-GEP ability to stratify pts who did not undergo SLNB for their risk of recurrence in an expanded cohort.
Study type and design	We analyzed formalin-fixed paraffin-embedded primary tumor samples of 930 pts with stage I/II CM diagnosed between 2000-2020, included in the Central Malignant Melanoma Registry, who did not receive SLNB. Study hypothesis and protocol were prospectively formulated.
Follow-up period	Five years after melanoma diagnosis
Inclusion and exclusion criteria	Patients eligible for SLNB surgery based on national clinical guidelines.
Intervention	CP-GEP model ("Merlin"), a model that combines clinicopathologic features patient's age and Breslow thickness, with the expression of eight genes from the primary tumour. Genes included are: *ITGB3, PLAT, SERPINE2, GDF15, TGFBR1, LOXL4, CXCL8 and MLANA*
Comparator	Sentinel Lymph Node Biopsy (SLNB)
Baseline characteristics	Baseline characteristics were limited reported in abstract Gender = 59.2% male Median age = 64 years Breslow Thickness = 0.5 mm Ulceration present = 4.7%

Primary and secondary outcome measures	Primary outcome measures: Risk of recurrence (RFS, DMFS, MSS)
Analysis method	• Survival was assessed by Kaplan-Meier curves and Cox proportional hazard analysis • The Wald test was used to assess the statistical significance of the difference in survival between groups. • The median follow-up was calculated based on reverse Kaplan-Meier estimator
Relevant sub-group analysis	No subgroup analysis have been conducted in this study
Supplementary articles based on the same study	Amaral 2023 & Amaral 2024

Study identification no.	**Enhanced Risk Stratification for Sentinel Lymph Node Biopsy in Head and Neck Melanoma Using the Merlin Assay (CP-GEP)**
Link to abstract	Link
Reference (first author, year)	Pazhava, 2025
Overall objective of the study	Investigate CP-GEP ability to stratify pts who did not undergo SLNB for their risk of recurrence in an expanded cohort.
Study type and design	We analyzed formalin-fixed paraffin-embedded primary tumor samples of 250 pts with stage I/III CM in the head and neck region diagnosed between 2004-2021, from the Mayo Clinic and University Hospitals Cleveland Medical Center.
Follow-up period	Five years after melanoma diagnosis
Inclusion and exclusion criteria	Patients eligible for SLNB surgery based on national clinical guidelines.
Intervention	CP-GEP model (“Merlin”), a model that combines clinicopathologic features patient's age and Breslow thickness, with the expression of eight genes from the primary tumour. Genes included are: *ITGB3, PLAT, SERPINE2, GDF15, TGFBR1, LOXL4, CXCL8 and MLANA*
Comparator	Sentinel Lymph Node Biopsy (SLNB)
Baseline characteristics	Baseline characteristics were limited reported in abstract Gender = 79.2% male Median age = 68 years Breslow Thickness = 1.6 mm Ulceration present = 19.6%
Primary and secondary outcome measures	Primary outcome measures: SLNB reduction rate (proportion of Low Risk assigned patients by the model, that theoretically could have forgone SLNB surgery) Negative Predictive Value (NPV) Risk of recurrence (RFS, DMFS, MSS) Secondary outcome measures: Positive Predictive Value (PPV) Sensitivity Specificity
Analysis method	• SLNB RR = (TN +FN)/ (TP + TN + FN + FP) • NPV = TN/ (TN + FN) • PPV = TP/ (TP +FP) • Sensitivity = TP/ (TP + FN)

	• Specificity = TN/ (TN +FP) • Survival was assessed by Kaplan-Meier curves and Cox proportional hazard analysis • The Wald test was used to assess the statistical significance of the difference in survival between groups. • The median follow-up was calculated based on reverse Kaplan-Meier estimator
Relevant sub-group analysis	No subgroup analysis have been conducted in this study
Supplementary articles based on the same study	Not applicable

11.3 Results per study included in the analysis of Clinical effectiveness and safety

Table 38. Overview study results per study – Nodal metastasis prediction

Reference: author (year) country	Cohorts & substages	Sample size	SLNB positivity prevalence (%)	SLNB RR, % [95% CI]	Sensitivity, % [95% CI]	Specificity, % [95% CI]	Negative predictive value, % [95% CI]
Bellomo (2020)	T1-T4	754	17.0	42.4	92.2	49.5	96.9
United States	T1-T2	549184	10.0	56.1	83.6	60.5	97.1
	pT1b	290	3.3	80 [74-86]	41	82	98 (95-100)
	pT2a	66	13.1	48 [42-54]	80	53	95 (91-98)
	pT2b	124	16.7	24 [14-34]	94	27	96 (91-100)
	pT3a	79	31.4	8 [3-12]	99	12	98 (95-100)
	pT3b		43.0	4 [0-9]	100	7	100 (97-100)
Mulder (2020)	T1-T3	175	26.9	24.0	91.5 [80.1-96.6]	29.7 [22.5-38.1]	90.5 [77.9-96.2]
Netherlands	pT1	11	0	90.9	0	90.9 [62.3-98.4]	100 [72.2-100]
	pT2	94	18.1	29.8	82.4 [59.0-93.8]	32.5 [23.1-43.5]	89.3 [72.8-96.3]
	pT3	70	42.9	5.7	96.7 [83.3-99.4]	7.5 [2.6-19.9]	75 [30.1-95.4]
Johansson (2021)	T1-T3	358	10.6	24 [19.7-28.8]	92.1 [78.6-98.3]	25.9 [21.2-31.1]	96.5 [90.1-99.3]
Sweden	T1-T2	240	8.3	35.4 [29.4-41.8]	85.0 [62.1-96.8]	37.3 [30.9-44.0]	96.5 [90.0-90.3]
	pT1	30	3.3	53.3 [34.3-71.7]	0 [0-97.5]	51.7 [32.5-70.6]	93.8 [69.8-99.8]
	pT2	210	9.0	32.9 [26.5-39.7]	89.5 [66.9-98.7]	35.1 [28.3-42.3]	97.1 [89.9-99.6]
	pT3	118	15.3	0.8 [0-4.6]	100 [81.5-100]	1 [0-5.4]	100 [2.5-100]
Yousaf (2021)	T1-T3	193	20.7	33.7 [27.1-40.8]	90.0 [76.3-97.2]	39.9 [32.1-48.1]	93.8 [85.0-98.3]
United States	T1-T2	153	16.3	41.8 [33.9-50.1]	84.0 [63.9-95.5]	46.9 [38.0-55.9]	93.8 [84.8-98.3]
	pT1	74	10.8	60.8 [48.8-72.0]	62.5 [24.5-91.5]	63.6 [50.9-75.1]	93.3 [81.7-98.6]
	pT2	79	21.5	24.1 [15.1-35.0]	94.1 [71.3-99.9]	29.0 [18.2-41.9]	94.7 [74.0-99.9]
	pT3	40	37.5	2.5 [0.1-13.2]	100 [78.2-100]	4.0 [0.1-20.4]	100 [2.5-100]
Stassen (2023)	T1-T4	222	15.8	39.2 [32.7-45.9]	91.4 [76.9-98.2]	45.8	96.7 [90.6-99.3]
Netherlands	T1-T2	162	11.7	53	84	57	96.0
	pT1	62	4.8	83.9 [72.3-92.0]	0 [0-70.8]	83.1 [71.0-91.6]	94.2 [84.1-98.8]
	pT2	100	16.0	33.0 [23.9-43.1]	100 [78.2-100]	38.8 [28.4-50.0]	100 [89.4-100]
	pT3	16	25.0	4.5 [0.6-15.5]	100 [71.5-100]	6.1 [0.74-20.2]	100 [15.8-100]
Hieken (2025)*	T1-T3	1701	17.0	36.4 [31.1-38.7]	85.8 [81.2-89.6]	40.9 [38.4-43.6]	93.4 [91.1-95.2]
United States	T1-T2	1323	12.5	45.7 [42.9-48.4]	76.5 [69.3-82.7]	48.8 [45.9-51.8]	93.5 [91.3-95.4]
	pT1a	23	0	82.6 [61.2-95]	N/A	82.6 [61.2-95]	100 [82.4-100]
	pT1b	448	7.6	68.5 [64-72.8]	58.8 [40.7-75.4]	70.8 [66.1-75.1]	95.4 [92.5-97.5]

	pT2a	683	14.9	36.2 [32.6-39.9]	81.4 [72.4-88.4]	39.2 [35.2-43.3]	92.3 [88.2-95.3]
	pT2b	163	17.8	17.2 [11.7-23.9]	82.8 [64.2-94.2]	17.2 [11.2-24.6]	82.1 [63.1-93.9]
	pT3a	216	29.6	5.6 [2.9-9.5]	98.4 [91.6-100]	7.2 [3.7-12.6]	91.7 [61.5-99.8]
	pT3b	162	36.4	1.9 [0.4-5.3]	98.3 [90.9-100]	1.9 [0.2-6.8]	66.7 [9.4-99.2]
Weitemeyer (2025)	T1-T3	536	18.1	40.9%	80.4 [71.1-87.7]	45.6 [40.8-50.4]	91.3 [86.8-94.7]
Denmark	pT1	176	7.4	72.7%	46.2 [19.2-74.9]	74.2 [66.8-80.8]	94.5 [89.1-97.8]
	pT2	251	19.5	35.5%	77.6 [63.4-88.2]	38.6 [31.9-45.7]	87.6 [79.0-93.7]
	pT3	109	32.1	1.8%	97.1 [85.1-99.9]	1.4 [0-0.1]	50 [0-98.7]
Pazhava (2025)	T1-T4	250	14.0	40.8 [34.6-47.2]	94.3 [80.8-99.3]	46.5 [39.7-53.4]	98 [93.1-99.8]
United States	T1-T3	235	13.2	43 [36.6-49.6]	93.5 [78.6-99.2]	48.5 [41.5-55.6]	98 [93-99.8]
	T1-T2	167	8.4	56.3 [48.4-63.9]	92.9 [66.1-99.8]	60.8 [52.6-68.6]	98.9 [94.2-100]
	pT1	52	0	73.1 [59-84.4]	-	73.1 [59-84.4]	100 [90.7-100]
	pT2	115	12.2	48.7 [39.3-58.2]	92.9 [66.1-99.8]	54.5 [44.2-64.4]	98.2 [90.4-100]
	pT3	68	25.0	10.3 [4.2-20.1]	94.1 [71.3-99.9]	11.8 [4.4-23.9]	85.7 [42.1-99.6]
	pT4	15	26.7	6.7 [0.2-31.9]	100 [39.8-100]	9.1 [0.2-41.3]	100 [2.5-100]
Wong (2025)	T1-T4	1061	17.8	27 [20-35]	93 [88-96]	32 [23-41]	95 [92-97]
Multiple countries	pT1	177	6.8	72 [55-85]	22 [1-93]	72 [56-84]	94 [89-97]
	pT2	483	14.3	31 [27-35]	91 [82-96]	35 [30-39]	96 [91-98]
	pT3	272	27.2	3 [1-6]	99 [91-100]	4 [2-8]	87 [46-98]
	pT4	129	26.4	0 [0-100]	100 [0-100]	0 [0-100]	-
Whole cohort analysis	T1-T3	3403	[redacted]				
of 7 studies: Mulder,	T1-T2	2577					
Johansson, Yousaf,	pT1a	105					
Stassen, Hieken,	pT1b	770					
Weitemeyer, Pazhava	pT2a	1407					
(unpublished)	pT2b	283					

*Hieken et al., 2025 updated data after WLE.

Table 39. study results per study – abstracts - safety

Reference: author (year) country	Cohort & Sample size	Recurrence-free survival, % (5-year)	Distant metastatic free survival, % (5-year)	Overall survival/ Melanoma-specific survival, % (5-year)	Hazard Ratio (95% CI, p-value)
Yu (2023)	All patients (583)	79.1 [75.1-82.6]	87.7 [84.4-90.3]	91.7 [87.9-94.3]	Stage I-III
United States	CP-GEP Low Risk (196)	92.4 [86.4-95.9]	96.9 [92.7-98.7]	98.2 [93-99.6]	RFS: 4.89; P<0.001
	CP-GEP High Risk (387)	72.2 [66.7-77]	82.9 [78.2-86.6]	88 [82.3-91.9]	DMFS: 6.37; P<0.001
					MSS: 7.49; P<0.01
	Stage I/II (476)	85.5 [81.4-88.7]	92.4 [89.4-94.6]	96.3 [92.9-98]	Stage I-II
	CP-GEP Low Risk (180)	92.5 [86.2-96]	97.4 [93.1-99]	99.1 [93.5-99.9]	RFS: 3.27; P<0.001
	CP-GEP High Risk (296)	81.1 [75.5-85.5]	89.3 [84.9-92.5]	94.3 [88.7-97.1]	DMFS: 4.56; P<0.005
					MSS: 6.27; NS
	Stage III (107)	51.4 [39.6-61.9]	67.3 [56.1-76.3]	67.9 [52.6-79.3]	Stage III
	CP-GEP Low Risk (16)	91.7 [53.9-98.8]	91.7 [53.9-98.8]	80 [20.4-96.9]	RFS: 10.05; P<0.05
(MSS)	CP-GEP High Risk (91)	44.6 [32.2-56.2]	63.1 [50.7-73.2]	66 [49.3-78.3]	DMFS: 6.02; NS
					MSS: 2.34; NS
Hill (2024)	Stage I-III	84.9 [78.4-89.5]	88.2 [82.1-92.4]	94 [88.7-96.9]	Stage I-III
United States	CP-GEP Low Risk (65)	95 [85.3-98.4]	98.1 [87.1-99.7]	98 [86.6-99.7]	RFS: 4.87; P<0.05
	CP-GEP High Risk (111)	78.7 [69.4-85.5]	82.3 [73.3-88.5]	91.5 [83.6-95.7]	DMFS: 11.89; P<0.05
(MSS)					MSS: 5.14; NS

11.4 Results per evaluation question

Not applicable

11.5 List of existing health economic analysis

Table 40. Overview cost evaluation Thao et al., 2023

Reference (author, year)	Peer-reviewed (yes/no)	Year	Country of origin	Analysis type	Patient population	Comparator	Time horizon	ΔC	ΔE*	ICER*	Used as inspiration for structure of analysis
Thao et al., 2023 (28)	Yes	2023	United States	Cost-minimization study	Patients with T1b or T2 primary cutaneous melanoma	SLNB	1 year	Break-even: T1b: $7,350 T1b-T2: $4,600	Not applicable	Not applicable	Yes

CEA: Cost-effectiveness analysis, CUA: Cost-utility analysis, ICER: Incremental cost-effectiveness ratio.

11.6 Description of the model design

A cost analysis was performed to compare the Merlin strategy with a no-testing (usual care) approach prior to SLNB. The Merlin strategy is designed to identify high-risk patients, allowing SLNB to be performed only when clinically necessary, thereby reducing overall costs and minimizing the risk of procedure-related complications. A decision tree model was constructed to estimate the cost implications of the Merlin testing strategy compared to usual care. The model incorporated the costs of Merlin testing, WLE, SLNB procedures, and potential surgical complications related to SLNB. A diagram of the decision tree is presented in **Figure 30**.

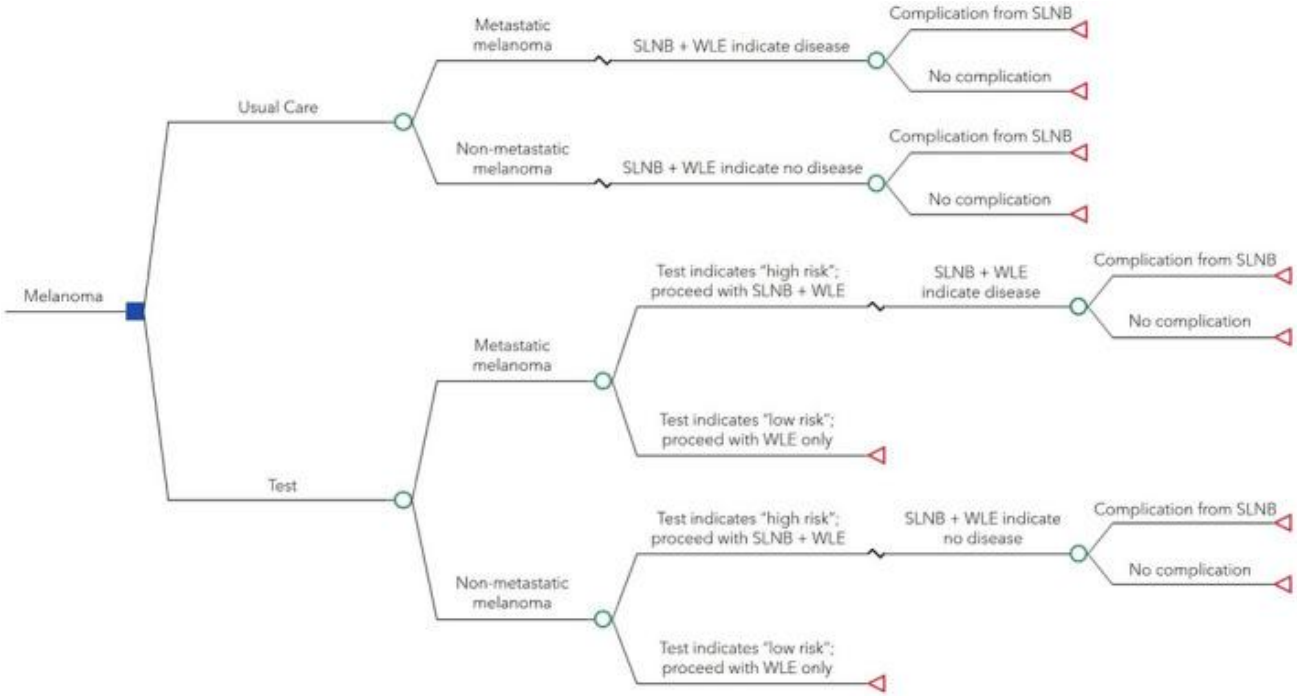


Figure 30. Decision-tree model that is used for cost evaluation of Merlin (CP-GEP) (28)

11.7 Transformation of data

To model background mortality transforming these into survival estimates, conversion to probabilities is required. The formula that was used is $1 - EXP^{(-rate)}$. Herein, the rate represents the mortality rate per unit of time (e.g., per year) to estimate the probability of surviving each subsequent year. As such, as per the formula, mortality rates are transformed into probabilities for survival for the provided time horizon.

11.8 Data extrapolation

See section 8.2.5.6.

11.9 Studies regarding health-related quality of life

Not applicable, external studies were used with utility or disutility values.

11.10 Annuitization and use of allocation keys

The current cost-effectiveness model does not incorporate annuitization of capital costs, meaning that upfront expenditures are treated as one-time costs rather than being spread over the useful life of the investment. Additionally, shared or overhead costs are not allocated using formal allocation keys.

11.11 Budget impact analysis

The budget impact model (BIM) for a Merlin testing strategy was developed in Microsoft Excel and includes a graphical and tabular presentation of results to aid decision-making aimed to estimate the budgetary implications of implementing Merlin within the Danish healthcare system, compared to the current standard of care (SLNB referral for eligible patients with T1–T2 melanoma).

The BIM was structured as a cohort-based, static model that estimates the annual cost impact over a defined time horizon of 5 years from the Danish healthcare system perspective, capturing direct medical costs relevant to payers and providers for Denmark and societal (patient) costs. Calculations include the total and per-patient budget impact per cost category (diagnostic cost, follow-up cost, therapy cost, opportunity cost, complication cost and out-of-pocket cost) and per diagnostic route. The model compares two different scenarios:

- Current practice (without Merlin): All SLNB-eligible patients undergo SLNB.
- Future scenario (with Merlin): SLNB is selectively performed based on a Merlin Low or High Risk test result.

Key model inputs:

- Target population: number of newly diagnosed T1–T2 melanoma patients eligible for SLNB, based on national cancer incidence data from the Danish Melanoma Database.
- Test performance: rates of Merlin Low and High Risk results implemented as SLNB RR
- Cost and time associated with SLNB procedure based on Danish tariffs, DRG rates, published literature, or expert input
- Cost and time associated with WLE based on Danish tariffs, DRG rates, published literature, or expert input
- Cost of Merlin testing
- Postoperative care and management of complications
- Downstream cost savings (e.g., avoided time spent in hospital, travel costs)
- Complication rates and costs: Incidence and treatment costs for SLNB-related adverse events such as seroma, hematoma, lymphedema.
- Unit costs: Based on Danish tariffs, DRG rates, published literature, or expert input (all stated in DKK, adjusted to a common base year).

11.11.1 Treatment pathway

The BIM follows the treatment pathway included in **Figure 4** and **Figure 7** including complications, potential follow-up and relapse.

11.11.2 Patient population

Patient population was estimated using data from the Danish melanoma database using distribution of patients over the specific substages T1a to T3b adapted from **Table 2**. The average increase or decrease in incidence of melanoma was calculated based on incidence from 2017 to 2023 averaging to a 2.2% yearly increase in melanoma incidence. Initially the DHTC requested data on T1-T3. However, in current clinical practice, T3 and T4 patients' risk of having nodal metastases is so significant that these patients are generally referred to SLNB. Merlin validation studies support these findings as most T3-T4 patients receive a Merlin High Risk label. Thus, the Merlin test adds the most value in the group T1-T2Hence, the analysis focuses on

the patient group where Merlin adds the most value: T1-T2 melanomas. The BIM is able to consider T3 patients, but results were not highlighted here.

Country	Denmark		Reference
Total population with melanoma:	2,820		Dansk Melanom Database
pT-stage distribution pT1a	4.3%		Dansk Melanom Database
pT-stage distribution pT1b	6.3%		Dansk Melanom Database
pT-stage distribution pT2a	17.2%		Dansk Melanom Database
pT-stage distribution pT2b	1.5%		Dansk Melanom Database
pT-stage distribution pT3a	5.9%		Dansk Melanom Database
pT-stage distribution pT3b	3.4%		Dansk Melanom Database
Prop. patients that are SNLB eligible pT1a	100.0%		Dansk Melanom Database
Prop. patients that are SNLB eligible pT1b	100.0%		Dansk Melanom Database
Prop. patients that are SNLB eligible pT2a	100.0%		Dansk Melanom Database
Prop. patients that are SNLB eligible pT2b	100.0%		Dansk Melanom Database
Prop. patients that are SNLB eligible pT3a	100.0%		Dansk Melanom Database
Prop. patients that are SNLB eligible pT3b	100.0%		Dansk Melanom Database
Total eligible patients per cohort pT1a	121	14.628%	Calculation
Total eligible patients per cohort pT1b	179	21.564%	Calculation
Total eligible patients per cohort pT2a	486	58.638%	Calculation
Total eligible patients per cohort pT2b	43	5.2%	Calculation
Total eligible patients per cohort pT3a	0	0%	Calculation
Total eligible patients per cohort pT3b	0	0%	Calculation
Total eligible population for model	828		
Yearly increase in incidence	2.2%		Dansk Melanom Database

Figure 31. Stepwise algorithm for calculating the target population.

11.11.3 Resource use & costs

To understand the costs associated with WLE and SLNB during the full trajectory of the patient, associated DRGs have been identified and summarized. Costs from the first melanoma diagnosis up until consultation about further follow-up or treatment plans would incur 26,539 kr. or 80.980 kr. depending on the length of stay in the hospital, with the higher price for patients staying longer than 12 hours. As per expert opinion, we assumed that approximately 10% of the patients would stay longer than 12 hours within the hospital. In contrast, if a WLE is to be carried out without further pathological research, the cost it would incur would amount to 11,845 kr. For a full breakdown of the associated DRG codes, types of specialists involved, and time associated with specific activities for SLNB and WLE, see cost breakdown below. This breakdown is similar to that of the cost-effectiveness model. The [redacted]

SLNB cost calculation budget impact model

Steps	Specific steps in clinical pathway	Type of specialist(s), or other health care personnel	Time in hours	Associated DRG codes	2025 cost (DKK)
1	Melanoma diagnosis (Referred to department of plastic surgery from GP or private Dermatologist OBS MM Plastic surgeon performs excision biopsy)	Plastic surgeon	30 min	09PR04	5,879 kr.
2	Consultation (patient informed about the diagnosis and SLNB + planning of the SLNB)	Plastic surgeon	45-60 minutes	09MA98	1,578 kr.
3	Pre-operative anesthesiologic visit	Anesthesiologist	10-15 minutes	36PR06 (same DRG code for dynamic and static lympho-scintigraphy)	4,565 kr.
4	Pre-operative plastic surgical visit	Plastic surgeon (trainee)	10-15 minutes		
5	Lymphoscintigraphy (0-1 days before SLNB)	Nuclear medicine	45-60 minutes		
6	Pre-operative tracer injection (on the day of SLNB)	Nuclear medicine	15 minutes	Included in SLNB codes	
7	Surgery (SLNB + wide local excision [WLE])	Plastic surgeon, anesthesiologist, nurse anesthesiologist, surgical assistant	1.5 hours*	36PR06 05PR01	8,374 kr. Or 62,815 kr.*
8	Consultation (patient informed about the SLNB result and further follow up)	Plastic surgeon	30 min	09MA98	1,578 kr.
	Total		HCP's ~9.5 hours Patient ~4.5 hours + 5 visits	90% of the cases 10% of the cases	<12 hours hospital stay after SLNB = 21.974 >=12 hours= 76.415** Weighted average=27,418

*HCP, healthcare practitioner; SLN, sentinel lymph node; SLNB, sentinel lymph node biopsy; WLE, wide local excision; *Four different specialists would be required for this activity, and time is associated with removal of one lymph node, generally 1-2 lymph nodes are removed; **depends on length of hospital stay (<12 hours versus ≥12 hours).*

WLE cost calculation budget impact model

Steps	Specific steps in clinical pathway	Type of specialist(s), or other health care personnel	Time in hours*	Associated DRG codes	2025 cost (DKK)
1	Melanoma diagnosis (Referred to department of plastic surgery from general practitioner or private Dermatologist + Plastic surgeon performs excision biopsy)	Plastic surgeon	30 min	09PR04	5,879 kr.
2	Consultation (patient informed about Merlin result and further follow up) + WLE under local anesthesia	Plastic surgeon	45-60 min	09PR04	5,879 kr.
3	Biopsy results by letter	Plastic surgeon	15	65TE01	87 kr.
	Total		~1.5 hour		11,845 kr.

WLE, wide local excision

If a positive node is encountered after SLNB, patients would be eligible for immunotherapy. In Denmark, patients with stage IIIA would not be eligible for therapy. To understand the cost impact of Merlin on further immunotherapy in patients with primary cutaneous melanoma, the cost of immunotherapy was calculated using information from: https://medicinpriser.dk/. To estimate the full price, a patient's body weight of 80 kg was assumed using the following schedules for 1) Nivolumab; 240 mg every 2 weeks over 30 minutes or 480 mg every 4 weeks over 60 minutes or over 30 minutes (3mg/kg, EPAR) continued as long as clinical benefit is observed or until treatment is no longer tolerated by the patient (and up to maximum duration of therapy if specified for an indication). For adjuvant therapy, the maximum treatment duration with OPDIVO is 12 months. 2) Pembrolizumab; 160 mg/kg bodyweight (up to a maximum of 200 mg), every 3 weeks administered as an intravenous infusion over 30 minutes (2 mg/kg, EPAR) and should be administered until disease recurrence, unacceptable toxicity, or for a duration of up to one year. For a breakdown of the costs for Nivolumab and Pembrolizumab see the table below. For the model, we assumed that patients were treated with pembrolizumab based on expert opinion amounting for: 749,953 kr. As not all patients would receive immunotherapy due to frailty or comorbidities or are categorized as stage IIIA, we assumed that 25% would not receive upfront immunotherapy as per discussions with experts.

Therapy cost calculation budget impact model

Immunotherapy	Price per vial	Per dose	Dose per patient	Total dose per year	Total price	Source
Nivolumab	20,457.13 kr.	240 mg	480 mg per 4 weeks	6,240 mg	531,885 kr.	Link
Pembrolizumab	21,573.58 kr.	100 mg	400 mg per 6 weeks	3,467 mg	747,884 kr.	

Immunotherapy	Price per administration	Number of administrations per year	Yearly price of admin	Total price	Source
Nivolumab	477.43 kr.	13	6,207 kr.	538,092 kr.	EPAR
Pembrolizumab	238.72 kr.	8.67	2,070 kr.	749,953 kr.	EPAR

Position	Effective hourly salary	Source
Ikke-ledende sundhedsplejersker (non-executive nurse)	**477.43**	**krl**

11.11.3.1 Costs associated with health conditions and events

Costs for adverse effects were estimated for complications following SLNB and costs of adverse events following immunotherapy. Find an overview of the costs for specific adverse events below. Specific Danish costs for complications were not identified. As such, complication costs from the UK for infection and lymphedema were considered. To ensure that costs were not driving model's model outcomes we kept complication costs relatively low. In addition, we assumed that if a patient was identified that required immunotherapy due to regional or distant metastasis additional costs would be incurred for immunotherapy of 749,953 kr.

Complication cost budget impact model

Adverse effect	Cost year	Country of origin & currency	Original cost	Converted cost	Source
Infection	2019	United Kingdom, £	£333.76	4298.58 kr.	(69)
Seroma	2019	United Kingdom, £	£333.76	4298.58 kr.	Proxy (69)
Hematoma	2019	United Kingdom, £	£333.76	4298.58 kr.	Proxy (69)
Lymphedema	2021	United Kingdom, £	£723.00	8788.68 kr.	(70)

Costs were converted using the following tool: CCEMG – EPPI-Centre Cost Converter. Accessed at: https://eppi.ioe.ac.uk/costconversion/default.aspx

11.11.4 Market share

A breakdown of the patient population and market shares for a Merlin testing strategy and standard of care as per:

- The treatment pathway with recommendation of health technology under examination
- The treatment pathway without recommendation of health technology under examination

Intervention and comparator(s)	Current year (Year 1)	Year 2	Year 3	Year 4	Year 5	Total
Annual patient population	828	846	865	884	904	
Merlin testing strategy	10%	20%	35%	55%	70%	
SLNB	90%	80%	65%	45%	30%	

Intervention and comparator(s)	Current year (Year 1)	Year 2	Year 3	Year 4	Year 5	Total
Annual patient population	828	846	865	884	904	
Merlin testing strategy	0%	0%	0%	0%	0%	
SLNB	100%	100%	100%	100%	100%	

11.11.5 Likely five-year budget impact

Disaggregated overview of the estimated budget impact per cost category and per testing strategy over a five-year time horizon can be found in the main text in section 8.3.2.

11.11.6 Benefits and savings

Savings related to staff time and theatre capacity resulting from a reduced surgical burden may not translate into immediate budget reductions. Instead, these may be reflected as freed-up capacity rather than actual savings. With the cost category “opportunity costs” we aimed to make this more visible. Efficiency gains like reduced staff time and reduced operating room capacity are relevant as it can alleviate pressure on overstretched surgical and nursing teams and potentially improve patient throughput in other clinical areas. Furthermore, a publication by Hieken et al. reported that, in addition to a reduction in procedure-related complications, if Merlin test decision was applied fewer post-procedural emergency department visits and hospital readmissions would have taken place. These downstream effects were not accounted for in the current budget impact model and may represent additional, unquantified areas of potential cost savings.

[redacted] Other relevant materials

[redacted] [redacted]

Version 1

[redacted]

[redacted]

[redacted]

[redacted]

