HTA Austria
Austrian Institute for
Health Technology Assessment
GmbH

Genetic Testing in Austria

Part C: Detection of PIK3CA/AKT1/PTEN/ESR1 genetic alterations in HR+/HER2– breast cancer

Final report

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Part C: Detection of *PIK3CA/AKT1/PTEN/ESR1* genetic alterations in HR+/HER2– breast cancer

Vienna, March 2026

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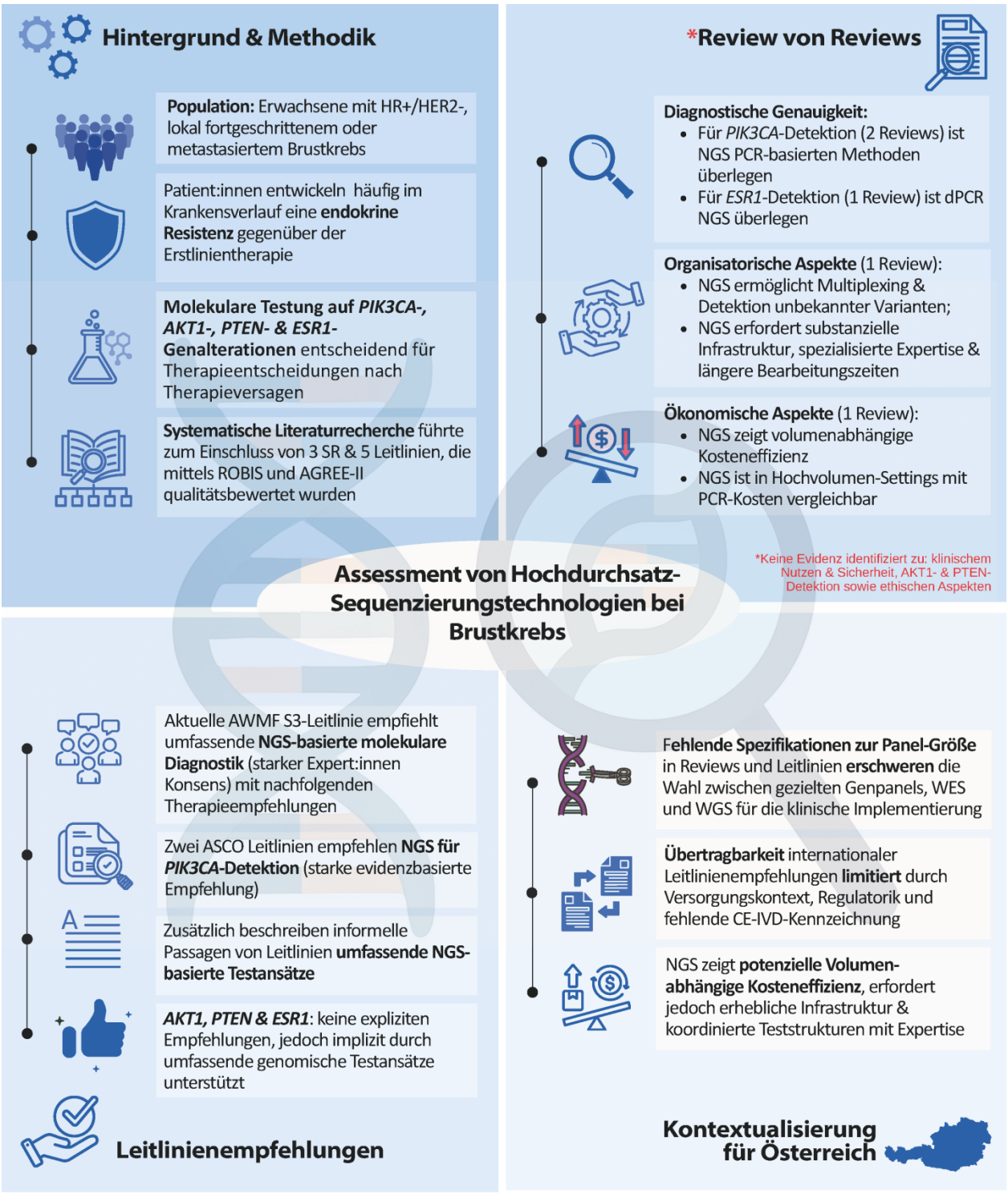
List of abbreviations

ACC ... Accuracy (proportion of all correctly identified cases)
AGREE II ... Appraisal of Guidelines for Research & Evaluation II
AIHTA ... Austrian Institute for Health Technology Assessment
AJCC ... American Joint Committee on Cancer
AKT1 ... AKT Serine/Threonine Kinase 1
ASCO ... American Society of Clinical Oncology
AT ... Austria
AUC ... Area Under the Curve
AWMF ... Arbeitsgemeinschaft der Wissenschaftlichen Medizinischen Fachgesellschaften
BC ... Breast Cancer

BEAMing ..... Beads, Emulsion, Amplification, and Magnetics
CDK4/6 ..... Cyclin-Dependent Kinase 4/6
CE-IVD ..... Conformité Européenne – In Vitro Diagnostic
cfDNA ..... Circulating Free DANN
CI ..... Confidence Interval
CLIA ..... Clinical Laboratory Improvement Amendments
ctDNA ..... Circulating Tumor DNA
ddPCR ..... Droplet Digital Polymerase Chain Reaction
DOR ..... Diagnostic Odds Ratio
dPCR ..... Digital Polymerase Chain Reaction
ECO ..... Economical Domain
EF ..... Effect
EGAPP ..... Evaluation of Genomic Applications in Practice and Prevention
ER ..... Estrogen Receptor
ESR1 ..... Estrogen Receptor 1
ETH ..... Ethical Domain
EUnetHTA ... European Network for Health Technology Assessment
FFPE ..... Formalin-Fixed Paraffin-Embedded
GER ..... Germany
GIN ..... Guidelines International Network
GRADE ..... Grading of Recommendations Assessment, Development and Evaluation
HER2 ..... Human Epidermal Growth Factor Receptor 2
HR ..... Hormone Receptor
HTA ..... Health Technology Assessment
IPD ..... Individual Patient Data
LL ..... Leitlinie (Guideline)
LKF ..... Leistungsorientierte Krankenanstaltenfinanzierung
MBC ..... Metastatic Breast Cancer
mTOR ..... Mechanistic Target of Rapamycin
n ..... Number of studies
N ..... Number of patients
NGS ..... Next-Generation Sequencing
NICE ..... National Institute for Health and Care Excellence
NIPH ..... Norwegian Institute of Public Health
NLR ..... Negative Likelihood Ratio
NOK ..... Norwegian Kroner
NPV ..... Negative Predictive Value
NR ..... Not Reported
OCEBM ..... Oxford Centre for Evidence-Based Medicine
ORG ..... Organisational Domain
OSF ..... Open Science Framework
PATA ..... Norwegian Pathology Activity Code
PCR ..... Polymerase Chain Reaction
PI3K ..... Phosphatidylinositol 3-Kinase
PICO ..... Population, Intervention, Comparison, Outcome
PIK3CA ..... Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha
PLR ..... Positive Likelihood Ratio
PPV ..... Positive Predictive Value
PTEN ..... Phosphatase and Tensin Homolog
qPCR ..... Quantitative Polymerase Chain Reaction
QUADAS-2 .. Quality Assessment of Diagnostic Accuracy Studies-2
RCT ..... Randomised Controlled Trial
REPL ..... Read-Eval-Print Loop
ROBIS ..... Risk of Bias in Systematic Reviews
RoB ..... Risk of Bias
RT-PCR ..... Real-Time Polymerase Chain Reaction
S3 ..... Highest level of evidence-based clinical practice guideline development (German classification)
SE ..... Sensitivity
SEER ..... Surveillance, Epidemiology, and End Results (Program)
SITC ..... Society for Immunotherapy of Cancer
SP ..... Specificity
SR ..... Systematic Review
STARD ..... Standards for Reporting of Diagnostic Accuracy
TAT ..... Turnaround Time
TMB ..... Tumor Mutational Burden
NM ..... Tumour, Node, Metastasis (Staging-System)
US ..... United States
WES ..... Whole Exome Sequencing
WGS ..... Whole Genome Sequencing

Visual Abstract



Abkürzungen: AGREE II…Appraisal of Guidelines for Research & Evaluation II; AKT1…AKT Serin/Threonin Kinase 1; ASCO…American Society of Clinical Oncology; AWMF…Arbeitsgemeinschaft der Wissenschaftlichen Medizinischen Fachgesellschaften; BK…Brustkrebs; dPCR…digitale Polymerase-Kettenreaktion; ESR1…Estrogen Receptor 1; HER2−…Humaner epidermaler Wachstumsfaktor-Rezeptor 2 negativ; HR+…Hormonrezeptor-positiv; nar…narrative; NGS…Next-Generation Sequencing; PCR…Polymerase-Kettenreaktion; PIK3CA…Phosphatidylinositol-4,5-bisphosphat-3-kinase catalytic subunit alpha; PTEN…Phosphatase and Tensin Homolog; ROBIS…Risk of Bias in Systematic Reviews tool; SR…Systematischer Review; WES…Whole Exome Sequencing; WGS…Whole Genome Sequencing.

Executive Summary

This assessment evaluates the clinical effectiveness and safety as well as the economic and organisational aspects of molecular tests for detection of *PIK3CA-/AKT1-/PTEN-/ESR1*-genetic alterations in adults diagnosed with HR+/HER2–, locally advanced or metastatic breast cancer.

Introduction

Breast cancer (BC) is a common cancer, affecting one in eight women over their lifetime. The HR+/HER2− subtype represents the most prevalent form and is characterised by an indolent clinical course with favourable response to endocrine therapy. Five-year survival rates for HR+/HER2– patients vary significantly according to disease stage: 90.1% for regional disease compared to 31.9% for distant metastatic spread. Endocrine therapy (hormone therapy) is typically the first-line treatment for HR+/HER2– advanced or metastatic BC. Endocrine resistance commonly develops during hormone therapy through alterations in the *PI3K/AKT/mTOR* pathway and genetic changes in the estrogen receptor (ER). Comprehensive molecular testing for *PIK3CA, AKT1, PTEN*, and *ESR1* genetic alterations has therefore become critical for guiding treatment decisions following first-line therapy failure.

This assessment evaluates the diagnostic accuracy of high-throughput sequencing technologies for detecting *PIK3CA, AKT1, PTEN*, and *ESR1* genetic alterations, including targeted-gene panel sequencing, whole exome sequencing (WES), whole genome sequencing (WGS), and virtual gene panel sequencing. Austrian clinical laboratories predominantly employ Illumina and Thermo Fisher Scientific platforms for targeted-gene panel sequencing, while WES and WGS are applied less frequently. These platforms are typically used without CE-IVD (Conformité Européenne In Vitro Diagnostic) designation and undergo local validation by laboratory experts. Molecular testing is performed at seven specialised centers, predominantly located at university hospitals or tertiary care facilities. Relevant comparators for this assessment include standard of care without molecular testing or without genetic alteration testing, and alternative molecular methods such as PCR or Sanger sequencing.

Methods

A systematic literature search was conducted in five databases covering publications from June 2020 to August 2025. The search focused on systematic reviews, HTA reports, and evidence-based clinical guidelines addressing *PIK3CA, AKT1, PTEN*, and *ESR1* genetic testing in HR+/HER2– BC. Guideline identification was supplemented by manual searches of four additional databases. Following deduplication, screening was performed by two independent researchers based on predefined PICO criteria. Diagnostic accuracy was evaluated in the available evidence against conventional diagnostic reference standards (e.g., PCR, Sanger sequencing) and linked to patient-relevant outcomes and safety aspects using the Linked Evidence Approach. Quality assessment employed the ROBIS tool for systematic reviews and the AGREE-II checklist for guidelines. Data extraction focused on diagnostic accuracy (review of reviews) and therapeutic recommendations (guideline synopsis). Furthermore, organisational, economic, and ethical implications were considered where evidence was available.

Results

Available evidence

Three high-quality systematic reviews (SRs), including two meta-analyses and one health technology assessment (HTA) report comprising over 40 primary studies, evaluated clinical validity. The two meta-analyses evaluated diagnostic test accuracy for *PIK3CA* and *ESR1* genetic alteration detection against tissue biopsy as a reference; the HTA report did a head-to-head comparison between methods for *PIK3CA* detection. No evidence was found for clinical utility outcomes, safety outcomes, or *AKT1* and *PTEN* testing. Additionally, the HTA report addressed organisational and economic aspects based on the Norwegian healthcare context. None of the identified reviews included ethical aspects.

For the guideline synopsis, five guideline recommendations/informal guidance passages from three international organisations of high methodological quality addressed next generation sequencing (NGS) for genetic alteration detection: the German Association of the Scientific Medical Societies (Arbeitsgemeinschaft der Wissenschaftlichen Medizinischen Fachgesellschaften, AWMF), the American Society of Clinical Oncology (ASCO), and the Society for Immunotherapy of Cancer (SITC).

Results: Review of reviews

For *PIK3CA*, NGS-based ctDNA testing achieved highest sensitivity and specificity among evaluated methods, with concordance between testing methods. For *ESR1*, dPCR demonstrated higher sensitivity than NGS, though substantial heterogeneity limited definitive conclusions. Both SRs referenced NGS technology without specifying length, but it is assumed to be targeted-gene panel sequencing.

The HTA report based on the Norwegian healthcare context identified organisational and economic considerations. Organisationally, NGS enables multiplexing (simultaneous sequencing of multiple samples in one run) and unknown variant detection but requires substantial infrastructure, specialised bioinformatics expertise and longer turnaround times. NGS shows high sensitivity with tissue samples but higher failure rates with liquid biopsies, while PCR shows opposite characteristics. Economically, NGS demonstrates volume-dependent cost-efficiency, with per-patient costs decreasing when multiple patients are tested simultaneously, becoming comparable to PCR in high-volume settings.

Results: Guideline synopsis

Five guideline recommendations/informal guidance passages from three international organisations (AWMF, ASCO, SITC) addressed NGS for genetic alteration detection. Three formal recommendations were identified. AWMF S3 2025 recommends comprehensive NGS-based molecular diagnostics with subsequent therapy recommendations based on strong expert consensus. ASCO 2021 provides an evidence-based strong recommendation for *PIK3CA* detection using sequential testing (cfDNA first, then tissue) for postmenopausal and male patients with HR+ metastatic BC. ASCO 2022 provides the same recommendation but for a broader population including patients with locally recurrent unresectable or metastatic HR+/HER2– BC. Two informal guidance passages describe comprehensive NGS testing: ASCO 2024 mentions large-panel NGS for targetable genetic alterations including *PIK3CA, AKT1, PTEN,* and *ESR1*, while SITC 2021 references "actionable gene genetic alterations" with *PIK3CA* explicitly mentioned. All guidelines referenced NGS technology without specifying length, but it is assumed to be targeted-gene panel sequencing.

Discussion and conclusion

This pilot assessment suggests that a focused review-of-reviews and guideline-synopsis approach can support the evaluation of genetic testing, while also revealing evidence limitations. Across the included reviews, study quality and reporting of the underlying primary studies were often inadequate, and substantial heterogeneity, particularly in *ESR1* analysis, limits interpretability. Current evidence supports superior clinical validity of NGS over PCR for *PIK3CA* detection and higher sensitivity of dPCR for *ESR1*, yet no data exist on clinical utility, safety outcomes, or *AKT1/PTEN* testing. International guidelines support NGS for *PIK3CA* and implicitly for broader genomic targets, but their transferability to Austria is constrained by differing healthcare contexts, regulatory requirements, and the absence of CE marking for many tests.

Moreover, implementation challenges arise from the lack of clear distinctions between gene panel, WES, and WGS approaches, varying target populations, and infrastructural needs for cost-efficient NGS use. Local data generation and stakeholder engagement will therefore be essential to determine feasibility within Austrian healthcare structures. The findings should be interpreted considering methodological limitations, including the absence of Austrian-specific primary analyses or consultation.

Zusammenfassung

Das folgende Assessment evaluiert die klinische Wirksamkeit und Sicherheit sowie die ökonomischen und organisatorischen Aspekte molekularer Tests zur Detektion von *PIK3CA-, AKT1-, PTEN-* und *ESR1*-Genalterationen bei Erwachsenen mit HR+/HER2–, lokal fortgeschrittenem oder metastasiertem Brustkrebs.

Einleitung

Beschreibung der Erkrankung

Brustkrebs ist mit einem Lebenszeitrisiko von etwa 12 % (jede achte Frau) die häufigste Krebserkrankung bei Frauen. Die Erkrankung entsteht durch die Ansammlung genetischer Alterationen, die zu unkontrolliertem Wachstum von Brustepithelzellen führen. Brustkrebs wird molekular in vier Hauptsubtypen nach Hormonrezeptor (HR)- und humanem epidermalen Wachstumsfaktorrezeptor 2 (HER2)-Status klassifiziert, die sich in Risikofaktoren, klinischer Präsentation, therapeutischem Ansprechen und Prognose unterscheiden. Der HR+/HER2− Subtyp ist die häufigste Form und zeigt einen günstigen Verlauf mit gutem Ansprechen auf endokrine Therapie. Die Fünf-Jahres-Überlebensraten variieren jedoch erheblich nach Krankheitsstadium: 90,1 % bei regionaler Erkrankung versus 31,9 % bei Fernmetastasen.

Bei HR+/HER2– Brustkrebs stellt die endokrine Therapie (Hormontherapie) den Standard in der Erstlinienbehandlung dar. Im Krankheitsverlauf entwickeln Tumore jedoch häufig eine Resistenz gegen diese Hormontherapie, ein Phänomen bekannt als endokrine Resistenz. Diese beruht oft auf Alterationen im *PI3K/AKT/mTOR*-Signalweg und genetische Veränderungen des Östrogenrezeptors (ER), zwei Schlüsselsignalwege, die Zellwachstum und -überleben regulieren. Umfassende molekulare Testung auf *PIK3CA-, AKT1-, PTEN-* und *ESR1*-Genalterationen ist daher entscheidend für Therapieentscheidungen nach Versagen der endokrinen Therapie. Diese molekulare Diagnostik ermöglicht die Identifikation von Patient:innen, die von zielgerichteten Therapien wie *PI3K*-Inhibitoren (bei *PIK3CA*-Mutationen) oder alternativen endokrinen Strategien (bei *ESR1*-Mutationen) profitieren können, und trägt somit zur Personalisierung der Behandlung bei.

Beschreibung der genetischen Tests

Das Assessment evaluiert folgende Hochdurchsatz-Sequenzierungstechnologien zur Detektion von *PIK3CA-, AKT1-, PTEN-* und *ESR1*-Genalterationen:

- Targeted-Gene Panel Sequencing: Panels mit 20–500+ krebsrelevanten Genen,
- Whole Exome Sequencing (WES): Sequenzierung von ca. 20.000 protein-kodierenden Genen,
- Whole Genome Sequencing (WGS): Umfassende Sequenzierung des gesamten Genoms,
- Virtual Gene Panel Sequencing: Gezielte Analyse von WES- oder WGS-Daten.

Klinische Labore in Österreich verwenden überwiegend Illumina- und Thermo Fisher Scientific-Plattformen für Targeted-Gene Panel Sequencing, während WES und WGS seltener eingesetzt werden. Diese Plattformen werden typischerweise ohne CE-IVD (Conformité Européenne In Vitro Diagnostic) Kennzeichnung verwendet und unterliegen lokaler Validierung durch Laborexpert:innen, was dem Standardansatz für laborentwickelte Tests (LDTs) in Österreich entspricht. Die molekulare Testung wird an sieben spezialisierten Zentren durchgeführt, überwiegend an Universitätskliniken oder tertiären Versorgungseinrichtungen. Der LKF-Leistungskatalog enthält einen allgemeinen Dokumentationscode (ZV680) für Molekularpathologie zur Dokumentation.

Relevante Komparatoren umfassen die Standardversorgung ohne molekulare Testung oder ohne spezifische Mutationstestung sowie andere molekulare Testmethoden, wie folgt:

- Real-Time PCR (RT-PCR): PCR-Technologie, die gezielt DNA-Sequenzen vervielfältigt und diese gleichzeitig mithilfe fluoreszierender Moleküle in Echtzeit misst,
- Digital PCR (dPCR): Weiterentwickelte PCR-Technologie, die DNA-Moleküle durch Aufteilung der Probe absolut quantifizieren kann,
- Sanger-Sequenzierung: Klassische Sequenzierungsmethode der ersten Generation, die DNA vervielfältigt und mittels Kapillarelektrophorese die Abfolge der Bausteine bestimmt.

Methoden

Das Assessment folgt dem European Network for Health Technology Assessment (EUnetHTA) Core Model® Framework zur Strukturierung der Bewertung. Eine systematische Literatursuche wurde in fünf Datenbanken für Publikationen von Juni 2020 bis August 2025 durchgeführt. Die Suche fokussierte auf systematische Reviews, HTA-Berichte und evidenzbasierte klinische Leitlinien zur *PIK3CA-, AKT1-, PTEN*- und *ESR1*-Gentestung bei HR+/HER2– Mammakarzinom. Die Leitlinienidentifikation wurde durch manuelle Suchen in vier zusätzlichen Datenbanken ergänzt.

Nach der Deduplizierung erfolgte das Screening durch zwei unabhängige Wissenschaftler:innen anhand vordefinierter PICO-Kriterien. Die diagnostische Genauigkeit wurde in der verfügbaren Evidenz gegen konventionelle diagnostische Referenzstandards (z. B. PCR, Sanger Sequencing) bewertet und mittels Linked Evidence Approach mit patient:innenrelevanten Outcomes sowie Sicherheitsaspekten verwendet. Die Qualitätsbewertung verwendete das ROBIS-Tool für systematische Reviews und die AGREE-II-Checkliste für Leitlinien. Die Datenextraktion fokussierte auf diagnostische Genauigkeit (Review von Reviews) und Therapieempfehlungen (Leitliniensynopse). Darüber hinaus wurden organisatorische, ökonomische und ethische Implikationen bei verfügbarer Evidenz berücksichtigt.

Ergebnisse

Verfügbare Evidenz

Insgesamt wurden acht Publikationen nach der systematischen Literatursuche eingeschlossen: drei systematische Reviews (SRs), darunter zwei Meta-Analysen und ein HTA-Bericht mit über 40 Primärstudien, und fünf relevante Leitlinienempfehlungen/Textpassagen von drei Organisationen.

Die beiden Meta-Analysen bewerteten die diagnostische Testgenauigkeit für *PIK3CA*- und *ESR1*-Detektion gegenüber Gewebebiopsie als Referenzstandard; der HTA-Bericht führte einen Vergleich verschiedener molekularer Tests (NGS vs. konventionelle Methoden) für *PIK3CA*-Detektion durch. Keine Evidenz wurde für klinische Nutzenbewertungen, Sicherheitsendpunkte oder *AKT1*- und *PTEN*-Detektion gefunden. Für die organisatorischen und ökonomischen Domänen adressierte der eingeschlossene HTA-Bericht diese Aspekte basierend auf dem norwegischen Gesundheitskontext. Keine Evidenz wurde für die ethische Domäne identifiziert.

Für die Leitliniensynopse wurden fünf relevante Leitlinienempfehlungen/Textpassagen von drei Organisationen (AWMF, ASCO, SITC) hoher methodologischer Qualität zur Next-Generation Sequencing (NGS)-basierten Genalteration-Detektion identifiziert. Sowohl SRs als auch Leitlinien bezogen sich dabei auf NGS-Technologien ohne Spezifizierung der Panellänge, wobei laut klinischen Expert:innen anzunehmen ist, dass Targeted-Gene Panel Sequencing gemeint ist.

Ergebnisse: Review von reviews

Zur klinischen Validität für *PIK3CA* erreichte NGS-basierte ctDNA-Testung die höchste Sensitivität und Spezifität unter den evaluierten molekularen Tests, mit guter Konkordanz zwischen verschiedenen Testmethoden. Für *ESR1* zeigte digitale PCR (dPCR) höhere Sensitivität als NGS, wobei substanzielle Heterogenität zwischen Studien definitive Schlussfolgerungen einschränkte.

Der HTA-Bericht, basierend auf dem norwegischen Gesundheitskontext, identifizierte zusätzlich zentrale organisatorische und ökonomische Aspekte:

- Organisatorisch ermöglicht NGS das Multiplexing (parallele Sequenzierung mehrerer Proben in einem Sequenzierlauf) und die Detektion unbekannter Varianten, erfordert jedoch substanzielle Infrastruktur, spezialisierte bioinformatische Expertise und längere Bearbeitungszeiten (3 Tage versus 4 Stunden bis 1 Tag für PCR). NGS zeigt hohe Sensitivität mit Gewebeproben, aber erhöhte Fehlerraten bei Flüssigbiopsie, während PCR gegenteilige Charakteristika aufweist.
- Ökonomisch demonstriert NGS volumenabhängige Kosteneffizienz: Die Kosten pro Patient:in sinken um ca. das Siebenfache, wenn 10 oder mehr Patient:innen simultan getestet werden, wodurch die Kosten von NGS in Hochvolumen-Settings mit jenen von PCR vergleichbar sind.

Ergebnisse: Leitliniensynopsis

Fünf Leitlinienempfehlungen/Textpassagen von drei internationalen Organisationen (AWMF, ASCO, SITC) adressierten NGS zur Genalteration-Detektion. Drei formale Empfehlungen wurden identifiziert:

- Die AWMF S3-Leitlinie 2025 empfiehlt umfassende NGS-basierte molekulare Diagnostik mit nachfolgenden Therapieempfehlungen basierend auf starkem Expertenkonsens.
- ASCO 2021 gibt eine evidenzbasierte starke Empfehlung für *PIK3CA*-Detektion mittels sequenzieller Testung (zunächst cfDNA, dann Gewebe) für postmenopausale und männliche Patient:innen mit HR+ metastasiertem Brustkrebs.
- ASCO 2022 erweitert diese Empfehlung auf eine breitere Population einschließlich Patient:innen mit lokal rezidivierendem, nicht resezierbarem oder metastasiertem HR+/HER2– Brustkrebs.

Zusätzlich beschreiben zwei informelle Passagen umfassende NGS-basierte Testansätze: ASCO 2024 adressiert umfassendes Tumor-Genomtesting mittels Large-Panel-NGS zur Identifikation targetierbarer genetischer Alterationen, wobei *PIK3CA, AKT1, PTEN* und *ESR1* genannt werden. SITC 2021 beschreibt die Detektion von „actionable gene genetic alterations" (therapeutisch nutzbare genetische Alterationen), wobei unter den betrachteten Genalterationen *PI3K* explizit erwähnt wird.

Diskussion

Kontextualisierung für Österreich

Dieses Pilot-Assessment demonstriert, dass ein fokussierter Review-of-Reviews- und Leitliniensynopse-Ansatz die Bewertung genetischer Testverfahren unterstützen kann, während gleichzeitig kritische Evidenzlücken offengelegt werden.

Die Analyse der Evidenz aus den eingeschlossenen systematischen Reviews zeigte mehrere Limitationen, die bei der Kontextualisierung mitbetrachtet werden sollten:

- Unzureichende Evidenzqualität und schlechte Berichterstattung in den Primärstudien, wobei aufgrund spärlicher Head-to-Head-Vergleiche nur drei geeignete Konkordanzstudien vorlagen.
- Erhebliche Heterogenität in der ESR1-Subgruppenanalyse schränkte die Möglichkeit ein, definitive Schlussfolgerungen zu ziehen.
- Für mehrere Domänen empfehlt Evidenz, darunter klinischer Nutzen, Sicherheits-Outcomes, Detektion von AKT1- und PTEN-Genveränderungen sowie ethische Implikationen.

Trotz dieser Limitationen zeigten die verfügbaren Daten, dass die diagnostische Genauigkeit je nach Zielgen variierte. Für PIK3CA übertraf NGS PCR-basierte Methoden durch Abdeckung von bis zu 60 Genveränderungen über mehrere Exons, verglichen mit 11 Hotspot-Genveränderungen bei PCR. Für ESR1 zeigte dPCR eine höhere Sensitivität als NGS aufgrund größerer Toleranz gegenüber niedrigen cfDNA-Konzentrationen. Die Auswahl der optimalen Testmethode hängt vom spezifischen Zielgen ab.

Über die diagnostische Genauigkeit hinaus ist eine organisatorische und ökonomische Kontextualisierung im österreichischen Gesundheitssystem essentiell. Mehrere Herausforderungen schränken die Implementierung molekularer Testung in Österreich ein:

- Keine Unterscheidung zwischen gezielten Gen-Panels, WES und WGS, obwohl diese Ansätze erheblich unterschiedliche technische Anforderungen, Kosten und klinische Implikationen haben.
- Zielpopulationen unterscheiden sich zwischen den Leitlinien, wobei einige Empfehlungen auf bestimmte Subgruppen beschränkt sind, während andere breiter anwendbar sind.
- Vielen molekularen Tests fehlt die CE-Kennzeichnung als Companion Diagnostics (CDx), was die HTA-Bewertung der Test-Medikament-Verknüpfung erschwert.

Die Übertragbarkeit der Evidenz auf das österreichische Gesundheitssystem ist eingeschränkt:

- Nur die AWMF-S3-Leitlinie ist direkt relevant, da sie für deutschsprachige Länder mit ähnlichen Gesundheitsstrukturen entwickelt wurde. ASCO und SITC repräsentieren US-amerikanische klinische Kontexte, die sich vom österreichischen Setting unterscheiden.
- Die einzige Evidenz zur organisatorischen und ökonomischen Domäne stammt aus dem norwegischen Gesundheitskontext mit begrenzter Übertragbarkeit aufgrund unterschiedlicher Gesundheitsstrukturen und Kosten.
- Es wurde keine primäre Datenerhebung oder Stakeholder-Konsultation durchgeführt, die notwendig gewesen wäre, um internationale Befunde für die lokale Implementierung zu kontextualisieren.

Die identifizierte Evidenz bietet ein erstes Verständnis, kann jedoch nicht direkt auf das österreichische Gesundheitssystem angewendet werden. Die Implementierung erfordert die Erhebung lokaler Daten und die Einbindung relevanter Stakeholder, um die Durchführbarkeit innerhalb der österreichischen Gesundheitsstrukturen sicherzustellen.

Limitationen des Assessments

Das vorliegende Assessment sollte im Kontext seiner Limitationen betrachtet werden. Es wurde ein Review-of-Reviews-Ansatz anstelle eines de novo systematischen Reviews verwendet. Die Literatursuche war auf englisch-, deutsch- und italienischsprachige Publikationen beschränkt. Es wurde keine dedizierte systematische Suche nach ökonomischen, organisatorischen oder ethischen Analysen durchgeführt, und es erfolgten keine Primärdatenerhebung, Kostenmodellierung oder Stakeholder-Konsultation für das österreichische Setting. Die Leitliniensynopse umfasste Textpassagen neben formal graduierten Empfehlungen, die jedoch in der gesamten Synthese transparent unterschieden wurden.

Schlussfolgerung

Die verfügbare Evidenz deutet daraufhin, dass NGS-basierte Ansätze höhere Sensitivität und Spezifität gegenüber PCR-basierten Methoden für *PIK3CA*-Detektion überlegen sind. Für ESR1-Mutationen zeigt digitale PCR (dPCR) höhere Sensitivität gegenüber NGS. Technische Parameter wie Panel-Größe oder WES/WGS werden nicht spezifiziert. Evidenz zu klinischem Nutzen, Sicherheit sowie zu *AKT1*- und *PTEN*-Detektion fehlt vollständig.

Internationale Leitlinien von drei Organisationen sprechen sich für den Einsatz von NGS-Testung bei *PIK3CA*-Detektion zur Therapiesteuerung aus. Für *AKT1, PTEN* und *ESR1* existieren keine expliziten Testempfehlungen, deren Detektion wird jedoch implizit im Rahmen umfassender genomischer Testansätze unterstützt. Technische Parameter wie Panel-Größe oder WES/WGS werden nicht spezifiziert.

NGS zeigt potenzielle volumenabhängige Kosteneffizienz, erfordert jedoch erhebliche Infrastruktur und koordinierte Teststrukturen mit entsprechender Expertise. Konkrete ökonomische Daten für den österreichischen Kontext liegen allerdings nicht vor.

Angesichts der schnellen Entwicklung der Präzisionsonkologie und limitierter Evidenz zum klinischen Nutzen können evidenzbasierte Leitlinienempfehlungen einen pragmatischen Ausgangspunkt für Erstattungsentscheidungen bieten. Diese sollten durch lokale Kontextbewertung und Machbarkeitsanalyse im österreichischen Gesundheitssystem ergänzt werden.

1 Background

Humangenetik-Projekt 2025 – Teil C: Bewertung molekularer Tests für ausgewählte Genalterationen beim Brustkrebs

This review is part of a comprehensive AIHTA project on genetic testing, with a focus on sequencing technologies. The topic of this report is to evaluate the clinical effectiveness and safety as well as the economic and organisational aspects of molecular tests for detection of *PIK3CA-/AKT1-/PTEN-/ESR1*-genetic alterations[1] in adults with locally advanced or metastatic breast cancer. Accurate genetic alterations detection is essential for selecting appropriate targeted therapies and optimising treatment outcomes. The topic was prioritised by Austrian decision makers, based on findings from a scoping review and results from a stakeholder workshop (see Part A [1]).

1.1 Overview of the disease and current clinical practice

Overview and classification

Brustkrebs: häufigste Krebsform bei Frauen

Breast cancer (BC) is a carcinoma of the mammary gland and represents the most common form of cancer in women [2, 3], it affects one in eight women [4]. Although BC predominantly affects women, men account for approximately 1% of all cases. The lower incidence in men is attributed to differences in breast tissue structure and hormonal profiles, particularly the substantially lower estrogen levels [5].

Ursprung meist in Milchgängen oder Läppchen

BC most frequently originates from cells of the milk ducts (ductal carcinoma) and lobules (lobular carcinoma) [6]. The disease develops through accumulation of genetic alterations[2] that disrupt normal cellular regulation, leading to uncontrolled proliferation of breast epithelial cells. Development may be due to inherited genetic defects or various predisposing factors such as prolonged hormonal exposure, unhealthy diet, lack of physical activity, alcohol consumption, and smoking [2].

molekulare Klassifikation in vier Hauptsubtypen nach HR und HER2-Status

BC is molecularly classified into four major subtypes according to hormone receptor (HR) and human epidermal growth factor receptor 2 (HER2) status. HR (oestrogen and progesterone receptor) are proteins that indicate the cancer's potential responsiveness to hormone-blocking therapies, while HER2 is a growth-promoting protein that, when overexpressed, drives more aggressive tumour growth. The plus (+) and minus (–) signs indicate whether specific receptors are present or absent in the BC cells. Based on these markers, BC is divided into the following molecular subtypes:

- Luminal A (HR+/HER2–),
- Luminal B (HR+/HER2+),
- Basal-like/Triple negative (HR–/HER2–), and
- HER2-enriched (HR–/HER2+).

[1] Abbreviations of the genetic alterations: Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PIK3CA); AKT serine/threonine kinase 1 (AKT1); Phosphatase and tensin homolog (PTEN); Estrogen receptor 1 (ESR1).

[2] Genetic alterations encompass all types of genetic changes, including point mutations, deletions, insertions, and amplifications.

jeder Subtyp zeigt unterschiedliche Charakteristika

Each subtype demonstrates distinct characteristics regarding risk factors, clinical presentation, therapeutic response, and prognosis. The HR+/HER2− subtype, constitutes the most common form of BC and is characterised by a typically indolent clinical course and response to endocrine-based treatments [6, 7].

Stages and clinical manifestation

AJCC-Staging-System: international anerkannte Klassifikation nach klinischen Stadien

BC follows a progressive disease course characterised by distinct clinical stages. The American Joint Committee on Cancer (AJCC) has developed the internationally recognised TNM staging system, which classifies cancer based on three factors: tumour (T), regional lymph node involvement (N), and distant metastasis (M). Early-stage disease (ductal carcinoma in situ and stages I–IIIA) refers to cancer that has not spread beyond the breast or the axillary lymph nodes, whereas advanced disease (stages IIIB onwards) indicates regional spread, and metastatic disease (stage IV) indicates that the cancer has spread to distant organs, most commonly the bones, liver, lungs, and brain [8].

klinische Präsentation variiert nach Krankheitsstadium

Clinical presentation varies significantly depending on disease stage and extent of spread; metastatic disease causes organ-specific symptoms such as bone pain, liver dysfunction, breathing difficulties, or neurological deficits. An important aspect of staging is assessing whether the tumour remains operable; in metastatic BC, surgical removal is typically no longer possible, which influences both treatment options and prognosis [9, 10].

Target population[3]

Inzidenz in AT mit HR+/HER2–

The age-standardised incidence rate of BC in Austria during 2021-2023 was 130.5 per 100,000 in women and 1.7 per 100,000 in men [11]. Regional data from the Salzburg Tumour Registry (2016-2019) show annual incidence rates of 13.5 per 100,000 for BC in stage III and 8.7 per 100,000 in stage IV across all molecular subtypes [12]. Based on US SEER subtype distribution data, it is estimated that approximately 63% of stage III and 61% of stage IV cases are HR+/HER2–, corresponding to approximately 8.5 and 5.3 cases per 100,000 women annually, respectively [13].

Prävalenz in AT mit HR+/HER2–

By the end of 2022, a total of 89,188 women and 742 men were living with a BC diagnosis, making it the largest cancer survivor group in Austria [14]. Given that around 70% of BC cases are HR+/HER2–, it is estimated that roughly 63,000 individuals in Austria are currently diagnosed with this subtype [15].

Prognosis

5-Jahres-Überlebensraten (HR+/HER2–) von Stadium abhängig

Prognosis is influenced by several factors, including patient age, overall health, HR and HER2 expression, tumour size, and the type and extent of tissue affected. The HR+/HER2− molecular subtype, generally carries the most favourable prognosis. Five-year survival rates (in patients with HR+/HER2–) differ significantly by disease stage: 90.1% for regional disease involving nearby lymph nodes, compared to 31.9% for distant metastatic spread [16].

Mortalitätsrate in AT 2022

The mortality rate for BC by the end of 2022 was 29.5 per 100,000 in women and 0.4 per 100,000 in men. BC remains the second leading cause of cancer-related death among women, accounting for 16.3% of all female cancer deaths in 2022 [17].

[3] These estimates combine Austrian incidence data with international distribution data and may therefore not fully reflect the Austrian patient population.

Endocrine resistance

bei HR+/HER2– häufig Verlust des Ansprechens auf Hormontherapie

In HR+/HER2– BC, it is common for the disease to eventually stop responding to hormone therapy, a phenomenon known as endocrine resistance. This resistance often arises from alterations in key signalling pathways within cancer cells that regulate their growth and survival [18].

PI3K/AKT/mTOR-Signalweg als zentraler Mechanismus der Resistenz

The *PI3K/AKT/mTOR* pathway represents a central mechanism in this resistance. This pathway controls cellular processes including proliferation, protein synthesis, and apoptosis evasion. If certain genes in this pathway become altered, such as *PIK3CA*, which produces the *PI3K* enzyme, the whole system can become overactive, encouraging cancer cells to grow. Other important genes include *AKT1*, which acts as a central hub in the pathway, and *PTEN*, which normally acts as a brake but may stop working properly if mutated. Changes in these genes can lead to uncontrolled signalling, contributing to tumour growth and treatment resistance [18].

Genalterationen im ER können zu permanenter Rezeptoraktivierung führen

In addition to these pathway changes, genetic alterations in the estrogen receptor (ER) itself also play a major role. The *ESR1* gene, which codes for the ERα, can acquire genetic alterations during treatment with aromatase inhibitors, a common type of hormone therapy. These genetic alterations, found in about 20-30% of patients with metastatic BC, make the receptor permanently active. Consequently, the cancer can no longer respond to therapies designed to block or reduce ER, making treatment more difficult [19].

Identification of resistance mechanisms

bei Stadium III & IV: molekulare Testung essenziell

The median age at diagnosis is approximately 65 years for women and 71 years for men, although breast cancer can also occur in younger women [4]. Approximately 7% of women are diagnosed with advanced disease and 4.4% with metastatic disease at initial BC presentation [12], while up to 30% of women with early-stage disease will subsequently develop metastatic progression [20]. At locally advanced and metastatic stages, molecular testing becomes essential to identify actionable genetic alterations and other genetic alterations in genes such as *PIK3CA*, *AKT1*, *PTEN,* and *ESR1*, which guide targeted treatment decisions [20, 21].

molekulare Testung via Gewebebiopsie oder Flüssigbiopsie

Molecular testing can be performed using tissue biopsy or liquid biopsy approaches. Tissue biopsy, obtained through core needle biopsy or surgical excision, remains the gold standard for comprehensive tumour characterisation, though it cannot fully capture spatial and temporal tumour heterogeneity. Liquid biopsy provides a non-invasive alternative by detecting circulating tumour DNA (ctDNA) from blood samples, which represents the tumour-specific fraction of cell-free DNA (cfDNA). This approach offers value for monitoring disease progression and identifying treatment-emergent genetic alterations [22]. The distinction between cfDNA and ctDNA is particularly relevant for ESR1 mutation detection, as these alterations are typically somatic and acquired under therapeutic pressure. Current German guideline from the Association of the Scientific Medical Societies (AWMF, Arbeitsgemeinschaft der Wissenschaftlichen Medizinischen Fachgesellschaften) recommends liquid biopsy specifically for *ESR1* detection, as these alterations frequently emerge during endocrine therapy and may be absent from archived primary tumour tissue [23].

Current clinical management

bei Krankheitsprogression: Therapiewahl direkt abhängig vom Genalterationsstatus

For patients with HR+/HER2– advanced or metastatic BC, current standard first-line treatment consists of endocrine-based therapy [23]. As mentioned above, patients commonly develop endocrine resistance during treatment. When disease progression occurs, comprehensive molecular testing for *PIK3CA*, *AKT1*, *PTEN*, and *ESR1* genetic alterations becomes critical for guiding subsequent treatment decisions. The treatment algorithm is presented in Figure 1-1 below, it was developed based on the AWMF guideline [23] and was validated by an Austrian clinical expert with experience in this field [24].

Behandlungsalgorithmus basierend auf AWMF S3 Leitlinie & klinischer Expert:in in AT

For patients whose tumours harbour activating *PIK3CA*, *AKT1*, or *PTEN* alterations detected at the time of disease progression, treatment with capivasertib plus fulvestrant is indicated. Specifically, for *PIK3CA* genetic alterations, additional therapeutic options exist. In patients who progress under or within 12 months after adjuvant endocrine therapy, inavolisib plus fulvestrant plus palbociclib represents a targeted option for metastatic disease. Alternatively, alpelisib plus fulvestrant can be considered for *PIK3CA*-mutated tumours after progression on endocrine therapy. For patients who have received at least two prior lines of endocrine therapy including a Cyclin-Dependent Kinase 4/6 (CDK4/6) inhibitor and whose tumours harbour activating *ESR1* genetic alterations detected at progression, elacestrant monotherapy is indicated. These agents target molecular alterations within the *PI3K/AKT/mTOR* and *ESR1* signalling pathways, which are key drivers of endocrine resistance. By inhibiting these pathways, the therapies aim to restore endocrine sensitivity and delay disease progression.

akkurate und zuverlässige Diagnostik ist essenziell

Given that treatment pathways are directly determined by genetic alteration status, accurate and reliable diagnostic testing is essential to enable precision oncology and optimise patient outcomes.

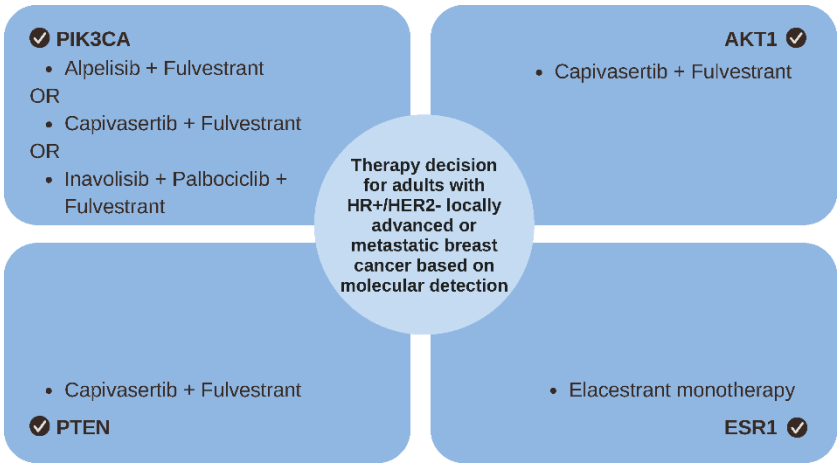


Figure 1-1: Gene-specific treatment algorithm for adults with HR+/HER2- locally advanced or metastatic BC.

1.2 Description of genetic tests

The following chapter provides an overview of the relevant terminology related to the selected topic. For a more detailed explanation of genetics terminology, please refer to the glossary in Part A of the AIHTA project on genetic testing [1].

siehe Glossar in Teil A des Human-Genetik-Projekts

Selected sequencing technologies

For the purpose of this assessment, the following high-throughput sequencing technologies are considered relevant for detecting *PIK3CA*, *AKT1*, *PTEN*, and *ESR1* genetic alterations. These methods allow simultaneous analysis of multiple genes or the entire exome/genome, enabling detection of both known and novel genetic alterations for comprehensive tumour profiling. They are listed according to their scope of analysis:

Fokus auf ausgewählte Sequenzierungs-technologien

- **Targeted-gene panel sequencing:** Targeted sequencing panels typically covering 20 to 500+ genes relevant for cancer profiling and targeted therapy selection [25].
- **Whole exome sequencing (WES):** Sequencing of approximately 20,000 protein-coding genes covering the exome [26].
- **Whole genome sequencing (WGS):** Comprehensive sequencing of the entire genome including coding and non-coding regions [27, 28].
- **Virtual gene panel sequencing:** Targeted analysis to comprehensive WES or WGS data, analysing only genes relevant to the patient's condition while retaining the full dataset for potential future reanalysis [29].

Table 1-1 provides a short overview of these sequencing technologies.

Table 1-1: Description of selected sequencing technologies [26, 27]

Technology	Targeted-gene panel sequencing	Whole Exome Sequencing (WES)	Whole Genome Sequencing (WGS)
Method	Targeted sequencing	Exome-wide sequencing	Genome-wide sequencing
Panel Size/Coverage	20–500+ genes	Approx. 20,000 coding genes (exome)	Entire genome (~3 billion base pairs)
Detection Method	Targeted genetic alterations	Coding region variants	Coding + non-coding variants
Regulatory status	Mostly LDTs with local clinical validation; some CE-IVD products (e.g., FoundationOne®Liquid CDx) available	LDTs with local clinical validation	LDTs with local clinical validation

Note: Virtual gene panel sequencing is not listed in the table, as it is a digital approach that uses data from WES and WGS.

Terminology regarding next-generation sequencing

The term "next-generation sequencing" (NGS) lacks a standardised definition in scientific literature. Originally referring to post-Sanger sequencing methods (second-generation sequencing), its scope became ambiguous with the emergence of long-read sequencing technologies (third-generation sequencing). Nevertheless, NGS remains widely used in clinical practice, where it typically refers to targeted-gene panel sequencing, as confirmed by clinical

NGS-Begriff wird trotz fehlender Standarddefinition im Ergebnis- & Diskussionsteil verwendet

experts [24]. Although this report generally refrains from using the term NGS due to the lack of a consistent definition, the term is applied in the results and discussion sections to reflect the terminology used in the included systematic reviews and clinical guidelines, where NGS is often employed without precise specification.

Relevant comparators

Komparatoren: Standard-Diagnostik und andere molekulare Testmethoden

Relevant comparators in this assessment include standard care without molecular testing, conventional diagnostic methods (e.g., imaging, histopathology) without specific genetic alteration testing, and other molecular testing methods that guide targeted treatment decisions (e.g., eligibility for alpelisib or other genetic alteration-specific therapies). The following molecular testing methods are compared in this assessment for detecting genetic alterations in BC:

- **Real-Time Polymerase Chain Reaction (RT-PCR)**, also known as quantitative PCR (qPCR), amplifies and simultaneously detects targeted DNA sequences using fluorescent reporter molecules to monitor the amplification process in real-time [30].
- **Digital PCR (dPCR)** represents an advanced PCR technology enabling absolute quantification of target DNA molecules through sample partitioning. Two main platforms exist: Droplet Digital PCR (ddPCR), which partitions samples into thousands of individual droplets, and BEAMing (Beads, Emulsion, Amplification, and Magnetics), which uses magnetic beads for digital counting of DNA molecules [31].
- **Sanger Sequencing** is a traditional first-generation sequencing method that uses DNA amplification followed by capillary electrophoresis to determine nucleotide sequences [32].

Sequencing platforms and regulatory status

In AT: gezielte Genpanel-Sequenzierung oft verwendet, WES & WGS seltener

According to information provided by our clinical expert, Austrian clinical laboratories primarily use platforms from Illumina and Thermo Fisher Scientific to perform targeted-gene panel sequencing, with WES and WGS applied less frequently [24]. These platforms are frequently used without an CE-IVD (Conformité Européenne – In Vitro Diagnostic) designation for companion diagnostics[4], with validation conducted locally by laboratory and clinical experts, a standard regulatory approach for laboratory-developed tests (LDTs) in Austria [33].

FDA-zugelassene Plattformen in AT

FoundationOne® Liquid CDx (Roche Diagnostics) [34] and Guardant360® CDx (Guardant Health) [35] are FDA-approved liquid biopsy companion diagnostic products with CE marking in Europe that utilize plasma samples for ctDNA analysis. FoundationOne Liquid CDx is CE-marked under IVDD and analyses >300 cancer-relevant genes, including PIK3CA; Guardant360 CDx is certified under the newer IVDR. The IVDR transition status of FoundationOne Liquid CDx remains unclear. For the remaining genetic alterations, no other CE-marked tests were identified.

[4] A companion diagnostic (CDx) is a regulatory-defined medical test that is specifically approved for use with a particular drug to identify patients who are likely to benefit from the therapy or who may be at increased risk of adverse effects.

For a detailed overview of the distinction between sequencing approach, platform, and product, see Figure 1-2 in the Appendix.

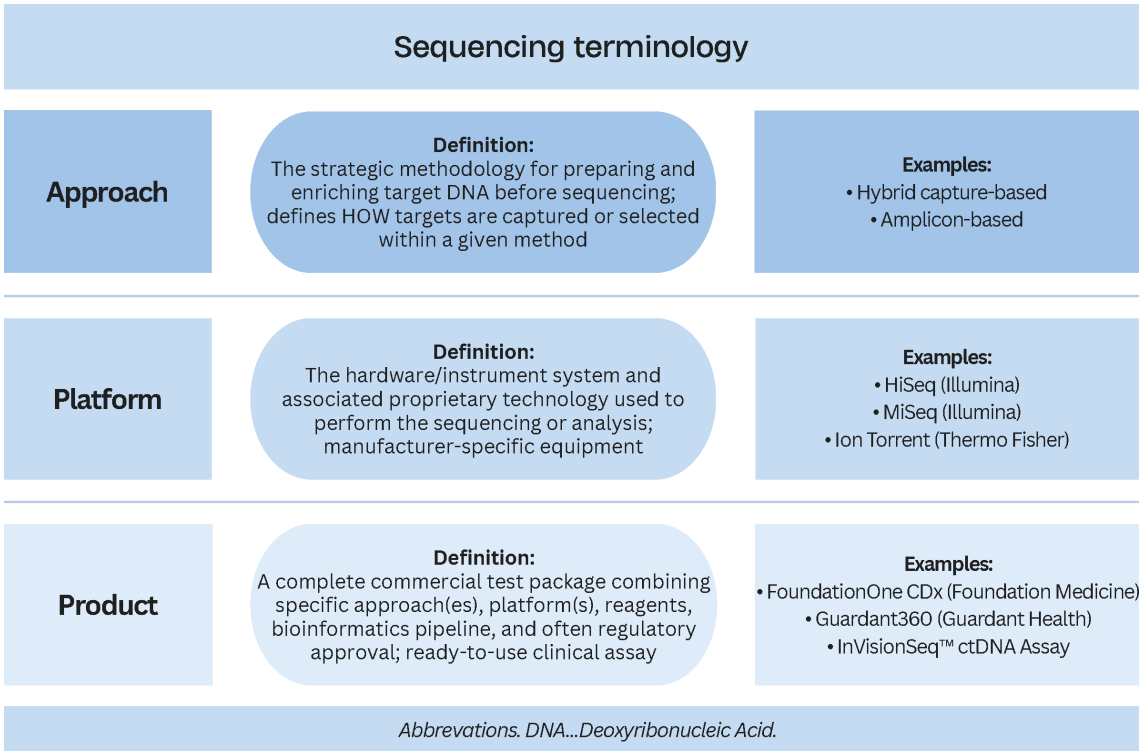


Figure 1-2: Distinction between sequencing approach, platform, and product.

Administration, resources, and clinical implementation

molekulare Testung in molekularpathologischen/ genomischen Laboren

Molecular testing for *PIK3CA*, *AKT1*, *PTEN*, and *ESR1* genetic alterations using targeted-gene panel sequencing, WES, or WGS is predominantly performed in specialised molecular pathology or genomic laboratories, typically affiliated with university hospitals or large tertiary care centers. These services are generally coordinated by molecular pathologists or geneticists in collaboration with oncologists [36]. PCR-based comparator assays are more widely available in routine hospital or private pathology laboratories equipped of molecular diagnostics.

Testing infrastructure and reimbursement status

in AT:
7 spezialisierte Zentren

In Austria, molecular diagnostic testing is provided by seven specialised humangenetic centers and a larger number of molecular pathology laboratories affiliated with pathology institutes. The pathology laboratories are exempt from mandatory reporting requirements under Type 1 of the Gene Technology Act for tumour tissue and liquid biopsy testing in oncological patients [24].

Although the performance-oriented hospital financing catalogue (LKF, Leistungsorientierte Krankenanstaltenfinanzierung) [37] of benefits includes a general documentation code (ZV680) for molecular pathology, this code is intended solely for documentation purposes and provides a flat-rate reimbursement that is independent of the specific laboratory services performed. La-

boratory costs are within the global laboratory budget. In practice, there are often further individual contracts with these centers [36, 38].

2 Scope of assessment

2.1 Research questions

Forschungsfragen zu Wirksamkeit, Sicherheit, ECO/ORG/ETH-Aspekten & Leitlinienempfehlungen ausgewählter molekularer Tests

This report aims to address the following research questions:

- What is the relative effectiveness and safety of selected high-throughput sequencing technologies (targeted-gene panel sequencing, WES, WGS, virtual gene panel sequencing) for detecting *PIK3CA*, *AKT1*, *PTEN*, and *ESR1* genetic alterations compared to standard care without molecular testing or conventional diagnostic methods in adults with HR+/HER2– locally advanced or metastatic breast cancer at disease progression on endocrine therapy?
- What organisational, economic, and ethical implications of these molecular tests are reported in identified HTA reports and systematic reviews?
- What do current evidence-based clinical practice guidelines recommend regarding these molecular testing strategies for *PIK3CA*, *AKT1*, *PTEN*, and *ESR1* genetic alterations in this population?

2.2 Inclusion criteria

Einschlusskriterien für relevante Studien

Inclusion criteria for relevant studies are summarized in Table 2-1.

Table 2-1: Inclusion criteria

Population	Adults diagnosed with HR+/HER2–, locally advanced or metastatic breast cancer
Intervention	Molecular tests with high-throughput sequencing technologies for detection of *PIK3CA*-/*AKT1*-/*PTEN*-/*ESR1*-genetic alterations, including: ■ Targeted-gene panel sequencing ■ Whole Exome Sequencing (WES) ■ Whole Genome Sequencing (WGS) ■ Virtual gene panel sequencing using WES or WGS
Control	■ Standard of care without molecular testing or other conventional diagnostic methods (e.g., imaging, histopathology) without specific testing of genetic alterations ■ Other molecular testing methods (e.g., polymerase chain reaction, single-gene tests, sanger-sequencing, immunohistochemestry) *Rationale*: informed by Austrian clinical expert
Outcomes	Clinical effectiveness: ■ *Clinical utility*[1] (e.g. progression free survival, overall survival, quality of life, change in management) ■ *Clinical validity as linked evidence* (sensitivity, specificity, predictive values, likelihood rations) and concordance (i.e., agreement) Safety: any adverse events and consequences from false-positive/false-negative test results Organisational, economic, and ethical implications; E.g., amount and type of biological material needed, turnaround time, coverage, challenges related to the analysis or interpretation of test results, implementation aspects and economic considerations[2]. Evidence based guideline recommendations with regard to any of the mentioned aspects, e.g., test criteria for when NGS tests are used and in which circumstances more resource intense tests (e.g., WES/WGS) are recommended (level of evidence/LoE, Grade of Recommendation/GoR) *Rationale*: Appropriate outcomes have been informed by recent reviews, EUnetHTA guidelines and clinical expert input

Time point	At the time of disease progression while on endocrine therapy.[3]
Study design	Systematic reviews, HTA reports, reviews with modelling studies (e.g., benefit-harm; if available) Evidence-based clinical guidelines from professional organisations to provide a synopsis of recommendations
Setting	Review of systematic reviews: no restriction Guideline synopsis: countries from the Global North[4]
Language	English, German, Italian
Publication period	From 6/2020 until 8/2025

Abbreviations: AKT1 … AKT Serine/Threonine Kinase 1; ER … Estrogen Receptor; ESR1 … Estrogen Receptor 1; GoR … Grade of Recommendation; HER2 … Human Epidermal Growth Factor Receptor 2; HR … Hormone Receptor; HR+ … Hormone Receptor Positive; HER2– … Human Epidermal Growth Factor Receptor 2 Negative; HTA … Health Technology Assessment; LoE … Level of Evidence; NGS … Next-Generation Sequencing; PCR … Polymerase Chain Reaction; PICO … Population, Intervention, Comparison, Outcomes; PIK3CA … Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha; PTEN … Phosphatase and Tensin Homolog; WES … Whole Exome Sequencing;
WGS … Whole Genome Sequencing.

Notes:

[1] *While PFS, OS, and QoL etc. are conventionally classified as clinical efficacy outcomes, they are considered in this context*
as patient-relevant endpoints that could be indirectly influenced by treatment decisions informed by the genetic test.

[2] *No cost-effectiveness analysis will be performed. Yet, results will be narratively described if cost-effectiveness analyses were performed within an identified HTA report.*

[3] *This refers to the timing of test administration, i.e., when the molecular test (for PIK3CA, AKT1, PTEN, ESR1 genetic alterations) should be performed. Patients initially receive endocrine therapy. When the disease progresses during this endocrine therapy, this represents the relevant time point for testing.*

[4] *Evidence-based guideline recommendations will be considered with attention to their geographical applicability and resource context.*

3 Methods

EUnetHTA Core Model® als Grundlage für Berichtsstruktur

Assessment elements from the European Network for Health Technology Assessment (EUnetHTA) Core Model® were used to guide the reporting structure (see guiding questions in the Appendix) and were customised to the specific objectives of the report [39].

3.1 Systematic literature search

systematische Literatursuche in 6 Datenbanken

The systematic literature search was conducted from July 18-25, 2025, building on the search strategy developed by the Norwegian Institute of Public Health (NIPH) Health Technology Assessment (HTA) [15], focusing solely on *PIK3CA* genetic alterations. An extended search was performed from August 11-12, 2025, to include additional genetic alterations (*AKT1*, *PTEN*, and *ESR1*) in the following databases:

- Medline via Ovid
- Embase
- The Cochrane Library
- Epistemonikos
- International HTA Database

nach Deduplizierung: 982 Zitate für Abstract-Screening

The systematic search was limited to the years 6/2020 to 8/2025, and to systematic reviews (including meta-analyses), HTA reports and guidelines. After deduplication, overall, 982 citations were included for abstract screening. The Medline search strategy is provided as an example in the Appendix. The other searches are published on Open Science Framework (OSF) [40].

2 zusätzliche Publikationen durch Handsuche

Furthermore, the following databases were manually searched to identify current evidence-based guidelines:

- National Institute for Health and Care Excellence (NICE)
- Trip medical database
- Guidelines International Network (GIN)
- Arbeitsgemeinschaft der Wissenschaftlichen Medizinischen Fachgesellschaften (AWMF)

insgesamt 984 Publikationen identifiziert

By hand-search, an additional two records were identified, resulting in overall 984 screened hits.

3.2 Flow chart of study selection

Literaturauswahl

Overall, 984 hits were identified. The references were screened by two independent researchers (AC, GG, or BY) and in case of disagreement a third researcher (SGG) was involved to solve the differences. The selection process is displayed in Figure 3-1.

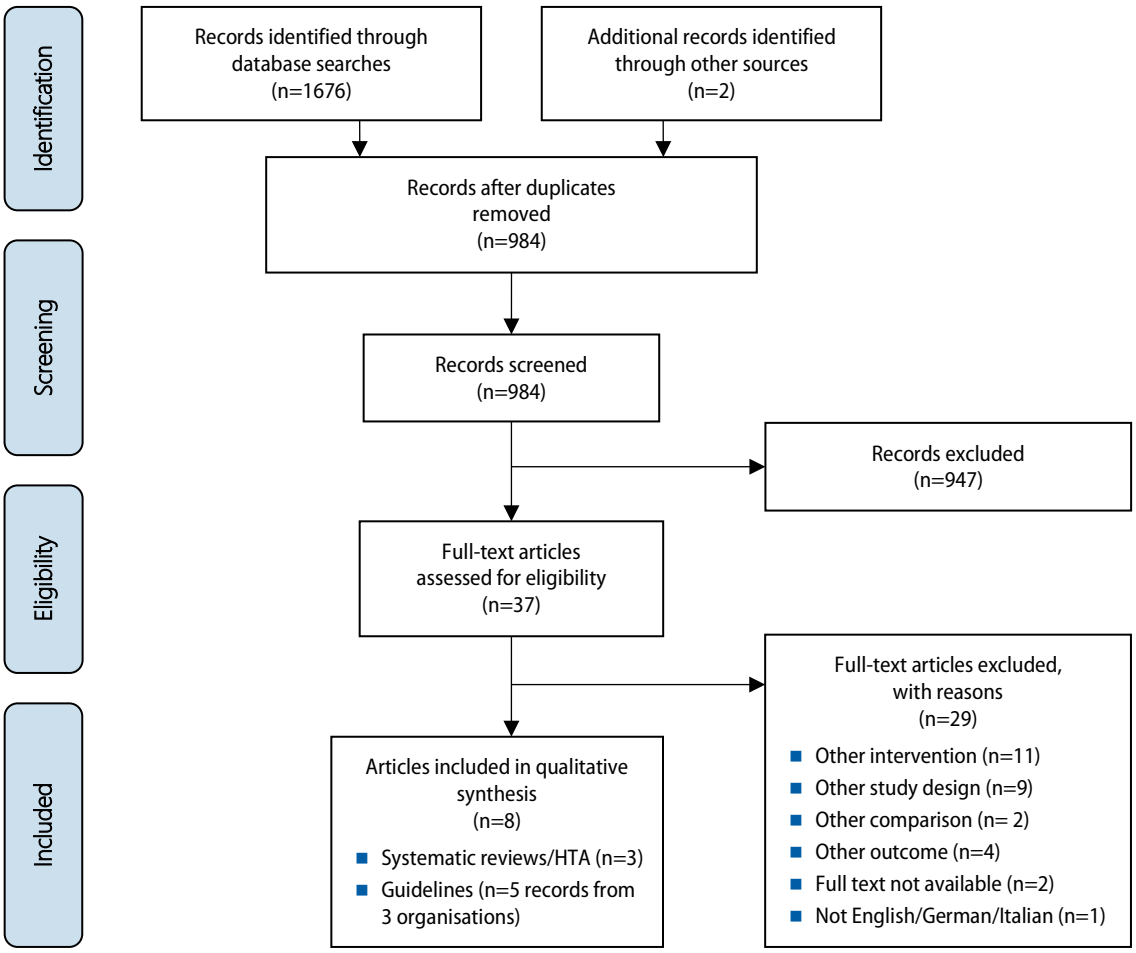


Figure 3-1: Flow chart of study selection (PRISMA Flow Diagram)

3.3 Data extraction and risk of bias

Vier-Augen-Prinzip bei allen Arbeitsschritten

We used single-data extraction with verification by a second reviewer. For included systematic reviews and HTA reports, data extraction focused on review methodology, study characteristics, test specifications (including genetic sequencing techniques), reference standards, and clinical outcomes as detailed in Table A-1 in the Appendix.

Extraktion Leitlinienempfehlungen

From the guidelines included, we extracted evidence-based recommendations relevant to our PICO (Population, Intervention, Comparison, Outcomes) scheme, including recommendation strength and certainty of evidence as detailed in Table A-2 in the Appendix.

Qualitätsbewertung durch 2 unabhängige Autor:innen

Quality assessment was conducted using the ROBIS (Risk Of Bias In Systematic reviews) tool [41] for systematic reviews and the AGREE-II (Appraisal of Guidelines for Research & Evaluation) checklist [42] for clinical guidelines by two independent authors (AC, GG), see Table A-3 and Table A-4 in the Appendix.

3.4 Evidence synthesis

deskriptive Synthese der Ergebnisse aus SR & HTAs

A descriptive synthesis of findings from systematic reviews and HTA reports was performed. Guideline recommendations were summarised to identify areas of consensus and divergence across different sources.

monetäre Werte bei Bedarf in € konvertiert

Where necessary, monetary values were converted to euros (€) using the conversion rates as of October 14, 2025 from the Austrian National Bank [43].

4 Results: systematic reviews

This chapter presents the findings of systematic reviews assessing the diagnostic accuracy of next generation sequencing (NGS) against relevant comparators for the detection of *PIK3CA*, *AKT1*, *PTEN*, and *ESR1* genetic alterations.

Ergebnisse zur diagnostischen Genauigkeit molekularer Tests aus SRs

4.1 Included systematic reviews

For the evaluation of molecular tests detecting *PIK3CA*, *AKT1*, *PTEN*, and *ESR1* genetic alterations in adults with HR+/HER2– locally advanced or metastatic breast cancer (BC), three reviews were included:

3 SRs eingeschlossen (2 Meta-Analysen & 1 HTA-Bericht)

- Galvano et al. 2022 [44]: A meta-analysis assessing the diagnostic test accuracy of NGS, different polymerase chain reaction (PCR) methods, Sanger sequencing against a tissue biopsy reference standard for *PIK3CA* detection.
- Norwegian Institute of Public Health (NIPH) publication 2022 [15]: A health technology assessment evaluating the concordance between NGS platforms, PCR methods, and NGS vs. PCR through direct head-to-head comparisons for *PIK3CA* detection;
- Raei et al. 2024 [19]: A meta-analysis assessing the diagnostic test accuracy of NGS and one PCR method against a tissue biopsy reference standard for *ESR1* detection.

Throughout this report, the Galvano et al. 2022 [44] and Raei et al. 2024 [19] are referred to as systematic reviews (SR) with their respective citation numbers. The NIPH report published in 2022 is referred to as "NIPH report" to reflect its broader scope beyond systematic review methodology, which includes economic evaluation and organisational implementation considerations.

Study characteristics and outcomes of included systematic reviews are presented in Table A-1 in the Appendix.

Characteristics of included systematic reviews

Literatursuchen zwischen 12.2020 bis 04.2022

The systematic searches of the included reviews were conducted between December 2020 and April 2022 [15, 19, 44].

klinische Wirksamkeit überwiegend durch diagnostische Kohortenstudien bewertet

Clinical effectiveness was evaluated based on the evidence reported in the included SRs, which predominantly identified diagnostic cohort studies. The number of primary studies included across the reviews ranged from 3 to 24 [15, 19, 44]. One SR [44] included 24 primary studies (7 randomised controlled trials and 17 cohort studies) investigating the detection of *PIK3CA* genetic alterations. The NIPH report [15] included three studies (one case-control or cross-sectional study and two cohort studies) assessing the analytical validity, clinical validity, and clinical utility of PIK3CA detection. Another SR [19] identified 13 diagnostic cohort studies focusing on *ESR1* detection. The total number of patients included was 1,966 in one SR [44], 674 in the NIPH report [15], and 389 in the second SR [19].

Population der SRs

The population of both SRs [19, 44] and the NIPH report [15] were predominantly composed of patients with advanced or metastatic BC with HR+/ HER2– status. Detailed population characteristics are provided Table A-1 in the Appendix.

Selected outcome domains

relevante PICO-Endpunkte

According to the PICO (Population, Intervention, Comparator, Outcome) framework, relevant outcomes included clinical effectiveness (clinical utility and clinical validity), safety, and other domains such as organisational, economic, and ethical implications. In the context of genetic testing, clinical validity refers to how accurately and reliably a genetic test identifies or predicts the presence, absence, or risk of a specific disease or condition. Clinical utility, in contrast, refers to the extent to which the results of a genetic test provide useful information that can improve patient management, influence treatment decisions, or lead to better health outcomes. A test with high clinical utility has a proven impact on clinical practice or patient-relevant outcomes.

klinische Wirksamkeit: nur klinische Validität

Sicherheit: keine Evidenz

Clinical effectiveness was assessed in both SRs [19, 44] and the NIPH report [15], although outcomes were limited to clinical validity measures. Safety outcomes were not defined in the eligibility criteria of any of the included reviews. Notably, the NIPH report [15] mentioned in their assessment that none of the included primary studies reported adverse events related to the testing procedures.

ECO & ORG bewertet von NIPH-Bericht; ETH: keine Evidenz

Concerning other domains, economic considerations were addressed through micro-costing analysis in the NIPH report [15], and organisational considerations were described based on a focus search for reviews on test feasibility. No evidence was found regarding ethical implications [15].

An overview of the two included SR [19, 44] and the NIPH report [15], the tested genetic alterations, and the assessed outcome domains is provided in Table 4-1, all outcomes will be described in detail in their respective chapters.

Table 4-1: Overview of included systematic reviews, tested genetic alteration and assessed outcome domains.

Name, year	Target genetic alteration	EFF/SAF	ECO	ORG	ETH
Galvano, 2022 [44]	PIK3CA	X			
NIPH, 2022 [15]	PIK3CA	X	X	X	
Raei, 2024 [19]	ESR1	X			

Abbreviations: EFF/SAF … efficacy and safety domain, ECO … economical domain, ETH … ethical domain, NIPH … Norwegian Institute of Public Health, ORG … organisational domain.

Molecular testing methods and reference standards

PIK3CA-Detektion in 2 SRs mit unterschiedlichen analytischen Ansätzen

Detection of *PIK3CA* was assessed in one SR [44] and the NIPH report [15], though they differed in their analytical framework. Individual molecular testing methods (NGS, ddPCR, BEAMing, RT-PCR, standard PCR, Sanger sequencing) were assessed against tissue biopsy as the reference standard in the SR [44]. In contrast, head-to-head comparisons between methods and platforms were performed in the NIPH report [15]. Although the NIPH report [44] originally planned to include Sanger sequencing and liquid chip technology in their PICO, their systematic search identified evidence only for NGS and PCR methods.

ESR1-Detektion in 1 SR (Subgruppenanalyse)

For *ESR1* detection, one SR [19] primarily assessed whether cell-free DNA (cfDNA) analysis (using dPCR or NGS) is superior to tissue biopsy as reference standard (see Table A-1 in the Appendix), which deviated from our PICO framework. This primary analysis did not compare the diagnostic accuracy of different molecular testing methods. However, the SR included a subgroup analysis providing head-to-head comparisons of PCR versus NGS for cfDNA analysis against tissue biopsy as reference standard, which aligned with our PICO and was therefore relevant for this assessment.

An overview of the different analytical frameworks across all reviews is presented in Table 4-2.

Approach, platform and product for NGS described in reviews

NGS-Kategorisierung

To facilitate comparison across reviews, NGS technologies were categorised into three hierarchical levels: approach (sequencing methodology), platform (sequencing instrument), and product (commercial assay). A broader explanation of these definitions is provided in Table A-1 in the Appendix.

eingeschlossene SRs unterscheiden sich in Ansatz, Plattform, Produkt

Levels of NGS specification differed between both SRs [19, 44] and the NIPH report [15]. Comprehensive reporting was provided in one SR [19], detailing NGS approaches (hybrid capture-based, amplicon-based), multiple platforms (HiSeq, MiSeq, Ion Torrent – all Illumina systems except Ion Torrent), and various commercial products (FoundationOne/FoundationACT, Guardant-360, AmpliSeq HD/Oncomine Pan-Cancer). NGS approaches (hybrid capture-based, amplicon-based) were similarly reported in the other SR [19], but platforms or commercial products were not specified. In contrast, a different approach was taken in the NIPH report [15], focusing exclusively on platforms and commercial products (Access Array™ system by Fluidigm, Oncomine™ Breast cfDNA Assay, InVisionSeq™ cfDNA Assay) without specifying the underlying NGS approaches. An overview of the NGS approaches, platforms, and products described in the included reviews is provided in Table 4-2.

Table 4-2: Comparison of molecular testing methods across reviews.

	Galvano et al., 2022 [44]	NIPH, 2022 [15]	Raei et al., 2024 [19]
Comparison of methods	Each molecular testing method: ■ NGS ■ ddPCR ■ BEAMing ■ RT-PCR ■ standard PCR ■ Sanger Sequencing vs. reference standard	Head-to-head comparison: ■ NGS vs. NGS on different platforms ■ ddPCR vs. BEAMing ■ NGS vs. ddPCR	Each molecular testing method: ■ NGS ■ dPCR vs. reference standard (*Note*: Subgroup analysis)

	Galvano et al., 2022 [44]	NIPH, 2022 [15]	Raei et al., 2024 [19]
Approach	■ Hybrid capture-based ■ Amplicon-based (implied)[1]	■ Hybrid capture-based (implied)[2] ■ Amplicon-based (implied)[2]	■ Hybrid capture-based ■ Amplicon-based-based
Platform	■ HiSeq (Illumina) ■ MiSeq (Illumina) ■ Ion Torrent	■ Access Array™ system (Fluidigm)	*Not specified at platform level*
Product	■ FoundationOne/FoundationACT (Foundation Medicine) ■ Guardant360 ■ AmpliSeq HD/Oncomine Pan-Cancer	■ Oncomine™ Breast cfDNA Assay ■ InVisionSeq™ ctDNA Assay	*Not specified at product level*

Abbreviations: BEAMing … Beads, Emulsion, Amplification, and Magnetics; ddPCR … droplet digital polymerase chain reaction; dPCR … digital polymerase chain reaction; NGS … Next-Generation Sequencing; PCR … Polymerase Chain Reaction; RT-PCR … real-time polymerase chain reaction.

Notes:

[1] *Amplicon-based NGS is not explicitly named as an approach category by SR of Galvano but is implied through the inclusion of amplicon-based platforms and products (Ion Torrent, MiSeq Illumina, AmpliSeq HD/Oncomine Pan-Cancer).*

[2] *NGS approaches are not explicitly specified by NIPH but are implied through the included platforms and products: amplicon-based NGS for Access Array™ system (Fluidigm) and Oncomine™ Breast cfDNA Assay, and hybrid capture-based NGS for InVisionSeq™ ctDNA Assay.*

Specification of NGS panel size and sequencing depth

NGS-Begriff nicht klar definiert in SRs

Regarding the specification of NGS panel size, none of the included reviews did clearly define the term "NGS," although it is likely that the term refers to targeted-gene panel sequencing rather than whole exome sequencing (WES) or whole genome sequencing (WGS) [24].

Risk of bias assessment and methodological quality

ROBIS: hohe Qualität in allen SRs

High quality according to the ROBIS (Risk Of Bias In Systematic reviews) tool [41] was reached in both SR [19, 44] and the NIPH report [15] (see Table A-3 in the Appendix).

QUADAS-2: überwiegend low RoB in Primärstudien

Risk of bias of included primary studies was assessed using the QUADAS-2 (Quality Assessment of Diagnostic Accuracy Studies) tool [45] for diagnostic accuracy studies in both SR [19, 44]. Overall low risk of bias was reported in one SR [44], with one study presenting a high risk of bias in patient selection. Predominantly low risk across all QUADAS-2 domains was found in the other SR [19], suggesting high-quality evidence with minimal applicability concerns.

NIPH-Bericht bewertet Evidenz- und Berichtqualität

The NIPH report [15] employed the EGAPP (Evaluation of Genomic Applications in Practice and Prevention) framework [46] to assess the quality of evidence across included primary concordance studies, an approach specifically developed for evaluating genomic and diagnostic test applications. In addition, the STARD (Standards for Reporting of Diagnostic Accuracy Studies) checklist [47] was used to assess the quality of reporting in the included studies.

Synthesis

Quantitative analyses to synthesise diagnostic accuracy data in the form of an individual patient data (IPD) meta-analyses were conducted in both SR [19, 44]. A narrative analysis of head-to-head comparisons of different testing methods was presented in the NIPH report [15], as formal meta-analysis was not performed due to limited available studies (n=3). NIPH's strict requirement for head-to-head comparisons between testing methods (rather than comparisons against a reference standard) resulted in only three eligible studies.

zwei SRs: IPD-Metaanalysen

NIPH-Bericht: narrative Analyse

None of the two SRs [19, 44] or the NIPH report [15] used a standardised tool such as GRADE (Grading of Recommendations, Assessment, Development and Evaluation) to assess the certainty of evidence across studies. Instead, the NIPH report applied the EGAPP framework, which is specifically designed for assessing genomic and diagnostic test applications and was therefore appropriate for this assessment.

Evidenzbewertung ohne GRADE

Funding

None of the SRs [19, 44] or the NIPH report [15] reported external funding. The funding sources of the individual primary studies included in these systematic reviews were not systematically reported across the reviews.

keine externe Finanzierung

4.2 Clinical effectiveness and safety

4.2.1 Clinical effectiveness and safety outcomes

Clinical validity was the focus in both SRs [19, 44], particularly diagnostic test accuracy metrics. Standard test accuracy metrics were defined in the reports, including [48, 49]:

klinische Validität: 2 SRs berichten Standardmaße für die Testgenauigkeit

- *Sensitivity (SE):* proportion of correctly identified positive cases
- *Specificity (SP):* proportion of correctly identified negative cases
- *Positive predictive value (PPV):* probability that a positive test result is truly positive
- *Negative predictive value (NPV):* probability that a negative test result is truly negative
- *Positive likelihood ratio (PLR):* ratio indicating how much more likely a positive test result occurs in patients with the condition versus those without
- *Negative likelihood ratio (NLR):* ratio indicating how much more likely a negative test result occurs in patients with the condition versus those without
- *Diagnostic odds ratio (DOR):* ratio of the odds of positive test results in diseased versus non-diseased individuals
- *Area under the curve (AUC):* overall measure of test performance across all possible thresholds
- *Accuracy (ACC):* proportion of all correctly identified cases (both positive and negative)

Head-to-head comparisons between different molecular testing methods and NGS platforms were conducted in the NIPH report [15], reporting only concordance (= agreement between two tests or methods) for clinical validity measurements, as sensitivity or specificity of the tests were not reported in any included study of the NIPH report.

klinische Validität: HTA-Bericht berichtet nur Konkordanz zwischen Testmethoden

Clinical utility outcomes were specified in the PICO framework only by the NIPH report [15], however, none of the included studies by NIPH report provided data on these outcomes.

klinischer Nutzen: keine Daten wurden identifiziert

For safety, none of the SRs [19, 44] or the NIPH report [15] predefined adverse events in their methodology. Nevertheless, the NIPH report explicitly noted in their discussion that none of the included studies reported adverse events related to the testing procedures themselves.

Sicherheit: keine Evidenz wurde identifiziert

4.2.2 Results on clinical effectiveness for testing *PIK3CA*

Given the distinct comparative frameworks used for *PIK3CA* testing in the SR [44] and NIPH report [15], these findings are presented separately in Table 4-3.

klinische Validität zur Detektion von PIK3CA

NGS-based ctDNA testing demonstrated the highest sensitivity and specificity among the three methods (NGS, ddPCR/BEAMing, PCR) evaluated against the reference standard. The positive and negative likelihood ratios, diagnostic odds ratio, and area under the curve all favored NGS over the other methods. Digital PCR methods (ddPCR/BEAMing) showed lower sensitivity compared to NGS while maintaining reduced specificity. The positive and negative likelihood ratios as well as the diagnostic odds ratio reflected diminished performance relative to NGS, though the area under the curve remained high. Standard PCR demonstrated the lowest sensitivity among the three methods, though specificity remained comparable to NGS. The confidence intervals for the positive and negative likelihood ratios as well as the diagnostic odds ratio were considerably wide. The area under the curve was notably lower than both NGS and digital PCR methods.

NGS-basierte ctDNA-Testung: höhere SE & SP gegenüber Referenzstandard

Concordance between molecular tests for *PIK3CA* detection ranged from 0.80 to 0.87, with discordance rates of 5-10%. When comparing two different NGS platforms (Oncomine™ Breast cfDNA Assay vs. InVisionSeq™ ctDNA Assay), concordance was lower compared to the comparison between the two digital PCR methods. Cross-methodology comparison between ddPCR and the NGS-based Access Array™ system showed concordance between the two previous comparisons. However, NIPH explicitly noted that, according to the EGAPP framework, the quality of reporting was poor, and the overall evidence was considered inadequate.

mittlere Übereinstimmung zwischen Testmethoden

Table 4-3: Clinical validity measures from one SR [44] and the NIPH report [15]

Galvano et al., 2022 [44] – Diagnostic accuracy of NGS, ddPCR/BEAMing and PCR vs. reference standard							
ctDNA test methods	n studies[1] & N patients	SE (95% CI)	SP (95% CI)	PLR (95% CI)	NLR (95% CI)	DOR (95% CI)	AUC
NGS	n = 9 N = 307	0.83 (0.75-0.89)	0.98 (0.94-0.99)	11.65 (5.43-24.99)	0.23 (0.09-0.62)	59.80 (14.29-250.23)	0.98
ddPCR/BEAMing	n = 12 N = 1 485	0.74 (0.70-0.78)	0.84 (0.82-0.86)	6.63 (3.97-11.08)	0.31 (0.22-0.43)	28.84 (13.45-61.86)	0.92
standard PCR	n = 5	0.51	0.96	9.30	0.54	20.61	0.77

	N = 174	(0.39-0.64)	(0.91-0.99)	(0.64-136.17)	(0.31-0.96)	(1.57-270.46)	

NIPH, 2022 [15] – Head-to-head comparison between different molecular tests				
Tested method 1	Tested method 2	n studies & study design	N of samples tested with both methods	Concordance
NGS (Oncomine™ Breast cfDNA Assay)	NGS (InVisionSeq™ ctDNA Assay)	1 case-control or cross-sectional study	30	0.80 (95% CI 0.59-1.00)
PCR (BEAMing)	PCR (ddPCR)	1 cohort study	363	0.87 (95% CI 0.81-0.93)
PCR (ddPCR)	NGS (Access Array™ system (Fluidigm))	1 cohort study	162	0.85 (95% CI NR)

Abbreviations: AUC … area under the curve, CI … confidence interval, ddPCR … droplet digital polymerase chain reaction, DOR … diagnostic odds ratio, NGS … next-generation sequencing, NLR … negative likelihood ratio, PCR … polymerase chain reaction, PLR … positive likelihood ratio, SE … sensitivity, SP … specificity.

Note: [1] The study designs were not clearly specified; only the distribution of 7 RCTs and 17 cohort studies was reported.

4.2.3 Results on clinical effectiveness for testing ESR1

klinische Validität zur Detektion von ESR1

For *ESR1* testing, evidence on clinical validity was limited to a subgroup analysis [19]; findings are provided in Table 4-4 below.

Referenzstandard höhere Sensitivität als NGS; vergleichbare Spezifität und Genauigkeit

dPCR demonstrated higher sensitivity compared to NGS, while specificity remained comparable between both methods. The positive and negative predictive value showed similar patterns, with dPCR achieving higher positive predictive value though with wide confidence intervals for negative predictive value in both methods. Accuracy was comparable between both methods.

The subgroup analysis revealed substantial heterogeneity between studies (see Table 4-4 for I^2), and no statistically significant difference between methods was demonstrated.

Table 4-4: Diagnostic accuracy of cfDNA test methods against tissue biopsy from SR [19]

cfDNA test method		SE	SP	PPV	NPV	PLR	NLR	ACC
NGS	EF (95% CI, I^2)	56.78 (13.89 to 99.67, 75.66)	90.14 (79.17 to 101.10, 33.98)	55.39 (28.17 to 82.61, 56.27)	94.74 (94.73 to 94.74, 12.87)	1.75 (−1.41 to 4.92, 4.95)	1.008 (0.94 to 1.07, 4.95)	88.35 (76.87 to 99.82, 0.00)
	n studies	4	5	3	3	4	3	4
dPCR	EF (95% CI, I^2)	81.01 (64.04 to 97.99, 75.74)	90.44 (82.55 to 98.33, 87.72)	61.76 (38.40 to 85.11, 84.84)	90.99 (21.78 to 81.84, 77.67)	1.61 (1.21 to 2.01, 78.54)	0.42 (−0.004 to 0.85, 0.00)	89.81 (81.70 to 97.93, 84.53)
	n studies	8	9	4	3	4	4	8

Abbreviations: ACC … accuracy; CI … confidence interval; dPCR … digital polymerase chain reaction; NGS … next-generation sequencing; NLR … negative likelihood ratio; NPV … negative predictive value; PLR … positive likelihood ratio; PPV … positive predictive value; SE … sensitivity; SP … specificity.

Note: Reference standard: tissue biopsy. Subgroup analysis limited by high heterogeneity between studies; no statistically significant difference between methods was demonstrated.

4.2.4 Results on clinical effectiveness and safety for testing *AKT1* and *PTEN*

keine Evidenz zu AKT1- & PTEN-Detektion

No publications meeting the PICO criteria were identified that evaluated the relative effectiveness or safety of *AKT1* or *PTEN* detection compared with standard diagnostic approaches or alternative tests.

4.3 Results for the organisational domain

narrative Synthese zu organisatorischen Aspekten von NIPH-Bericht

Organisational aspects of *PIK3CA* detection were addressed in the NIPH report [15], which narratively synthesised advantages and limitations of RT-PCR, NGS, and Sanger sequencing for HR+/HER2– advanced or metastatic BC, based on the information of one included narrative report and expert consultation (see Table 4-5), with the source specified after each paragraph below.

Type of samples and material requirements

Testmethoden zeigen unterschiedliche Performance je nach Probenmaterial

The three molecular testing methods show different characteristics depending on the type of sample used. NGS demonstrates high sensitivity when tissue samples such as formalin-fixed paraffin-embedded (FFPE) specimens are analysed but faces a greater risk of failures and false-negative results when liquid biopsies containing circulating tumour DNA (ctDNA) are employed. In contrast, RT-PCR performs very sensitively with liquid biopsies, making it particularly suitable for plasma-based testing, though it shows an increased risk of failures and false-negative results with tissue samples. Sanger sequencing is not recommended for liquid biopsies and shows similar limitations to RT-PCR when tissue samples are used, with risks of failures and false-negative results (source: included review of NIPH report).

plasmabasierte und gewebebasierte Tests nicht austauschbar

It must be emphasized that plasma-based tests (liquid biopsies) and tissue-based tests were developed for distinct clinical purposes and are not interchangeable. While RT-PCR performs sensitively with liquid biopsies for detecting known hotspot mutations, NGS is the required standard for comprehensive discovery of resistance markers [24].

Multiplexing capabilities and genetic alteration detection

Multiplexing-Kapazität: NGS detektiert am meisten Genalterationen

NGS offers substantial advantages in terms of multiplexing capability and coverage of genetic alterations. It can be easily multiplexed for numerous genetic alterations, thereby avoiding the need for sequential testing, and has the capacity to detect both known and unknown genetic alterations. RT-PCR, while capable of being multiplexed, is limited to a smaller number of genetic alterations and primarily detects known genetic alterations, which may necessitate sequential testing for comprehensive analysis. Sanger sequencing is not easily multiplexed and often requires sequential testing. However, Sanger sequencing has a notable advantage in read length, producing much longer reads than NGS, which can be beneficial for certain applications despite its other limitations (source: Norwegian expert consultation). It should be noted that this read length advantage is not relevant for plasma-based ctDNA detection in metastatic breast cancer, where DNA fragments are typically short (~160bp) [24].

Turnaround Time

RT-PCR: schnellste Methode (4h-1 Tag)

Turnaround time (TAT)[5] varies between testing methods. RT-PCR offers the fastest analysis duration, typically requiring between 4 hours and 1 day, making it highly advantageous for time-sensitive clinical decisions. NGS requires approximately 3 days for analysis, though in some cases this can extend to 1-3 weeks (source: included review of NIPH report). In the Norwegian hospital setting, current NGS platforms have a TAT of 7-12 days, although newer systems promise to reduce this to 24 hours for some gene panel assays. No specific information on turnaround time is available for Sanger sequencing, which is no longer commonly used in many settings including Norway (source: Norwegian expert consultation).

Infrastructure and expertise required

NGS: hoher Infrastruktur-/Expertise-Bedarf

NGS is a complex procedure requiring significant infrastructure and bioinformatics capabilities. PCR analysis also demands specialised expertise. The review did not include information on the time and expertise needed to interpret test results or communicate them to clinicians, nor did it discuss the use of data analysis pipelines for NGS (source: included review of NIPH report).

Table 4-5: Comparison of molecular testing methods for PIK3CA detection [15].

Aspect	NGS	RT-PCR	Sanger Sequencing
Sample type & material			
Tissue samples (FFPE)	✓ High sensitivity	⚠ Increased risk of failures and false-negative results	⚠ Risk of failures and false-negative results
Liquid biopsies (ctDNA)	⚠ Greater risk of failures and false-negative results	✓ Very sensitive	✗ Not recommended
Multiplexing & genetic alteration coverage			
Multiplexing capability	✓ Easily multiplexed for numerous genetic alterations	⚠ Possible, but limited number	✗ Not easy to multiplex
Detection of genetic alterations	✓ Known and unknown genetic alterations	⚠ Mostly known genetic alterations	NR
Sequential testing	✓ Not required	✗ Often required	✗ Often required
Read length	✗ Short read length	NR	✓ Much longer read length than NGS
Turnaround time (TAT)			
Analysis duration	✗ ~3 days, sometimes 1-3 weeks	✓ 4 hours to 1 day	No information available
TAT in Norway	⚠ 7-12 days (current), 24h (new systems)	NR	No longer common
Infrastructure & expertise			
Complexity	✗ Complex, requires high level of infrastructure and bioinformatics	⚠ Special expertise required	No specific information
Availability	⚠ Increasingly widespread	✓ Cheap and widely available	✗ Rarely used in Norway
Sample processing	✓ Many patients can be combined in parallel	✓ 96 or 384-well plates for parallel analysis	✗ Not easily combined (4-32 samples)

Abbreviations: ctDNA … Circulating tumour Deoxyribonucleic Acid; FFPE … Formalin-Fixed Paraffin-Embedded; NGS … Next generation sequencing; NR … Not reported; RT-PCR … Real-Time Polymerase Chain Reaction; TAT … Turnaround time.

[5] defined as the time between test request and treatment decision

4.4 Results for the economic domain

Mikrokostenanalyse für norwegische Settings von NIPH-Bericht

Economic considerations of diagnostic testing methods for HR+/HER2– advanced or metastatic BC were addressed in the NIPH report [15], which conducted a micro-costing analysis relevant for Norwegian clinical settings. Results are illustrated in Table 4-6. In collaboration with experts from Norwegian regional health authorities, all underlying resources required for PCR and NGS testing were identified, including equipment, consumables, and staff time, with unit costs attached to generate overall costs per test. The analysis considered both single-patient testing scenarios and parallel testing of multiple patients with multiple biomarkers, reflecting current oncology practice where several molecular tests are performed simultaneously.

Kosten stark volumenabhängig, NGS bei hohem Durchsatz kosteneffizient

The micro-costing analysis revealed substantial differences in cost-efficiency depending on testing volume. RT-PCR testing for a single biomarker cost 238 € per patient. NGS testing showed markedly different cost profiles depending on the number of patients tested: when performed for a single patient, costs reached 1,385 € per patient but decreased dramatically to 198 € per patient when testing 10 or more patients at the same time.

Table 4-6: Results of micro-costing analysis comparing RT-PCR and NGS [15].

Cost category	RT-PCR[1]	NGS[2]
Test costs per patient (micro-costing analysis)		
Single biomarker/patient	238 €/patient	1,385 €/patient 198 €/10+ patients
Reagent costs[3]	117 €/patient	1,230 €/patient 147 €/10+ patients
Personnel costs	124 €/patient	155 €/patient 50 €/10+ patients
Infrastructure and maintenance costs		
Initial equipment and supplies	39,074 €	254,831-339,775 €
Annual maintenance	2,124 €/year	12,742 €/year
External quality control	425 € per biomarker/year	Included in maintenance
Validation costs (one-time)	NR	2,548-4,247 €/kit
Additional costs		
Biopsy preparation	23 €/preparation	23 €/preparation

Abbreviations: NGS … next-generation sequencing; NOK … Norwegian kroner; NR…not reported; PATA … Norwegian Pathology Activity Code; PCR … polymerase chain reaction; RT-PCR … real-time polymerase chain reaction.

Notes: Current exchange rate (14.10.2025) of NOK: 1 € = 11,7725 NOK

[1] *The estimated costs are associated with testing one sample;*

[2] *The costs are estimated for Oncomine Focus panel. The panel can analyse 8 samples and 6 biomarkers simultaneously;*

[3] *The reagent costs are varied between 119–212 € per patient, depend on the type of the commercial kit.*

Infrastruktur- und Wartungskosten unterschiedlich

Infrastructure and maintenance costs also differed between methods. NGS implementation required initial equipment and supply investments of 254,831 € to 339,775 € with annual maintenance costs of approximately 12,742 €. The validation of the method is time-consuming and requires various experts, such as bioengineers, pathologists, and engineers. At least one NGS kit is used, costing between 2,548 € and 6,795 €. This validation is only done once when

the method is first established. In comparison, PCR infrastructure costs were substantially lower at 55,213 €, with annual maintenance costs of 2,124 €. In addition, external quality control rounds were estimated at 425 € per biomarker per year for PCR. At the time of the assessment, approximately eleven pathology departments in Norway had NGS equipment, while PCR was available at most university hospitals and some regional hospitals.

The costs associated with preparing the biopsy are the same for both diagnostic methods.

Biopsie-Vorbereitungskosten identisch für beide Methoden

4.5 Results for the ethical domain

The ethical domain was included in this assessment to evaluate potential equity issues such as access disparities across Austrian regions and fairness in patient selection for molecular testing. However, no publications meeting the PICO criteria for ethical outcomes were identified in the systematic literature search.

keine Evidenz zu ethischen Aspekten

5 Results: guideline recommendations

This chapter presents recommendations from clinical guidelines on testing of genetic alterations with next generation sequencing (NGS) for treatment decision-making in patients with advanced and metastatic breast cancer (BC).

Leitlinienempfehlungen zu Genetic alterationstestung

5.1 Included guidelines

For the role of different sequencing technologies in detection of *PIK3CA-/AKT1-/PTEN-/ESR1*-genetic alterations in adults with HR+/HER2–, locally advanced or metastatic BC, guideline recommendations/informal guidance passages from three international organisations were considered: These included the German Association of the Scientific Medical Societies (AWMF, Arbeitsgemeinschaft der Wissenschaftlichen Medizinischen Fachgesellschaften) S3 guideline [23] and the Society for Immunotherapy of Cancer's clinical practice guidelines on immunotherapy (SITC) [50], both published as comprehensive standalone guidelines. Additionally, focused updates from the American Society of Clinical Oncology (ASCO) were included, which represent partial revisions to earlier ASCO guidelines and cannot be interpreted independently of their original versions. These ASCO updates comprise one update [51] to a 2015 guideline on biomarkers for systemic therapy in metastatic BC [52] and two updates [53, 54] to a 2016 guideline on endocrine and targeted therapy for HR+/HER2– MBC [55].

Leitlinienempfehlungen von 3 internationalen Organisationen eingeschlossen

Methodological quality of included guidelines

All guidelines meet high methodological standards according to the appraisal using the AGREE-II checklist. We found recommendations suitable for clinical use in guiding molecular testing for HR+/HER2– advanced BC. The AWMF [23] guideline is directly relevant to the Austrian context, while the ASCO [51, 53, 54] and SITC [50] guidelines are only partially applicable to Austrian clinical settings. The AWMF [23] and ASCO [51, 53, 54] guidelines demonstrated strong methodology across all domains, with comprehensive stakeholder involvement and good development processes. The SITC guideline [50], while generally of good quality and recommended for use, showed some limitations primarily in the rigor of its systematic literature search and evidence synthesis methodology, which should be considered when interpreting its recommendations (the full AGREE-II quality appraisal can be found in Table A-4).

laut AGREE-II: alle Leitlinien hohe methodische Qualität

Recommendation classification systems of included guidelines

The AWMF S3 guideline [23] distinguishes between evidence-based and consensus-based recommendations, with consensus-based recommendations reflecting expert agreement when evidence is missing. It uses recommendation grades A (strong: "shall/shall not"), B (moderate: "should/should not"), and 0 (open recommendation: "can"), combined with GRADE (Grading of Recommendations Assessment, Development and Evaluation) evidence quality ratings ranging from high quality (⊕⊕⊕⊕) to very low quality (⊕⊖⊖⊖).

unterschiedliche Klassifikationssysteme für Empfehlungen der einbezogenen Leitlinien

ASCO guidelines [51, 53, 54] similarly distinguish between evidence-based and consensus-based recommendations. ASCO applies a two-dimensional rating system that assesses evidence quality (high, intermediate, low, or insufficient) and recommendation strength (strong, moderate, or weak). The SITC guideline [50] employs the Oxford Centre for Evidence-Based Medicine (OCEBM) Levels of Evidence (2016 version), ranging from Level 1 (systematic review/meta-analysis) to Level 5 (mechanism-based reasoning), with recommendations developed through expert panel consensus requiring a minimum 75% agreement threshold.

5.2 Guideline synopsis

Synopse umfasst formale Empfehlungen & relevante Textpassagen ohne formale Graduierung

This synthesis includes both formally graded recommendations and relevant guidance passages where NGS is mentioned without formal grading (labeled "informal guidance"). Comprehensive molecular testing is an emerging area, and guidelines increasingly address NGS in the clinical context without formal recommendation grading. In the narrative synthesis below, recommendations and informal guidance passages are indicated by their evidence grading shown in brackets. Guideline positions are synthesised separately for *PIK3CA* (5.2.1) and *AKT1*, *PTEN*, *ESR1* genetic alterations (5.2.2).

The recommendations and informal guidance passages from the guidelines for NGS testing are presented in Figure 5-1; while verbatim guideline recommendations are provided in Table A-2 in the Appendix.

5.2.1 Comparison of guideline recommendations for NGS-testing for *PIK3CA* detection

Testing methodology

Für PIK3CA-Detektion:

3 Leitlinienempfehlungen, die NGS empfehlen

2 Textpassagen, die NGS adressieren

All guidelines recommend NGS for detection of genetic alterations, though with varying levels of specificity. AWMF S3 2025 (strong expert consensus) recommends comprehensive NGS-based molecular pathological diagnostics. ASCO 2024 (informal guidance) specifically requires tumour genomic testing via large-panel NGS in CLIA-certified laboratories. ASCO 2021 (evidence-based of high quality and strong strength), ASCO 2022 (evidence-based of high quality and strong strength) and SITC 2021 (informal guidance) recommend NGS. ASCO 2021 and 2022 emphasize sequential testing strategies: cfDNA first, then tumour tissue if negative, to maximise genetic alteration detection. No guideline addresses WES or WGS, or clearly defines what is meant by NGS, though targeted-gene panel NGS is most likely intended.

Target populations

Populationen zwischen Leitlinienempfehlungen leicht unterschiedlich

Target populations vary in specificity across guidelines. AWMF 2025 (strong expert consensus) defines the broadest population: patients with metastatic or treatment-resistant BC without expected survival benefit from standard therapies, without restriction to molecular subtype. ASCO 2024 (informal guidance) refers to metastatic HR+/HER2– BC. ASCO 2021 (evidence-based of high quality and strong strength) specifically addresses postmenopausal

and male patients with HR+ MBC. ASCO 2022 (evidence-based of high quality and strong strength) includes patients with locally recurrent unresectable or metastatic HR+/HER2– BC. SITC 2021 (informal guidance) specifies only ER+/HER2– disease without stage specification.

Genetic alterations detected and therapeutic context

AWMF S3 Empfehlung: umfassende NGS-Diagnostik ohne deutliche Spezifizierung der Genalterationen

AWMF S3 2025 (strong expert consensus) recommends comprehensive NGS-based molecular diagnostics without specifying genetic alterations. However, subsequent therapy recommendations within the same guideline provide specific treatment guidance for *PIK3CA* genetic alterations (Inavolisib plus Fulvestrant plus Palbociclib; Capivasertib plus Fulvestrant; Alpelisib plus Fulvestrant), and for *PIK3CA*, *AKT1*, *PTEN*, and *ESR1* genetic alterations (Capivasertib plus Fulvestrant), implicitly supporting the inclusion of these genes in the comprehensive NGS approach. AWMF also recommends molecular tumour board presentation and considering re-biopsy to capture variants developed during tumour progression.

Empfehlungen und Textpassagen die PIK3CA-Detektion adressieren

ASCO 2024 (informal guidance) addresses comprehensive tumour genomic testing to identify multiple targetable genetic alterations including *PIK3CA*, *AKT1*, *PTEN*, and *ESR1*, with treatment pathways for these genetic alterations mentioned later in the same table. ASCO 2021 (evidence-based of high quality and strong strength) explicitly recommends *PIK3CA* detection in cfDNA or tissue DNA to guide alpelisib/fulvestrant therapy. ASCO 2022 (evidence-based, high quality, strong) similarly recommends PIK3CA detection in cfDNA or tissue DNA (sequential testing: cfDNA first, then tissue if negative) for alpelisib/fulvestrant therapy. SITC 2021 (informal guidance) addresses detection of actionable gene genetic alterations including PI3K alterations, linked to alpelisib/fulvestrant therapy.

Timing of testing

Testzeitpunkt variiert, bzw. wird nicht spezifiziert

Timing recommendations vary between guidelines. AWMF 2025 (strong expert consensus) recommends testing when standard therapies no longer offer survival benefit. ASCO 2024 (informal guidance) recommends testing at time of progression or using archival tissue after first-line treatment. ASCO 2021 and 2022 (both evidence-based of high quality and strong strength) do not specify the timing of testing. SITC 2021 (informal guidance) also does not specify the timing.

5.2.2 Comparison of guideline recommendations for NGS-testing for *AKT1*, *PTEN*, *ESR1* genetic alteration detection

AKT1-, PTEN-, ESR1-Detektion: deutlich weniger Empfehlungen als für PIK3CA

Explicit recommendations for *AKT1*, *PTEN*, and *ESR1* detection are considerably more limited than those for *PIK3CA* detection. Only the AWMF 2025 and ASCO 2024 guidelines address these genetic alterations, whereas ASCO 2021 and 2022 focus solely on *PIK3CA* genetic alterations. SITC 2021 references "actionable gene mutations including *PI3K* alterations" without specifying *AKT1*, *PTEN*, or *ESR1*.

Testing methodology

AWMF 2025 (strong expert consensus) recommends comprehensive NGS-based molecular pathological diagnostics without specifying genetic alterations. ASCO 2024 (informal guidance) describes tumour genomic testing via large-panel NGS in CLIA-certified laboratories using tissue or plasma samples. ASCO 2021 and 2022 (both evidence-based of high quality and strong strength) do not address *AKT1*, *PTEN*, or *ESR1* detection. SITC 2021 (informal guidance) does not explicitly specify these genetic alterations. No guideline addresses WES or WGS for detection of these genetic alterations.

AWMF S3 Empfehlung: umfassende NGS-Diagnostik ohne Spezifizierung der Genalteration

Target populations

Target populations for *AKT1*, *PTEN*, and *ESR1* detection align with those described for *PIK3CA* detection.

Zielpopulationen für AKT1/PTEN/ESR1 identisch mit PIK3CA-Detektion

Genetic alterations detected and therapeutic context

AWMF S3 2025 (strong expert consensus) does not explicitly mention *AKT1*, *PTEN*, or *ESR1* genetic alterations in its initial NGS recommendation. However, it implicitly supports comprehensive testing through subsequent formal therapy recommendation. These recommendations link *ESR1* genetic alterations to Elacestrant monotherapy and *AKT1*/*PTEN* alterations to Capivasertib plus Fulvestrant therapy.

AWMF S3: implizite Empfehlung via Therapie-Genalteration-Verknüpfung

ASCO 2024 (informal guidance) identifies targetable genetic alterations including *AKT1* activating genetic alterations, *PTEN* inactivation, and *ESR1* genetic alterations in a treatment algorithm table. The guideline specifies technical criteria for *PTEN* inactivation (premature stop codons, frameshift alterations, splice site genetic alterations, homozygous deletions, rearrangements, and specific missense genetic alterations). However, this information appears in a treatment table and does not constitute a formal recommendation.

ASCO 2024: keine formale Empfehlung (nur Behandlungstabelle)

Timing of testing

AWMF 2025 (strong expert consensus) recommends testing when standard therapies no longer provide a survival benefit. ASCO 2024 (informal guidance) recommends obtaining samples at disease progression or using archival tissue samples after first-line treatment.

Testzeitpunkt variiert in Leitlinien

Guideline recommendations and informal guidance for NGS-based detection of PIK3CA, AKT1, PTEN and ESR1 mutations in breast cancer

Informal guidance from SITC, 2021, US

- **Population**: ER+/HER2- BC, stage not specified
- **Recommended test**: NGS, panel size and sequencing depth not specified
- **Mutation detected**: Actionable gene mutations including PI3K alterations
- **Recommended therapy**: Alpelisib/fulvestrant therapy
- **Time point:** Not specified
- **Type of recommendation**: Informal (textpassage)

Recommendation from ASCO, 2021, US

- **Population**: Postmenopausal & male patients with HR+ metastatic BC
- **Recommended test**: NGS, panel size and sequencing depth not specified
- **Mutation detected**: PIK3CA detection in cfDNA or tissue DNA (sequential testing: cfDNA first, then tissue if negative)
- **Recommended therapy**: Alpelisib/fulvestrant therapy
- **Time point**: Not specified
- **Type of recommendation**: Evidence-based
- **Evidence quality**: high
- **Strength**: strong

Recommendation from ASCO, 2022, US

- **Population**: Locally recurrent unresectable or metastatic HR+/HER2- BC
- **Recommended test**: NGS, panel size and sequencing depth not specified
- **Mutation detected**: PIK3CA detection in cfDNA or tissue DNA (sequential testing: cfDNA first, then tissue if negative)
- **Recommended therapy**: Alpelisib/fulvestrant therapy
- **Time point**: Not specified
- **Type of recommendation**: Evidence-based
- **Evidence quality**: high
- **Strength**: strong

Informal guidance from ASCO, 2024, US

- **Population**: Metastatic HR+/HER2- BC
- **Recommended test**: Tumor genomic testing via large-panel NGS in CLIA-certified laboratory, panel size and sequencing depth not specified
- **Mutation detected**: Targetable mutations including PIK3CA, AKT1, PTEN and ESR1
- **Recommended therapy**: Not specified, but treatment pathways for PIK3CA, AKT1, PTEN, and ESR1 mutations are mentioned later in the same table
- **Time point**: At time of progression or using archival tissue; after first-line treatment
- **Type of recommendation**: Informal (footnote in treatment table)

Recommendation from AWMF S3, 2025, Germany

- **Population**: Metastatic or treatment-resistant BC without expected survival benefit from standard therapies
- **Recommended test**: Comprehensive NGS-based molecular pathological diagnostics, panel size and sequencing depth not specified
- **Mutation detected**: Not specified
- **Recommended therapy**: Not specified, buttreatment pathways for PIK3CA, AKT1, PTEN, and ESR1 mutations are recommended later in the guideline
- **Time point**: when standard therapies no longer offer survival benefit
- **Type of recommendation**: Expert consensus
- **Strength**: strong consensus

Abbreviations: ASCO...American Society of Clinical Oncology; AWMF...Arbeitsgemeinschaft der Wissenschaftlichen Medizinischen Fachgesellschaften; BC...Breast cancer; cfDNA...Cell-free DNA; CLIA...Clinical Laboratory Improvement Amendments; ER+...Estrogen receptor-positive; HER2-...Human epidermal growth factor receptor 2-negative; HR+...Hormone receptor-positive; NGS...Next-generation sequencing; SITC...Society for Immunotherapy of Cancer; AKT1...AKT serine/threonine kinase 1; ESR1...Estrogen receptor 1; PIK3CA...Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; PTEN...Phosphatase and tensin homolog.

Legend: = Recommendation; = Informal guidance.

Note: The narrative text on guideline findings is divided into sections for PIK3CA and for the remaining genetic alterations (AKT1/PTEN/ESR1) to mirror the structure of the SRs results. The figure does not follow this subdivision since some of the guideline recommendations are identical for all genetic alterations.

Figure 5-1: Guideline recommendations for NGS-based detection of PIK3CA, AKT1, PTEN and ESR1 genetic alterations in breast cancer.

6 Discussion

Bewertung molekularer Tests (Wirksamkeit, Sicherheit, ORG/ECO/ETH-Aspekte) und Leitliniensynopse

This report aimed to evaluate the clinical effectiveness and safety of sequencing technologies (targeted gene panel sequencing, WES, WGS, and virtual gene panel sequencing) compared to no molecular testing or alternative molecular testing methods for detecting *PIK3CA*, *AKT1*, *PTEN*, and *ESR1* genetic alterations in adults with HR+/HER2– locally advanced or metastatic breast cancer (BC). Additionally, organisational, economic and ethical aspects were addressed based on the included reviews, and a guideline synopsis was conducted to clarify the role of sequencing technologies in the Austrian context.

terminologische Herausforderung: NGS ohne einheitliche Definition

This report uses specific method terms (e.g., targeted gene panel sequencing) wherever possible, as the term next-generation sequencing (NGS) lacks a standardised definition in scientific literature despite its widespread use in clinical practice. However, the included reviews and guidelines consistently employed the term NGS without providing precise definitions. Therefore, this terminology was necessarily adopted in the discussion section.

Summary of findings

drei hochqualitative SRs (2 Metaanalysen, 1 HTA-Bericht)

Three systematic reviews (SRs), including two meta-analyses [19, 44] and one Health Technology Assessment (HTA) report [15], of high quality (according to ROBIS assessment) comprising over 40 primary studies, evaluated clinical validity. The two meta-analyses evaluated diagnostic test accuracy for *PIK3CA* and *ESR1* detection against tissue biopsy as a reference; the HTA report did a head-to-head comparison between methods for *PIK3CA* detection. All studies mentioned next-generation sequencing (NGS) technology without specifying details, though it can be assumed to be targeted-gene panel sequencing [24].

Für PIK3CA: NGS-ctDNA beste Genauigkeit

Für ESR1: dPCR sensitiver als NGS

For *PIK3CA*, NGS-based ctDNA testing reached the highest sensitivity and specificity among evaluated methods [44]. Regarding concordance, the HTA report found good agreement between different testing methods (various PCR methods and NGS assays) for *PIK3CA* detection [15]. For *ESR1*, dPCR demonstrated higher sensitivity than NGS, though substantial heterogeneity between studies limited definitive conclusions [19]. No evidence was found regarding clinical utility outcomes, safety outcomes, or *AKT1* and *PTEN* detection.

ökonomische und organisatorische Aspekte (aus norwegischen HTA)

In addition, the included HTA report addressed organisational and economic aspects based on the Norwegian healthcare context [15]:

- Organisationally, NGS offers multiplexing capability for simultaneous genetic alteration testing and detection of unknown variants, but requires substantial infrastructure, specialised bioinformatics expertise, and longer turnaround times (approximately 3 days versus 4 hours to 1 day for PCR). Tissue sample type influences performance: NGS shows high sensitivity with tissue samples but increased failure rates with liquid biopsies, while PCR demonstrates opposite characteristics.
- Economically, NGS demonstrates volume-dependent cost-efficiency, with per-patient costs decreasing substantially with higher testing volume. A micro-costing analysis using Norwegian data shows that per-patient costs for NGS decrease approximately seven-fold when testing

10 or more patients simultaneously, becoming comparable to PCR costs in high-volume settings.

5 Leitlinienempfehlungen/ -textpassagen aus 3 internationalen Organisationen:

NGS for genetic alteration detection was addressed in five guideline recommendations/informal guidance passages [23, 50, 51, 53, 54] from three international organisations (AWMF, ASCO, SITC) of high methodological quality. No guideline specifies NGS panel size or addresses whole exome sequencing (WES)/whole genome sequencing (WGS), although targeted-gene panel sequencing is most likely intended. The guidelines differ slightly in population definitions and often do not specify time points for testing.

3 Leitlinienempfehlungen von AWMF S3 und ASCO, die NGS empfehlen

Three recommendations were identified:

- **AWMF S3 2025** (strong expert consensus) recommends comprehensive NGS-based molecular diagnostics with subsequent therapy recommendations implicitly supporting *PIK3CA*, *AKT1*, *PTEN*, and *ESR1* detection [23].
- **ASCO 2021** (evidence-based, high-quality evidence, strong recommendation strength) explicitly recommends *PIK3CA* detection using sequential testing strategies (cfDNA first, then tumour tissue if negative) to guide alpelisib/fulvestrant therapy in postmenopausal and male patients with HR+ metastatic BC [54].
- **ASCO 2022** (evidence-based, high-quality evidence, strong recommendation strength) provides the same recommendation as ASCO 2021 but for a broader population: patients with locally recurrent unresectable or metastatic HR+/HER2– BC [51].

und 2 Textpassagen von ASCO und SITC, die NGS adressieren

In two further guideline documents, informal guidance passages addressing NGS-based genetic alteration detection were identified: ASCO 2024 describes comprehensive tumour genomic testing using large-panel NGS to identify targetable genetic alterations including *PIK3CA*, *AKT1*, *PTEN*, and *ESR1* [53]; SITC 2021 describes detecting "actionable gene genetic alterations" with only PI3K alterations explicitly mentioned among the genetic alterations considered of interest [50].

Contextualisation

Review-of-Reviews & Leitliniensynopse als geeigneter Ansatz

This pilot assessment of genetic testing for *PIK3CA*, *AKT1*, *PTEN*, and *ESR1* genetic alterations, selected as a prioritised topic within a broader AIHTA genetics project [1, 56], highlights that a focused review-of-reviews and guideline synopsis approach can support the evaluation of genetic testing, while also revealing evidence limitations.

Limitationen der identifizierten Evidenz

The analysis of evidence from the included SRs [15, 19, 44] revealed several limitations:

- The HTA report noted inadequate evidence quality and poor reporting in included primary studies, with only three eligible concordance studies due to scarce head-to-head comparisons in the literature [15].
- Substantial heterogeneity in the *ESR1* subgroup analysis limited the ability to draw definitive conclusions [19].

Evidenzlücken

In addition to evidence limitations, several domains remained entirely unaddressed in the identified literature, including clinical utility (impact on patient management or outcomes), safety outcomes, *AKT1* and *PTEN* genetic alteration detection, and ethical implications.

klinischer Nutzen als zentraler, fehlender Evidenzparameter

According to European Union HTA guidance [57], clinical effectiveness is most convincingly demonstrated when the test-treatment combination shows improved outcomes compared with standard of care. The absence of such integrative evidence highlights the challenges in generating robust clinical utility data for novel molecular tests and, therefore, creates uncertainty for payers when evaluating the true added value of NGS testing.

optimale Testmethode abhängig vom Zielgen

Despite these evidence gaps and limitations, the available data demonstrated that diagnostic performance of testing methods varied by target gene. For *PIK3CA*, NGS outperformed PCR-based methods by covering up to 60 genetic alterations across multiple exons compared with PCR's 11 hotspot genetic alterations [44]. For *ESR1*, dPCR demonstrated higher sensitivity than NGS due to greater tolerance for low cfDNA concentrations [19]. These findings suggest that optimal testing method selection depends on the specific target gene.

österreichische Implementierung

Beyond evidence on diagnostic accuracy, organisational and economic contextualisation within the Austrian healthcare system is essential for implementation. Several technical and regulatory challenges were identified that limit the practical guidance available for implementing molecular testing in Austria:

technische & regulatorische Hürden

- No distinction between targeted-gene panel, WES, and WGS, despite these approaches having substantially different technical requirements, costs, and clinical implications.
- Target populations differ across guidelines, with some recommendations restricted to specific subgroups while others apply more broadly to all patients [23, 50, 51, 53, 54].
- Many molecular tests lack CE marking as companion diagnostics (CDx), complicating HTA evaluation of test-drug linkage [58].

The transferability of the identified evidence to the Austrian healthcare settings is substantially limited by several contextual factors:

organisatorische & ökonomische Hürden

- Only the AWMF S3 guideline is directly relevant, as it was developed specifically for German-speaking countries with similar healthcare structures [23]. In contrast, ASCO and SITC represent US clinical contexts, which differ considerably from the Austrian setting [50, 51, 53, 54].
- The only identified evidence on organisational and economical domain originates from Norwegian healthcare context [15] with limited transferability to the Austrian setting due to differences in healthcare structures, costs, and testing volumes.
- The absence of molecular subtype-specific data in Austrian national registries, such as the number of eligible patients, hampers local cost-effectiveness modelling and evidence-based pricing decisions.
- No primary data collection or stakeholder consultation (Austrian laboratories, oncologists, or healthcare administrators) was conducted, which would have been necessary to contextualise international findings for local implementation.

Implementierung erfordert lokale Datenerhebung und Stakeholder-Einbindung

The identified evidence provides an initial understanding but cannot be directly applied to Austrian healthcare setting. Implementation would require the collection of local data and engagement with relevant stakeholders to ensure feasibility within Austrian healthcare structures.

Limitations of HTA report

This report should be viewed in the context of its limitations. First, a review-of-reviews approach was employed rather than a de novo systematic review of primary studies. Second, the literature search was restricted to English, German, and Italian publications. Third, no dedicated systematic search for economic, organisational, or ethical analyses was conducted, and no primary data collection, cost modelling, or stakeholder consultation was performed for the Austrian setting. Fourth, the guideline synopsis included informal guidance passages alongside formally graded recommendations. To mitigate concerns, formal recommendations and informal guidance passages were transparently distinguished throughout the synthesis.

Limitationen: Review-of-Reviews, Spracheinschränkung, keine österreich-spezifischen Primäranalysen (ECO/ORG/ETH)

Conclusion

The evidence indicates superior clinical validity of NGS-based approaches compared to PCR-based methods for *PIK3CA* detection in HR+/HER2– advanced or metastatic BC. For *ESR1*, dPCR demonstrated higher sensitivity than NGS, though with substantial heterogeneity between studies. No evidence was found regarding clinical utility outcomes, safety outcomes, or for *AKT1* and *PTEN* detection.

klinische Validität: PIK3CA → NGS überlegen ESR1 → ZPCR sensitiver AKT1/PTEN → Evidenzlücke

Few guideline recommendations from three international organisations consistently support NGS testing for *PIK3CA* to guide targeted therapy. For *AKT1*, *PTEN*, and *ESR1*, no explicit testing recommendations exist, although their detection is implicitly supported within comprehensive genomic testing approaches (AWMF 2025 and ASCO 2024) through the provision of subsequent treatment pathways. No guideline specifies technical parameters, such as panel size, or addresses WES or WGS.

Empfehlungen/ Textpassagen aus 3 internationalen Leitlinien; technische Details/WES/ WGS nicht spezifiziert

Economic and organisational considerations addressed in one HTA report indicate potential volume-dependent cost-efficiency of NGS, as well as substantial requirements for infrastructure, specialised expertise, and staff training. While these findings may inform Austrian decision-makers, they are not directly transferable to the local context. No evidence was identified regarding ethical implications.

NGS kosteneffizient bei hohem Volumen; aber erhebliche Infrastruktur & Expertise erforderlich

Given the rapid evolution of precision oncology and the limited evidence on clinical utility, evidence-based guideline recommendations from directly applicable sources may provide a pragmatic starting point for reimbursement decisions. These should be complemented by an assessment of local context and the feasibility of implementation within the healthcare system.

evidenzbasierte Leitlinien als Grundlage für Erstattungsentscheidungen

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Appendix

Evidence tables of included systematic reviews for clinical effectiveness and safety

Table A-1: Results from systematic reviews and one HTA report for molecular tests for detection of PIK3CA-/AKT1-/PTEN-/ESR1-genetic alterations in adults with HR+/HER2–, locally advanced or metastatic breast cancer.

Author, year	Galvano et al., 2022 [44]	NIPH, 2022 [15]	Raei et al., 2024 [19]
Study Design and Methods	DTA SR and IPD meta-analysis	SR	DTA SR and meta-analysis
Study aim(s)	To compare plasma and tissue *PIK3CA* alterations and assess diagnostic accuracy of ctDNA for detecting *PIK3CA* genetic alterations in breast cancer	To assess analytical validity, clinical validity, and clinical utility of molecular tests for *PIK3CA* genetic alteration detection in HR+/HER2– locally advanced or metastatic breast cancer patients who progressed under endocrine treatment	to systematise the most recent evidence on the diagnostic value of cfDNA for *ESR1* genotyping in breast cancer patients to identify the accuracy of this approach for early diagnosis.
Population	Most patients (men and women) had advanced-stage disease (n=1,836, 93.4%) with matched tumor tissue and plasma ctDNA samples. The majority were HR+/HER2– (n=1,357), with smaller subgroups of HR+ (n=52) and unknown subtype (n=557).	Men and postmenopausal women with HR+/HER2– locally advanced or metastatic breast cancer that had progressed under endocrine treatment	Men and women with HR+ or HR+/HER2– metastatic breast cancer (implied[2])
Intervention	Diagnostic techniques: PCR, digital droplet PCR (ddPCR), BEAMing, and NGS for *PIK3CA* detection ctDNA as experimental procedure	Sanger sequencing, PCR, NGS and Liquid Chip Technology for *PIK3CA* detection	Detecting *ESR1* cfDNA as experimental procedure
Control and/or reference standard	Tissue PIK3CA mutational status as gold standard	Head-to-head comparisons of the listed tests	Primary or metastatic lesion tissue biopsy
Outcomes	Sensitivity (SE), specificity (SP), concordance, negative predictive value (NPV), positive predictive value (PPV), positive likelihood ratio (PLR), negative likelihood ratio (NLR), diagnostic odds ratio (DOR), area under the curve (ACU)	Analytic validity (SE, SP, assay robustness), clinical validity (SE, SP, predictive values, likelihood ratios, concordance), clinical utility (overall survival, quality of life), advantages and limitations	SE, SP, PPV, NPV, accuracy (ACC), PLR & NLR
Exclusion Criteria	Studies not matching the inclusion criteria and ongoing clinical trials	■ Studies that included case reports, conference abstracts, animal or cell-line studies ■ Studies without a full text or in an unlisted language ■ Studies without appropriate test comparisons or on irrelevant patient populations	■ Studies that reported duplicate data ■ Review papers, case reports, comments, letters, in vitro studies and non-human subjects ■ Studies lacking adequate data for calculating desired parameters
Number of Included Studies	24	3	13
Study designs of included studies	7 RCTs, 17 cohort studies	Unclear	Diagnostic cohort studies (not clearly specified)
Timeframe of Search	Until December 2020	Up to 10/2021	Up to 04/2022
Countries of Included studies	NR	Austrialia (n=1), United Kingdom (n=1), NR (n=1)	USA (n=7), Italy (n=2), Egypt (n=2), FR (n=1), KOR (n=1)
Settings of Included Studies	NR	NR	NR
Funding	None	None	None

Author, year	Galvano et al., 2022 [44]	NIPH, 2022 [15]	Raei et al., 2024 [19]
Databases/Sources Searched	MEDLINE, EMBASE, CCRTC, conferences, abstracts	Epistemonikos, MEDLINE (Ovid), Embase (Ovid), Cochrane Database of Systematic Reviews, Cochrane Central Register of Controlled Trials, ClinicalTrials.gov, WHO ICTRP, Current Controlled Trials, HTAi Vortal, PROSPERO, POP database	PubMed, Embase, and Web of Science
Total number of Patients	1,966	674	389
Type of Index Test	NGS, PCR, ddPCR/beaming	Sanger sequencing, NGS, PCR, and Liquid Chip Technology	NGS, PCR
Type of Reference Standard	tissue biopsy	Head-to-head comparisons of the tests listed above	tissue biopsy
Type of Genetic Testing	Methods ■ NGS ■ Sanger Sequencing ■ Digital droplet PCR (ddPCR) ■ BEAMing ■ RT-PCR (ARMS primers/Scorpion probes) ■ Standard PCR/MALDI-TOF ■ PCR (Pyromark Q24) Approach for NGS ■ Hybrid capture-based ■ Amplicon-based (implied) Platform for NGS ■ HiSeq (Illumina) ■ MiSeq (Illumina) ■ Ion Torrent Product for NGS ■ FoundationOne/FoundationACT (Foundation Medicine) ■ Guardant360 ■ AmpliSeq HD/Oncomine Pan-Cancer	Methods ■ NGS ■ ddPCR ■ BEAMing Approach for NGS NR Plaform for NGS ■ Access Array™ system (Fluidigm) Product for NGS ■ Oncomine™ Breast for cfDNA Assay ■ InVisionSeq™ for ctDNA Assay	Methods ■ NGS ■ dPCR Approach for NGS ■ Hybrid capture method ■ Amplicon-based method Platforms for NGS NR Product for NGS NR
Clinical utility (e.g. progression free survival, overall survival, quality of life, change in management)	NR	No data available	NR

Author, year	Galvano et al., 2022 [44]	NIPH, 2022 [15]	Raei et al., 2024 [19]
Clinical validity as linked evidence sensitivity, specificity, predictive values, likelihood rations) and concordance (i.e., agreement)	ctDNA (NGS) vs. tissue biobsy ■ SE: 0.83 (95%CI, 0.75-0.89) ■ SP: 0.98 (95%CI, 0.94-0.99) ■ PLR: 11.65 (95%CI, 5.43-24.99) ■ NLR: 0.23 (95%CI, 0.09-0.62) ■ DOR: 59.80 (95%CI, 14.29-250.23) ■ AUC: 0.98	NGS (Oncomine™ Breast cfDNA Assay) vs. NGS (InVisionSeq™ ctDNA Assay) in 1 study ■ Concordance: 0.80 (95%CI, 0.59-1.00) PCR (BEAMing) vs. PCR (ddPCR) in 1 study ■ Concordance: 0.87 (95% CI, 0.81-0.93) PCR (ddPCR) vs. NGS (Access Array™ system (Fluidigm) in 1 study ■ Concordance: 0.85 (95% CI, NR)	cfDNA (dPCR or NGS) vs. tissue biobsy in 13 studies ■ SE: 75.52 (95% CI 60.19-90.85) ■ SP: 88.20 (95% CI 80.99-95.40) ■ PPV: 56.94% (95% CI 41.70-72.18) ■ NPV: 88.53% (95% CI 82.61-94.44) ■ PLR: 1.60 (95%CI, 1.20-1.99) ■ NLR: 0.44 (95%CI, 0.09-0.79) ■ ACC: 88.96 (95% CI 83.23-94.69)
Clinical validity as linked evidence sensitivity, specificity, predictive values, likelihood rations) and concordance (i.e., agreement) *(continuation)*	ctDNA (ddPCR/BEAMing) vs. tissue biobsy ■ SE: 0.74 (95%CI, 0.70-0.78) ■ SP: 0.84 (95%CI, 0.82-0.86) ■ PLR: 6.63 (95%CI, 3.97-11.08) ■ NLR: 0.31 (95%CI, 0.22-0.43) ■ DOR: 28.84 (95%CI, 13.45-61.86) ■ AUC: 0.92 ctDNA (PCR) vs. tissue biobsy ■ SE: 0.51 (95%CI, 0.39-0.64) ■ SP: 0.96 (95%CI, 0.91-0.99) ■ PLR: 9.30 (95%CI, 0.64-136.17) ■ NLR: 0.54 (95%CI, 0.31-0.96) ■ DOR: 20.61 (95%CI, 1.57-270.46) ■ AUC: 0.77		Subgroup analysis NGS vs. dPCR2 ■ SE: 56.78 (13.89-99.67) vs. 81.01(64.04-97.99) ■ SP: 90.14 (79.17-101.10) vs. 90.44 (82.55-98.33) ■ PPV: 55.39 (28.17-82.61) vs. 61.76 (38.40-85.11) ■ NPV: 94.74 (94.73-94.74) vs. 90.99 (21.78-81.84) ■ NLR: 1.008 (0.94-1.07) vs. 0.42 (-0.004-0.85) ■ PLR: 1.75 (-1.41-4.92) vs. 1.61 (1.21-2.01) ■ ACC: 88.35 (76.87-99.82) vs. 89.81 (81.70-97.93)
Quality assessment	QUADAS-2 ■ Records were overall affected by a low risk of bias ■ One study (Perkins et al.) presented a high risk of bias in the patient selection.	EGAPP ■ All 3 studies provided inadequate evidence for test accuracy ■ Quality of reporting was poor	QUADAS-2 ■ Predominantly low risk across all domains, suggesting high-quality evidence, but with minimal applicability concerns.
Health Economic evidence (method and results)	NR	Micro costing analysis: ■ RT-PCR: NOK 2,800 per patient (one biomarker) ■ NGS (1 patient): NOK 16,300 per patient ■ NGS (10+ patients)NOK 2,330 per patient Infrastructure and maintenaince (NGS vs. PCR): ■ equipment and supplies: 3 to 4 million NOK vs. NOK 650,000 ■ annual maintenaince: NOK 150,000 vs. NOK 25,000	NR
Ethical or organisational aspects	NR	NR	NR

Abbrevations: ACC…accuracy, AUC … area under the curve, BEAMing … beads, emulsion, amplification, and magnetics, cfDNA … cell-free DNA, CI … confidence interval, ctDNA … circulating tumor DNA, ddPCR … droplet digital polymerase chain reaction, DNA … deoxyribonucleic acid, DOR … diagnostic odds ratio, dPCR … digital polymerase chain reaction, RGAPP… Evaluation of Genomic Applications in Practice and Prevention, ESR1 … estrogen receptor 1, HER2 … human epidermal growth factor receptor 2, HR … hormone receptor,
IPD … individual patient data, NLR … negative likelihood ratio, NOK … Norwegian kroner, NPV … negative predictive value, NR … not reported, PCR … polymerase chain reaction, PIK3CA … phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha, PLR … positive likelihood ratio, PPV … positive predictive value, PTEN … phosphatase and tensin homolog, QUADAS-2… Quality Assessment of Diagnostic Accuracy Studies-2, RT-PCR … real-time polymerase chain reaction, SE…sensitivity, SP…specificity, SR … systematic review,
VAF … variant allele fraction, WES … whole exome sequencing, WGS … whole genome sequencing.

Notes:

[1] *SR by Raei did not explicitly specify disease stage or molecular subtype, but patients can be assumed to have HR+ or HR+/HER2− metastatic breast cancer, as ESR1 detection is clinically relevant in the advanced/metastatic HR+ setting where endocrine therapy is used (refer to the background chapter for ulterior information about ESR1).*

[2] *Subgroup analysis is limited by high heterogeneity between studies, and it was not shown statistically significant difference between methods.*

Tables of guideline recommendations

Table A-2: Guideline recommendations for molecular tests for detection of PIK3CA-/AKT1-/PTEN-/ESR1-genetic alterations in adults with HR+/HER2–, locally advanced or metastatic breast cancer

Guideline	Date of issue	Country/ies to which applicable	Recommendation (verbatim)	Specific testing recommendation	Type, evidence quality, strength of recommendation
SITC clinical practice [50]	Accepted: June 30 2021 Published: August 13, 2021	US	Text passage: Beyond TMB assessment, NGS is also useful to identify other actionable gene genetic alterations, such *PI3K* alterations, for which alpelisib in combination with fulvestrant109 is a treatment option in ER+/HER2– disease.	NGS for PI3K alterations in addition to TMB assessment	Informal recommendation
ASCO Endocrine Treatment and Targeted [54]	Accepted: June 9, 2021 Published: July 29, 2021	US	Recommendation 2.1: To guide the decision to use alpelisib in combination with fulvestrant in postmenopausal patients, and in male patients with HR-positive MBC (metastatic breast cancer), clinicians should use next-generation sequencing in tumor tissue or cell-free DNA in plasma to detect *PIK3CA* genetic alterations. If no mutation is found in cell-free DNA, testing in tumor tissue, if available, should be used as this will detect a small number of additional patients with *PIK3CA* genetic alterations.	NGS in tumour tissue or ctDNA for PIK3CA genetic alterations. If ctDNA negative, test tumor tissue if available	Type: evidence-based, benefits outweigh harms, Evidence quality: high, Strength of recommendation: strong.
ASCO Biomarkers for Systemic Therapy [51]	Accepted: May 12, 2022 Published: June 27, 2022	US	Recommendation 1.1: Patients with locally recurrent unresectable or metastatic hormone receptor-positive and human epidermal growth factor receptor 2 (HER2)–negative breast cancer who are candidates for a treatment regimen that includes a phosphatidylinositol 3-kinase inhibitor and a hormonal therapy should undergo testing for *PIK3CA* genetic alterations using next generation sequencing of tumor tissue or circulating tumor DNA (ctDNA) in plasma to determine their eligibility for treatment with the phosphatidylinositol 3-kinase inhibitor alpelisib plus fulvestrant. If no mutation is found in ctDNA, testing in tumor tissue, if available, should be used as this will detect a small number of additional patients with *PIK3CA* genetic alterations.	NGS of tumour tissue or ctDNA for PIK3CA genetic alterations before alpelisib + fulvestrant	Type: evidence based, benefits outweigh harms, Evidence quality: high, Strength of recommendation: strong.
ASCO Endocrine and Targeted Therapy [53]	Accepted: February 8, 2024 Published: March 13, 2024	US	Foot note: Tumor genomic testing includes sequencing for targetable genetic alterations, accomplished through large panel tumor genomic testing in a CLIA-certified laboratory performed on tissue or plasma obtained either at the time of progression or from archival tissue. In addition to selecting patients whose tumors have increased *PIK3CA* or *AKT1* activity because of the presence of activating genetic alterations, it is also important to identify those whose tumors have inactivation of *PTEN* protein. *PTEN* inactivation can be identified based on the presence of premature stop codons, frameshift alterations, splice site genetic alterations, *PTEN* homozygous deletion, *PTEN* rearrangements that disrupt protein function, or specific missense genetic alterations (C124R, C124S, G129E, G129V, G129R, R130Q, R130G, R130L, R130P, C136R, C136Y, S170R, and R173C) on next-generation sequencing.	Large panel tumour genomic testing; PTEN inactivation can be identified through NGS detection of various genomic alterations	Informal recommendation
AWMF S3[1] [23]	Published: May 2025	Germany	Recommendation 4.93: Patients with metastatic or treatment-resistant breast cancer, for whom standard therapies are no longer ex-pected to provide a survival benefit, should be offered presentation at a molecular tumor board for comprehensive NGS-based molecular pathological diagnostics. An important addition is that a re-biopsy should be considered for this diagnostic process. This is to capture genetic variants that may have developed during the course of tumor progression.	Comprehensive NGS-based molecular diagnostics via tumour board. Consider re-biopsy	Type: expert consens, Strength of recommendation: Strong consensus.

Abbreviations: AKT1 … AKT Serine/Threonine Kinase 1, ASCO … American Society of Clinical Oncology, AWMF … Arbeitsgemeinschaft der Wissenschaftlichen Medizinischen Fachgesellschaften (Association of the Scientific Medical Societies), CDK4/6 … Cyclin-Dependent Kinase 4/6, CLIA … Clinical Laboratory Improvement Amendments, ctDNA … circulating tumor DNA,
ER … Estrogen Receptor, ESR1 … Estrogen Receptor 1, ET … Endocrine Treatment/Therapy, GRADE … Grading of Recommendations Assessment, Development and Evaluation,
HER2 … Human Epidermal Growth Factor Receptor 2, HR … Hormone Receptor, MBC … Metastatic Breast Cancer, NGS … Next-Generation Sequencing, PI3K … Phosphatidylinositol

3-Kinase, PIK3CA … Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha, PR … Progesterone Receptor, PTEN … Phosphatase and Tensin Homolog, S3 … Highest level of evidence-based clinical practice guideline development (German classification), SITC … Society for Immunotherapy of Cancer, TMB … Tumor Mutational Burden, US…United States,

VUS … Variant of Uncertain Significance, WES … Whole Exome Sequencing, WGS … Whole Genome Sequencing

Note: [1] Recommendations from the German AWMF S3 guideline have been translated into English while preserving the original meaning and clinical intent of the German text.

Risk of bias tables

Table A-3: Risk of bias of included systematic reviews/HTA report

Name, year	D1: Study Eligibility	D2: Identification/ Selection of studies	D3: Data collection and study appraisal	D4: Synthesis and Findings	RoB in the Review	Rationale
Galvano, 2022 [44]	LOW	LOW	LOW	LOW	LOW	No major concerns identified. Minor limitations include absence of sensitivity analyses, which could have been feasible given the 24 included studies. Additionally, it was not specified whether data screening was performed independently by two reviewers.
NIPH, 2022 [15]	LOW	LOW	LOW	LOW	LOW	No concerns identified.
Raei, 2024 [19]	LOW	LOW	LOW	LOW	LOW	No major concerns identified. Minor limitations include absence of published study protocol and lack of sensitivity analyses, which could have been feasible given the 13 included studies.

Note: The risk of bias of the included systematic reviews was assessed using the ROBIS tool [41].

Table A-4: AGREE II quality appraisal of the guidelines addressing NGS for detection of PIK3CA-/AKT1-/PTEN-/ESR1-genetic alterations in adults with HR+/HER2–, locally advanced or metastatic BC.

Domain	Item	AGREE II Score		
		AWMF S3, 2025 [23]	ASCO [51, 53, 54]	SITC, 2021 [50]
Scope and purpose	1. The overall objective(s) of the guideline is (are) specifically described.	7	7	7
	2. The health question(s) covered by the guideline is (are) specifically described.	7	7	7
	3. The population (patients, public, etc.) to whom the guideline is meant to apply is specifically described.	7	7	7
Stakeholder involvement	4. The guideline development group includes individuals from all the relevant professional groups.	7	7	6.5
	5. The views and preferences of the target population (patients, public, etc.) have been sought.	7	7	6.5
	6. The target users of the guideline are clearly defined.	7	7	7
Rigor of development	7. Systematic methods were used to search for evidence.	7	7	3
	8. The criteria for selecting the evidence are clearly described.	7	7	3
	9. The strengths and limitations of the body of evidence are clearly described.	7	7	6.5
	10. The methods for formulating the recommendations are clearly described.	7	7	6
	11. The health benefits, side effects and risks have been considered in formulating the recommendations.	7	7	5
	12. There is an explicit link between the recommendations and the supporting evidence.	7	7	4.5
	13. The guideline has been externally reviewed by experts prior to its publication.	7	6.5	6
	14. A procedure for updating the guideline is provided.	7	6	4.5
Clarity of presentation	15. The recommendations are specific and unambiguous.	7	7	4
	16. The different options for management of the condition or health issue are clearly presented.	7	7	4
	17. Key recommendations are easily identifiable.	4.5	7	3.5
Applicability	18. The guideline describes facilitators and barriers to its application.	5.5	6	4.5
	19. The guideline provides advice and/or tools on how the recommendations can be put into practice.	4.5	5.5	4
	20. The potential resource implications of applying the recommendations have been considered.	7	6	5
	21. The guideline presents monitoring and/or auditing criteria.	7	6	6
Editorial independence	22. The views of the funding body have not influenced the content of the guideline.	7	7	7
	23. Competing interests of guideline development group members have been recorded and addressed.	7	7	7
Overall Guideline Assessment	1. Rate the overall quality of this guideline.	7	7	5.25
Overall Guideline Assessment	2. I would recommend this guideline for use.	*Yes*	*Yes*	*Yes, with modification (lack of systematic search)*

Applicability table

Table A-5: Applicability of included studies to the Austrian healthcare context.

Domain	Description of applicability of evidence
Population	Systematic reviews: The included systematic reviews focused on adults with HR+/HER2– locally advanced or metastatic breast cancer. The proportion of patients potentially benefiting from genetic testing depends on genetic alteration prevalence (PIK3CA: 20-40%; ESR1: 14-40% in metastatic disease; AKT1 and PTEN: <5%). Clinical guidelines: International guidelines (ASCO, AWMF, SITC) address similar target populations of HR+ BC in an advanced or metastatic stage. Guidelines provide applicable recommendations for HR+/HER2– locally advanced or metastatic BC in the Austrian population.
Intervention	Systematic reviews: All included systematic reviews evaluated NGS tests without specifying DNA length or technical specifications. It is assumed that NGS refers to targeted gene panel sequencing, which is most commonly used in clinical practice. Clinical guidelines: All guidelines provided recommendations for NGS without describing sequencing technologies, panel size, or sequencing depth. It is assumed that NGS refers to targeted gene panel sequencing, which is most commonly used in clinical practice.
Comparators	Systematic reviews: Two reviews assessed the diagnostic accuracy of NGS against a gold standard reference, with one review conducting this as a subgroup analysis only. One HTA report compared two NGS technologies head-to-head and additionally compared NGS with PCR. Clinical guidelines: No comparators were searched for guidelines, as the focus was solely on presenting and summarising guideline recommendations for NGS sequencing.
Outcomes	Systematic reviews: Reviews primarily reported diagnostic accuracy outcomes (sensitivity, specificity, predictive values) and concordance between testing methods. Clinical utility outcomes (PFS, OS, QoL) were not assessed. One HTA additionally addressed organisational and economic aspects. Clinical guidelines: Guidelines emphasize clinical utility outcomes, particularly treatment selection and patient management. ASCO recommends testing to guide alpelisib therapy decisions for PIK3CA-mutated disease. Guidelines acknowledge gaps in direct evidence linking testing to improved patient outcomes but focus on actionable genetic alterations enabling access to targeted therapies.
Setting	Systematic reviews: Studies were predominantly conducted in Global North settings (Europe, North America, Asia). Applicability to Austrian settings is generally high, though country-specific differences in resource availability, reimbursement systems, and clinical practice patterns require consideration. Clinical guidelines: Guidelines originate from international (ASCO, SITC) and European (AWMF) professional organisations representing diverse healthcare settings. AWMF guidelines directly apply to German-speaking countries including Austria. ASCO and SITC guidelines reflect US practice patterns but provide broadly applicable recommendations. Settings where interventions are typically used (specialised molecular pathology laboratories) may limit generalisability to broader care structures.

Research questions

Table A-6: Health problem and current use

Element ID	Research question
A0001	For which health conditions, and for what purposes is the technology used?
A0002	What is the disease or health condition in the scope of this assessment?
A0003	What are the known risk factors for the disease or health condition?
A0004	What is the natural course of the disease or health condition?
A0005	What is the burden of disease for the patients with the disease or health condition?
A0006	What are the consequences of the disease or health condition for the society?
A0024	How is the disease or health condition currently diagnosed according to published guidelines and in practice?
A0025	How is the disease or health condition currently managed according to published guidelines and in practice?
A0007	What is the target population in this assessment?

A0023	How many people belong to the target population?
A0011	How much are the technologies utilised?

Table A-7: Description of the technology.

Element ID	Research question
B0001	What is the technology and the comparator(s)?
A0020	For which indications has the technology received marketing authorisation or CE marking?
B0002	What is the claimed benefit of the technology in relation to the comparators?
B0003	What is the phase of development and implementation of the technology and the comparator(s)?
B0004	Who administers the technology and the comparators and in what context and level of care are they provided?
B0008	What kind of special premises are needed to use the technology and the comparator(s)?
B0009	What supplies are needed to use the technology and the comparator(s)?
A0021	What is the reimbursement status of the technology?

Table A-8: Clinical effectiveness

Element ID	Research question
D0001	What is the expected beneficial effect of the technology on mortality?
D0003	What is the effect of the technology on the mortality due to causes other than the target disease?
D0005	How does the technology affect symptoms and findings (severity, frequency) of the disease or health condition?
D0006	How does the technology affect progression (or recurrence) of the disease or health condition?
D0011	What is the effect of the technology on patients' body functions?
D0016	How does the use of technology affect activities of daily living?
D0012	What is the effect of the technology on generic health-related quality of life?
D0013	What is the effect of the technology on disease-specific quality of life?
D0017	Was the use of the technology worthwhile?

Table A-9: Safety

Element ID	Research question
C0008	How safe is the technology in comparison to the comparator(s)?
C0002	Are the harms related to dosage or frequency of applying the technology?
C0004	How does the frequency or severity of harms change over time or in different settings?
C0005	What are the susceptible patient groups that are more likely to be harmed through the use of the technology?
C0007	Are the technology and comparator(s) associated with user-dependent harms?
B0010	What kind of data/records and/or registry is needed to monitor the use of the technology and the comparator?

Literature search strategies

Example: Search strategy for Medline via Ovid

Search Name: Ovid MEDLINE(R) ALL <1946 to July 24, 2025>	
Search date: 25.07.2025	
ID	Search
1	(pik3ca or phosphatidylinositol* or phosphoinositide or phospholipids or phosphatidyl or phosphoglycerides or phosphatidyl or phosphoglycerides or phosphoinositides or phosphatidyl or phosphoinositides or ptdIns or phosphatidylisitide).ti,ab,kw,kf. (141427)
2	exp Phosphatidylinositol 3-Kinases/ (56752)
3	1 or 2 (171838)
4	exp Breast Neoplasms/ (371275)
5	(ER positive or HER2 negative or ((breast* or mammary or mamma) adj3 (neoplasm* or cancer* or tumor* or tumour* or carcinoma* or onco* or adenocarci noma* or metastas* or cancerogen*))).mp. (543286)
6	4 or 5 (543296)
7	3 and 6 (7003)
8	limit 7 to (meta analysis or "systematic review") (57)
9	(((comprehensive* or integrative or systematic*) adj3 (bibliographic* or review* or literature)) or (meta-analy* or metaanaly* or "research synthesis" or ((information or data) adj3 synthesis) or (data adj2 extract*))).ti,ab. or (cinahl or (cochrane adj3 trial*) or embase or medline or psyclit or (psycinfo not "psycinfo database") or pubmed or scopus or "sociological abstracts" or "web of science").ab. or ("cochrane database of systematic reviews" or evidence report technology assessment or evidence report technology assessment summary).jn. or Evidence Report: Technology Assessment*.jn. or ((review adj5 (rationale or evidence)).ti,ab. and review.pt.) or meta-analysis as topic/ or Meta-Analysis.pt. (891370)
10	7 and 9 (176)
11	8 or 10 (176)
12	limit 11 to yr="2020 - 2025" (123)
Total hits: 123	

Search Name: Ovid MEDLINE(R) ALL <1946 to August 08, 2025>	
Search date: 11/08/2025	
ID	Search
1	(pik3ca or phosphatidylinositol* or phosphoinositide or phospholipids or phosphatidyl or phosphoglycerides or phosphatidyl or phosphoglycerides or phosphoinositides or phosphatidyl or phosphoinositides or ptdIns or phosphatidylisitide).ti,ab,kw,kf. (141624)
2	exp Phosphatidylinositol 3-Kinases/ (56843)
3	1 or 2 (172100)
4	(AKT1 or AKT or CWS6 or PKB or PKB-ALPHA or PRKBA or RAC or RAC-ALPHA or serine threonine protein kinas*).ti,ab,kw,kf. (157626)
5	exp Protein Serine-Threonine Kinases/ (398894)
6	4 or 5 (481965)
7	(PTEN or 10q23del or BZS or CWS1 or DEC or GLM2 or MHAM or MMAC1 or PTEN1 or TEP1 or "phosphatase and tensin homolog" or PTENbeta or phosphatidylinositol-3,4,5-trisphosphate 3-phosphatas*).ti,ab,kw,kf. (42279)
8	exp PTEN Phosphohydrolase/ (12110)
9	7 or 8 (43288)
10	(ESR1 or ESR or ESRA or ESTRR or Era or NR3A1 or ?estrogen receptor 1 or ?estrogen receptor alpha or eralpha).ti,ab,kw,kf. (162943)
11	exp Estrogen Receptor alpha/ (14771)
12	10 or 11 (165876)
13	3 or 6 or 9 or 12 (783860)
14	exp Breast Neoplasms/ (371754)
15	(ER positive or HER2 negative or ((breast* or mammary or mamma) adj3 (neoplasm* or cancer* or tumor* or tumour* or carcinoma* or onco* or adenocarci noma* or metastas* or cancerogen*))).mp. (544442)
16	14 or 15 (544452)

17	13 and 16 (35767)
18	limit 17 to (meta analysis or "systematic review") (311)
19	(((comprehensive* or integrative or systematic*) adj3 (bibliographic* or review* or literature)) or (meta-analy* or metaanaly* or "research synthesis" or ((information or data) adj3 synthesis) or (data adj2 extract*))).ti,ab. or (cinahl or (cochrane adj3 trial*) or embase or medline or psyclit or (psycinfo not "psycinfo database") or pubmed or scopus or "sociological abstracts" or "web of science").ab. or ("cochrane database of systematic reviews" or evidence report technology assessment or evidence report technology assessment summary).jn. or Evidence Report: Technology Assessment*.jn. or ((review adj5 (rationale or evidence)).ti,ab. and review.pt.) or meta-analysis as topic/ or Meta-Analysis.pt. (896056)
20	17 and 19 (883)
21	18 or 20 (886)
22	limit 21 to yr="2020 - 2025" (502)
23	remove duplicates from 22 (501)
Total hits: 501	

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