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The Pitfalls of Next-Generation Sequencing: Revisiting the Importance of Clinical Actionable Results in Targeted Genomic Testing and Precision Oncology

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ABSTRACT

With Next-Generation Sequencing having transformed genomic testing, its increased implementation has exposed major limitations, including signal degradation, variant miscallings in low-frequency signals, and errors in reading regions with Copy Number Variations (CNVs). As a result of this, data collected by such sequencing methods often are clinically inactionable and are vulnerable to misinterpretations, reflected in the disparity between the 7.4% of breast cancer patients who have undergone MP-guided treatment despite 62% of cases involving targetable genomic alterations identified through NGS. In response to this concern, Mammaprint has reinforced the value of targeted genomic profiling in precision oncology, highlighting the importance of more focused gene panels for obtaining results that provide clinical insight into suitable treatment pathways for patients. By translating data to binary results of "low risk" and "high risk", leveraging Distant Free Metastasis Survival (DFMS) calculations as a key metric to display the expected outcomes and levels of efficacy in different treatment pathways, genomic testing tools like Mammaprint have displayed the importance of clinically actionable interpretation over broad genomic complexity in assisting informed decision-making in oncology.

INTRODUCTION

With the synergy of novel technological innovations and rapid industrialisation, the experimental endorsement of various tools in healthcare has transformed patient care. The Next Generation Sequencer (NGS) is a current paradox, adapted for DNA/RNA sequencing and variant mutation analysis. A pressing challenge that medicine faces today is translating the information obtained from new technological advancements in precision medicine. Being able to detect germline and somatic mutations, NGS is a common tool used in breast cancer diagnosis, where 62% of cases involving NGS had targetable genomic alterations, despite only 7.4% of patients undergoing MP-guided treatment (Suh et al., 2022). The uncertainty of NGS arises as many question its ability to translate molecular basis into clinically actionable significance.

In genomic testing, clinical actionability acts as an important metric to understand the efficacy of molecular profiling in guiding clinicians to make treatment decisions for patients. It refers to the capacity that such genomic tests have in influencing clinical decisions and providing new informed insight that can create another layer of extra consideration when designing treatment pathways. Visually, clinically actionability can be associated with targeted treatment recommendations or applications of molecular profiles to a patient's specific case, whether that might be eligibility for targeted therapies, recommended drugs, prognostic stratification, etc. This concept can be bridged with the idea of interpretability, involving the assessment of genomic profiling data and the ability to evaluate its significance in every patient's unique case and scenario. In the midst of state-of-the-art technology being introduced to elevate the current progress made in precision oncology, the crucial variable of clinical actionability of these tools is often disregarded. especially when Variants of Unknown Significance (VUS) are flagged, off-label treatments are suggested (Figure 1), and tumour heterogeneity with low-frequency variants is identified, without any comprehensive understanding of its relevance to the dominant tumour microenvironment and molecular profile (Moon et al., 2025). Such cases of tumour heterogeneity are particularly prevalent in metastatic breast cancer, where a specific microenvironment may exhibit divergent genomic profiles as cancer cells proliferate and evolve, resulting in genetic instability. These main challenges stem from sample bias, where single biopsies result in genomic profiling results being an incomplete representation of the biomarkers in the tumour. Consequently, potentially actionable alterations that can be treated by targeted therapy are often overlooked, thereby making NGS weak as a standalone mechanism for molecular profiling.

Whilst technology such as NGS can conduct genomic testing across a multitude of clinical scenarios, most of the data remains uncertain and challenging to interpret. Figure 1 depicts this by highlighting the disparity of the large volume of targetable genomic alterations that NGS can pick up and its limited impact on supporting treatment decisions. A large subset of NGS results were associated with off-label drugs, resulting in a high degree of uncertainty about whether the established, unapproved treatment pathway can directly target the alteration. With NGS results also recommending further clinical trials, this also shows that many alterations identified are still only considered a murky area of concern and are still under investigation. Hence, they cannot be directly linked to standard therapeutic pathways. Although this data may direct patients to therapies, further validation is required from other clinical trials. NGS is often inadequate in determining suitable treatment for patients and must be supported with the required contextual details of clinicopathological findings, laboratory investigations, etc. The most actionable data patients can receive lies in the 12.6% of patients receiving on-label therapy recommendations and the small 5.9% proportion that receive information on on-label resistance. By directing patients to approved drugs to target specific alterations and identifying therapies that may be ineffective, this demonstrates that sequencing may still provide meaningful information for clinicians, although the more substantial proportion of patients with data that lack immediate clinical implementation still remains a loophole that must be addressed.

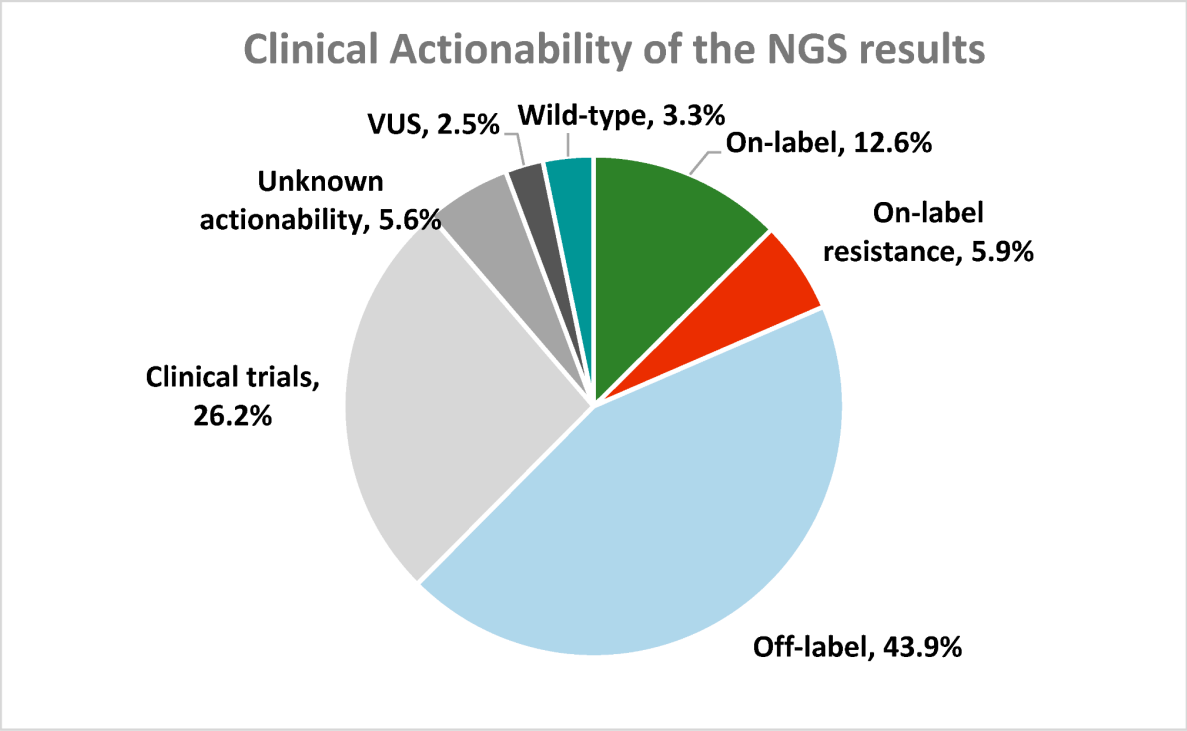

Figure 1 - Clinical Actionability from NGS Results (Meintani et al., 2025)

The Pitfalls of Next Generation Sequencing

In Next Generation Sequencing, DNA samples are first prepared through random fragmentation via enzymatic digestion or sonication to primarily obtain shorter DNA fragments. From this, the targeted genes are isolated and amplified using biotinylated probes to extract specific oncogenes and to wash away the unused fragments (Qin, 2019). With the Illumina flow cell, sequencing will correspondingly occur with fluorescent tags being added into each strand via complementary base pairing, with each base having a different colour, ergo the distinct base identification when the tag undergoes excitation by the laser. After imaging, bioinformatic analyses allow the fragment to be compared to the reference human genome for variant identification, base calling, and read alignment.

False negatives and inaccurate results can arise from its susceptibility to bias as the quality of signals degrades over multiple cycles, with Nucleotide Per Cycle bias occurring due to signals cross-talking before cycles, resulting in higher margins of error, missed signals for low-frequency variants and miscallings (Schwartz et al., 2011). Signal crosstalks occur when multiple samples are run together simultaneously, resulting in barcode cross-contamination and misinterpretation of artefacts that are consequently formed as identified alterations (Scharf et al.) Whilst this error can be reduced by optimising the library preparation process to ensure greater precision and more stringent bioinformatics pipelines that can filter and identify specific patients' reads more accurately, other factors, such as signal degradation, can be another source of error that results in false positives. Signals could degrade due to many factors,

one being the specimen preparation with FFPE introducing artefacts (Zhang et al.). Some tumour samples also lack tumour cellularity, compromising sequencing depth and limiting the detection of specific low-frequency variants. Specifically, slow-growing tumours in early stages of breast cancer display fewer chromosomal abnormalities overall, reducing the ability to pick up these signals, leading to false positives. Even though NGS has a very broad panel and can often detect irrelevant genes, its database ironically still has limited database diversity, increasing the probability of identifying rare, uncharacterized DNA changes that make genomic results contain Variants of Uncertain Significance. These errors not only serve as inaccuracies of data that could result in false positives and negatives but also reduce the clinical interpretability of results. An example of this is TP53, a gene that commonly presents aggressive behaviour, which accounts for almost 30% of breast cancer cases, and is common in Triple Negative Breast Cancer. With these mutations having a low Variant Allele Frequency of <5%, NGS often faces difficulties in distinguishing these low-frequency signals from noise (Pavlova et al., 2025). Furthermore, mappability errors can often lead to the corresponding issue of misinterpretation and false negatives. Since hereditary breast cancer genes like BRCA1 and BRCA2 often undergo structural rearrangements and Copy Number Variations (CNVs) with large deletions or duplications, NGS has difficulty reading repetitive sequences, leading to alignment errors or discarding them as low-frequency data (Concolino et al., 2019). With the diagnosis landing in a murky region where it cannot be concluded if cells are benign or malignant, this results in incorrect histopathological conclusions, overtreatment and clinical mismanagement in handling the patient's specific condition.

Although inaccuracies in NGS may arise due to many factors that have already been discussed, including sequencing errors, the presence of artefacts, signal degradation and challenges in variant identification, the core limitation in NGS is how it is often treated as an isolated tool in targeted therapy. Biomarkers are used as a determinant of the therapy that patients undergo, when in reality, there are many other factors other than identification of genomic alterations that must be taken into consideration when designing a patient's specific treatment pathway. This further highlights the need to contextualise findings. Identification of molecular alterations must be considered alongside patient history, tumour microenvironment and other clinicopathological factors that must be taken into account in precision oncology (Ahmad et al.). Even if this may not decrease the inaccurate findings NGS infers, integrating systems that acknowledge the broader clinical frameworks for data interpretation may increase the efficacy of treatment decisions and reduce the risk of treatment decisions being guided by molecular findings alone.

DISCUSSION:

Switching the Lens: Reevaluating the Role of NGS as a Complementary Tool

While NGS ultimately is an accurate, cutting-edge tool for genomic testing, clinical decisions cannot be oversimplified to prescribing patients with targeted drugs to treat the variants and mutations detected. This concept was strongly supported by findings from the randomised phase 2 SHIVA trial in 2015, in which molecular-target therapy based on molecular profiling was compared with conventional therapy

based on standard clinical judgement (Dureau et al.). The experiment included patients who had molecular alterations in three specific pathways, including the hormone receptor, PI3K/AKT/mTOR, and RAF/MEK (Le Tourneau et al.). According to the molecular profile obtained, these patients would be treated by eleven pre-selected, available targeted agents (erlotinib, lapatinib plus trastuzumab, sorafenib, imatinib, dasatinib, vemurafenib, everolimus, abiraterone, letrozole, tamoxifen). All patients had solid tumours that were resistant to standard care and tumours that were accessible for biopsies.

195 selected patients that fit this criteria were then separated into two separate groups, the experimental group and the control group. The patients in the experimental group were assigned one of the eleven targeted therapeutic drugs aligned with the specific pathways that the molecular profile identified as the most critical abnormality. On the other hand, the control group were assigned treatment pathways according to clinical judgement without utilising the SHIVA matching algorithm, relying on the holistic review of patient history, pathology, imaging and other standard guidelines to direct patients to different treatment pathways. The median follow-up duration was 11.3 months, where patient progress was monitored closely to obtain the results that the experimental group had a median progression-free survival result of 2.3 months, while for the control group, the median was approximately 2.0 months (Le Tourneau et al.). Statistical inferences with a p-value of 0.41 proved that this difference is statistically insignificant, meaning that the length of time at which the tumour persists without worsening is not comparatively different when genomic profiling is used and when it is not. Besides measuring the efficacy of genomic profiling as an obsolete method in making clinical decisions for targeted therapy, researchers also assessed the safety of both groups' treatment pathways, with 43% of the demographic of the experimental group experiencing adverse side effects and 32% of patients from the control group undergoing cytotoxic chemotherapy experiencing grade 3-4 side effects. (Tsimberidou and Kurzrock). The consistent statistical insignificance that the bivariate data represent emphasises how overreliance on molecular profiling and corresponding molecular targeted therapy does not improve progression-free survival. A key takeaway is the inherent understanding that designing the specific treatment pathways for patients cannot be oversimplified to the simple process of utilising genomic profiling and assigning targeted therapy and drugs. This finding suggests the important fact that genomic information should not be considered as an isolated determinant for treatment but as a complementary tool in molecular profiling to integrate precision in decision-making. Hence, the key limitation in implementing NGS is not the small margins of error that the technology possesses, but the way in which such technology is interpreted for decision-making and the importance of utilising this broad targeted assay as a foundation for new genomic testing technology in oncology.

Despite this, it is important to recognise that NGS has been an essential complementary tool in many genomic testing applications. The most intrinsic value of such technology is the transformation process where NGS is applied, emphasising how the data that NGS has generated undergoes an essential synthesis process in which more interpretable outputs are formed. An example of this is FoundationOne, a genomic testing tool that utilises bioinformatics through NGS panels, which specifically utilises hybridisation-based capture technology for the detection of alterations in 324 genes (Milbury et al.). Like the SHIVA algorithm, this platform detects the specific biomarkers and matches them with approved

targeted, therapeutic products. However, every patient gets a specific FoundationOne CDx report where inferences of the data obtained are showcased with the interpretation engine and software of the tool, allowing a more comprehensive summary of the patient's results and recommendations for clinical actions. Besides identifying variants, FoundationOne utilises tumour-type context stratification, resulting in the targeted therapy it recommends being more appropriate, as the tumour microenvironment and tissue location can change a drug's efficacy (Takeda et al.).

Furthermore, a critical pitfall in pure NGS technology for genomic profiling is the stagnant conclusion of monotherapy approaches and blurring the lines between "targeted therapy" and oversimplified treatment pathways. Tumours are highly complex and can easily bypass pathways that are singly blocked. FoundationOne takes a "combination approach" by incorporating a more holistic treatment program and plan to target specific tumours. The FoundationOne core database is also continuously updated by computational biologists, regularly screening the clinical database and inserting new peer-reviewed studies and recording newly FDA-approved companion diagnostic drugs ("US FDA Approves FoundationOne®CDx"). This real-time update not only improves the efficacy and impact that such reports have on patients' treatment plans by providing holistic treatment options but also ensures accuracy by ensuring the database is not outdated. The end-product of FoundationOnce core tests are extensive reports with a professional summary of biomarkers detected, key findings with inclusive details of potential resistance, germline implications, non-targeted therapy implications, etc., specific to each patient case. The report identifies potential germline or clonal hematopoiesis alterations that may require attention for follow-up testing and a list of FDA-approved claims aligned with the diagnostics retrieved from the data. When implemented beneficially, NGS tools can be maximised to generate outcomes that can provide more concrete information for clinicians in making clinical decisions.

The Rise of Mammaprint in Informed Decision-Making

To prioritise the clinical actionability in genomic testing, technological methods in healthcare should shift from tools being universally applied or a one-size-fits-all approach to targeted applications that cater to specific patient populations and market segments. By tailoring NGS as a grounding platform instead of fully relying on it in clinical settings, the incorporation of NGS is essential for the synergised union that interpretation engines and targeted assay tests can create. Mammaprint uses a microarray with 70 specific genes to assess the probability of recurrence within a 10-year time window in early breast cancer stages (Audeh et al., 2019). This reduces the probability of identifying irrelevant or uncertain variants, a prevalent concern with NGS. Instead of cataloguing every possible mutation, Mammaprint aims to clinically identify specific genes to validate the risk of recurrence in early breast cancer according to its expression-based assays; this shows the advantage of designing tools for specific clinical contexts rather than broad-panel technology for reducing the likelihood of generating uncertain findings.

Compared with NGS, where a targeted sequence is amplified for PCR, the DNA microarray in Mammaprint amplifies the entire transcript pool. Then it uses DNA probes to capture nucleic-acid target molecules via sequence capture (Mittempergher et al., 2019). Furthermore, the fluorescent signal released

from bound targets reflects the abundance of target molecules in the entire pool, known as the direct hybridisation signal, which eliminates inaccuracies that may arise from NGS due to cross-hybridisation and biases in de novo sequencing (Bumgarner, 2013). With this targeted approach in identifying specific genomes, it reduces noise and the need to filter irrelevant genes. As Mammaprint is a transcriptome-focused assay, it does not rely on structural integrity or the identification of specific alterations; instead, it measures the level of expression of the RNA, which eliminates misinterpretations arising from CNVs.

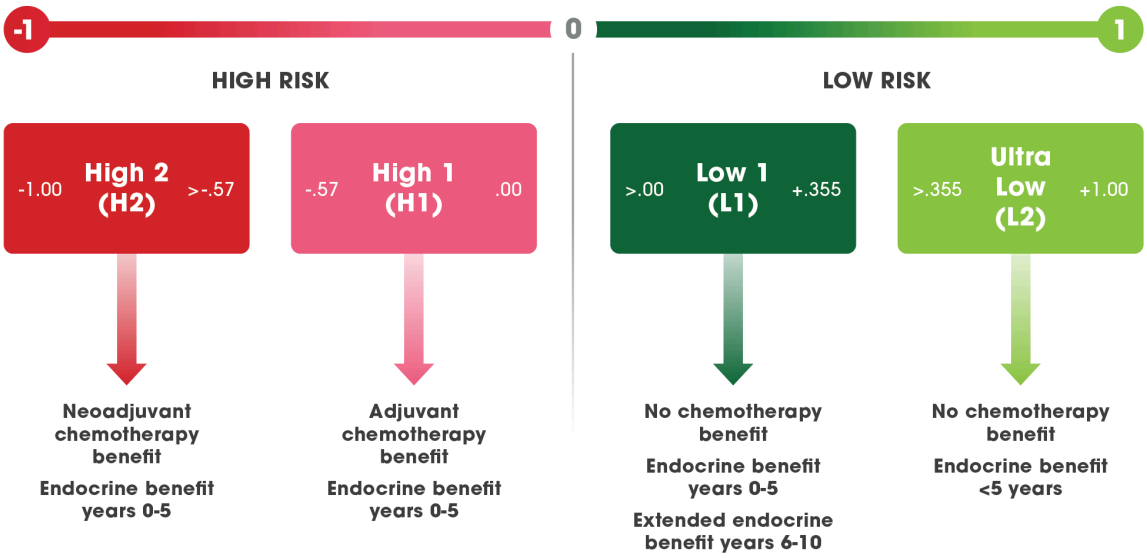

Figure 2: Interpretation of Mammaprint's results - assessing the significance of chemotherapy (Kevin) - this figure is taken from the format of an official Mammaprint report. It is included solely to highlight the clinical representation of the genomic findings from Mammaprint.

Once the results have been obtained, they are interpreted into binary conclusions, with the categories "low risk" and "high risk" (Figure 2) to determine the treatment suitable for the patient, based on the cancer's capacity to metastasise. This image further illustrates how Mammaprint index scores can be interpreted with increasing values between -1 and +1, indicating gradually lowering risks of recurrence. High-risk group emphasises the additional benefit patients can gain from chemotherapy, while L1 and L2 groups experience insignificant additional benefit from selecting chemotherapy for their treatment pathway, preventing overtreatment and allowing clinicians to select a treatment pathway that is only necessary and appropriate for each patient's specific case. Generally, low-risk results indicate that patients have 10% chance of recurrence within 10 years, whilst high-risk results indicate that patients have a 29% chance of recurrence (Manjili et al., 2012). To further demonstrate Mammaprint's role in supporting clinical decisions, Distant-Free Metastasis Survival (DFMS) is used as a key metric to validate Mammaprint as a prognostic tool. In terms of adjuvant responses to therapy, taking chemotherapy as a treatment pathway has resulted in a 99% DFMS, whilst endocrine therapy has a 93% DMFS (Yao et al., 2013). With a

p-value of 0.20, these DFMS values are not statistically significant, emphasising that low-risk patients do not necessarily need chemotherapy, supporting its omission in treatment decisions. If the clinical assessment and Mammaprint risk result are concordant, meaning that they similarly show high-risk or low-risk results, this consolidates the clinical decisions or treatment pathway that is determined. For discordant results, such as when Mammaprint shows low risk whilst clinical assessment indicates high risk of metastasis and recurrence, Mammaprint can override these results once all clinical contexts have been considered (Sun et al.). By approaching and handling different treatment pathways in a more "essential" approach, this will not only support practitioners in making appropriate decisions for their patients but will also provide insight to patients, allowing them to be more cost-efficient in the long term by only selecting the treatment pathway that is relevant.

Limitations of Technology Centred in Clinical Actionability

Despite Mammaprint's clear clinical actionability, its narrow scope limits its applicability to very niche clinical settings. Especially with its specific 70-gene panel, Mammaprint is not able to detect new genes or variants and has circumscribed power in bringing discoveries in genomic testing by identifying new variants. With the discovery of new genes and variants in breast cancer, the Mammaprint microarray requires continuous upgrading to remain clinically validated as mutations change. A contributor to endocrine therapy resistance in breast cancer has recently been recognised with ES31 mutations, causing estrogen deprivation by inhibiting aromatase, affecting the outcomes of estrogen receptor-targeting and combination therapies (Reinert et al.). However, as ES31 is not one of the 70 genes that Mammaprint can assess, it measures the profile of gene expression of gene alterations according to its pre-defined gene signature instead of comprehensive DNA sequencing. With novel therapeutic agents and biomarkers emerging, Mammaprint would have to continuously adapt to recent genetic discoveries, resulting in the need for continuous revalidation of updated fixed panel assays. Meanwhile, NGS can identify uncharacterized alterations, fusion gene alterations and other point mutations that are excluded from Mammaprint's 70-gene signature. The lack of elasticity that the Mammaprint tests have in response to emerging biomarkers becomes the core limitation of this targeted technology, where genomic alterations that can strongly influence treatment selection or eligibility are not accordingly identified, making its targeted but limited scope diminish the reliability of its usage, considering the rapid evolution of precision oncology.

Mammaprint is particularly appraised for its ability to provide interpretable and applicable conclusions from genomic profiling, but a consequence of this targeted and specific tool is its exclusivity and inaccessibility for a significant population of breast cancer patients. This tool is only accessible for patients who are considered "eligible" under the terms and conditions of the FDA-cleared test and the IVDR-CE mark (Zeevi). Mammaprint has the specific aim to measure the recurrence risk of breast cancer patients, ultimately, patients in earlier stages of breast cancer (I and II); these tests are deemed irrelevant for patients with metastatic breast cancer. With the cancer already spreading to distant organs, the main concern that clinicians prioritise in addressing is no longer the risk of the cancer recurring after surgery but the specific therapeutic targets, targetable mutations and potential resistance that may be met with

specific drugs. In this context, NGS provides more pertinent support for clinicians where the monitoring of the proliferation of cancer cells and the mutations that evolve is crucial for treatment selection.

Despite the plausible solution for Mammaprint's panels in being continuously updated to include the emergence of novel biomarkers, the expenses incurred from constant R&D and feature upgrades result in high development costs. Costs do not only arise from upgrading its technology but also from Mammaprint's nature as a molecular diagnostic tool for a specific niche and market segment. As a consequence, Mammaprint is considered a premium and expensive genomic test, costing approximately 4200 USD (Yang et al., 2012). In a holistic view, introducing technology for every specific clinical niche can be seen as time-consuming, expensive, and unsustainable.

CONCLUSION

Whilst measuring the limitations of these specific technologies in precision oncology, it can be confidently inferred that these targeted technologies are no panacea. NGS may be capable of discovering rare variants and can be applied to diverse diseases, including complex and early-stage cancers. However, the core concern of clinical inactionability and the futility of these tools when implemented to assist in making informed clinical decisions must be evaluated. As healthcare continues to evolve, future clinicians and doctors should shift their values in precision medicine from technological complexity to its ability to translate genomic information into meaningful actions and recommendations to elevate patient care.

REFERENCES

Ahmad, Zubair, et al. "The Age of Molecular Biomarkers: Cancer in the Era of Personalized Medicine. What Do Pathologists in Developing Countries Need to Know and Understand?" International Journal of General Medicine, vol. Volume 19, Mar. 2026, pp. 1–35, https://doi.org/10.2147/ijgm.s590285. Accessed 20 May 2026.

Audeh, William, et al. "Prospective Validation of a Genomic Assay in Breast Cancer: The 70-Gene MammaPrint Assay and the MINDACT Trial." *Acta Medica Academica*, vol. 48, no. 1, 1 Apr. 2019, pp. 18–34, pubmed.ncbi.nlm.nih.gov/31264430/, https://doi.org/10.5644/ama2006-124.239. Accessed 21 Feb. 2022.

Bumgarner, Roger. "Overview of DNA Microarrays: Types, Applications, and Their Future." *Current Protocols in Molecular Biology*, vol. 101, no. 1, Jan. 2013, www.ncbi.nlm.nih.gov/pmc/articles/PMC4011503/, https://doi.org/10.1002/0471142727.mb2201s101.

Concolino, Paola, et al. "BRCA1 and BRCA2 Testing through next Generation Sequencing in a Small Cohort of Italian Breast/Ovarian Cancer Patients: Novel Pathogenic and Unknown Clinical

Significance Variants." *International Journal of Molecular Sciences*, vol. 20, no. 14, 1 Jan. 2019, p. 3442, www.mdpi.com/1422-0067/20/14/3442/htm, https://doi.org/10.3390/ijms20143442.

Dureau, Sylvain, et al. "SHIVA: Randomized Phase II Trial Comparing Molecularly Targeted Therapy Based on Tumor Molecular Profiling versus Conventional Therapy in Patients with Refractory Cancer—Overall Survival (OS) Analysis." *Journal of Clinical Oncology*, vol. 35, no. 15_suppl, 20 May 2017, pp. 11515–11515, https://doi.org/10.1200/jco.2017.35.15_suppl.11515. Accessed 8 June 2026.

Kevin. "Endocrine Utility." *Agendia*, 18 Feb. 2023, agendia.com/endocrine-utility/. Accessed 29 Mar. 2026.

Le Tourneau, Christophe, et al. "Molecularly Targeted Therapy Based on Tumour Molecular Profiling versus Conventional Therapy for Advanced Cancer (SHIVA): A Multicentre, Open-Label, Proof-of-Concept, Randomised, Controlled Phase 2 Trial." *The Lancet Oncology*, vol. 16, no. 13, Oct. 2015, pp. 1324–1334, https://doi.org/10.1016/s1470-2045(15)00188-6.

Manjili, Masoud H, et al. "Signatures of Tumor–Immune Interactions as Biomarkers for Breast Cancer Prognosis." *Future Oncology*, vol. 8, no. 6, June 2012, pp. 703–711, https://doi.org/10.2217/fon.12.57. Accessed 13 June 2020.

Milbury, Coren A., et al. "Clinical and Analytical Validation of FoundationOne®CDx, a Comprehensive Genomic Profiling Assay for Solid Tumors." *PLOS ONE*, vol. 17, no. 3, 16 Mar. 2022, p. e0264138, https://doi.org/10.1371/journal.pone.0264138.

Mittempergher, Lorenza, et al. "MammaPrint and BluePrint Molecular Diagnostics Using Targeted RNA Next-Generation Sequencing Technology." *The Journal of Molecular Diagnostics*, vol. 21, no. 5, 1 Sept. 2019, pp. 808–823, https://doi.org/10.1016/j.jmoldx.2019.04.007. Accessed 23 June 2023.

Moon, Youngbeen, et al. "Evaluation of False Positive and False Negative Errors in Targeted next Generation Sequencing." *Genome Biology*, vol. 26, no. 1, 1 Dec. 2025, pmc.ncbi.nlm.nih.gov/articles/PMC12670792/?utm_source=chatgpt.com, https://doi.org/10.1186/s13059-025-03882-2. Accessed 24 Feb. 2026.

Pavlova, Sarka, et al. "Detection of Clinically Relevant Variants in the *TP53* Gene below 10% Allelic Frequency: A Multicenter Study by ERIC, the European Research Initiative on CLL." *HemaSphere*, vol. 9, no. 1, Jan. 2025, https://doi.org/10.1002/hem3.70065.

Qin, Dahui. "Next-Generation Sequencing and Its Clinical Application." *Cancer Biology & Medicine*, vol. 16, no. 1, Feb. 2019, pp. 4–10, https://doi.org/10.20892/j.issn.2095-3941.2018.0055.

Reinert, Tomas, et al. "Clinical Implications of ESR1 Mutations in Hormone Receptor-Positive Advanced Breast Cancer." *Frontiers in Oncology*, vol. 7, 15 Mar. 2017, https://doi.org/10.3389/fonc.2017.00026.

Scharf, Sebastian A., et al. *Barcode Crosstalk in ONT Multiplex Sequencing: Quantification and Mitigation Strategies*. 29 May 2026, https://doi.org/10.21203/rs.3.rs-9711921/v1. Accessed 8 June 2026.

Schwartz, Schraga, et al. "Detection and Removal of Biases in the Analysis of Next-Generation Sequencing Reads." *PLoS ONE*, vol. 6, no. 1, 31 Jan. 2011, p. e16685, pmc.ncbi.nlm.nih.gov/articles/PMC3031631/, https://doi.org/10.1371/journal.pone.0016685. Accessed 27 Feb. 2026.

Suh, Koung Jin, et al. "Clinical Application of Next-Generation Sequencing in Patients with Breast Cancer: Real-World Data." *Journal of Breast Cancer*, vol. 25, 2022, https://doi.org/10.4048/jbc.2022.25.e30.

Sun, Lulu, et al. "Molecular Testing in Breast Cancer: Current Status and Future Directions." *The Journal of Molecular Diagnostics*, vol. 23, no. 11, 1 Nov. 2021, pp. 1422–1432, www.sciencedirect.com/science/article/pii/S1525157821002622#tbl2, https://doi.org/10.1016/j.jmoldx.2021.07.026.

Takeda, Masayuki, et al. "Clinical Application of the FoundationOne CDx Assay to Therapeutic Decision‐Making for Patients with Advanced Solid Tumors." The Oncologist, vol. 26, no. 4, 6 Jan. 2021, https://doi.org/10.1002/onco.13639.

Tsimberidou, Apostolia M, and Razelle Kurzrock. "Precision Medicine: Lessons Learned from the SHIVA Trial." The Lancet Oncology, vol. 16, no. 16, Dec. 2015, pp. e579–e580, https://doi.org/10.1016/s1470-2045(15)00397-6. Accessed 20 Nov. 2019.

"US FDA Approves FoundationOne®CDx and FoundationOne®Liquid CDx as Companion Diagnostics for TALZENNA® (Talazoparib) in Combination with XTANDI® (Enzalutamide) to Identify Patients with HRR Gene-Mutated Metastatic Castration-Resistant Prostate Cancer." *Foundation Medicine*, 2026, www.foundationmedicine.com/press-release/fda-approval-tissue-blood-therapy-mcrpc. Accessed 8 June 2026.

Yang, Mo, et al. "Cost Effectiveness of Gene Expression Profiling for Early Stage Breast Cancer." *Cancer*, vol. 118, no. 20, 22 Feb. 2012, pp. 5163–5170, https://doi.org/10.1002/cncr.27443.

Yao, K, et al. "Abstract P2-11-23: MammaPrint and BluePrint in Early Breast Cancer: Clinical Implications of Prognostic Stratification and Molecular Subtyping." *Cancer Research*, vol. 73, no. 24_Supplement, 15 Dec. 2013, pp. P2-1123-P211–23, https://doi.org/10.1158/0008-5472.sabcs13-p2-11-23. Accessed 29 Mar. 2026.

Zeevi, Daniel. "Who Is Eligible for MammaPrint Testing?" Agendia, 2026, agendia.com/faq-items/who-is-eligible-for-mammaprint-testing-2/. Accessed 8 June 2026.

Zhang, Yifan, et al. "Deep Oncopanel Sequencing Reveals within Block Position-Dependent Quality Degradation in FFPE Processed Samples." Genome Biology, vol. 23, no. 1, 29 June 2022, https://doi.org/10.1186/s13059-022-02709-8. Accessed 8 June 2026.