A Case Study of Next-Generation Sequencing Operationalization in an Oncology Companion Diagnostic Environment

A Case Study of Next-Generation Sequencing Operationalization in an Oncology Companion Diagnostic Environment

Caitlin McMahon Patriquin

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Abstract

The rise of utilization of next-generation sequencing (NGS) tests in the clinical practice and drug development settings has increased the need for additional regulatory oversight and company practices to support the growth. The operational effects of next-generation sequencing tests, from both the regulatory and growth burden are largely undervalued during the test development process. As such, if the operational components that affect the scalability and success of a product can be classified, there is a higher likelihood of the overall clinical adoption and FDA approval success. This study focuses on the regulatory oversight of next-generation sequencing tests and the components of the product that can affect the operational outcomes. Through the review of current FDA Medical Device regulations and comparison to other categories of regulated products, the overall regulatory needs and opportunities for improved oversight can be achieved. Furthermore, by review of the technical, performance, and operational criteria of currently approved NGS tests, an overall predictive model can be generated to offer perspectives into the regulatory, development, and operational components of non-FDA approved tests. Through this review, via a comprehensive analysis of the regulatory requirements, assay specifications, and operational components of next-generation sequencing tests, a viable model for the prediction of clinical adoption and FDA approval was generated.

Dedication

This effort is dedicated to my family – thank you for sticking it out with me

Acknowledgments

This thesis would not have been possible without the patience and understanding of my family. My daughter, Lucy, has not experienced a "school-free" mom yet, even finishing a final while in labor with her. My husband, Drew, allowed me the time I needed to fulfill this goal and supported me, endlessly, while I fought through it. And Bruce, my trusty thesis buddy, who kept my feet warm and provided white noise snores for concentration. Thank you to my Mom and Dad for your unwavering support of my dreams and helping me achieve them in every way possible.

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Chapter I.

Introduction

Next-Generation Sequencing in the Biotechnology and Medical Fields

Over the past five years, the capabilities of sequencing technologies have dramatically increased, while at the same time, the costs associated with the sequencing technology have decreased (Baker, 2017). A common depiction of the advances of DNA sequencing costs is the comparison to Moore's Law (Figure 1), which describes the long-term trend of computing power doubling every two years. In application to sequencing technologies, it shows the dramatic effect of the exceedingly well-performing pace of the technology advances and the decreases in cost of human genome sequencing over the years (Wetterstrand 2019).

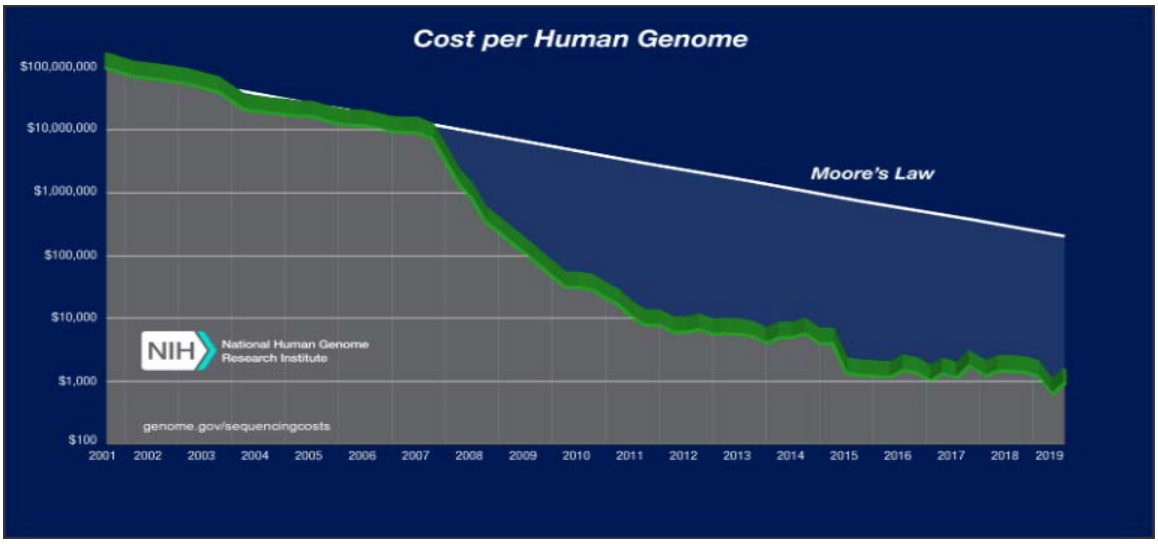

Figure 1. Human Genome Sequencing Cost Over Time (2001–2019)

Cost per human genome over time in comparison to Moore's Law. (Wetterstrand 2019).

The precipice of the reduction in sequencing costs, occurring in 2007, was the adoption of Next-Generation Sequencing (NGS) over the traditional Sanger sequencing technologies. Sanger sequencing was first developed in the 1970s and was the preferred sequencing technology for approximately 30 years, as well as set the stage for the future of DNA sequencing. The principles behind Sanger and NGS sequencing are similar in that the DNA polymerase add fluorescent nucleotides to a growing DNA template strand, however the critical difference between Sanger and NGS is the sequencing volume (Illumina, Inc., 2020). While traditional Sanger methods add sequences one DNA fragment at a time, NGS methods allow for sequencing of millions of fragments simultaneously (Illumina, Inc., 2020). Due to the developments in time and cost effectiveness of the NGS technologies, it quickly became the preferred methodology for large genomic sequencing analyses. Notably, there are many times Sanger sequencing

may remain the preferred technology, as shown in Figure 2, documenting the known benefits and challenges to Sanger and NGS sequencing.

	Sanger Sequencing	Targeted NGS
Benefits	• Fast, cost-effective sequencing for low numbers of targets (1–20 targets) • Familiar workflow	• Higher sequencing depth enables higher sensitivity (down to 1%) • Higher discovery power* • Higher mutation resolution† • More data produced with the same amount of input DNA‡ • Higher sample throughput
Challenges	• Low sensitivity (limit of detection ~15–20%) • Low discovery power • Not as cost-effective for high numbers of targets (> 20 targets) • Low scalability due to increasing sample input requirements	• Less cost-effective for sequencing low numbers of targets (1–20 targets) • Time-consuming for sequencing low numbers of targets (1–20 targets)

* Discovery power is the ability to identify novel variants.
† Mutation resolution is the size of the mutation identified. NGS can identify large chromosomal rearrangements down to single nucleotide variants.
‡ 10 ng DNA will produce ~1 kb with Sanger sequencing or ~300 kb with targeted resequencing (250 bp amplicon length × 1536 amplicons with TruSeq Custom Amplicon workflow)

Figure 2. Comparison of Sanger Sequencing and NGS.

The known benefits and challenges of Sanger sequencing and NGS technologies (Illumina, Inc., 2020).

The cost of sequencing technologies was a driving factor towards the adoption of NGS methods over Sanger sequencing for large genomes. Due to the time and costs associated with Sanger sequencing, the technology had limited applicability to large scale human genome sequencing. As a result, when the NGS methods introduced methodologies to decrease the sequencing time and ability to interrogate larger regions of

the genome, the associated cost of NGS sequencing was reduced. As of 2019, current costs for the raw sequencing costs of the human genome is roughly $1,000. However, the sequencing costs alone are typically not indicative of the true cost of a next-generation sequencing test as there are laboratory preparatory components, bioinformatics analyses, and medical interpretation reports that are typically included in the cost of a product. Regardless, despite the additional costs associated with a test, the relatively low cost of sequencing technology has dramatically shifted the momentum in clinical practice towards the utilization of the technology.

Aside from the cost benefit of the technology, the sequencing data and efficiency abilities of next-generation technologies have been on a steady incline since 2005 (Mardis, 2011). The benefits of next-generation sequencing in the oncology field are changing the way cancer diagnoses, monitoring, and treatments are made. With the combination of advancements in the technology and decreasing cost, increasing numbers of pharmaceutical companies, academic medical centers, and clinicians are looking to expand their use of next-generation sequencing for drug development and treatment decisions. The drug development process has contributed to the increase of next-generation sequencing use through requirement of precision medicine for companion diagnostic claims. As shown in Figure 3, several drugs (Crizotinib, Dabrafenib, Rucaparib) exclusively use NGS as the required technology to detect the biomarker in certain indications. Therefore, as the number of drugs developed via precision medicine techniques increases, physicians will need to adapt to the technology in order to get their patients access to the drugs they may need.

Drug (brand name)	Indication	Biomarker	Technology (year PMA first approved)
Afatinib (Gilotrif)	NSCLC	*EGFR* exon 19 deletions or exon 21 (L858R) substitution mutations	RT-PCR (2013)
Cetuximab (Erbitux) and panitumumab (Vectibix)	Colorectal cancer	*KRAS* mutation negative or *KRAS* and *NRAS** mutation negative	IHC (2004); RT-PCR (2012); NGS* (2017)
Crizotinib (Xalkori)	NSCLC	ALK overexpression or gene fusion	FISH (2011); IHC (2015)
Crizotinib (Xalkori)	NSCLC	*ROS1* fusions	NGS (2017)
Dabrafenib (Tafinlar) and trametinib (Mekinist)	Melanoma	$BRAF^{V600E}$ or $BRAF^{V600K}$ mutations	RT-PCR (2013); NGS (2017)
Dabrafenib (Tafinlar) and trametinib (Mekinist)	NSCLC	$BRAF^{V600E}$ or $BRAF^{V600K}$ mutations	NGS (2017)
Deferasirox (Exjade)	Non-transfusion-dependent thalassaemia	Liver iron concentration	MRI (2013)
Enasidenib (Idhifa)	AML	*IDH2* mutation positive	PCR (2017)
Erlotinib (Tarceva)	NSCLC	*EGFR* exon 19 deletions or exon 21 (L858R) substitution mutations	RT-PCR (2013)
Gefitinib (Iressa)	NSCLC	*EGFR* exon 19 deletions or exon 21 (L858R) substitution mutations	RT-PCR (2015); NGS (2017)
Imatinib (Gleevec)	Gastrointestinal stromal tumours	KIT expression	IHC (2005)
Midostaurin (Rydapt)	AML	*FLT3* mutation-positive	PCR (2017)
Olaparib (Lynparza)	Ovarian cancer	*BRCA1* or *BRCA2* mutation	PCR and Sanger sequencing (2014)
Osimertinib (Tagrisso)	NSCLC	$EGFR^{T790M}$ mutations	RT-PCR (2016)
Pembrolizumab (Keytruda)	NSCLC	PDL1 expression	IHC (2016)
Rucaparib (Rubraca)	Ovarian cancer	*BRCA1* or *BRCA2* mutation	NGS (2016)
Trastuzumab (Herceptin)	Breast cancer	HER2 expression and/or *ERBB2* amplification	ISH (2011); CISH (2011); IHC (2012)
Trastuzumab (Herceptin); pertuzumab (Perjeta) and ado-trastuzumab emtansine (Kadcyla)	Breast cancer, gastric cancer	*ERBB2* amplification	FISH (2005); ICC (1998)
Vemurafenib (Zelboraf)	Melanoma	$BRAF^{V600E}$ mutations	RT-PCR (2011)
Venetoclax (Venclexta)	B cell chronic lymphocytic leukaemia	Deletion of 17p (which contains *TP53*)	FISH (2016)

Figure 3. Companion Diagnostics Approved by the FDA pre-2018.

List of FDA approved companion diagnostic drugs and the technologies used to detect the biomarkers (Dugger, Platt, & Goldstein, 2017).

Overall, Next-Generation Sequencing (NGS) refers to the technology that performs high throughput sequencing of DNA to detect variations in the DNA sequences as compared to known reference genome sequences. Currently, there are two primary sequencing instruments, the Ion Torrent sequencers by Life Technologies and the Illumina platform, which both require and utilize the same general process of template

preparation, sequencing, and data analysis (Resta & Ferrari, 2018). The advantage to NGS tests, those that are accurate and reliable, is to accelerate precision medicine and tailor specific treatments based on the individual characteristics of each patient, as identified through the NGS testing (U.S. Food & Drug Administration, 2015). Consequently, clinicians and pharmaceutical companies are realizing the impact NGS testing can have on the benefits to patients and more efficient drug development.

While there are obvious advantages to next-generation sequencing, there remain barriers in the adoption of the technology. The main argument around lack of adoption tends to stem over the associated costs of the technology from the insurance perspective. While comprehensive cost-effectiveness reports are sparse for this research, studies have shown very low overall cost for insurance companies in certain cancers ($0.0072/member/month over 5 years) compared to costs associated with multiple single-gene tests (Yu, et. al., 2018). Additionally, Haslem et al. (2017) showed that in patients with advanced cancers, progression-free survival was improved for those using precision medicine, without any increased health costs. As sequencing continues to become mainstream and integrates into standard of care in the clinical setting, there will be additional opportunities for research into the cost-benefit of the technology. At the same time, the sequencing technologies and drug development process will continue to progress, further impacting clinical and economic decisions.

Overview of the U.S. Food and Drug Administration

The U.S. Food and Drug Administration (FDA) is the federal agency branch of the Department of Health and Human Services. The FDA's mission is to protect the public health by ensuring the safety, efficacy, and security of the products that it regulates (U.S. Food & Drug Administration, 2018d). In addition to the regulation of current products, the FDA also claims responsibility for the advancement of public health and speed of innovation of new and existing products (U.S. Food & Drug Administration, 2018d). The FDA regulates several categories of products, all which have their own subcategories and classes, and regulatory strategies and protocols. The eight product categories the FDA regulates are: Food; Drugs; Medical Devices; Radiation-Emitting Products; Vaccine, Blood, and Biologics; Animal and Veterinary; Cosmetics; Tobacco Products. Each category of regulated products is unique and therefore has its own regulations and oversight needs by the agency.

While the main functions of the FDA have been around since the early 1900s, the FDA is continually evolving and adding sectors to the organization as needed. The 1906 Pure Food and Drugs Act was the first modern law that gave the FDA its oversight and functions of providing basic protection to consumers (U.S. Food & Drug Administration, 2018c). As a testament to their continually evolution of the organization, on March 31, 2019, the FDA announced that a reorganization of the FDA is underway, with the future structure of the agency outlined in Appendix 1. Each main category of FDA oversight continues to have an overall center for review, however additional offices, including 'Oncology Center of Excellence,' and 'Office of Regulatory Affairs' would have direct impact on NGS and the oncology community. As of early 2020, the NGS market

continues to be categorized under the Center for Devices and Radiological Health (CDRH).

FDA Regulation of Next-Generation Sequencing Panels

The adoption of next-generation sequencing technologies by the clinical and pharmaceutical partners was contributing to new knowledge to the field, however there was little formal oversight of the technology until late 2017 when the FDA approved the first comprehensive NGS panel in the oncology setting (Allegretti et al., 2018). This gap in oversight has only recently been addressed, as the regulations are attempting to keep up with the scientific advances in the field. While regulations may manifest as additional operational burden on existing and upcoming NGS testing platforms, there will inevitably be sample volume implications as increased clinical adoption of the tests occurs. At the same time, regulatory approval by the FDA can advance the use of genomic-based precision medicine through the prescription of standard of care treatment for patients (Dy et al., 2019). Overall, given the implications of FDA regulation on both the companies who create the products and their standard operating procedures (SOP) and the clinical utility in the market, the current and future FDA regulations are a key component of the next-generation sequencing process.

Next-generation sequencing tests are categorized under Medical Devices under FDA regulation. The classification of the medical device will determine what type of premarketing submission and application is required for FDA clearance (U.S. Food & Drug Administration, 2018a). The classification levels for Medical Devices are Class I, II, and III, each with general controls and with and without exemptions. The device

classification depends on the intended use, indications for use, and risk associated with the device, with Class I posing the least risk to the patient and Class III having the greatest risk (U.S. Food & Drug Administration, 2018a). Depending on the classification level of the medical device, for FDA approval, a 510(k) or Pre-Market Approval (PMA) submission is required. For Class I and II devices, the standard approval route is through the 510(k) submission, while Class III devices require a PMA submission. Given the risks associated with Class III devices, the FDA utilizes the more rigorous PMA submission requirement to ensure there is sufficient valid scientific evidence to ensure safety and effectiveness of the device (U.S. Food & Drug Administration, 2019c).

Within the category of Medical Devices, and with specific focus on next-generation sequencing tests, the CDRH has additional levels of regulation, which largely depend on the level of supporting evidence accompanying the tests (Figure 4).

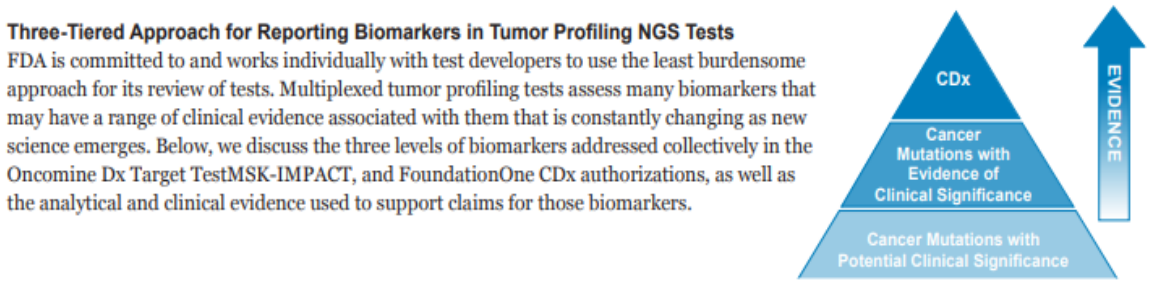

Figure 4. FDA Approach to NGS Biomarker Testing

CDRH's three-tiered approach to tumor profiling next-generation sequencing tests (U.S. Food and Drug Administration, n.d.).

Level three regulation refers to tests which report on cancer mutations with potential clinical significance, which largely corresponds to clinical trials for detected mutations. Level two regulation refers to cancer mutations with evidence of clinical significance and can enable physicians to use the information for prescription of therapies. Finally, level one regulation refers to companion diagnostic tests, which provide information on the therapeutic use of safe and effective products for a specific drug target. Level one Companion Diagnostic tests require the highest amount of supporting evidence and analytical validation and are therefore a smaller subset of next-generation sequencing tests have applied and received approval for companion diagnostic designations. An important consideration for a next-generation sequencing test is the level of application of the test and consequently the amount of analytical validation required and its potential effect on the operational considerations of such regulation.

History of FDA Regulation of NGS in the Oncology Setting

In November 2017, the first FDA approval of comprehensive NGS panels for oncology testing occurred, setting the stage for future test developments. The following year, the FDA released a guidance document on NGS testing to establish its regulatory approach for the technology (Luh & Yen, 2018). For the NGS tests that are approved for companion diagnostic use, the FDA has released guidance documents over the course of the years to help companies implement and develop their product per the FDA regulations. While the draft guidance documents were helpful as recommendations to companies developing tests, the guidance disposition of the reports was not the regulation

that was needed. Additionally, as shown in Figure 5, prior to 2017, the FDA documents did not have NGS tests as a specific focus for non-germline NGS-based tests. The FDA guidance documents came as a needed response from the FDA and as guidance to new and existing testing methods, however only until recently did the guidance become formal regulation.

2005	*Pharmacogenomic Data Submissions* (final guidance)
2007	*Pharmacogenomic Tests and Genetic Tests for Heritable Markers* (final guidance)
2007	*In Vitro Diagnostic Multivariate Index Assays* (draft guidance)
2008	*E15 Definitions for Genomic Biomarkers, Pharmacogenomics, Pharmacogenetics, Genomic Data, and Sample Coding Categories* (final guidance)
2011	*E16 Guidance on Biomarkers Related to Drug or Biotechnology Product Development: Context, Structure, and Format of Qualifications Submissions* (final guidance)
2012	*Enrichment Strategies for Clinical Trials to Support Approval of Human Drugs and Biological Products* (draft guidance)
2013	*Clinical Pharmacogenomics: Premarket Evaluation in Early-Phase Clinical Studies and Recommendations for Labeling* (final guidance)
2013	*Clinical Pharmacogenomics: Premarket Evaluation in Early-Phase Clinical Studies and Recommendations for Labeling* (final guidance)
2014	*Qualification Process for Drug Development Tools* (final guidance)
2014	*In Vitro Companion Diagnostic Devices* (final guidance)
2014	*Framework for Regulatory Oversight of Laboratory Developed Tests (LDTs)* (draft guidance)
2014	*FDA Notification and Medical Device Reporting for Laboratory Developed Tests (LDTs)* (draft guidance)
2016	*Use of Standards in FDA Regulatory Oversight of Next Generation Sequencing (NGS)-Based In Vitro Diagnostics (IVDs) Used for Diagnosing Germline Diseases* (draft guidance)
2016	*Use of Public Human Genetic Variant Databases to Support Clinical Validity for Next Generation Sequencing (NGS)-Based In Vitro Diagnostics* (draft guidance)
2016	*Principles for Codevelopment of an In Vitro Companion Diagnostic Device with a Therapeutic Product* (draft guidance)
2017	*Discussion Paper on Laboratory Developed Tests (LDTs)* (discussion paper)

Figure 5. Personalized Medicine Draft Guidance Reports 2005 – 2017.

Collection of draft guidance documents issued by the FDA from 2005–2017 related to personalized medicine and associated testing methods (Personalized Medicine Coalition, 2017).

In April 2018, The FDA issued two additional guidance documents on streamlining the submission and review of data for clinical and analytical validation of Next-Generation Sequencing tests (U.S. Food & Drug Administration, 2018b). These more recent guidance documents are meant to provide recommendations to streamline the regulatory process, given that the landscape and technology advances in NGS testing can progress quickly in the field. The newest, as of August 2019, guidance documents are based around clinical database guidance and analytical validation guidance. For their current approach to the regulatory oversight, the FDA aims to create standards for design, development, and validation, however, is also aware of the value of bioinformatics tools and databases and the need for regulatory input (Figure 6).

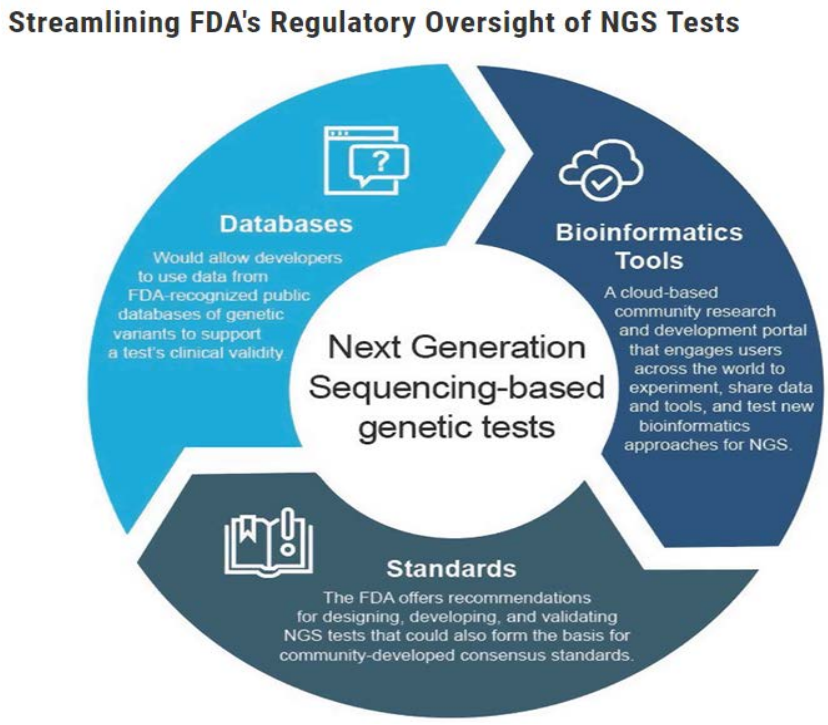

Figure 6. FDA Oversight Methods of NGS Tests.

FDA's approach to regulatory oversight of NGS tests (U.S. Food & Drug Administration, 2018b).

Tumor Profiling Next-Generation Sequencing Landscape

The NGS test landscape is constantly evolving as companies competitively push each other to progress the science of the technology. The breadth of available *in vitro* companion diagnostic devices and wave of new companies interested in NGS testing shows the progression of the testing options and the increasing competition in the field. In the U.S. Food & Drug Administration's (n.d.) fact sheet on CDRH's approach to tumor profiling next-generation sequencing tests, three tests are explicitly called out: Thermo Fischer Scientific's Oncomine Dx Target Test, Memorial Sloan Kettering's IMPACT test, and Foundation Medicine's FoundationOne CDx. These three tests are the first-to-market NGS tests approved by the FDA and will serve as the main comparators in this case study.

Thermo Fischer's Oncomine Dx Target Test

The first FDA approved NGS-based test was approved on June 22, 2017 and was Thermo Fischer's Oncomine Dx Target Test. The test is categorized as an *in vitro* diagnostic and is approved for use in non-small cell lung cancer (NSCLC). Upon FDA initial submission, the test is designed to test for 23 genes, in which three of the genes can be used as a companion diagnostic for treatment using: combined therapy of Tafinlar® and Mekinist®, XALKORI®, or IRESSA® (ThermoFischer Scientific, 2017). The companion diagnostic mutations are conveyed in Figure 7:

Gene	Variant status	Targeted therapies
BRAF	*BRAF* V600E	TAFINLAR® + MEKINIST® (dabrafenib in combination with trametinib)
ROS1	*ROS1* fusions	XALKORI® (crizotinib)
EGFR	L858R, exon 19 deletions	IRESSA® (gefitinib)

Figure 7. Oncomine Dx Target Test Companion Diagnostic Variant and Claims.

Companion diagnostic indications for the Oncomine Dx Target NGS test (ThermoFischer Scientific, 2017).

The key advantage of the Oncomine Dx tests over others is the reduction in tumor requirement (10ng) for testing (Macdonald, 2017). The assay specifications and technical information, company profile, and FDA submission documents will be fully analyzed in this review.

Memorial Sloan Kettering's IMPACT Test

Memorial Sloan Kettering (MSK)'s IMPACT test was approved by the FDA in November 2017 and is currently available for patients exclusively treated at the Memorial Sloan Kettering Cancer Center (MSKCC). The IMPACT test analyzes 468 genes that are known to play a critical role in the development and behavior of tumors (Memorial Sloan Kettering Cancer Center, 2019). The IMPACT test is not considered a companion diagnostic as there are no corresponding therapeutic targets that the FDA authorized to match the detected mutations. A key feature of the IMPACT test is the sequencing and comparison of a tumor sample and a normal tissue sample. A known limitation of the

IMPACT test is the availability of the test to patients treated at hospitals and cancer centers outside of MSKCC, as well as the lack of companion diagnostic designation. While a potential disadvantage from the accessibility standpoint, this may prove to be an operational and scalability advantage.

Foundation Medicine's Foundation One CDx Test

Foundation Medicine's FoundationOne CDx product received FDA approval on November 30, 2017. The laboratory test is designed to detect genetic mutations in 324 genes and two genomic signatures, Microsatellite Instability (MSI) and Tumor Mutational Burden (TMB), in any solid tumor (Foundation Medicine, Inc., 2017). The test was initially approved as a companion diagnostic for non-small cell lung cancer, melanoma, breast cancer, colorectal cancer, and ovarian cancer. Foundation Medicine's FoundationOne CDx test was the first broad coverage companion diagnostic NGS test and was initially approved for companion diagnostic indications for identifiable biomarkers and related FDA-approved therapies listed in Figure 8:

INDICATIONS	BIOMARKER	FDA-APPROVED THERAPY*
Non-Small Cell Lung Cancer (NSCLC)	*EGFR* exon 19 deletions and *EGFR* exon 21 L858R alterations	Gilotrif® (afatinib), Iressa® (gefitinib), or Tarceva® (erlotinib)
	EGFR exon 20 T790M alterations	Tagrisso® (osimertinib)
	ALK rearrangements	Alecensa® (alectinib), Xalkori® (crizotinib), or Zykadia® (ceritinib)
	BRAF V600E	Tafinlar® (dabrafenib) in combination with Mekinist® (trametinib)
Melanoma	*BRAF* V600E	Tafinlar® (dabrafenib) or Zelboraf® (vemurafenib)
	BRAF V600E or V600K	Mekinist® (trametinib) or Cotellic® (cobimetinib), in combination with Zelboraf® (vemurafenib)
Breast Cancer	*ERBB2* (HER2) amplification	Herceptin® (trastuzumab), Kadcyla® (ado-trastuzumab-emtansine), or Perjeta® (pertuzumab)
Colorectal Cancer	*KRAS* wild-type (absence of mutations in codons 12 and 13)	Erbitux® (cetuximab)
	KRAS wild-type (absence of mutations in exons 2, 3 and 4) and *NRAS* wild-type (absence of mutations in exons 2, 3 and 4)	Vectibix® (panitumumab)
Ovarian Cancer	*BRCA1/2* alterations	Rubraca® (rucaparib)

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Figure 8. FoundationOne CDx Companion Diagnostic Biomarkers and Claims.

Companion diagnostic indications for the FoundationOne CDx NGS test (Foundation Medicine, 2018b).

The FoundationOne CDx tests' key advantages are the breadth of clinical and analytical validation, comprehensive results which evaluate multiple types of genomic alternations (short variants, copy number alterations, rearrangements, biomarkers), and payer coverage due to the companion diagnostic designation.

Future Candidates for NGS Test FDA Regulation

The competitive landscape in the NGS market is quickly evolving as new tests receive the necessary funding and academic centers continue to evolve home grown tests into marketable products. A comprehensive list of oncology NGS tests is difficult to amass given the constantly changing landscape, funding abilities, economy impact on the

companies, and changing markets. A sampling of tests, either FDA-approved, or currently in development is found in Table 1.

Table 1. Next-Generation Sequencing Oncology Test Landscape.

Sampling of currently commercially available next-generation sequencing tumor profiling tests.

Company	Test
ArcherDx	VariantPlex
Ashion	GEM ExTra
Caris Molecular Intelligence	MI Tumor Seek
Foundation Medicine	FoundationOne CDx
Illumina	TruSight Oncology 500
Integrated Oncology	OmniSeq Comprehensive
Kew	Cancerplex
Memorial Sloan Kettering (MSK)	IMPACT
Nantheath	GPS Cancer
NeoGenomics	NeoType Discovery Profile
Novogene	Proband
OncoDNA	OncoDeep
Paradigm	PCDx
Personal Genome Diagnostics	CancerSELECT 125
Qiagen	QIAseq
Quest	OncoVantage
Sema4	Solid Tumor Panel
Strata	StrataNGS
Tempus	xT
ThermoFischer	Oncomine Dx Target Test

The cancer and tumor profiling market, which includes NGS testing, is expected to rise from 7.56 billion to 12.47 billion by 2024, driven mainly by an increase of incidence of cancer rates and the increasing demand for personalized medicine (Markets and Markets, 2019). Therefore, it is predicted that the competition among NGS tests will continue to grow and evolve as companies attempt to gain the market share of the tumor profiling industries. Not only will the upcoming NGS tests need to be equal or better than

the current tests from a test performance standpoint, the operational considerations from the company standpoint may provide a scalable and efficiency advantage over competitors.

Operational Considerations for Next-Generation Sequencing Laboratories

There are many studies and technical reviews performed that compare the sequencing platforms for next-generation sequencing, however the operational effects related to staffing, training, quality and validation, data, and the customer needs, of the technology advancements related to companion diagnostic NGS panels are much less understood nor evaluated during the product development process. Detailed analysis of the test specifications and company profiles could shed light into the operational workflows relevant to the tests and companies. Some of the more important elements of an operational processes in a biotechnology industry are scalability of the process, pre-launch/launch planning, process improvements, and hiring considerations. From the quality standpoint there are a host of other considerations that must be taken into account, including error detection and incident reporting, documentation practices, and quality assurance integration into an operational workflow. With the oversight of the FDA in play, error detection methods are a necessary component of the quality process and if an error is detected, incident and non-conformance reporting is required by the FDA. Overall, for the FDA to approve a test product, there needs to be well-documented quality plans in place, including an overall plan and specific standard operating procedures for each step in the operational process. Finally, aside from the companies themselves and the operational plans in place, the introduction of NGS-based tests in a companion

diagnostic setting can have market advantages and global implications that can significantly impact a company if these considerations are not accounted for upfront.

For a business, laboratory or otherwise, to be successful, the operations department must be one of the main considerations in the development of a new business or new product within an existing platform. Often, the operational components can be overlooked as the excitement of the product or service is envisioned by the Product Development or Research and Development teams and the operational specifics may be inflexible or less appealing to work through. However stringent the operational aspects may be, for the business or product to succeed it must have high quality, be scalable, and meet the customer needs.

Quality Considerations in the Operational Process

A main consideration in the operational workflow should be the quality procedures and their impacts on the overall test deliverables including error rate, turnaround time, and personnel requirements to meet the quality demands. The components of a quality plan include quality planning, quality assurance, and quality control (Centers for Medicare & Medicaid Services, 2019). Quality affects the end product and services provided but additionally, the quality aspect of a product relates to the way the employees perform the work processes and those should be as efficient as possible and continually improving (Manghani, 2011). Within the larger quality processes, the more specific responsibilities related to auditing processes, standard operating procedures (SOPs) creation and maintenance, quality control systems,

documentation, and metrics gathering all play a part in the operational workflow of a product and can impact the customer needs around turnaround time and quality.

Aside from the operational efficiency aspects in the quality management, the risks and errors of a product must be well understood. Work by Ma et al. (2019) discovered several opportunities for error in the NGS workflow, including DNA polymerases fidelity, substitution bias, and sub-optimal handling/storage conditions. Additionally, aside from the laboratory chemistry and computational errors, the risks associated with manual intervention steps and overall operational workflows can ultimately affect the end result of a patient report. Whether the error is laboratory, computational, or manual in nature, the effect of the error can result in incorrect therapies being recommended, or lack thereof for false negative errors, and ultimately may negatively affect patient outcomes.

Customer Requirements of a Next-Generation Sequencing Test

While quality is arguably the most important aspect of a product, in the laboratory testing environment, turnaround time (TAT) is one of the most easily measurable performance metrics in the laboratory setting. A laboratory may measure product success based on the analytical validation, or ongoing quality of the product, while clinicians may perceive turnaround time as an indicator of the quality of the lab or test. In addition to the impact of TAT on the patients and clinicians, poor TAT is a source of customer complaints and requires time and effort from the laboratory staff for complaint resolution and service improvement (Hawkins, 2007). In addition to the customer effects of turnaround time, there are many additional considerations regarding turnaround time that

may impact market adoption, including definition agreement of turnaround time, effects of unsatisfactory TAT, the market acceptable TAT for NGS testing, and methods to improve TAT.

Laboratory Hiring Considerations in Next-Generation Sequencing

The importance of hiring to plan and scaling a business to meet the customer demands are obvious and necessary aspects of a successful company. However, there currently exists little information on the role of obtaining laboratory personnel talent and scalability of a hiring model for next-generation sequencing tests. While limited on the personnel aspect, it is well-studied that the output of next-generation sequencing has created an unforeseen burden related to computing and processing, analytics, quality, storage, and tracking. The three overall categories related to the data produced in an NGS assay that impact the operational success of a test are the volume of data, the variety of data formats with multiple users, and the velocity in which the data is amassed (Roy, 2016). Interestingly, the departments that manage the computational aspects of the results are more often part of the Technology structure than within the Operations, despite the impact it may have on the day to day operations. Therefore, the organizational structure or interdepartmental collaborations of a company may shed additional insight into the long-term success of a company through the importance placed on the cross-functional operational impacts.

With specific focus on FDA-regulated next-generation sequencing (NGS) tests, there are unforeseen operational considerations related to the adoption of the technology,

the scalability (hiring and infrastructure), and regulatory oversight, that can impact the productivity of the operations, the customer needs, and the overall performance of the company. If the benefits and limitations of NGS technologies from an operational perspective can be appropriately characterized for its current use in the oncology field, this could lead to faster adoption of the technology by operational teams and allow for further efficiency improvements as current limitations are addressed.

Chapter II.

Materials and Methods

The following section details the techniques used throughout the case study review. Briefly, the overall FDA regulations and classifications for Medical Devices were reviewed and compared to a subset of FDA regulated products to review opportunities for additional oversight or new workflows for approvals and ongoing regulation. The current FDA-approved oncology tumor profiling NGS tests were investigated and compared, with focus on the company profile and performance specifications that could impact laboratory operational considerations. Finally, future NGS test candidates for FDA approval were evaluated for their predicted success of approval and clinical adoption.

Review and Comparison of FDA Product Regulation

The regulation of products in different categories of FDA oversight was reviewed for three categories of regulation: Medical Devices, Cosmetics, and Radiation-emitting electronic products. The FDA resources for each category were reviewed and compared to one another. The regulatory oversight and approval pathways for each category were investigated. Similarities and differences, and potential avenues for future improvements of the regulations in relation to Medical Devices and next-generation sequencing tests, were noted in this review.

Review and Comparison of Current FDA Approved Tumor Profiling NGS Tests

The NGS tests in the oncology field that have received FDA approval are: Foundation Medicine's FoundationOne CDx, ThermoFischer's Oncomine Dx Target Test, and Memorial Sloan Kettering's MSK-IMPACT. For the technical and performance specifications, using FDA approval as the benchmark for success of an NGS test, each FDA approved test was reviewed on the criteria listed in Table 2.

Table 2. Technical and Performance Method Criteria

Criteria used to evaluate the technical and performance specifications for next-generation sequencing assays.

Analysis Criteria
Test Methodology
Number of Baited Genes (DNA/RNA)
Preferred Specimen Type
Minimum Sample Size
Required DNA Input
Minimum Tissue Surface Area Requirement
Minimum Tumor Content
Variant Detection Classes
Test Precision
Accuracy of the assay
Concordance Testing
Limit of Detection

The criteria were determined using the product labeling information for each product that was submitted to the FDA as part of the application process. This information is publicly available from the FDA Medical Device database. For the operational considerations, if available, data from each company that developed the tests, were reviewed on the criteria of turnaround time (TAT), sequencer type, and distribution mode.

Additionally, to determine the operational components of the tests, the operational workflow processes and report templates, if available, were determined through the technical information and company website material. The workflows and clinical reports between tests were evaluated and compared.

Review of Non-FDA Approved Tumor Profiling NGS Tests

Using the framework created in review of the existing FDA-approved tests, future candidates for possible submission for FDA approval were reviewed and compared using the same criteria as outlined in Table 2. Due to the lack of regulatory submission materials, the information available for review is much more limited and possibly intentionally withheld for competitive reasons, therefore for certain tests that were evaluated, criteria were not specified from the publicly available information. This information is likely to be required if, or when, FDA approval of the test is pursued.

The technical and operational metrics for 12 next-generation sequencing tests were reviewed and compared to the current FDA approved tests. Due to the expansive and rapidly growing landscape of oncology NGS tests, the more rigorous review of the performance specifications and operational workflows were evaluated for two next-generation sequencing tests that are currently commercially available, yet non-FDA approved. The Tempus xT and Caris Molecular Insights MI Tumor Seek test were evaluated for their full performance and operational criteria, equivalently to the analysis performed on the currently FDA approved tests. The two tests selected were chosen given

the ability to directly compare with the FDA-approved tests due to their similarity in test characteristics and distribution mechanisms of the tests.

Predictive Model for NGS Test FDA Approval and Clinical Adoption

Benchmark Generation

Using the FDA-approved NGS tests as the baseline, the maximum approved requirements for sample size, surface area, and tumor content were considered as the benchmark needed for FDA approval. The benchmark for the number of baited DNA genes and the turnaround time metrics in the model were determined by taking an average of the baited genes and published turnaround time values across the three currently approved tests. The variant detection requirements were determined by assessing the categories of variants approved by the FDA under medical device next-generation sequencing oncology panels with multiplexed variant detection systems. If greater than one FDA-approved test detected the variant type, it was considered a necessity for clinical adoption of the test.

The assay performance criterion was determined by reviewing the results of the analytical studies for the PMA submissions and the minimally accepted values (lowest percentage) between the two tests were used for the accuracy, precision, and concordance. The minimum LoD was separated by SNVs and indels and an average of the LoD values was used as the benchmark. Analysis for the model for performance was limited to the SNVs and indel variant bins due to the differences in additional variant detection methodologies. The 510(k) submission was excluded from the benchmark for

assay performance due to the decreased requirements for validation testing for Class II devices.

Test Comparison

Once the benchmark metrics were determined, the technical, performance, and operational metrics for the comparator, non-FDA approved tests were inputted into the model and compared to the benchmark. The model was tested for two comparator tests, in which the operational and performance metrics were fully evaluated for, the Tempus xT test and Caris Molecular Information MI Tumor Seek test.

Clinical Adoption and FDA Approval Scores

The test criteria generated in the model was used to generate clinical adoption and FDA approval scores. If the criterion was purely categorical, it was excluded from score calculations. For the benchmark score creation, a score of '1' was used to classify the minimum criteria needed. A score of '0' in the benchmark score was used for optional criterion, as classified during the benchmark generation. A score of '1' for the comparator tests indicates the benchmark criterion was met. A score of '2' indicated the comparator test outperformed the benchmark. A score of '0' for the test comparators indicated the test underperformed in comparison to the benchmark. If all the benchmark criteria were met, the score would match the benchmark, and suggest similar clinical adoption/FDA approval success to currently approved NGS tests. A score below the benchmark score would suggest lower clinical adoption/FDA approval success until potential gaps in criteria are addressed. Finally, a score greater than the benchmark would suggest a higher probability of clinical adoption/FDA approval.

Chapter III.

Results

FDA Product Regulation

The individual sections of each regulated product type for Medical Devices, Cosmetics, and Radiation-emitting electronic products showed distinct differences from each other, with little overlap other than common topics that would be applicable across all regulated products. Table 3 shows a comparison of the overall product regulation criteria obtained from information available on the FDA website.

The regulatory oversight for cosmetic products is generally limited to the regulations outlined in the Federal Food, Drug, and Cosmetic Act, prohibiting the use of adulterated or misbranded products, and oversight of the labeling of cosmetics. (U.S. Food & Drug Administration, 2017). The overall requirements for cosmetic registration, pre-market requirements, inspections, reporting, and alerts to the general public are limited in comparison to other FDA-regulated products, as summarized in Table 3.

The FDA regulation of radiation-emitting products shows an increased oversight responsibility in comparison to cosmetics regulation (Table 3). The requirement of registration programs and a defined set of pre-market requirements based on performance standards provide a quality standard for the products. Additionally, mandatory recalls, safety communications, and FDA alerts show an increased communication requirement to the general public for radiation-emitting products in comparison to cosmetics. There

are certain requirements under radiation-emitting products that fall under the medical device oversight, including databases, establishment registration, and reporting.

In comparison to cosmetics and radiation-emitting products, the requirements for medical devices are the most comprehensive. In every category of FDA oversight, the medical device regulations require the highest level of registrations, compliance, and reporting. While comprehensive, the requirements and regulations are difficult to interpret from a public perspective. While in comparison to the cosmetics and radiation-emitting products, the medical device regulation appears thorough, a simplistic pathway may be needed to better streamline the review and oversight of medical device products, while maintaining the focus on compliance, quality, and performance.

There were few direct comparison mechanisms available from the FDA to review the various regulated products. However, common information and requirements criteria was found when a product was being imported (U.S. Food & Drug Administration, 2020). These requirements are summarized from the FDA import program and provided in Table 4.

While the importation of regulated products is outside of the scope of this review, the common requirements by the FDA provided a comparison route for the overall components of each product and may provide input on the important aspects of each product. Additionally, this route of comparison may provide a baseline template for future clarification and streamlining of overall FDA regulation instead of individual requirements by product type.

Table 3. Product regulation overview for Cosmetics, Radiation-emitting products, and Medical Devices.

	FDA Regulation	Registration	Pre-market Requirements	Inspections	Reporting	Databases	Alerts
Cosmetics	(1) Laws of the Federal Food, Drug, and Cosmetic Act (Adulterated, misbranded, exemptions) (2) Labeling	Voluntary	For color additive products only	Allowable at manufacturing sites	Not required; customer complaint reporting through MedWatch	CFSAN Adverse Event Reporting System (CAERS)	(1) Request of recall to company (2) Safety alerts
Radiation-emitting products	(1) Distribution of Products (2) Manufacturing or importing products	Initial Product Report Establishment Registration (applicable for Medical Device products)	(1) Product is compliant with performance standards (2) Adequate quality control and testing procedures (3) Certification of performance standard compliance (4) Product report submitted to FDA - CFR citation	For mammography products only - MQSA (Mammography Quality Standards Act)	(1) Accidental exposures (2) MDR regulations	Medical Device Database	(1) FDA Notices (2) Safety Communications (3) Recalls
Medical Devices	(1) Establishment registration (2) Medical Device Listing (3) Premarket Notification 510(k) (unless exempt), or Premarket Approval (4) Investigational Device Exemption (IDE) for clinical studies (5) Quality System (QD) regulation (6) Labeling requirements (7) Medical Device Reporting	Establishment Registration (Annual)	(1) Classification of device and applicable regulatory controls (2) Selection and Preparation of premarket submission reports (3) Premarket submission to FDA and FDA review (4) Compliance with regulatory controls and establishment of registration and device listings	For PMA devices: (1) Premarket Approval (2) Post market Inspections	(1) Mandatory reporting (Manufacturers, Importers, Device User Facilities) - Medical Device Reporting (MDR) (2) Voluntary reporting (health care professionals, patients, caregivers, consumers) - MedWatch	Appendix 3	(1) Safety Communications (2) Recalls (3) Letters to Health Care Providers (4) Medical Device Bans

Data collected and summarized from U.S. Food and Drug Administration (2020a).

Table 4. FDA Import Requirements for Medical Devices, Radiation-emitting products, and Cosmetics.

Summary of import requirements for three FDA-regulated products: Medical Devices, Radiation-emitting electronic products, and Cosmetics. Information obtained from U.S. Food and Drug Administration (2020b)

	Product Category (imports)		
	Medical Device	**Radiation-emitting electronic products**	**Cosmetics**
Responsible FDA Center	CDRH	CDRH	Office of Cosmetics and Colors
Definition	(1) an instrument, apparatus, implement, machine, contrivance, implant, in vitro reagent, or other similar or related article, including a component part or accessory which is: recognized in the official National Formulary, or the United States Pharmacopoeia, or any supplement to them (2) intended for use in the diagnosis of disease or other conditions, or in the cure, mitigation, treatment, or prevention of disease, in man or other animals, or (3) intended to affect the structure or any function of the body of man or other animals, and which does not achieve its primary intended purposes through chemical action within or on the body of man or other animals and which is not dependent upon being metabolized for the achievement of any of its primary intended purposes	Any electrically powered product that can emit any form of radiation on the electromagnetic spectrum	A product (excluding pure soap) intended to be applied to the human body for cleansing, beautifying, promoting attractiveness, or altering the appearance
Examples of products	Next-generation sequencing tests; implants; contact lenses; syringes	MRI; laser pointers; LEDs	Shampoo, make-up, face cream

Requirements verified at time of Importation	Registration Listing Premarket submission (510(k)/PMA) Labeling Import alert database	Import alert database Manufacturer Report accession number Model designation Annual report	Import alert database Ingredients Color additives (color identification number) Labeling Voluntary cosmetic registration
Method of verification of requirements	Comparison of entry declarations to FDA internal data systems	Comparison of entry declarations to FDA internal data systems	Field examinations Sample collection
Verification of Compliance	Declared Manufacturer Declared importer/consignee Product Description Affirmations of Compliance	Declared Manufacturer Product Description Affirmations of Compliance	Declared Manufacturer Declared importer/consignee Product Description Affirmations of Compliance
Additional Requirements	Premarket submission verification	Subject to Medical Device Regulations Form FDA-2877 for products subject to performance standards	Not specified

Regulation

The results of the PMA (FoundationOne CDx and Oncomine Dx Target Test) and 510(k) (MSK-IMPACT) information submitted for FDA review highlight the difference in regulation pathways of tests with companion diagnostic designations and those that do report claims (Table 5).

Table 5. Next-Generation Sequencing Product Regulation Summary Results

Summary data of the product regulation and FDA approval decisions for FoundationOne CDx, Oncomine Dx Target Test, and MSK-IMPACT FDA-approved tumor profiling next-generation sequencing test

	Medical Device		
	FoundationOne CDx	**MSK-IMPACT**	**Oncomine Dx Target Test**
FDA Identifier #	P170019	DEN170058	P160045
Date Received	6/2/2017	9/25/2017	10/17/2016
FDA Decision Date	11/30/2017	11/15/2017	6/22/2017
FDA Decision Duration (Days)	181	51	248
Product Code	PQP	PZM	PQP
Advisory Committee	Pathology	Pathology	Pathology
Classification	III	II	III
Regulation	21 CFR 801.109	21 CFR 866.6080	21 CFR 801.109

Although the three devices have similar descriptions and technical methods, the difference in product code are the result of the distinct device classification, resulting in the differences in timing of FDA approval decisions and overall federal regulations. The classification of the test is the first step in determining what the product code of the test

and the associated regulation pathway, and ultimately Code of Federal Regulation (CFR) adherences required. Additionally, the device classification consequently affects the time to approval of the device, with Class II devices having a significantly lower decision timeframe compared to Class III. The PMA approval process timeframe is designed to take six months, while the two currently approved FDA Class III NGS tests averaged 215 days, however, due to the limited number of tests, an estimate on timing is likely to be unique to the test specifications, validation study submissions, and the federal resources available for review.

The validation studies submitted as part of the PMA submission emphasize the breadth of validation required for approval. Due to the Class II designation for the MSK-IMPACT test, the amount of validation studies (Table 6) submitted for the 510(k) submission were significant less than the Class III tests. In general, the MSK-IMPACT test was largely evaluating the precision, accuracy, and limit of detect for the test.

Table 6. MSK-IMPACT 510(k) Validation Studies

Summary of the validation studies submitted to the FDA as part of the 510(k) submission package for the MSK-IMPACT Class II next-generation sequencing tumor profiling test.

MSK-IMPACT	Analytical Performance	Precision Studies
		Precision Panel
		Panel-Wide Reproducibility
		Per Specimen Precision
		Well-characterized reference material
		Microsatellite Instability
	Analytical Sensitivity	Limit of Detection (LoD)
		Dilution Series
		Confirmation of the LoD
		Microsatellite Instability
		DNA-Input
	Analytical Specificity	Interference
	Comparison Studies	Method Comparison
		Supplemental Method Comparison Study for Wildtype Calls
		Method Comparison for MSI Status
	Clinical Performance	Clinical Evidence Curation

The Oncomine Dx Target Test had a total of 10 Non-Clinical Laboratory validation studies, with more specific studies performed within each category. Additionally, for the support of companion diagnostic claims, there were three clinical validation studies performed (Table 7). Due to the mode of distribution of the Oncomine Dx Target Test, additional studies for the stability of the materials and transport of products were needed to demonstrate safety and effectiveness of the test.

Table 7. Oncomine Dx Target Test PMA Validation Study Summary

Oncomine Dx Target Test	Non-Clinical Studies	Laboratory Studies	Analytical Accuracy Analytical Sensitivity Limit of Blank Limit of Detection DNA/RNA Input Tissue Input Tumor Content Analytical Specificity Inclusivity/Cross-Reactivity Interference Endogenous Interference Exogenous Interference Anti-microbial Testing Precision and Reproducibility Assay Reproducibility Precision External Sample Processing Reproducibility Tissue Heterogeneity Extraction Method Equivalency DNA RNA Contrived Sample Functional Characterization Study Guard Band Studies Workflow Tolerances Tissue Fixation Study Contamination Stability Studies Shelf-Life Stability In-Use Stability Designated Hold Times Kit Lot Interchangeability Sample Stability - Extracted RNA and DNA Stored Slide Stability Stored Block Stability Transport Stability
		Animal Studies	Not Applicable

Clinical Studies	BRAF ROS1 EGFR	Study Design Accountability of PMA Cohort Study Population Demographics and Baseline Parameters Safety and Effectiveness Results

The FoundationOne CDx test validation studies (Table 8) were similar to the validation studies of the Oncomine Dx Target Test due to the requirements of a PMA submission, however FoundationOne CDx required additional clinical studies due to the increased number of companion diagnostic claims. Additionally, the validation for FoundationOne CDx required less stability studies due to the distribution type of a send out test versus a distributed kit.

Table 8. FoundationOne CDx PMA Validation Study Summary

FoundationOne CDx	Non-Clinical Studies	Laboratory Studies	Analytical Accuracy/Concordance Comparison to an Orthogonal Method Comparison to FoundationOne LDT Analytical Sensitivity Limit of Detection Limit of Blank Tumor Purity Analytical Specificity Interfering Substances Hybrid Capture Bait Specificity Carryover/Cross-Contamination Precision and Reproducibility Reagent Lot-to-Lot Reproducibility Instrument-to-Instrument Reproducibility Reagent Lot Interchangeability Stability Reagent Stability DNA Stability FFPE Slide Stability General Lab Equipment and Reagent Evaluation DNA Amplification DNA Extraction Guard banding/Robustness Tissue Comparability
		Animal Studies	Not Applicable
	Clinical Studies	EGFR ALK KRAS ERBB2 BRAF BRCA1/2	Study Design Concordance

Overall, there appears to be a minimum set of validation studies (Table 9) required for companion diagnostic next-generation sequencing tests, which would follow a Class III classification and require a PMA submission. The precise laboratory and clinical studies will depend on the specifications of the test and the proposed companion diagnostic claims.

Table 9. Overlap of PMA Validation Studies

Shared categories of validation studies for PMA submissions to the FDA between the FoundationOne CDx test and Oncomine Dx Target Test.

Validation Study Description
Analytical Accuracy
Analytical Sensitivity
Analytical Specificity
Interfering Substances
Precision and Reproducibility
Guard Band Studies
Lab Equipment/Method Evaluation Studies
Stability Studies
Tissue Comparability/Heterogeneity Studies
Contamination Studies
Clinical Studies for CDx Claims

Technical Specifications

The performance specifications of an NGS test define the analytical and technical components of the test. Through the analytical validation of the tests, each company has defined its performance. This material is the main component that is reviewed by the FDA upon submission. The turnaround time, laboratory hiring, and other operational aspects are part of the company, however in terms of the FDA oversight, the quality and

accuracy of the tests are the utmost importance. In this review, the technical specifications and summary of clinical validation studies were reviewed to define what the acceptable criteria for test specifications was for FDA approval. The test specifications will offer insight into the impact on an operations workflow needed to produce the assay specifications.

The results of the technical components of the currently FDA approved next-generation sequencing tests is summarized in Table 10. The methodology and preferred specimen type were the same across each test, being exclusively next-generation sequencing and formalin-fixed paraffin embedded (FFPE) respectively. Future FDA-approved tests may include other forms of testing along with next-generation sequencing, including whole genome sequencing (WGS), whole exome sequencing (WES), transcriptome sequencing, immunohistochemistry (IHC), and/or fluorescence in situ hybridization (FISH) testing. Likewise, the preferred specimen type may extend beyond the typical FFPE slide, and encompass liquid biopsies, frozen tissue, or other methodologies for collection and storage.

The number of genes tested was highest for the MSK-IMPACT test (N = 468) and lowest for the Oncomine Dx Target Test (N = 23). However, the Oncomine Dx Target Test boasted the lowest minimum DNA input (10 ng) and tumor content (10%). The minimum sample size was not specified for Oncomine Dx Target Test, most likely due to the alternate distribution mode (in-house, decentralized) opposed to FoundationOne CDx and MSK-IMPACT. The variant detection was most extensive for Foundation Medicine's FoundationOne CDx, including the typical variant calling (SNVs, Indels, CNVs, Rearrangements), in addition to several complex biomarkers (MSI, TMB, HRD). Both

Oncomine Dx Target Test and MSK-IMPACT had limitations in the variant detection in comparison to FoundationOne Dx, with Oncomine Dx having limited indel and copy number variation (CNV) calling, and no inclusion of complex biomarkers. Alternatively, although the MSK-IMPACT test includes biomarker (MSI) calling, upon FDA submission, did not include CNVs nor rearrangement calling in the IMPACT test. Taken together, there appears to be trade-offs made for lower input/sample size/tissue requirements for the variant detection results and number of genes interrogated.

Table 10. FDA-Approved NGS Tests - Summary of Technical Information.

Summary of technical criteria for the FoundationOne CDx, Oncomine Dx Target Test, and MSK-IMPACT NGS tests.

Test	**Methodology**	**Number of Genes (DNA)**	**Preferred Specimen Type**	**Minimum Sample Size**	**DNA input**	**Minimum Surface Area Tissue Requirements**	**Minimum Tumor Content**	**Variant Detection[1]**
Foundation Medicine FoundationOne CDx	Next-Generation Sequencing	324	Formalin-fixed paraffin embedded (FFPE)	10 unstained slides, 4–5 µm thick	≥ 50ng	25mm^2	20%	SNVs, Indels, CNVs, select Rearrangements, MSI, TMB, HRD (select)
ThermoFischer Oncomine Dx Target Test	Next-Generation Sequencing	23	Formalin-fixed paraffin embedded (FFPE)	Not specified	10ng	Not specified	10%	SNVs, deletions, select Rearrangements
MSK-IMPACT	Next-Generation Sequencing	468	Formalin-fixed paraffin embedded (FFPE)	5–20 unstained slides, 10 µm thick	100–250ng	Not specified	>10%	SNVs, Indels, MSI

[1]SNVs = Single-nucleotide variants; Indels = Short insertion/deletion events; CNVs = Copy Number Variation; MSI = Microsatellite Instability; TMB = Tumor Mutational Burden; HRD = homologous recombination deficiency

Performance Specifications

Each of the FDA-approved NGS tumor profiling tests had their own set of specific results based on the validation studies performed (summarized in Tables 6–8). However, the marketed and/or published material is often what is used for clinical adoption towards physicians or data results for biopharmaceutical partners to emphasize the performance of the test. Therefore, when available, the results of the performance tests, as presented in publicly available marking material, was used for the performance specification analyses. The performance results that are most commonly reported for performance specifications are concordance, accuracy, reproducibility, and limit of detection.

Oncomine Dx Target Test. The results of the Oncomine Dx Target Test concordance study demonstrate an overall percent agreement of 100% for BRAF V600E, 99% for EGFR alterations, and 96.5% for ROS1 between the Oncomine Dx Target test and the comparator methods (Figure 9).

Variants for therapy selection	Validated comparator methods	Excluding no-calls			Including no-calls		
		Positive percent agreement	Negative percent agreement	Overall percent agreement	Positive percent agreement	Negative percent agreement	Overall percent agreement
BRAF V600E	Validated *BRAF* V600E qPCR test	100% (67/67)	100% (114/114)	100% (181/181)	91.8% (67/73)	97.4% (114/117)	95.3% (181/190)
EGFR		98.6% (71/72)	99.2% (120/121)	99.0% (191/193)	81.6% (71/87)	96.8% (120/124)	90.5% (191/211)
EGFR exon 19 deletions	Validated *EGFR* PCR test	97.6% (41/42)	99.3% (147/148)	99.0% (188/190)	74.6% (41/55)	94.2% (147/156)	89.1% (188/211)
EGFR exon 21 L858R		100% (30/30)	100% (167/167)	100% (197/197)	93.8% (30/32)	93.3% (167/179)	93.4% (197/211)
ROS1 fusions	Validated *ROS1* FISH test	80% (20/25)	100% (119/119)	96.5% (139/144)	80% (20/25)	100% (119/119)	96.5% (139/144)

Figure 9. Oncomine Dx Target Test Concordance Results

Concordance results for the Oncomine Dx Target Test and comparator test methods, illustrated in the Oncome Dx Target Test brochure for laboratory professionals (ThermoFischer Scientific, 2017).

An accuracy study was performed using 290 FFPE tumor samples to demonstrate the ability of the Oncomine Dx Target Test to identify somatic variants in human patients (ThermoFischer Scientific, 2017). The results of the study are summarized in Figure 10 and demonstrate 96.8% and 100% overall percent agreement for exclusion and inclusion of no-calls respectively (ThermoFischer Scientific, 2017).

Variant level measure of agreement	Percent agreement (N) excluding no-calls	Percent agreement (N) including no-calls
Positive percent agreement	98.5% (195/198)	98.5% (195/198)
Negative percent agreement	100.0% (118,155/118,159)	96.8% (118,155/122,012)
Overall percent agreement	100.0% (118,350/118,357)	96.8% (118,350/122,210)

Figure 10. Oncomine Dx Target Test Accuracy Results.

Variant level accuracy study results for the Oncomine Dx Target Test as demonstrated in the laboratory professional brochure (ThermoFischer Scientific, 2017).

Upon further inspection of the Summary of Safety and Effectiveness data submitted to the FDA during PMA submission, the overall accuracy results are a summary of the four variant bin types: simple SNV, complex SNV, deletion, fusion. The results of the analytical validation separated by bin types are presented in Table 11, highlighting the sensitivity differences across the variant types.

Table 11. Oncomine Dx Target Test Accuracy Results by Bin Type.

Results of the analytical validation results for test accuracy for the Oncomine Dx Target Test, separated by variant bin type. From the Summary of Safety and Effectiveness Data (Life Technologies Corporation, 2017).

Bin	Agree-ment	No Calls Included			No Calls Excluded		
		# Comp	# ODx	% (95% CI)	# Comp	# ODx	% (95% CI)
Simple SNV	PPA	83	82	98.80% (93.47%, 99.97%)	83	82	98.80% (93.47%, 99.97%)
	NPA	200	65	32.50% (26.06%, 39.47%)	206	206	100.00% (98.23%, 100.00%)
Complex SNV	PPA	85	83	97.65% (91.76%, 99.71%)	85	83	97.65% (91.76%, 99.71%)
	NPA	203	82	40.39% (33.58%, 47.49%)	204	202	99.02% (96.50%, 99.88%)
Deletion	PPA	11	11	100.00% (71.51%, 100.0%)	11	11	100.00% (71.51%, 100.0%)
	NPA	278	252	90.65% (86.60%, 93.80%)	276	276	100.00% (98.67%, 100.0%)
Fusion	PPA	2	0	0.00% (0%, 84.2%)	2	0	0.00% (0%, 84.2%)
	NPA	258	258	100.00% (98.6%, 100.0%)	258	258	100.00% (98.6%, 100.0%)

The assay reproducibility test for the Oncomine Dx Target test was performed for 30 representative variants (18 DNA/9 RNA samples) and was designed to evaluate the repeatability and reproducibility of the test across runs, operators, sites, and instruments. The study demonstrated 95–99% performance for the mean call rates across the variant calls and are summarized in Figure 11 (ThermoFischer Scientific, 2017).

Description	No. of variant samples	Call rate excluding no-calls		Call rate including no-calls	
		Mean	Median	Mean	Median
DNA positive variants (positive calls)	46	96.60%	97.10%	94.50%	95.80%
RNA positive variants (positive calls)	6	94.80%	95.50%	94.80%	95.50%
WT DNA variant locations (negative calls)	872	96.10%	95.00%	96.10%	95.00%
WT DNA variant locations (negative calls)	170	99.30%	99.30%	99.30%	99.30%

Figure 11. Oncomine Dx Target Test Reproducibility Results.

Summary results of the Oncomine Dx Target test reproducibility validation study (ThermoFischer Scientific, 2017).

Finally, the limit of detection (LoD) study for the Oncomine Dx Target Test was performed on 26 specimens (19 FPPE tissue samples and 7 plasmid constructs) with representative variants in the categories of base substitutions, multi-nucleotide polymorphisms (MNP), short variant deletions, and RNA fusion variants (Life Technologies Corporation, 2017). The validation study demonstrated a limit of detection (LOD) of 6–13% for short variant events (base substitutions and indels) and 732 reads for RNA fusion events (Table 12, ThermoFischer Scientific, 2017).

Table 12. Oncomine Dx Target Test LoD Results

Gene	Variant	Variant Category	LoD[1] (%AF or # Reads)
BRAF	V600E	SNV	12% AF
EGFR	L858R	SNV	8% AF
EGFR	Ex. 19del	Deletion	6% AF
ROS1	ROS1 Fusion	RNA Fusion	732 Reads

[1]Clinical specimens were tested for all variants for which clinical claims are being sought.

MSK-IMPACT Test. Due to the lack of marketing material for the MSK-IMPACT test, the results of the main validation tests from the IMPACT test were retrieved from the FDA 510(k) submission.

To assess the accuracy of the MSK-IMPACT test, precision studies were performed using 10 samples, representing a variety of tumor types, mutation types, and allele frequencies (Memorial Sloan Kettering, 2017). For the panel-wide reproducibility study, there was a total of 82 mutations tested and demonstrated 100% concordance across replicates for all but seven mutations, due to issues of poor mapping quality in highly repetitive regions of the genome, or frequencies near the 2% allele frequency limit (Memorial Sloan Kettering, 2017). Therefore, the overall panel-wide precision was 75/82 (91.5%). Additionally, the per specimen precision was reported for the 10 samples. The results of the study are presented in Table 13, from the 510(k) submission for the IMPACT test. Since the MSK-IMPACT test was not intended for companion diagnostic use, concordance with a comparator testing method was not tested for the MSK-IMPACT initial FDA submission.

Table 13. MSK-IMPACT Precision Results.

Per specimen precision across all replicates for the MSK-IMPACT validation study.

Specimen	Total No unique mutations detected across all 5 replicates*	*Positive call rate per mutation	Positive call rate* (two-sided 95% CI)	Negative call rate (two-sided 95% CI)
M15-22924	5	5/5 for all	25/25 100.0% (86.3%, 100.0%)	-
M15-3038	3	5/5 for all	15/15 100.0% (78.2%, 100.0%)	-
M16-19000	10	5/5 for 9 4/5 for 1	49/50 98.0% (89.4%, 99.9%)	-
M1688-5C	18	5/5 for 17 1/5 for 1	86/90 95.6% (89.0%, 98.8%)	4/5 80.0% (28.4%, 99.5%)
M-1698-A9	5	5/5 for all	25/25 100.0% (86.3%, 100.0%)	-
M-1654-CA	6	5/5 for all	30/30 100.0% (88.4%, 100.0%)	-
M-1612-28	4	5/5 for all	20/20 100.0% (83.2%, 100.0%)	-
M1648-D5	10	5/5 for all	50/50 100.0% (92.9%, 100.0%)	-
M-1707-12	5	5/5 for 3 3/5 for 1; 2/5 for 1	20/25 80.0% (59.3%, 93.2%)	3/5 60.0% (14.7%, 94.7%)
Commercial sample	13	5/5 for 10; 4/5 for 1; 3/5 for 1; 2/5 for 1	59/65 90.8% (81.0%, 96.5%)	3/5 60.0% (14.7%, 94.7%)

*Positive call rate is calculated based on variants with majority call detected as positive
#Negative call rate is calculated based on variants detected at least once, but with majority call as negative. For all other locations, the negative call rates are 100%.

The limit of detection validation test was performed using a two-part dilution series, initially on 10 normal FFPE samples with five to eight dilution series, and then confirmed in a total of five replicates for each category using a 5% allele frequency cutoff (Memorial Sloan Kettering, 2017). The results of the LoD study show a lower bound of 2% allele frequency, with confirmation of calls using a 5% cutoff.

FoundationOne CDx Test. The FoundationOne CDx test performed multiple concordance studies, precision studies, sensitivity studies, as summarized previously in Table 8. For the concordance studies, the FoundationOne CDx test was validated against an externally validated NGS assay using 188 samples from 46 different tumor types (Foundation Medicine, Inc., 2017). The results of the NGS comparator study show and overall concordance rate of 94.6% PPA and 99.9% NPA, as summarized in Figure 12.

	POSITIVE-PERCENT AGREEMENT (PPA*)	NEGATIVE-PERCENT AGREEMENT (NPA*)
All short variants	94.6%	99.9%
Substitutions	96.6%	99.9%
Indels	83.4%	99.9%

Figure 12. FoundationOne CDx Concordance Results.

Results of the concordance study between FoundationOne CDx and an externally validated NGS test, summarized in Foundation Medicine marketing material (Foundation Medicine, Inc., 2018a).

In addition to concordance with an externally validated NGS assay, a concordance study between FoundationOne CDx and various comparator methods were performed for each of the clinical companion diagnostic claims. The summarized results of the comparator concordance studies are shown in Figure 13, demonstrating between 89.4–100% concordance for PPA and 86.1–100% concordance for NPA.

BIOMARKER	POSITIVE-PERCENT AGREEMENT (PPA)*	NEGATIVE-PERCENT AGREEMENT (NPA)	COMPARATOR METHOD†
EGFR Exon 19 Deletions and L858R	**98.1%** (106/108)	**99.4%** (153/154)	cobas® *EGFR* Mutation Test v2
EGFR T790M	**98.9%** (87/88)	**86.1%** (93/108)	cobas® *EGFR* Mutation Test v1 cobas® *EGFR* Mutation Test v2
ALK Rearrangements	**92.9%** (78/84)	**100%** (75/75)	Ventana *ALK* (D5F3) CDx Assay Vysis *ALK* Break-Apart FISH Probe Kit
KRAS	**100%** (173/173)	**100%** (154/154)	therascreen® *KRAS* RGQ PCR Kit
ERBB2 (HER2) Amplifications	**89.4%** (101/113)	**98.4%** (180/183)	Dako HER2 FISH PharmDx® Kit
BRAF V600 *BRAF* V600E	**99.4%** (166/167) **99.3%** (149/150)	**89.6%** (121/135)‡ **99.2%** (121/122)	cobas® *BRAF* V600 Mutation Test
BRAF V600 dinucleotide§	**96.3%** (26/27)	**100%** (24/24)	THxID® *BRAF* kit

Figure 13. FoundationOne CDx Biomarker Concordance Results.

Summarized results of the biomarker concordance studies between FoundationOne CDx and comparator methods (Foundation Medicine, Inc., 2018a).

The FoundationOne CDx precision and reproducibility studies were performed using a total of 47 samples and 717 alterations tested (Foundation Medicine, Inc., 2017). The results showed >99% for the PPA and NPA for the platform level study (all variants, Table 14). Within the assessment of repeatability and reproducibility for companion diagnostic alterations, PPA and NPA was 100% concordant (Foundation Medicine, 2017).

Table 14. FoundationOne CDx Reproducibility Results.

Summary results of the FoundationOne CDx reproducibility variant-bin study (Foundation Medicine, 2017).

Variant Bin	# of Variants	# of valid Comparisons	# of Agreements	Positive Percent Agreement	95% CI Lower Limit	95% CI Upper Limit
CNAs	68	67,524	67,300	99.67%	99.62%	99.71%
Rearrangements	18	17,874	17,851	99.87%	99.81%	99.92%
Substitutions	443	439,899	439,649	99.94%	99.94%	99.95%
Indels	188	186,684	186,319	99.80%	99.78%	99.82%
All Variants	717	711,981	711,119	99.88%	99.87%	99.89%

Focusing on the short variant analyses, the limit of detection studies for both specific companion diagnostic claims (Table 15), and overall platform results for all short variants (Table 16) were performed (Foundation Medicine, Inc., 2017).

Table 15. FoundationOne CDx LoD for CDx variants

Limit of Detection (LoD) results for the FoundationOne CDx test companion diagnostic short variant claims (Foundation Medicine, Inc., 2017).

Alteration	LoD[1] Allele Fraction (%) (100% Hit Rate)	LoD[2] Allele Fraction (%) (Probit)
EGFR L858R	2.4%	< 2.4% (all detected)
EGFR Exon 19 deletion	5.1%	3.4%
EGFR T790M	2.5%	1.8%
KRAS G12/G13	2.3%	< 2.3% (all detected)
BRAF V600E/K	2.0%	< 2.0% (all detected)
PIK3CA E542K	4.9%	Not Calculated
BRCA1/2[3] Alteration in non-repetitive or homopolymer <4 bp Deletion in 8 bp homopolymer	 N/A N/A	 5.9% 15.3%

[1] LoD calculations for the CDx variants were based on the hit rate approach, as there were less than three levels with hit rate between 10% and 90% for all CDx variants (not including *BRCA*1/2 variants). LoD from the hit rate approach is defined as the lowest level with 100% hit rate (worst scenario).
[2]LoD calculations for the CDx variants based on the probit approach with 95% probability of detection.
[3]See Summary of Safety and Effectiveness Data for P160018.

Table 16. FoundationOne CDx Platform Variant LoD Results.

Limit of Detection (LoD) results for the FoundationOne CDx test for all short variant categories (Foundation Medicine, Inc., 2017).

Variant Category	Subcategory	N	Range LoD[1] Allele Fraction (%)
Base Substitutions	known[3]	21[2]	1.8-7.9[2]
	other[4]	166	5.9-11.8
Indels at non-homopolymer context, including insertions up to 42bp and deletions up to 276bp	Known	3	4.5-6.5
	Other	17	6.0-10.2
Indels at homopolymer context	5bp repeat	8	10.0-12.2
	6bp repeat	2	13.6-13.7
	7bp repeat	4	16.3-20.4
	8bp repeat	3	17.0-20.0

[1]LoD calculations for the platform variants were based on the hit rate approach for variants with less than three levels with hit rate between 10% and 90% and probit approach for variants with at least three levels with hit rate between 10% and 90%. LoD from the hit rate approach is defined as the lowest level with 100% hit rate (worst scenario).
[2]Data includes an alteration in the *TERT* promoter, 124C>T (LoD of 7.9%). *TERT* is the only promoter region interrogated and is highly enriched for repetitive context of poly-Gs, not present in coding regions.
[3]Alterations classified as" known" are defined as those that are listed in COSMIC
[4]Alterations classified as "other" include truncating events in tumor suppressor genes (splice, frameshift and nonsense) as well as variants that appear in hotspot locations but do not have a specific COSMIC association, or are considered variants of unknown significance (VUS) due to lack of reported evidence and conclusive change in function.

The results of the LoD studies, with the specific focus on short variant events, demonstrate a 1.8% allele frequency LoD for base substitutions and 4.5% LoD for non-homopolymer indel events (Foundation Medicine, Inc., 2017).

Operational Considerations

Operational Specifications. Turnaround time and mode of distribution of a next-generation sequencing test are two of the main components and considerations in the output or processes needed for a test. Additionally, the type of sequencer(s) used in the workflow may prove to have a high impact on both the workflow and/or turnaround time, especially as the efficiencies and data generation of the technology continues to improve. A summary of the published turnaround time, sequencer type, and distribution mode of the commercially available tests are summarized in Table 17. Oncomine Dx Target Test

and FoundationOne CDx turnaround times were obtainable through published material on their respective websites, however MSK-IMPACT turnaround time was not readily available. The result of MSK-IMPACT turnaround time was obtained through a review of the work performed by Sabari et. al. (2019).

Table 17. FDA-Approved NGS Tests - Operational Specifications Results

Turnaround time, sequencer type, and distribution mode for FoundationOne CDx, Oncomine Dx Target Test, and MSK-IMPACT tests.

Company	Test	TAT (Days)	Sequencer Type	Distribution Mode
Foundation Medicine	FoundationOne CDx	<14	Illumina HiSeq 4000	Send out
ThermoFischer Scientific	Oncomine Dx Target Test	4	Ion PGM Dx	In-House (Kit)
Memorial Sloan Kettering	IMPACT	20	Illumina HiSeq 2500	Single site (In-House)

The minimum turnaround time for the current FDA approved tests is four days on the Oncomine Dx Target Test. The main difference between the Oncomine Dx Target Test and Foundation Medicine's FoundationOne CDx Test and MSKCC IMPACT test is the distribution mode being an in-house distributable kit versus a send out test. Therefore, the turnaround time for the Oncomine Dx Target Test only accounts for the workflow of the products supplied by ThermoFischer Scientific, any additional workflows created or maintained by the institution performing the workflow would be outside of the scope of the Oncomine Dx Target Test. Additionally, the type of sequencers between the tests differs, however the average sequencing time and output between Illumina and the Ion PGM Dx systems were not evaluated during this review but may contribute to the differences in overall turnaround time.

Test Workflows. The operational components of a test offer insight into the workflows and departments that may be a part of a particular test. The test workflow often defines the turnaround time, scalability needs, and quality components of a product. Therefore, as the first investigation into the operational aspects of the current FDA-approved tests, the laboratory, analytical, and reporting workflows were evaluated.

The Oncomine Dx Target Test is marketed as a four-day workflow, which includes laboratory preparation, analysis using the Ion PGM Dx sequencers, and an automated report generation (Figure 14).

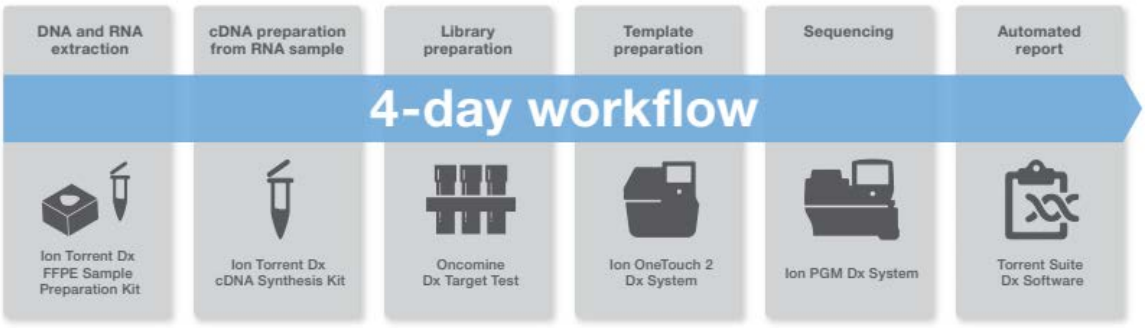

Figure 14. Oncomine Dx Target Test Workflow.

Workflow depiction for the Oncomine Dx Target Test (ThermoFischer Scientific, 2017).

One of the key characteristics of the Oncomine Dx Target Test workflow is that the test is performed in-house, meaning that the ordering physician must have the ability to perform each step in the workflow (laboratory preparatory steps and sequencing) at their own facility. Therefore, the operational components of the workflow are dependent on the infrastructure and hiring considerations of the facility instead of ThermoFischer. Due to the technical requirements for distribution of the product, the Oncomine Dx Target Test

workflow provides a detailed and technical outline of the workflow. The company does provide resources for the initiation of the workflow in the facility and is thoroughly documented in the available test kit user guides, which are supplied with the kits and available on the FDA PMA database as part of the device labeling.

Contrary to the Oncomine Dx Target Test, the Foundation Medicine FoundationOne CDx test is a send-off NGS test, comprised of a similar workflow in a 2-week timeframe (Figure 15), with an emphasis on coordination with the patients' doctors.

Figure 15. FoundationOne CDx Test Workflow.

General workflow for the Foundation Medicine FoundationOne CDx test from the Foundation Medicine, Inc. (2020) resources page.

The workflow depicted on the marketing material for the FoundationOne CDx test shows the process from initial doctor consultations through the testing process, ending with review of test results with the physician. In contrast to the Oncomine Dx Target Test workflow, the technical components of the testing process are not present in the FoundationOne CDx image. Possibly due to the distribution mode of the test, or to appeal to a more general community doctor viewpoint, the details of the operational process are more high level and include the pre and post technical analysis steps in the process. Additionally, the 2-week testing timeline is highlighted to show this timing is when Foundation Medicine receives the test through to when the doctor receives the results, excluding timing that may be necessary for initial consultation and patient follow-ups.

The sample process workflow for the MSKCC IMPACT test is not marketed for external parties nor the general public due to the limited availability of the product outside of the hospital. Despite the lack of marketable material, the IMPACT test has been reviewed by researchers and the workflow is summarized in Figure 16 (Hyman et al., 2015).

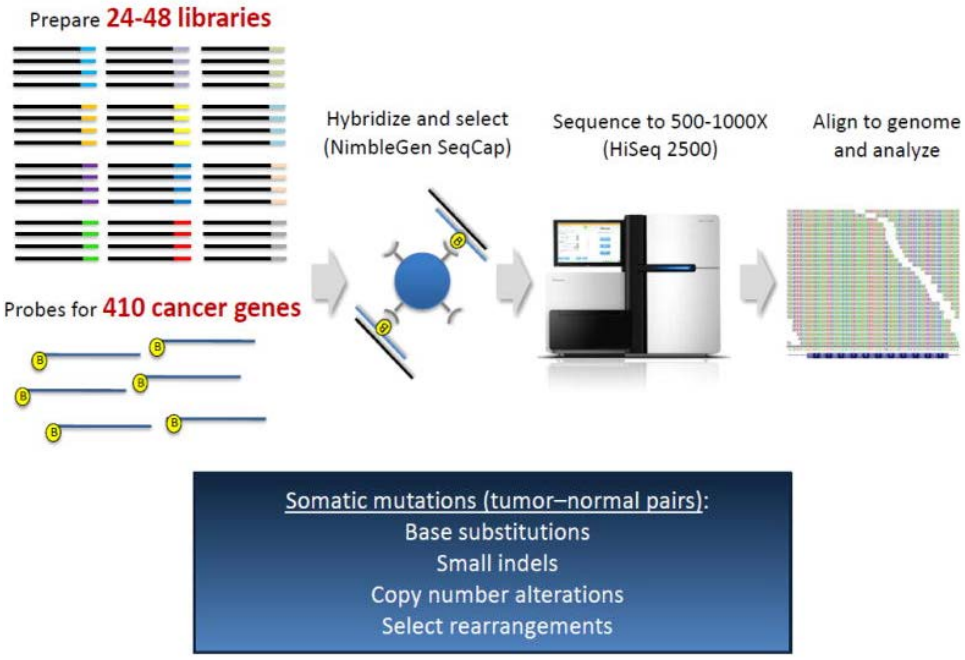

Figure 16. MSK-IMPACT Test Workflow.

Schematic overview of the MSK-IMPACT test summarized by Hyman et al. (2015).

Similarly to the Oncomine Dx Target Test, the workflow summarized in Hyman et al.'s (2015) review, focused on the technical aspects of the process, in contrast to the Foundation Medicine workflow depiction, which incorporated process steps about the physician and patient interactions. While the technical workflow of the FoundationOne CDx test can be summarized in similar workflows as the Oncomine Dx Target Test and the MSK-IMPACT test, the marketed material chosen to depict the testing workflow of the FoundationOne CDx test may shed light into the targeted physician and patient populations. The MSK-IMPACT test is an in-house test for use only at the Memorial Sloan Kettering Cancer Center, and therefore could only be used by physicians at that medical facility. Likewise, the Oncomine Dx Target Test is for use at medical facilities

with the capabilities of sequencing technologies. However, the FoundationOne CDx test, through its send out testing distribution mode, is marketed toward physicians who do not have current access to sequencing technologies or an FDA-approved test on site. In future review of non-FDA approved NGS tests, the distribution mode of the test may determine how the operational workflow is depicted to the targeted audience.

Clinical Report. One of the major, if not the most important outputs of each test, is the final medical report that is aggregated, interpreted, and transmitted to the physicians. For other business considerations, including pharmaceutical partnerships, the emphasis on the clinical report may be less on the clinical report than the available data types and applications. However, for the sake of an operational viewpoint and the impact on the physicians, the final medical report is a main consideration for physicians. Assuming the performance of the product is high quality and well tested, from an operational perspective, the display and transmission route of the report is an important factor for each test.

The clinical test report for the Oncomine Dx Target Test, as shown in a snapshot of the first page of the report (Figure 17), has three main sections, biographical and sample details, companion diagnostic results, and sequencing results for non-CDx variants.

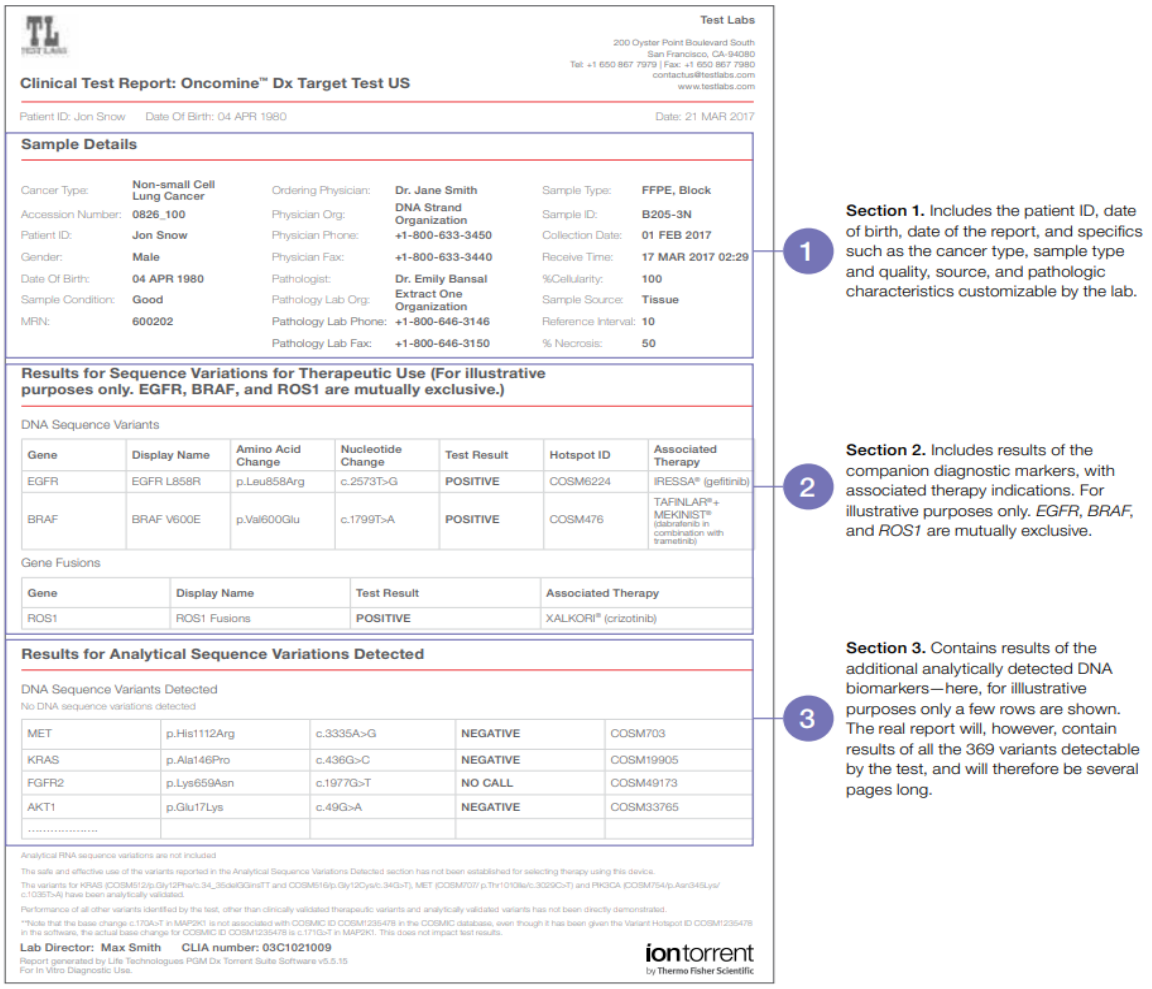

Figure 17. Oncomine Dx Target Test Report.

Screenshot of a sample report for the Oncomine Dx Target Test (ThermoFischer Scientific, 2017).

The layout of the report is in simple font with limited color, and headers and font changes to highlight the different sections of the report. Additionally, the report shows a test result call (Positive, Negative, No Call) for every mutation tested. Due to the comprehensive reporting of each variant call tested, the length of a report is expected to be at least 11 pages, as deduced from a full Oncomine Dx Target Test sample report. A snapshot of a

page from the report, showing the specific test results by gene/mutation is provided in Appendix 3.

The clinical test report for the FoundationOne CDx test has two main sections, an FDA-Approved Content page (Figure 18), with relevant FDA approved therapies and a list of non-CDx variants detected, and a Professional Services page (Figure 19).

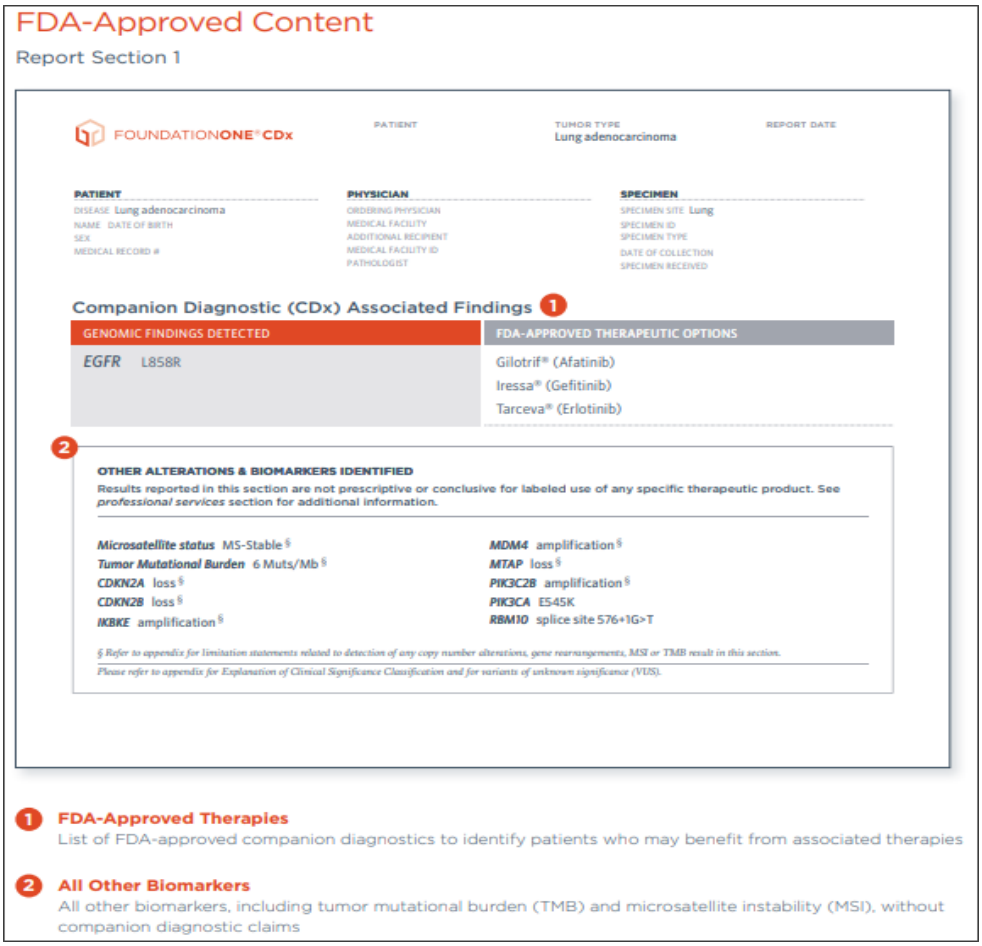

Figure 18. FoundationOne CDx Companion Diagnostic Report.

FDA-Approved Content page on a sample report for the FoundationOne CDx test (Foundation Medicine, Inc., 2019).

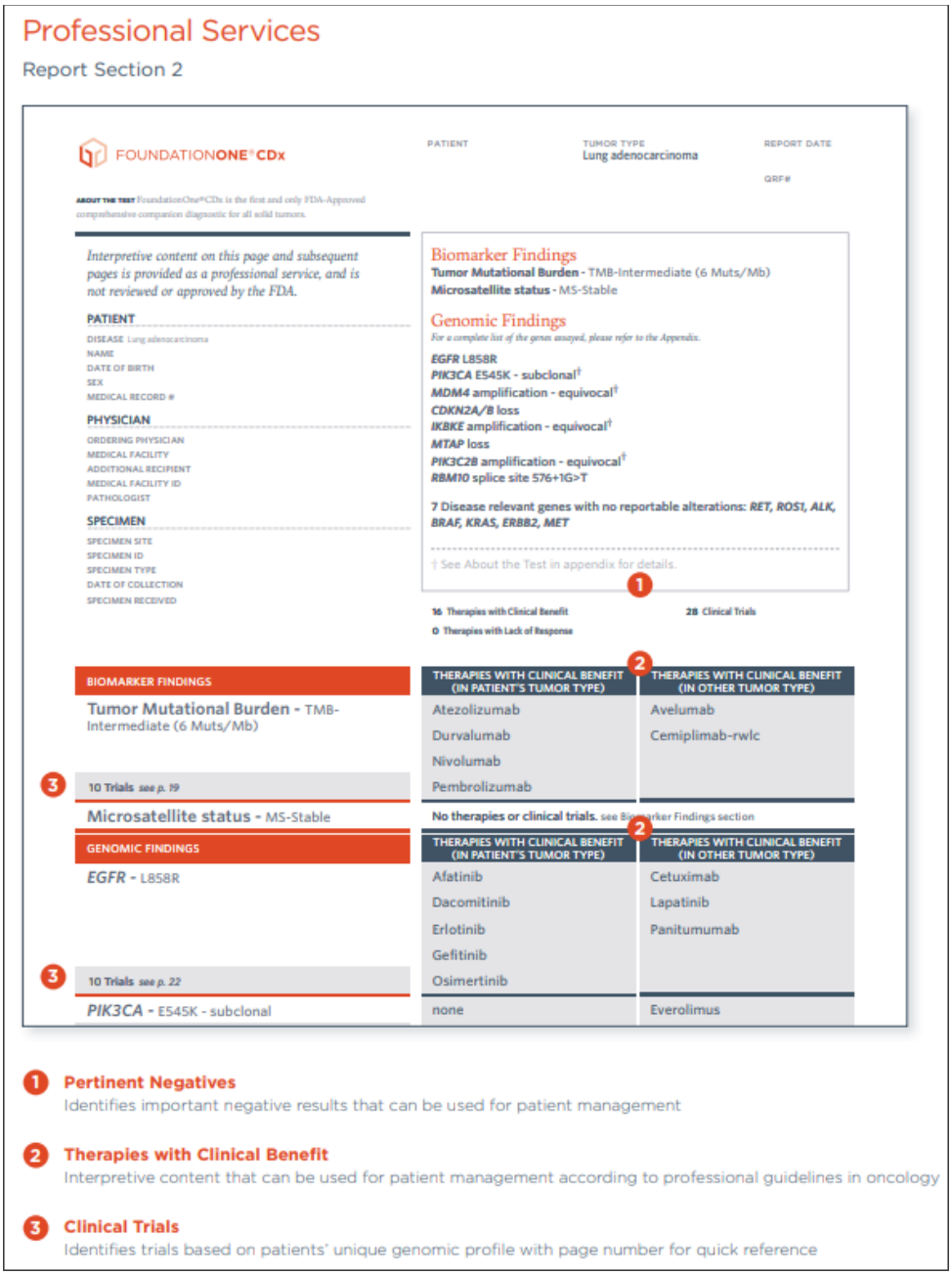

Figure 19. FoundationOne CDx Professional Services Report.

Professional Services page on a sample report for the FoundationOne CDx test (Foundation Medicine, Inc., 2019).

Both main pages on the FoundationOne CDx report contain overall patient and specimen information, as well as variant information, albeit for use in different contexts. The FDA-Approved Content page highlights the companion diagnostic variant calls and associated therapies. Meanwhile, the Professional Services page specifically calls out pertinent negatives, therapies with clinical benefit, and clinical trials. Alike to the Oncomine Dx Target Test report, font changes are used to highlight key pieces of information for the physician and patient. Alternatively, the FoundationOne CDx sample report shows usage of color, shading, and boxed sections to divide the report. The FoundationOne CDx report does not appear to call out each variant detected as the Oncomine Dx Target Test does, however, a comprehensive sample report was not able to be obtained through publicly available material.

Due to lack of publicly available information, the clinical test report for the MSK-IMPACT test could not be obtained. The IMPACT test is a test result within a single site hospital and is not a marketed test for outside physicians or biopharmaceutical partnerships.

Overall, the two evaluable clinical reports showed similarities of overall patient and sample information, variants associated with companion diagnostic claims, and other, non-CDx mutations detected. There are differences in the presentation and usage of color/shading, which would require further investigation on physician preference to the styling. A major difference between the Oncomine Dx Target Test and the FoundationOne CDx report was the comprehensive list of all mutations tested and their mutational status on the Oncomine Dx Target Test report. This difference may relate back to the technical specifications of each test, in that the Oncomine Dx Target Test

baits for 23 genes and specific mutations within those genes, whereas the FoundationOne CDx test covers 324 genes and a more comprehensive variant detection method. Therefore, it would seem unreasonable to specifically call out mutations for a test with a high number of genes tested and extensive variant calling. Finally, through various marketing campaigns, it is reasonable to assume the input from physicians and patients on the clinical report is being continually requested by companies. Therefore, today's clinical report for each company is likely to change over time with the feedback received from both the FDA and the consumers of the report.

Future Candidates for FDA Approval

Technical Specifications

Using the same criteria collected for the FDA approved next-generation sequencing tests, the technical specifications for 12 currently commercially available, non-FDA approved next-generation sequencing tests were collected and summarized in Table 18. For ease of comparison, the current FDA approved tests are included.

Table 18. Technical Specifications Summary

Technical specifications for 12 commercially available tumor profiling oncology tests. The three currently FDA-approved NGS tests (FoundationOne CDx, Oncomine Dx Target Test, MSK-IMPACT) are included for comparison. Technical and specimen information was obtained from publicly available company websites, retrieved from August through December 2019.

Test	**Methodology**	**Number of Genes (DNA/RNA)**	**Preferred Specimen Type[1]**	**Minimum Sample Size**	**DNA input**	**Minimum Surface Area Tissue Requirements**	**Minimum Tumor Content**	**Variant Detection[2]**
Foundation Medicine FoundationOne CDx	NGS	324	FFPE	10 unstained slides, 4–5 µm thick	≥ 50ng	25mm^2	20%	SNVs, Indels, CNVs, select Rearrangements, MSI, TMB, HRD (select)
ThermoFischer Oncomine Dx Target Test	NGS	23	FFPE	Not specified	10ng	Not specified	10%	SNVs, deletions, select Rearrangements
MSK-IMPACT	NGS	468	FFPE	5–20 unstained slides, 10 µm thick	100–250ng	Not specified	>10%	SNVs, Indels, MSI
Archer VariantPlex for Solid Tumors	NGS	67	FFPE	Not specified	≥ 10ng	Not specified	Not specified	SNVs, Indels, CNVs
Ashion GEM ExTra	Whole Exome/Transcriptome Sequencing	>190000	FFPE	10 unstained slides, 4 µm thick	≥ 150ng	25mm^2	Not specified	SNVs, Indels, CNVs, select Rearrangements, MSI, TMB
Caris Molecular Intelligence MI Tumor Seek	NGS	592/22000	FFPE	10 unstained slides, 4 µm thick	Not specified	25mm^2	20%	SNVs, Indels, CNVs, select Rearrangements, MSI, TMB
Illumina TruSight Oncology 500	NGS	523/55	FFPE	5 unstained slides	40ng	0.65mm^3	Not specified	SNVs, Indels, CNVs, Fusions, MSI, TMB

Integrated Oncology OmniSeq Comprehensive Assay	NGS	144	FFPE	10 unstained slides, 5 µm thick	1–30ng	25mm^2	Not specified	SNVs, Indels, CNVs, select Rearrangements
Kew Cancerplex	NGS	435	FFPE	10 unstained slides, 5 µm thick	>50ng	25mm^2	>20%	SNVs, Indels, Rearrangements, CNVs, MSI, TMB
NantHealth GPS Cancer	Whole Genome Sequencing	>190000	FFPE	10–14 unstained slides, 10 µm thick	Not specified	25mm^2	25%	SNVs, Indels, CNVs (highly expressed amplifications only), TMB, MSI
NeoGenomics NeoType Discovery	NGS+IHC+FISH	336	FFPE	5–10 unstained slides, 5 µm thick	Not specified	Not specified	Not specified	SNVs, Indels, Rearrangements, MSI, TMB, Other (Not Specified)
OncoDNA OncoDeep	NGS+IHC	313	FFPE	25 slides, 5 µm thick	Not specified	Not specified	Not specified	SNVs, Indels, IHC, MSI, select biomolecular tests ("Package Plus")
Paradigm PCDx	NGS+IHC	234	FFPE	6–10 slides, 10 µm thick	20–75ng	3mm^2/75mm^2	>=15%	SNVs, Indels, amplifications, TMB, IHC (non-NGS)
Sema4 Solid Tumor Panel	NGS	161	FFPE	5 unstained slides, 10 µm thick	200ng	>10mm^2	>=10%	SNVs, Indels, CNVs, select Rearrangements, MSI
Tempus xT	NGS	648	FFPE	10 unstained slides, 5 µm thick	Not specified	5mm^2/25mm^2	20%	SNVs, Indels, CNVs, select Rearrangements, MSI, TMB

[1]Formalin-fixed paraffin embedded

[2]SNVs = Single-nucleotide variants; Indels = Short insertion/deletion events; CNVs = Copy Number Variation; MSI = Microsatellite Instability; TMB = Tumor Mutational Burden; HRD = homologous recombination deficiency

Overall, the range of number of DNA genes tested (67–648), DNA input (1–200ng), and variants types detected, differed widely across all the evaluated non-FDA approved tests, with specific focus on tests using next-generation sequencing methods for primary detection. However, the minimum sample size and minimum tumor content largely remained constant across tests, with a minimum sample size of 5–10 slides (5 microns thick) and a typical tumor content of 10–20%, suggesting these inputs are currently the lower bounds for the technology. There appears to be a trade-off between variant detection and required DNA input and/or tumor content. When the DNA input or tumor content was specified, the test typically required a higher DNA input and/or tumor content in order for the detection of additional variant classes. Unfortunately, due to lack of FDA submission material or specified specimen requirement information, the DNA input, minimum surface area, and minimum tumor content were not specified for several tests and therefore did not allow for full interpretation of the products.

Performance Specifications

The performance specifications for two commercially available, non-FDA approved next-generation sequencing tests were evaluated and compared to the FDA-approved tests. Due to the lack of full validation study access, or lack of testing at this time, there were no publicly available information on concordance or precision validation studies for either the Caris Molecular Intelligence MI Tumor Seek test or the Tempus xT test. The information publicly available at this time is the accuracy of each test for positive and negative percent agreements.

The Caris Molecular Intelligence MI Tumor Seek test reports a PPA of >95% for base substitutions, >95% for indels, and >90% for copy number alterations tested through DNA sequencing (Caris Molecular Intelligence, 2019). The NPA, presumably for all variant types, is >99%. The LoD is reported at ≥ 5% allele frequency for both base substitutions and indel events. The full results of the performance specifications are shown in Figure 20.

Technical Information	Next-Generation Sequencing (DNA)	Whole Transcriptome Sequencing (RNA)
Sample Requirements	FFPE block or 10 unstained slides with a minimum of 20% malignant origin for DNA and 10% malignant origin for RNA. Needle biopsy is also acceptable (4-6 cores).	
Tumor Enrichment (when necessary)	Microdissection to isolate and increase the number of cancer cells to improve test performance and increase the chance for successful testing from small tumor samples	
Number of Genes	592 genes	~22,000 genes
Average Depth of Coverage (DNA) Average Read Count (RNA)	>750X	60 million
Positive Percent Agreement (PPA)	> 95% for base substitutions at ≥ 5% mutant allele frequency; > 95% for indels at ≥ 5% mutant allele frequency; >90% for copy number alterations (amplifications ≥ 6 copies)	>97%
Negative Percent Agreement (NPA)	>99%	>99%
Genomic Signatures	Microsatellite Instability (MSI), Tumor Mutational Burden (TMB) MI GPS™ (Genomic Profiling Similarity) Score	–

Figure 20. Caris Molecular Intelligence Technical Specifications.

Technical information for the Caris Molecular Intelligence MI Tumor Seek test (Caris Molecular Intelligence, 2019).

The Tempus xT test reports a 96.6% sensitivity and 99.95% specificity for single nucleotide variant calls at 5% allele frequencies. For indel events, with a LoD of 10%, the sensitivity is 93.4% and specificity of 99.9%. Short variant events as a variant bin (base substitutions + indels) were not reported in current (2020) validation material. The results

of the Tempus accuracy study are presented in Figure 21 from Tempus's (2020) performance specifications validation material.

Variant Class	Limit of Detection	Sensitivity (%)	Specificity (%)
Single Nucleotide Variants	5% VAF	96.6	99.95
Insertions and Deletions	10% VAF	93.4	99.99
Copy Number Alterations	30% tumor purity; loss—0 copies; gain—8 copies	94.7	99.99
Rearrangements/Fusions*	10% tumor purity	99.9	99.99
Microsatellite Instability Status	30% tumor	99.9	99.9

*Utilizing both DNA and RNA sequencing

Figure 21. Tempus xT Performance Specifications.

Performance specifications (version 3) for the Tempus xT next-generation sequencing assay (Tempus, 2020).

Tempus (2020) material on the performance specifications of the xT test separate the performance of SNVs and indel events. Previous validation material from Tempus (2019b) reported a combined sensitivity (>95%) and specificity (>99%) for DNA-derived variant calls.

Operational Considerations

Operational Specifications. For the 12 non-FDA approved NGS tests, when available, the operational specifications of turnaround time, sequencer model, and test distribution mode were evaluated and summarized in Table 19. The currently FDA-approved tests are included for ease of comparison.

Table 19. Operational Specifications Summary.

Turnaround time, sequencer type, and distribution mode for 12 commercially available NGS tests, along with the currently FDA-approved NGS tumor profiling tests. Operational information was obtained from publicly available company websites, retrieved from August through December 2019.

Company	**Test**	**TAT (Days)**	**Sequencer Type**	**Distribution Mode**
Foundation Medicine	FoundationOne CDx	<14	Illumina HiSeq 4000	Send out
ThermoFischer Scientific	Oncomine Dx Target Test	4	Ion PGM Dx	In-House
Memorial Sloan Kettering	IMPACT	20	Illumina HiSeq 2500	Single site (In-House)
Archer	VariantPlex	1	Illumina (Model not specified)	In-House
Ashion	GEM ExTra	14	Illumina NovaSeq 6000	Send out
Caris Molecular Intelligence	MI Tumor Seek	8–14	Not specified	Send out
Illumina	TruSight Oncology 500	3–4	Illumina NextSeq 500	In-House
Integrated Oncology	OmniSeq Comprehensive Assay	10–15	Not specified	Send out
Kew	Cancerplex	7–10	Illumina (MiSeq/NextSeq)	Send out
NantHealth	GPS Cancer	21	Illumina (Model not specified)	Send out
NeoGenomics	NeoType Discovery Profile	22	Not specified	Send out
OncoDNA	OncoDEEP	7	Ion PGM	Send out
Paradigm	PCDx	5	Illumina NextSeq 500	Send out
Sema4	Solid Tumor Panel	14	Ion PGM	Send out
Tempus	xT	14–21	Illumina HiSeq 4000	Send out

Overall, the average turnaround time across the evaluated tests (N = 10) was 11.42 days (Median = 11.75, Range = 1–22). If a range was given for the turnaround time, the median of the range was used during summary analyses. The mode of distribution of the tests highly impacted the turnaround time, with in-house testing having an average TAT of 2.25 days (Median = 2.25, Range 1–3.5), compared to the average of

13.25 days (Median = 13.25, Range = 5–22) for send out testing, however the number of in-house tests versus send out is low (N = 2) compared to send out testing (N = 10), therefore further investigation is needed on the impact of in-house testing turnaround times. The summary statistics for the turnaround time are presented in Table 20.

Table 20. Turnaround Time Summary Statistics.

Turnaround time (in days) summary statistics for the 12 commercially available sequencing tests.

	Distribution Mode		
	In House	Send Out	All
N	2	10	12
Mean	2.25	13.25	11.42
Median	2.25	13.25	11.75
Min	1	5	1
Max	3.50	22	22

Due to lack of data in three of the tests, and unspecified sequencer models in an additional two tests, the impact of sequencer type and model type could not be fully evaluated. Overall, there appears to be a selection preference for Illumina (N = 7) versus the Ion PGM Systems (N = 2).

Test Workflows. Similar to the FDA-approved NGS tests, the operational workflow of the Tempus xT and Caris MI Tumor Seek test were evaluated. Both evaluated tests have a distribution mode of send out testing, in which a physician consults, collects, and sends the tissue sample to the company's testing facility and awaits the results.

The Tempus xT operational workflow is depicted in Figure 22. The workflow touches on four main points in the process, initial sample collection at the clinic, receipt of the sample at the testing facility, sequencing and report generation, and follow-up with the patient's provider. The workflow is positioned for a patient's perspective and focuses on the necessity of paperwork, overall methods for testing, and physician contact. Additionally, the workflow specifically calls out the turnaround time of two to three weeks.

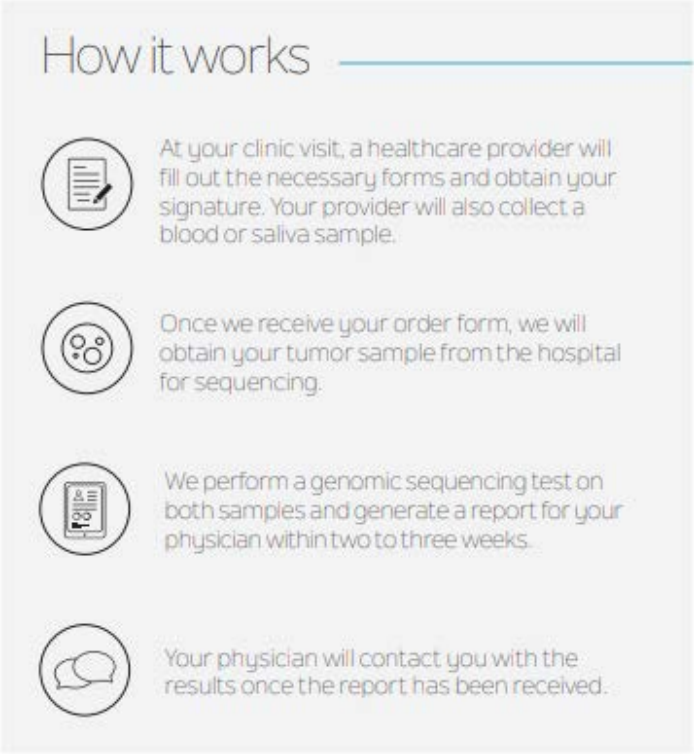

Figure 22. Tempus Test Workflow.

Operational workflow for the Tempus xT test (Tempus, 2018).

The Caris Molecular Intelligence MI Tumor Seek test workflow is a combination of the high-level operational workflow with more specific details on the sample details, test methodologies, analyses, and turnaround time (Figure 23). In line with the FoundationOne CDx and Tempus xT workflow, the MI Tumor Seek workflow outlines four major touchpoints of the sample including sample delivery, testing, analyses, and report transmittal. However, in comparison to FoundationOne CDx and Tempus xT workflows, the MI Tumor Seek workflow provides a snapshot of a portion of the technical information for the test. Aside from the general operational workflow outlined, the inclusion of technical information provides details aimed towards the laboratory professional or physician, or a more scientific patient or caregiver.

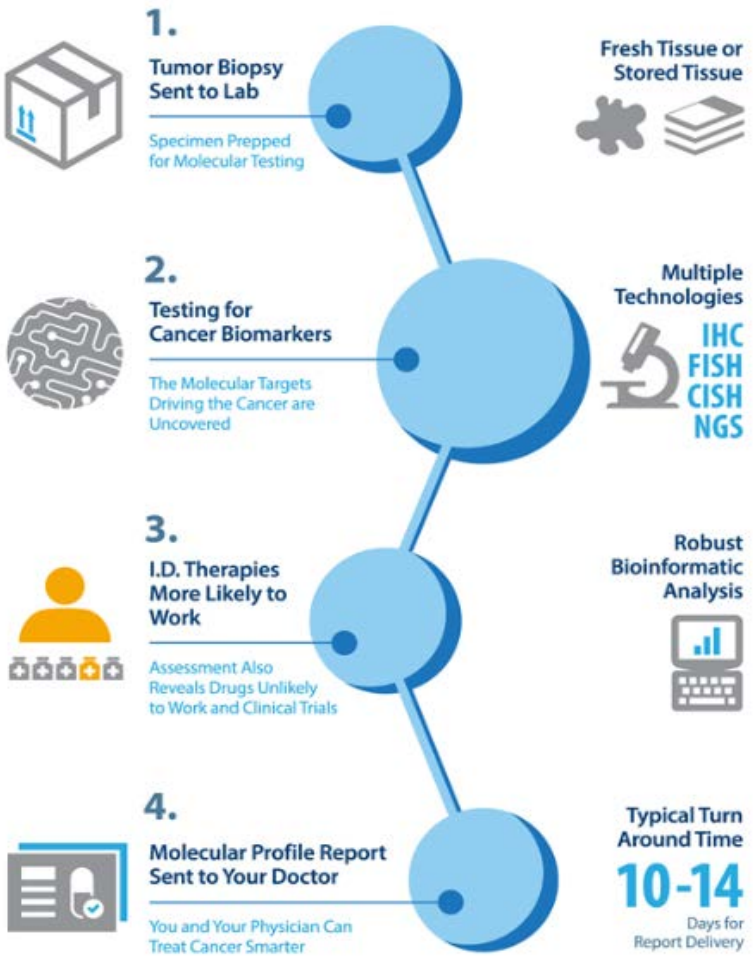

Figure 23. Caris Molecular Intelligence Test Workflow.

Caris Molecular Intelligence MI Tumor Seek test operational workflow (Caris Life Sciences, 2015).

Overall, the test workflows of the two non-FDA approved tests evaluated, revealed similarities most in line with the FoundationOne CDx workflow, which supplied a more high-level overview and likely was targeted toward a non-technical patient perspective. In contrast to the Oncomine Dx Target Test workflow, which focused on the technical laboratory workflows, and the MSK-IMAPCT workflow that highlighted the

sequencing and bioinformatic processes, the send out NGS tests focused on the patient interactions and high-level overview of the test workflow.

Clinical Report. The clinical report for the Tempus xT test (Figure 24) contains the patient and specimen information on the left of the report, with the genomic variants and therapy indications encompassing the majority of the front page. For the genomic variant sections, the mutations are displayed through the use of simple images and gene name. Additionally, the variants are sectioned by 'Somatic – Potentially Actionable,' 'Somatic – Biologically Relevant,' and 'Germline – Pathogenic/Likely Pathogenic.' The complex biomarkers are displayed as 'Immunotherapy Markers' and display a score, percentile, or scale for the result. Finally, the therapies are divided by 'FDA-Approved Therapies, Current Diagnosis' and 'FDA-Approved, Other Indications.' The report makes use of font styles and limited use of color to depict certain sections and/or results.

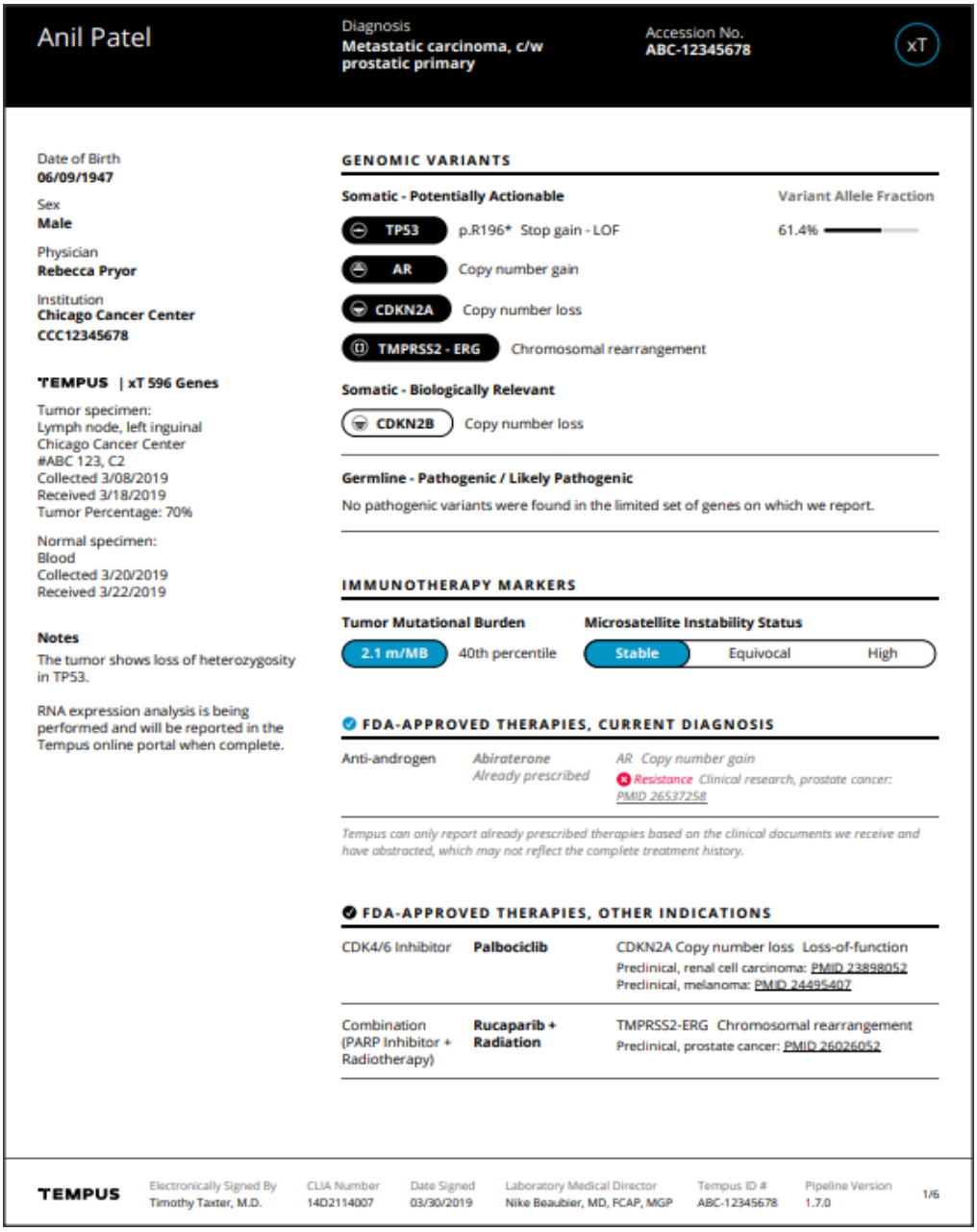

Figure 24. Tempus xT Test Report.

Screenshot of a sample report for the Tempus xT test (Tempus, 2019a).

Overall, the clinical report for the Tempus xT test is similar to the FoundationOne CDx report in relation to the modest use of color and sectioning of genomic variant calls. Key differences in the Tempus xT test report are reportability of the variant allele frequency, germline characterization of variant calls, and organization of the therapy indications. The lack of display of the variant allele frequency and/or germline characterizations on the Oncomine Dx Target test and FoundationOne CDx test reports are possibly the result of company preference, validation limitations, and/or regulations imposed by the FDA. Therefore, it would be necessary to re-evaluate the Tempus xT clinical report post FDA approval.

The Caris Molecular Intelligence MI Tumor Seek clinical report (Figure 25) utilizes a vertical layout with the patient, specimen, and physician information on top, the "high impact" results and therapy associations mid-level, and other notes and additional results at the end of the report. The MI Tumor Seek report utilizes a variety of font differences, shading, and bright color use to organize the report and highlight certain aspects of the results. Due to the variety of detection methods in the MI Tumor Seek test, the specific method of detection of a variant call is noted.

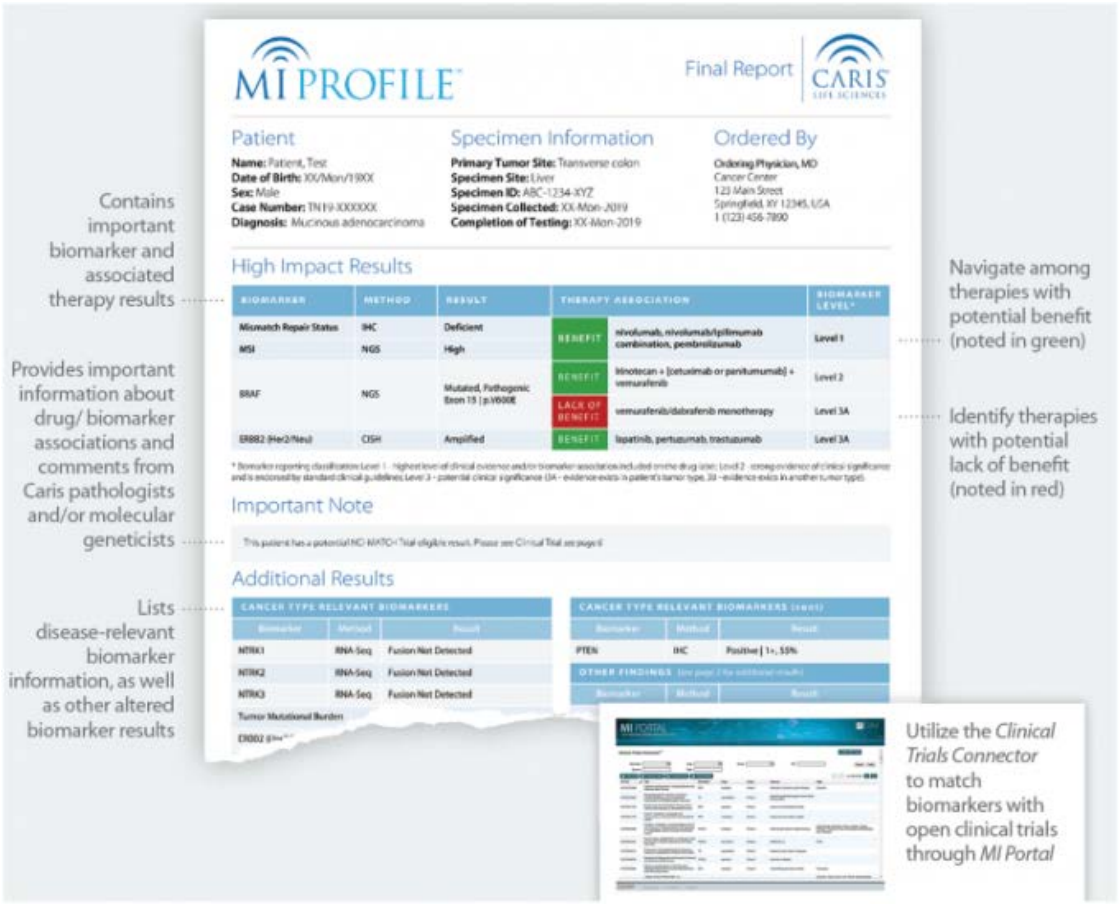

Figure 25. Caris Molecular Intelligence Test Report.

Snapshot of the Caris Molecular Intelligence MI Tumor Seek clinical report with specific references to the main sections of the report (Caris Molecular Intelligence, 2020).

Overall, the Caris MI Tumor Seek test clinical report displayed the most amount of visual differences via use of colors and font styles in comparison to the other evaluated reports. Additionally, the classifications of therapy associations (benefit/lack of benefit), the biomarker level, and the overall section titling of results with high impact, the MI Tumor Seek report displays the highest level of medical interpretation on the front page than the other evaluated test reports, which may contain the information on subsequent report pages. In addition to the Tempus xT report, these key differences observed in the

Caris Molecular Information MI Tumor Seek test may change upon FDA review of the report and will require re-review if the tests are submitted for FDA approval.

Probability of Success of NGS Tests in Development

The presumed goal of a next-generation sequencing test is approval from the FDA, due to the insurance coverage, quality, and scientific rigor considerations that come along with the federal coverage. Using the previously FDA approved NGS tests as a benchmark, future tests that may be in various stages of development can be evaluated to predict the success of the FDA approval. In addition to the success of the FDA coverage, the other operational considerations of NGS tests can be compared with the currently existing tests to determine the operational success of a test. In order for a test to flourish and be a candidate on the NGS market, a product must be operationally feasible.

A benchmark model for probability of FDA approval and clinical adoption was created and used for the assessment of two non-FDA next-generation sequencing tests: Tempus xT test and Caris Molecular Information MI Tumor Seek test. The data obtained for analysis was found on publicly available websites. For performance data, overall test accuracy and limit of detection (LoD) were the only data publicly available for use in the model. Upon PMA submission, a more comprehensive analysis can be performed using precision and concordance results. The results of the model are only predictive of an outcome. The actual results of the FDA approval are likely to be individual to the specific test and may be subject to the changing FDA regulations over time. The results of the overall test comparisons to the benchmark criteria are presented in Table 21.

Table 21. Test Comparison Results in Relation to a Benchmark Model of Next-Generation Sequencing Tests.

	Benchmark	**Tempus xT**	**Caris Molecular Intelligence MI Tumor Seek**
Test Methodology	NGS	NGS	NGS
Test Distribution Mode	Send out	Send out	Send out
Number of Genes (DNA)	271	648	592
Test Material	DNA	DNA, RNA	DNA, RNA
Specimen Type	FFPE	FFPE	FFPE
Sample Size	$25mm^2$	$25mm^2$	$25mm^2$
Surface Area Tissue Requirement	10 unstained slides, 4–10µm thick	10 unstained slides, 5µm thick	10 unstained slides, 4µm thick
Tumor Content	20%	20%	20%
Variant Detection			
SNVs	Required	Yes	Yes
Indels	Required	Yes	Yes
CNVs	Required	Yes	Yes
Rearrangements	Required	Yes	Yes
Biomarkers	Required	Yes	Yes
RNA Detection	Optional	Yes	Yes
Complementary Testing (IHC, FISH, etc.)	Optional	No	Yes
Turnaround Time	13	14–21	8–14
Assay Performance			
Precision	≥95%	Not Available	
Accuracy (PPA/NPA)	≥95%/≥99%	>95%/>99%	>95%/>99%
Concordance (PPA/NPA)	≥95%/≥99%	Not Available	
LoD (Allele Freq.) (SNVs/Indels)	5%/5%	5%/10%	5%/5%

The clinical adoption and FDA approval scores for the Tempus xT and Caris Molecular Information MI Tumor Seek test were inputted and compared against the benchmark. The benchmark clinical adoption score was determined to be 14, with two criteria (RNA detection, Complementary testing) being marked with an optional '0' designation. Test methodology, test distribution mode, test material type, and specimen type were determined to be categorical criteria and excluded from the score analysis. The clinical adoption score results of the Tempus xT and Caris Molecular Intelligence MI Tumor Seek tests as compared to the benchmark are presented in Table 22.

Table 22. Clinical Adoption Score Results

	Clinical Adoption Score		
Criteria	**Benchmark**	**Tempus xT**	**Caris Molecular Intelligence MI Tumor Seek**
Number of Genes (DNA)	1	2	2
Sample Size	1	1	1
Surface Area Tissue Requirement	1	1	1
Tumor Content	1	1	1
Variant Detection			
SNVs	1	1	1
Indels	1	1	1
CNVs	1	1	1
Rearrangements	1	1	1
Biomarkers	1	1	1
RNA Detection	0	1	1
Complementary Testing (IHC, FISH, etc.)	0	0	1
Turnaround Time	1	0	1
Assay Performance			
Precision	1	0[1]	0[1]
Accuracy	1	1	1
Concordance	1	0[1]	0[1]
LoD	1	0	1
Overall Score	**14**	**12**	**15**

Notes: [1]Unknown status of validation test: not performed or data not available for analysis

The goal and responsibility of the FDA is to ensure the safety and efficacy of products. Therefore, the FDA approval score was limited to test criteria that may impact, or improve, test safety or efficacy. Test criteria that was purely categorical or related to the clinical adoption only of a test was excluded from analysis. The benchmark FDA approval score was determined to be 10, with two criteria being marked with an optional '0' designation. The FDA approval score results of the Tempus xT and Caris Molecular

Intelligence MI Tumor Seek tests as compared to the benchmark are presented in Table 23.

Table 23. FDA Approval Score Results

	FDA Approval Score		
Criteria	**Benchmark**	**Tempus xT**	**Caris Molecular Intelligence MI Tumor Seek**
Tumor Content	1	1	1
Variant Detection			
SNVs	1	1	1
Indels	1	1	1
CNVs	1	1	1
Rearrangements	1	1	1
Biomarkers	1	1	1
RNA Detection	0	1	1
Complementary Testing (IHC, FISH, etc.)	0	0	1
Assay Performance			
Precision	1	0[1]	0[1]
Accuracy	1	1	1
Concordance	1	0[1]	0[1]
LoD	1	0	1
Total Score	**10**	**8**	**10**

Notes: [1]Unknown status of validation test: not performed or data not available for analysis

Overall, the results of the predictive models place the Tempus xT test below the benchmark for clinical adoption and FDA approval scores. The technical specifications of the assay meet or exceed the benchmark criteria. The Tempus xT test includes RNA sequencing for the detection of rearrangement events, adding to the variant detection methods. The published turnaround time for the Tempus xT test is 14–21 days (Tempus, 2018), which is above the range of the benchmark criterion (13 days). The accuracy study

presented the results divided by base substitution and indel events (Tempus, 2020), therefore the overall variant bin analyses could not be performed with current validation material. Due to this limitation, the combined data from Tempus (2019b) validation material was used in the model, which stated a >95% sensitivity. The limit of detection (LoD) for indel events is higher (10%) then the benchmark criteria of 5%. Precision and concordance studies were not publicly available and therefore could not be counted towards the score.

The Caris Molecular Information MI Tumor Seek test model results predict a high success of clinical adoption and expected FDA approval. The MI Tumor Seek test met or outperformed the technical, performance, and operational specification requirements. The only barrier to FDA approval may be the additional variant detection methods (RNA, IHC) that may require additional FDA input, oversight, and validation study requirements. Similarly to the Tempus xT test, precision and concordance studies were not publicly available and therefore could not be counted towards the score. Upon PMA submission or further test development, the results of the precision and concordance studies could be added to the model and better predict adoption and approval success for the Tempus xT and Caris Molecular Information MI Tumor Seek tests.

Further development of the model is warranted to advance the predictability of the model. For one, the various categories of criteria could benefit from a weighted algorithm approach to take into account preferences and importance of the metrics. A weighted approach would require physician input on the criteria most likely to contribute to the clinical adoption of the test. Furthermore, for the success of FDA approval, insight from the agency on the criteria most important to the safety and efficacy of a test would need

to be obtained. The model may also need to be adapted as complexity of NGS tests evolves. For the purposes of the generation and testing of the model, the assay performance specifications was limited to short variant (base substitutions and indels) events. A more precise and complex model could incorporate multiple performance criteria depending on the variant detection methods. Finally, the model serves as a blueprint for possible clinical adoption and FDA approval success. Given the tests previously approved and in clinical use, a benchmark was created and compared to two tests yet to receive FDA approval. As tests improve and evolve, the model will need to be updated to incorporate the latest success benchmarks and further developments of comparator tests.

Chapter IV.

Discussion

The next-generation sequencing market is quickly evolving and increasing competitive pressures; therefore, the operational considerations of a test may begin to provide a selective advantage over tests in order to have a higher likelihood of clinical adoption. This case study reviewed the technical, performance, and operational components of the three currently approved FDA next-generation sequencing tests and used the criteria as a benchmark for other upcoming tests for approval. The findings from this review showed a successful review and comparator method for review of FDA-regulated products in the same product and classification type, while highlighting the difficulties of comparison across products and within-product classification differences. Additionally, this review successfully generated a predictor model for FDA approval and clinical adoption for potential future companion diagnostic NGS tumor profiling tests through the review of the technical, performance, and operational components.

Several limitations were identified during the course of this case review, which highlighted the importance of publicly available information and the lack of shared data across competitor companies. The information that was readily available from the FDA medical device database allowed for data collection for the tests that have received FDA approval. However, the information that would need to be submitted to the FDA upon pre-market approval submission, is lacking for public accessibility for the non-approved tests. Presumably due to competitive reasons or lack of testing, the comprehensive results

of studies are typically not published for public view. Not only did the lack of available data and requirements impact this review, it impacts the physicians, patients, academic medical centers, biopharmaceutical companies, and the scientific community overall by having limited information in order to make comprehensive decisions on what test would be best for their use. The lack of open data sharing is a major barrier to clinical adoption and could gain more physician interest if companies were compelled to share data during FDA approval (Messner et al., 2017). Companies and collaborators could benefit overall if the data and information sharing is more widely shared upfront, and undoubtedly the patients would experience the benefits in the long run through the ever-increasing knowledge and advancements in the field.

In addition to the lack of publicly available data for in-development tests, the ways data is presented differs widely amongst tests. Aside from the tests which have received approval, which have full methods and data results from the validation tests presented in the FDA submission material, the non-FDA approved tests often do not present the comprehensive results nor methods for their technical and performance specifications. This methodology and data constraint create a barrier to comparison and interpretation of the results. Furthermore, the various presentations of data with differing thresholds adds to the comparison confusion. One test may present the accuracy results using an allele frequency cut-off of 10%, while others present the data for a 2% cutoff, or perhaps no cut-off at all. Therefore, there is risk that one is comparing the performance results using different baselines and may not come to an appropriate conclusion. The data is likely presented in a way that augments the performance of the test, however if there were industry standards, or FDA oversight, that standardized the presentation and

reporting of technical and performance specifications, the public would be able to make better informed decisions.

Finally, an additional limitation in this review was determined to be the scope of the case study. The current Medical Device regulation, aside from FDA regulation overall, is extensive and complex. That in its own creates a need for a more streamlined and simplistic methodology for FDA oversight. Work by Hines, Lurie, Yu, & Wolfe (2010) attempted to clarify and represent the medical device regulation pathways. However due to the various conditionals (predicates, de novo), the workflow highlights the potential areas of confusion and exception routes (Figure 26).

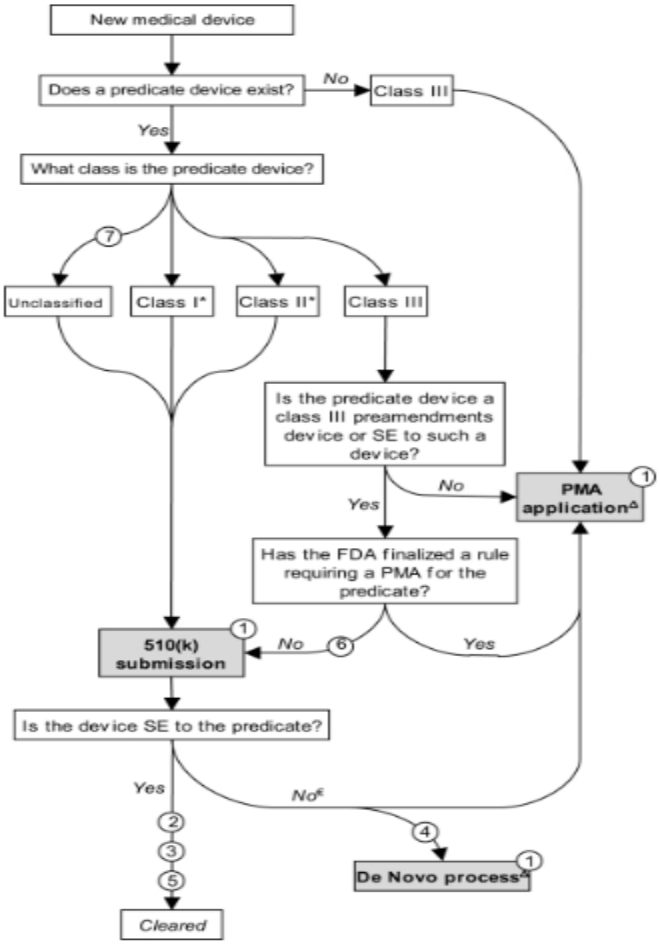

Figure 26. Schematic Representation of Medical Device PMA FDA Review

Workflow for FDA classification scheme for new medical devices (Hines et al., 2010).

Without a clear outline on regulatory pathways, between and within products, there is a need for specialized regulatory groups that are able to decipher the pathways and coordinate with the agencies on requirements. While these team are value-added to the companies, the impact will eventually trickle down to the operational teams and create burdens to the everyday work, ultimately affecting the patients. This review was only able to touch upon the components of the FDA specifically related to Medical

Devices and next-generation sequencing. Additional review of the field could bring to light opportunities for more efficient regulatory oversight and overall organization of the regulations.

Aside from the current barriers, future directions of next-generation sequencing tests will continue to add complexity to the FDA approval system. For solid based assays, the incorporation of additional testing, including IHC, cytogenetics, FISH, and RNA sequencing, will create additional regulatory and validation burden during review. The addition of complementary testing also introduces a wide range of possible operational burdens, given the additional workstreams that are required with the added test types. The current regulatory pathways for next-generation sequencing approvals follow either the PMA or 510(k) routes, however with the additional of complementary or add-on testing methods, the future of regulatory pathways could see combinations of the PMA and 510(k) submissions. The FDA may consider these types of devices as combination products, which may introduce a review gap and lead to safety risks to the patients (McDonough, 2020). Instead, if the regulatory requirement for NGS plus complementary testing requires two separate submissions, this would likely impact the accessibility to the patients in need, in addition to the added burdens for FDA regulatory review and test submissions by the company. Alternatively, if the strictest regulatory pathway for combination products is required, this may create a high, and unnecessary, test validation burden for tests that fall into the PMA route but may have additional methods that on their own would follow a 510(k) process.

As mentioned at the beginning of this care review, the complexity and computing power of sequencing is constantly increasing. As presented by Nederbragt (2014), for

every next-generation sequencing platform, there have been developments to increasing the read length and/or the number of gigabases per run over time (Figure 27).

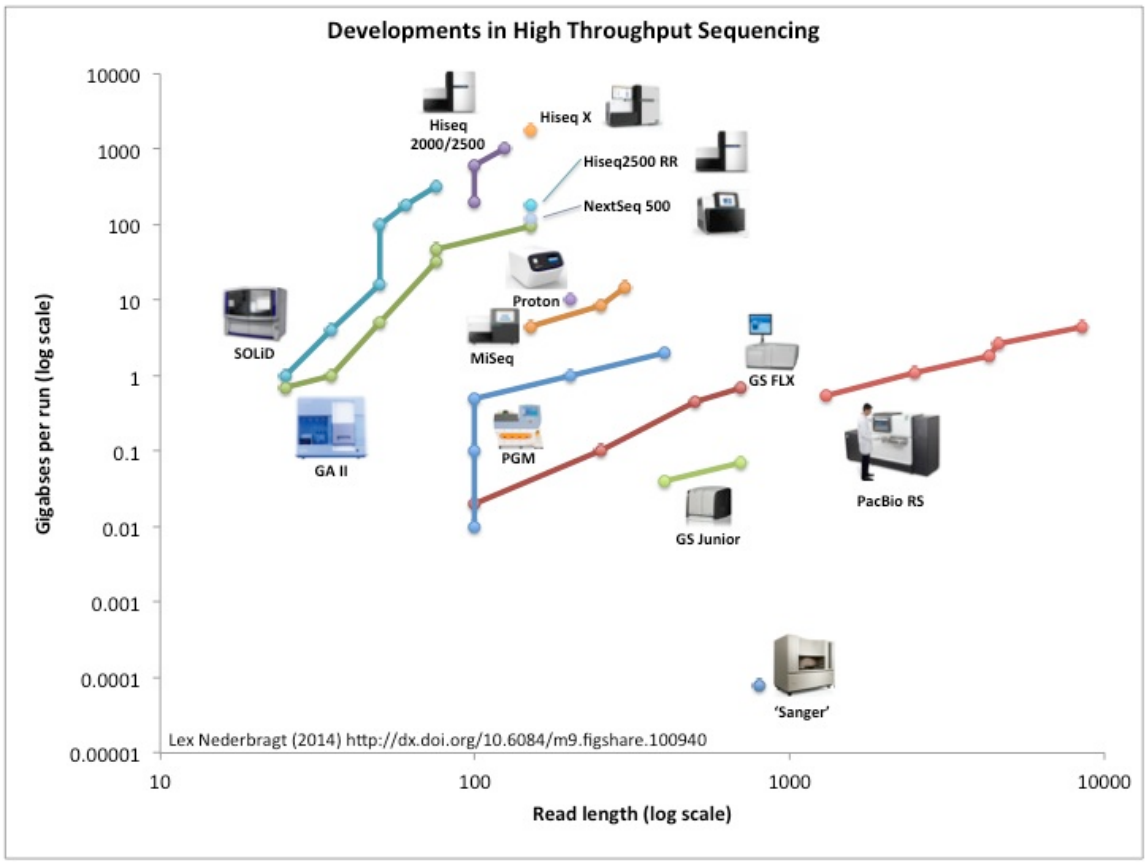

Figure 27. Read Length and Gigabases Per Run Developments in High Throughput Sequencing.

The developments of high throughput sequencing platforms by increasing read length and/or gigabases per run for a collection of sequencing technologies (Nederbragt, 2014).

The progression of the technologies has and will continue to push the limits of computing resources, making it a critical component of next-generation sequencing considerations.

The sequencing platform distribution shown by Nederbragt (2014) not only shows the further developments of individual systems but highlights the breadth of options available to customers. Therefore, in addition to the NGS tests themselves, the sequencing platforms used in each assay may emerge as a contributing factor to the overall performance and clinical adoption of a test.

The mode of distribution of next-generation sequencing tests may impact the regulatory pathways and operational considerations. Currently, tests which are sent off to a company for testing ("send out") and distributed kit test solutions ("in-house") follow the same regulatory pathways. Convenience kits, which refer to two or medical devices packaged together, are nonexempt from the PMA regulatory pathway if the kit is an *in vitro* diagnostic (IVD), which next-generation sequencing tests are classified as such in the oncology setting (U.S. Food & Drug Administration, 2019d). As the number and complexity of the tests increase, the need for separate validation and oversight may be needed, distinct from the overall Class III regulations. As seen in the differences between the Oncomine Dx Target Test and the FoundationOne CDx tests, the number and composition of validation studies differed due to the separate distribution modes. Therefore, it may benefit the regulatory agencies to separate the classifications in order to streamline review and approval.

The operational requirements and workflows for a distributed kit are different than a central lab model and may be challenging for certain testing facilities. The operational components of a kit are largely placed on the testing facility, instead of being absorbed by the company. In a community hospital setting, Akkari, Smith, Wetall, & Lupo (2019) reported significant challenges upon initial use of a distributed model in

house, related to technical expertise, bioinformatics, computing infrastructure, laboratory training, and integration into clinical decision making. The distributed model will inevitably allow for greater patient access to NGS testing and therefore improve quality of patient care, however the operational components of the tests must be understood by the testing facility to ensure success for the patient and product.

In addition to added complexities to solid-based tumor sequencing, the developments in liquid biopsy sequencing may prove to be an added regulatory burden on the FDA and operational workflow needs. In addition to the already complex nature of NGS testing, with additional avenues of testing methods being explored, a liquid biopsy NGS approach will introduce the need for new categories of medical device and next-generation sequencing categorization and classification. Similar to current NGS test categorizations, liquid biopsy NGS methods may fall into similar categories in the FDA space, however, if new pathways and categorizations are not created, it may cause further complications down the line from the regulatory and industry standpoint.

The liquid biopsy NGS test landscape is rapidly growing and becoming an area of interests to physicians due to ease of sample collection and concordance with tissue results (Kwapisz, 2017). Due to the biological and technical differences between tumor and liquid assays, the regulatory and operational aspects of the tests may also prove to be separate enough to deserve distinct classification differences. In general, there are separate challenges with liquid biopsy tests compared to tumor tests. The emerging computational challenges relating to test sensitivity and heterogeneity, and the clinical adoption challenges of understanding the underlying biological mechanisms create new barriers to execution for liquid biopsy adoption (Castro-Giner et al., 2018). Meanwhile,

the solid tumor market is faced with challenges of scalability, computing resources, and physician adoption. Despite the similarities in test objectives, the nature of the distinct test source will need to be further investigated as additional liquid biopsy tests are approved to understand the differences in operational impact of the emerging technology.

Overall, the findings of this review highlight the need for a streamlined regulatory approach both between products and within the specific classifications of medical devices. This was exemplified in the comparison review of FDA product regulation as it applied to Medical Devices and between regulated product categories. As the complexity of the overall scope of FDA regulation and breadth of NGS testing continues to grow and evolve, the need for clarification and FDA guidance will be needed more than ever. Not only will an organized approach to NGS testing benefit the companies seeking FDA approval, the additional testing options will ultimately serve in patients' best interests as more tests become available, with potentially better results.

In addition to the regulation review, the technical, performance, and operational review of next-generation sequencing tests provided a holistic review of the criteria critical to currently available NGS tests. The review of the currently FDA-approved tests and comparison to non-FDA approved commercially available tests, highlighted the discrepancies and challenges in data sharing and standardization. Despite the known limitations, through the review of the technical, performance, and operational criteria, a predictive model was successfully generated to serve as a blueprint to estimate the success of FDA approval and clinical adoption. Ultimately, the more information that is known, shared and utilized, the easier it is for physicians to make informed decisions on clinical care for their patients, as well as drive the scientific knowledge of the field to

further advance the development of future products.

Appendix 1.

FDA Organization Chart – March 31, 2019 Reorganization Plan

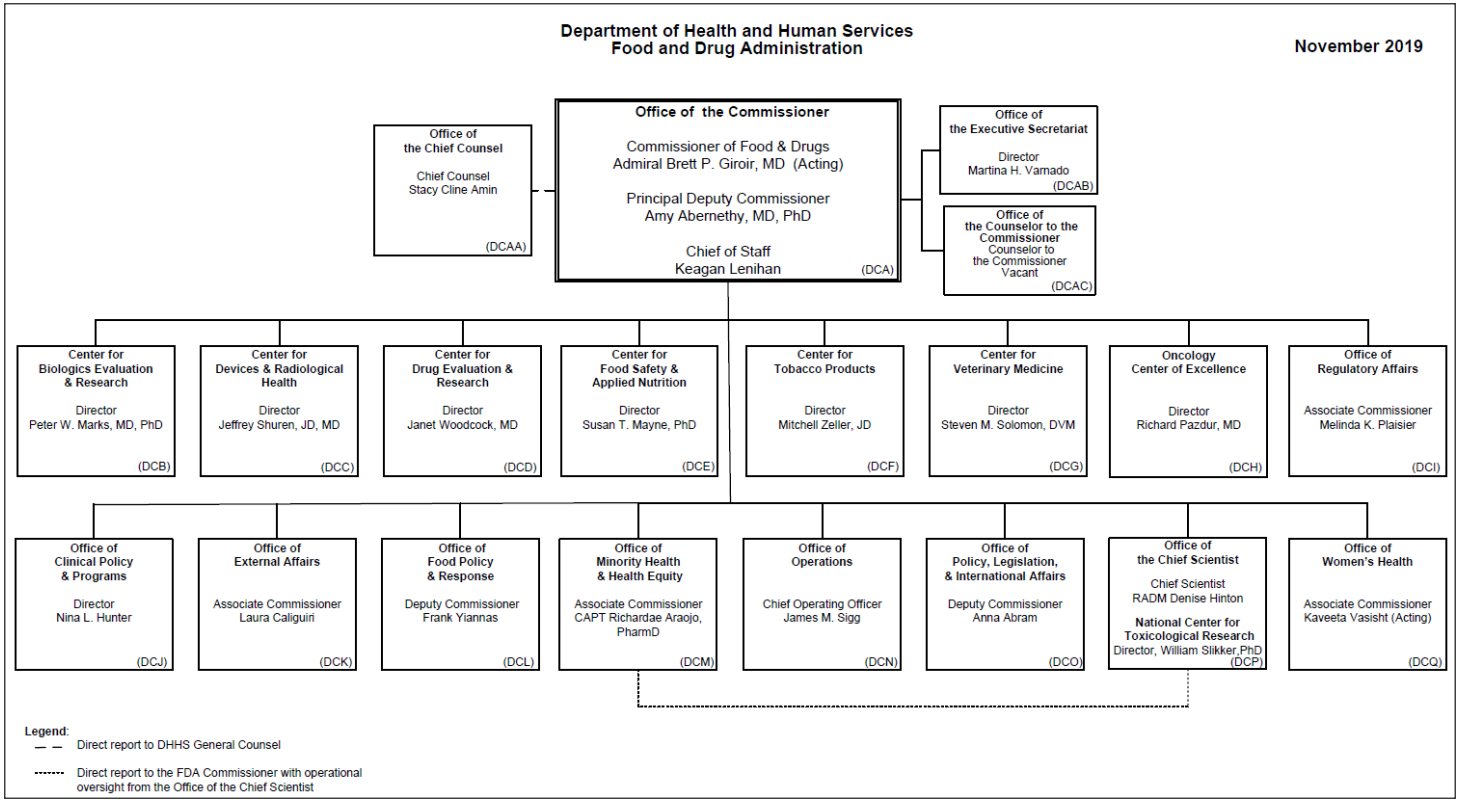

Figure 28. FDA Organization Chart.

FDA Organization Chart based on the March 2019 organization redesign (U.S. Food & Drug Administration, 2019a).

Appendix 2.

Medical Device Databases

Table 24. FDA Medical Device Databases.

Summary of historical and current FDA databases used to catalog Medical Devices (U.S. Food & Drug Administration, 2019b).

Title of Database	Description of Database	Update Frequency
AccessGUDID (Global Unique Device Identification Database)	This database contains key device identification information submitted to the FDA about medical devices that have Unique Device Identifiers (UDI).	Daily
Advisory Committee/Panel Meetings - CDRH	This database contains historical information about CDRH Advisory Committees and Panel meetings through 2008, including summaries and transcripts.	No longer being updated
CDRH Export Certificate Validation (CECV)	This searchable database contains valid (not expired) export certificates submitted electronically via CECATS (CDRH Export Certification Application and Tracking System) and issued by the Center for Devices and Radiological Health. The results displayed include the facility name, certificate type, expiration date, certificate number, and the number of pages per certificate.	Weekly
CFR Title 21 - Food and Drugs	This database contains the most recent revision from the Government Printing Office (GPO) of the Code of Federal Regulations (CFR) Title 21 - Food and Drugs.	Annually
Clinical Laboratory Improvement Amendments (CLIA)	This database contains the commercially marketed in vitro test systems categorized by the FDA since January 31, 2000, and tests categorized by the Centers for Disease Control and Prevention (CDC) prior to that date.	Weekly
CLIA Currently Waived Analytes disclaimer icon	This database contains the commercially marketed in vitro test systems categorized as CLIA waived by the FDA since January 31, 2000, and by the Centers for Disease Control and Prevention (CDC) prior to that date. CLIA waived test systems are waived from certain CLIA laboratory requirements (42 CFR Part 493).	Monthly
De Novo	De novo provides a possible route to classify novel devices of low to moderate risk. This database contains de novo classification orders.	Weekly

FDA Certified Mammography Facilities	A searchable listing by state and zip code of all mammography facilities certified by the Food and Drug Administration (FDA) as meeting baseline quality standards for equipment, personnel and practices under the Mammography Quality Standards Act of 1992 (MQSA).	Weekly
Humanitarian Device Exemption (HDE)	Searchable listing of Humanitarian Device Exemption (HDE) Class III medical devices.	Weekly
IVD Home Use Lab Tests (Over The Counter) Tests	Searchable listing of Over-the-Counter tests (OTC) and collection kits that have been cleared or approved by the FDA	Weekly
MAUDE (Manufacturer and User Facility Device Experience)	MAUDE data represents reports of adverse events involving medical devices. The data consists of all voluntary reports since June, 1993, user facility reports since 1991, distributor reports since 1993, and manufacturer reports since August, 1996.	Weekly
MDR (Medical Device Reporting)	This database allows you to search the CDRH's database information on medical devices which may have malfunctioned or caused a death or serious injury during the years 1992 through 1996.	No longer being updated
MedSun Reports	The Medical Product Safety Network (MedSun) is an adverse event reporting program launched in 2002 by the U.S. Food and Drug Administration's Center for Devices and Radiological Health (CDRH). The primary goal for MedSun is to work collaboratively with the clinical community to identify, understand, and solve problems with the use of medical devices.	Daily
Post-Approval Studies (PAS) Database	This database contains information about current Post-Approval Studies (PAS). Manufacturers required to conduct PAS must complete the study as a condition of approval. This database allows you to search PAS information by applicant or device information. This database is updated once a week.	Weekly
Premarket Approvals (PMA)	Premarket approval by FDA is the required process of scientific review to ensure the safety and effectiveness of all devices classified as Class III devices. An approved Premarket Approval Application (PMA) is, in effect, a private license granted to the applicant for marketing a particular medical device. This database may be searched by a variety of fields and is updated once a week.	Weekly

Premarket Approval (PMA) Summary Review Memos for 180-Day Design Changes	A 180-day supplement is a request for a significant change in components, materials, design, specification, software, color additive, and labeling to an approved premarket application or premarket report. As a pilot program under the CDRH Transparency Initiative, FDA has begun releasing some summary review memos for 180-day PMA supplements relating to design changes.	Weekly
Premarket Notifications (510(k)s)	Medical device manufacturers are required to submit a premarket notification or 510(k) if they intend to introduce a device into commercial distribution for the first time or reintroduce a device that will be significantly changed or modified to the extent that its safety or effectiveness could be affected. This database of releasable 510(k)s can be searched by 510(k) number, applicant, device name or FDA product code. Summaries of safety and effectiveness information is available via the web interface for more recent records. The database is updated once a week.	Weekly
Product Classification	This database contains medical device names and associated information developed by the Center. It includes a three letter device product code and a Device Class that refers to the level of CDRH regulation of a given device.	Weekly
Radiation-emitting Electronic Product Codes	This database contains product names and associated information developed by the Center for all products, both medical and non-medical, which emit radiation. It includes a three letter product code, a descriptor for radiation type, applicable performance standard(s), and a definition for the code.	Weekly
Radiation Emitting Product Corrective Actions and Recalls	This database provides descriptions of radiation-emitting products that have been recalled under an approved corrective action plan to remove defective and noncompliant products from the market. Searches may be done by manufacturer name, performance standard, product name, description, or date range.	Weekly

Recalls of Medical Devices	This database contains Medical Device Recalls classified since November 1, 2002. Beginning January 3, 2017, the database may also include correction or removal actions initiated by a firm prior to review by the FDA. The status of the action is updated if the FDA identifies a violation and classifies the action as a recall and again when the recall is terminated. FDA recall classification may occur after the firm recalling the medical device product conducts and communicates with its customers about the recall and provides contact information for customers with questions. Therefore, the recall information posting date ("create date") indicates the date FDA classified the recall, it does not necessarily mean that the recall is new. CBER recall information is available here.	Frequently as items become available
Recognized Consensus Standards	This database consists of those national and international standards recognized by FDA which manufacturers can declare conformity to and is part of the information the Center can use to make an appropriate decision regarding the clearance or approval of a submission. Information submitted on conformance with such standards will have a direct bearing on safety and effectiveness determinations made during the review of IDEs, HDEs, PMAs, and PDPs. Conformance with recognized consensus standards in and of itself, however, may not always be a sufficient basis for regulatory decisions.	Quarterly
Registration & Listing	This searchable database contains establishments (engaged in the manufacture, preparation, propagation, compounding, assembly, or processing of medical devices intended for human use and commercial distribution) and listings of medical devices in commercial distribution by both domestic and foreign manufacturers. Note: This database is updated once a week.	Weekly
Total Product Life Cycle (TPLC)	The Total Product Life Cycle (TPLC) database integrates premarket and postmarket data about medical devices. It includes information pulled from CDRH databases including Premarket Approvals (PMA), Premarket Notifications (510[k]), Adverse Events, and Recalls. You can search the TPLC database by device name or procode to receive a full report about a particular product line.	Weekly

X-Ray Assembler Data	Federal regulations require that an assembler who installs one or more certified components of a diagnostic x-ray system submit a report of assembly. This database contains the releasable information submitted including Equipment Location, General Information and Component Information. Note: Data does not include dental system installations.	Annually

Appendix 3.

Oncomine Dx Target Test Example Clinical Report

Results for Analytical Sequence Variations Not Detected

Analytical DNA Sequence Variants Not Detected

Note: Results for negative variants are listed first, followed by variants that were reported as no calls.

Gene	Amino Acid Change	Nucleotide Change	Test Result	Hotspot ID
AKT1	p.Glu17Lys	c.49G>A	NEGATIVE	COSM33765
ALK	p.Arg1275Gln	c.3824G>A	NEGATIVE	COSM28056
ALK	p.Arg1275Leu	c.3824G>T	NEGATIVE	COSM28060
ALK	p.Cys1156Tyr	c.3467G>A	NEGATIVE	COSM99136
ALK	p.Gly1128Ala	c.3383G>C	NEGATIVE	COSM98475
ALK	p.Gly1202Arg	c.3604G>A	NEGATIVE	COSM144250
ALK	p.Ile1171Asn	c.3512T>A	NEGATIVE	COSM28498
ALK	p.Ile1171Thr	c.3512T>C	NEGATIVE	COSM4381100
ALK	p.Leu1152Arg	c.3455T>G	NEGATIVE	COSM97185
ALK	p.Leu1152Pro	c.3455T>C	NEGATIVE	COSM1407659
ALK	p.Leu1196Gln	c.3587T>A	NEGATIVE	COSM1169447
ALK	p.Leu1196Met	c.3586C>A	NEGATIVE	COSM99137
ALK	p.Phe1174Cys	c.3521T>G	NEGATIVE	COSM28059
ALK	p.Phe1174Ile	c.3520T>A	NEGATIVE	COSM28491
ALK	p.Phe1174Leu	c.3522C>G	NEGATIVE	COSM28061
ALK	p.Phe1174Leu	c.3522C>A	NEGATIVE	COSM28055
ALK	p.Phe1174Leu	c.3520T>C	NEGATIVE	COSM28057
ALK	p.Phe1174Ser	c.3521T>C	NEGATIVE	COSM53063
ALK	p.Phe1174Val	c.3520T>G	NEGATIVE	COSM28054
ALK	p.Phe1245Cys	c.3734T>G	NEGATIVE	COSM28500
ALK	p.Phe1245Ile	c.3733T>A	NEGATIVE	COSM28492
ALK	p.Phe1245Leu	c.3735C>G	NEGATIVE	COSM28062
ALK	p.Phe1245Leu	c.3735C>A	NEGATIVE	COSM28493
ALK	p.Phe1245Val	c.3733T>G	NEGATIVE	COSM28499
ALK	p.Ser1206Tyr	c.3617C>A	NEGATIVE	COSM144251
ALK	p.Val1180Leu	c.3538G>C	NEGATIVE	COSM4381101
BRAF	p.Asp594Asn	c.1780G>A	NEGATIVE	COSM27639
BRAF	p.Asp594Gly	c.1781A>G	NEGATIVE	COSM467
BRAF	p.Gly466Glu	c.1397G>A	NEGATIVE	COSM453
BRAF	p.Gly466Val	c.1397G>T	NEGATIVE	COSM451
BRAF	p.Gly469Ala	c.1406G>C	NEGATIVE	COSM460
BRAF	p.Gly469Arg	c.1405G>A	NEGATIVE	COSM457
BRAF	p.Gly469Val	c.1406G>T	NEGATIVE	COSM459
BRAF	p.Lys601Glu	c.1801A>G	NEGATIVE	COSM478
BRAF	p.Val600Arg	c.1798_1799delGTinsAG	NEGATIVE	COSM474
BRAF	p.Val600Lys	c.1798_1799delGTinsAA	NEGATIVE	COSM473
BRAF	p.Val600_Lys601delinsGlu	c.1799_1801delTGA	NEGATIVE	COSM1133
CDK4	p.Arg24Cys	c.70C>T	NEGATIVE	COSM1677139
CDK4	p.Arg24His	c.71G>A	NEGATIVE	COSM1989836
CDK4	p.Arg24Leu	c.71G>T	NEGATIVE	COSM363684
CDK4	p.Arg24Ser	c.70C>A	NEGATIVE	COSM3463914
CDK4	p.Lys22Arg	c.65A>G	NEGATIVE	COSM232013
CDK4	p.Lys22Gln	c.64A>C	NEGATIVE	OM3153

Figure 29. Snapshot of Full Sample Report for the Oncomine Dx Target Test.

Example of negative variant calls taken from a page of a sample clinical report for the Oncomine Dx Target Test (ThermoFischer Scientific, n.d.).

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