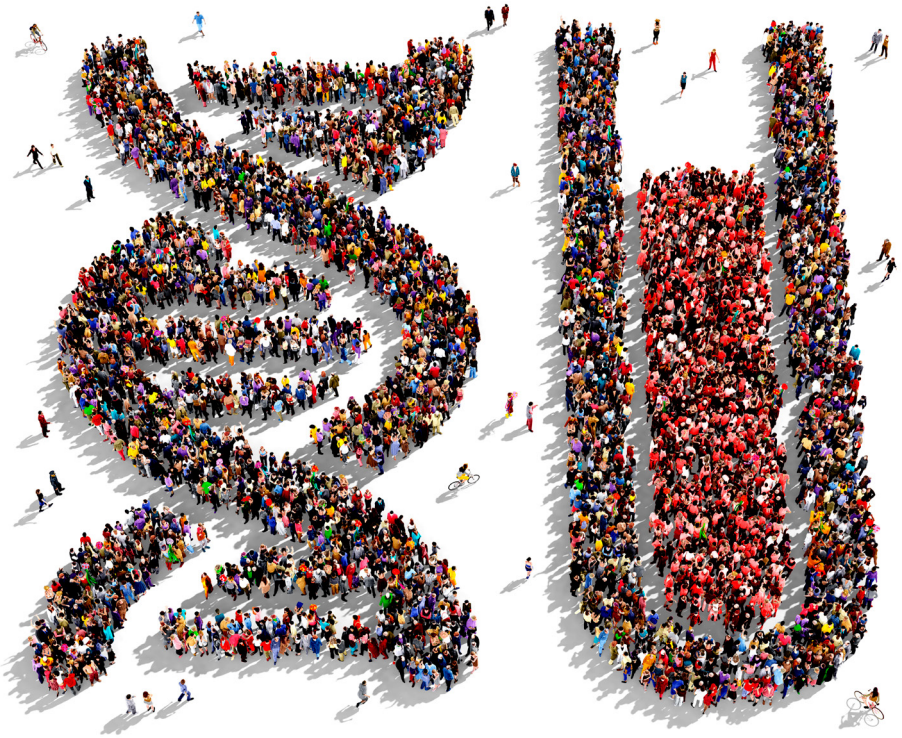
DNV·GL

Implementing NGS-based diagnostics in cancer care:

Technical and organizational factors in the Nordics



2021

Design, layout and print production of this report: ETN Grafisk
BigMed visual identity: Melkeveien
All images from Shutterstock, unless specified.

DNV GL

Driven by our purpose to safeguard life, property, and the environment, DNV GL works with key partners to address the technical, organizational, quality, and regulatory challenges that prevent the implementation of precision medicine approaches in routine clinical care.

https://www.dnvgl.com/research/precision-medicine/index.html

Table of contents

Summary

Next-generation sequencing (NGS)-based molecular diagnostics are a critical part of precision medicine, and are growing in importance for cancer care as companion diagnostics for targeted therapies. The transfer of these technologies into routine clinical use is not straight-forward, as these diagnostics rely on complex laboratory, clinical, and informatics infrastructure which may not be in place. The use of these tests in a clinical context intersects and challenges other aspects of the health system, such as reimbursement discussions and the development of clinical guidelines.

BigMed is a Norwegian Research Council-funded project with the aim of addressing the challenges surrounding the clinical implementation of precision medicine approaches in Norway. This work, led by DNV GL, examines the implementation of NGS-based molecular diagnostics at clinical trials and molecular pathology units. Through process mapping, interviews, and site visits with labs across Norway and the other Nordic countries, this work explores the technical and organizational factors that have both hindered and promoted the adoption of NGS-based diagnostics in clinical pathways at these institutions.

The implementation of NGS-based cancer diagnostics in many hospitals is driven at a local level, often hinging largely on the efforts or involvement of single individuals. While this approach builds local competence and clinician buy-in, it leads to heterogeneous implementation on a national scale. Robust diagnostic guidelines and national coordination groups could help harmonize these services.

The challenges at large university hospitals and smaller institutions differ. Within large university hospitals, complex organizational structures with overlapping units, unclear mandates, and complicated funding and incentive systems can lead to an environment where technologies are actively developed and used in clinical research, but where these innovations are not available for routine patient care, which often evolves separately.

This work examines the implementation of NGS-based molecular diagnostics at clinical trials and molecular pathology units. Through process mapping, interviews, and site visits with labs across Norway and the other Nordic countries, this work explores the technical and organizational factors that have both hindered and promoted the adoption of NGS-based diagnostics in clinical pathways at these institutions.

Conversely, regional hospitals and units offering diagnostics in standard patient pathways often face challenges obtaining funds to develop and maintain diagnostic pipelines. IT infrastructure in these settings is often a key challenge, and is often implemented without integration with other lab systems, leading to inefficient workflows. Outside of diagnostics for clinical trials, labs are also reliant on testing guidelines and national reimbursement decisions, which in some cases are slow to adapt.

A key issue identified by stakeholders interviewed for this work was the verification, validation, and quality assurance of NGS-based tests. This becomes critical as Europe approaches the end of the transition period for the 2017 *In Vitro* Diagnostic Regulation (IVDR)[1]. which places substantial new requirements on health institutions. Nordic hospitals are heavily reliant on lab-developed tests, which are addressed by IVDR, but efforts to ensure compliance are only now beginning.

In addition to this work, which describes the organizational and technical factors faced by laboratories in the Nordics, various other projects address some of the topics raised here. Of particular note in Norway, a recent mapping of amplicon-based cancer diagnostics[2] serves to provide a comprehensive overview of this heterogeneous landscape, and the national DRUP-style IMPRESS oncology trial has the potential to promote clinical trials involvement for regional hospitals, a goal many stakeholders identified in this work.

Introduction

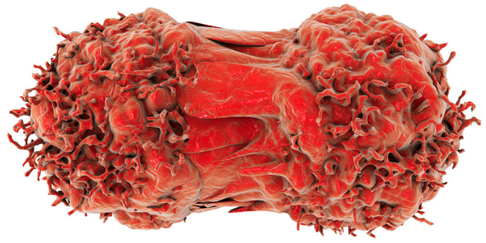
Rates of cancer are rising globally, with an estimated 18.1 million new cancer cases and estimated 9.6 million deaths in 2018[3]. On average, 1 in 5 men and 1 in 6 women will develop cancer at some point in their lifetime, and cancer is already the first or second leading cause of death of individuals under age 70 in over half of all countries.

Traditional treatment modalities include radiotherapy, chemotherapy, and surgical interventions, depending on the stage and type of Tumor and other clinical factors. Recently, using a precision medicine approach to treat cancer has become more common. Under this strategy, therapies are designed to take advantage of very specific molecular weaknesses in Tumors. These therapies may be more effective than existing modalities, but only against Tumors with a specific genetic profile. To determine if a targeted therapy is appropriate, biopsies of the candidate Tumor or blood samples are examined with molecular assays, so-called companion diagnostics, often based on complex workflows comprising molecular biology, high-throughput DNA sequencing, and bioinformatics.

The spread of precision medicine approaches for cancer has been rapid. In 2018, 42% of all new drugs approved by the US FDA were personalized medicines, and 10 out of 25 of these have indications for cancer[4], including the first ever cancer drug approved for a molecular indication, regardless of Tumor type.

Early meta-analyses[5] indicate that the precision medicine approach correlates broadly with higher median response rate and longer median progression-free survival, and mounting evidence on the effectiveness of precision medicines mean that global clinical practice guidelines (ie. NCCN[6], ESMO[7], Cochrane[8]) now routinely incorporate targeted therapies and molecular testing into their recommended standard-of-care.

The Norwegian Health Directorate strategy for personalized medicine in healthcare[9] recognizes the role for precision medicine approaches in the national healthcare system, and sets out high-level guiding principles and recommendations. Numerous other actors in the health space have issued similar roadmaps and strategies, however little structured data is available on the uptake of precision medicine approaches. How has the healthcare system begun to integrate precision medicine approaches alongside existing patient pathways? Has the implementation of prerequisite tools and infrastructure been successful? And more importantly, what are the factors that have enabled or hindered the use of precision medicine in the clinic?



Cancer is the first or second leading cause of death of individuals under age 70 in over half of all countries

9.6 mill
estimated deaths in 2018

18.1 mill
estimated new cancer cases in 2018

42%
of all new drugs approved by the US FDA in 2018 were personalized medicines

10 of 25
of these have indications for cancer

Background

As more medicines become available that are based on molecular indications, the role of companion diagnostics and molecular testing in the health system becomes more critical. This report explores how health systems across the Nordics have integrated sequencing-based molecular diagnostics into clinical care within the broad context of cancer. Evidence from clinical trials relying on NGS-based diagnostics including SHIVA[10], MATCH[11], FOCUS4[12] and others highlight the clinical utility of broad molecular testing and targeted therapies, and the increasing proportion of new therapies with molecular indications suggest that in the future, molecular testing will take a more central role in cancer care.

As many of these diagnostics rely on relatively new technologies and require specialized knowledge to develop, assess, and conduct, it cannot be taken for granted that these tests are ubiquitously available, even if the medicines requiring their use are approved and available. NGS-based diagnostics differ from other laboratory tests in that they can routinely analyze thousands of targets, including biomarkers for which there are not yet approved therapies. The technical capacity to run these diagnostics is critical to modern basket or umbrella trials, and data produced from these tests is valuable both to supporting Nordic involvement in clinical trials, but also to support health economics decisions and ongoing safety and performance monitoring.

Our goal has not been to provide a comprehensive overview of all organizations conducting NGS-based molecular diagnostics for cancer across the Nordics, but rather to provide a series of in-depth snapshots from different countries, health systems, and hospitals. By exploring a wide variety of settings, we can compare and contrast approaches to test development, implementation, and uptake, and uncover common trends, challenges, and enabling factors. Within a Norwegian context, this work should be considered alongside the recent comprehensive mapping[2] of Amplicon-based gene panel availability.

As more medicines become available that are based on molecular indications, the role of companion diagnostics and molecular testing in the health system becomes more critical. This report explores how health systems across the Nordics have integrated sequencing-based molecular diagnostics into clinical care within the broad context of cancer.

Method

Between August 2019 and September 2020, DNV GL led a process together with the network of BigMed partners to contact stakeholders in hospital management, diagnostics laboratories, and clinical units to join the project, aiming to enlist a mix of larger and smaller laboratories across different Nordic countries. Not all labs contacted were able to participate; commonly citing a lack of time or that their diagnostics were still undergoing development and were not yet mature.

Interviews for each site were conducted over 1 or 2 days, and typically included discussions with individuals involved in lab management, diagnostic workflows, bioinformatics, and clinical interpretation. Initial sessions were held as workshops at each lab, however COVID-19-related travel and hospital access restrictions meant that some were held virtually.

Lines of questioning were pre-designed to address four main subject areas: organizational factors, technical details, strategic ambitions, and operational considerations. Questions were designed to gather sufficient information to map out in detail the clinical workflows for diagnostics at that location, the organization and broader context which the unit interacts with, NGS- and hospital-specific IT systems, clinical interfaces, and specific enabling factors, barriers, and trends identified by staff. Participants were encouraged to discuss items freely, including off-record topics and information to be held in confidence. The same two interviewers, with backgrounds in NGS product development and risk management, were involved at every site.

After on-site or virtual interviews, data were merged and cross-checked, and areas where information was unclear or not addressed were used to generate a set of follow-up questions. Interactive visualizations of the diagnostic workflow and organizational structure at each site were used to structure information, and this was sent along with follow-up questions to participants to identify incorrect or missing information.

Short descriptive summaries were developed from these interactive maps with the goal of structuring information and promoting site-to-site comparison. These were shared with participants to check for accuracy, and are presented in the appendix of this report.

In addition to these interviews, in November 2019 a workshop was held in conjunction with the Nordic Alliance for Clinical Genomics (NACG) with the goal of mapping out cancer-related diagnostics activities across the Nordics. Based on initial findings from the first on-site mappings described above, a program was developed to gain a broader understanding of the activities and stakeholders in each Nordic country. First, this workshop identified the specializations and roles of participants, to help identify sampling bias. Participants then worked through a series of activities designed to map out institutions and connections involved in developing and providing cancer diagnostics, and worked together to identify and present country-specific needs and challenges. The results from this workshop are available on the NACG website[13].

Using data from the in-depth site mappings and Nordic conference, a set of preliminary findings were generated. These were designed to be broad enough in scope to reflect input from all sites, and were supported by specific examples. The preliminary findings were presented to each participating unit, as well as to stakeholders from within BigMed, to refine viewpoints and gather feedback. This document was prepared based on this feedback, and was further distributed to these same participants for review.

There are limitations to this approach that should be considered when evaluating the findings. Firstly, this work considers a relatively small number of sites which conduct different activities, ranging from routine Molecular Diagnostics (MDx) for solid Tumors, through hematological malignancies or MDx for clinical trials. While this approach provides an in-depth understanding at each site as well as a broad overview, this work should not be viewed as a comprehensive overview of either clinical research or diagnostics units. Recent work[2] from Oslo University Hospital has mapped out current panel offerings in Norwegian hospitals, and this provides a valuable companion resource to this paper.

Secondly, it must be stressed that the area of molecular diagnostics for cancer is rapidly evolving: available technologies, guidelines, available biomarkers and medicines, and policy and reimbursement decisions all impact these units and the factors identified here, and practices change more quickly than in other areas of medicine. As such, technologies, workflows, guidelines and practices are likely to change at many of these sites, so the information presented here should be viewed as a baseline.

Overview of Units

Participants represented units from both regional and university hospitals, and conducted a broad range of activities, including high-throughput testing of solid Tumors, diagnostics for clinical trials, technology evaluation, and screening. The scope and focus of units differed, as did the challenges faced by each. The factors presented in this work are meant to be relevant in some aspect to each of the units interviewed, although of course the specifics will vary.

University Hospital North Norway
Universitetssykehuset Nord-Norge (UNN).
Department for Clinical Pathology.

unn.no

St. Olavs University Hospital
Universitetssykehuset i Trondheim.
Department for Pathology.

stolav.no

Haukeland University Hospital (HUS)
Haukeland Universitetssykehus (HUS)
Section for Cancer Genetics

helse-bergen.no

Oslo University Hospital
Oslo Universitetssykehus (OUS)
Experimental Pathology

oslo-universitetssykehus.no

Vestfold Hospital (SiV)
Sykehuset i Vestfold (SiV)
Pathology Unit

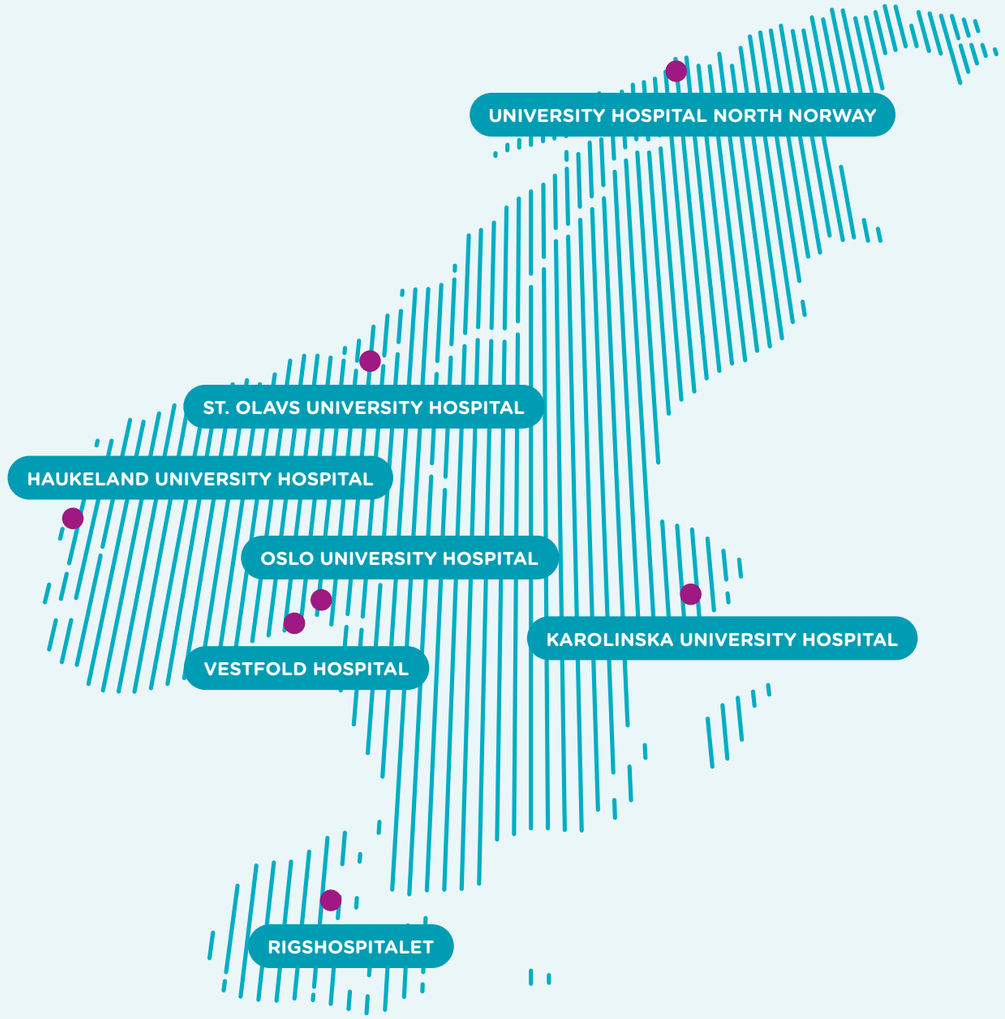
siv.no

Karolinska University Hospital and SciLifeLab
Karolinska Universitetssjukhuset (KUS/SLL)

karolinska.se / scilifelab.se

Rigshospitalet
Center for Genomic Medicine (CGM)

rigshospitalet.dk



General notes on institutions

The institutions we mapped generally fell into two categories: either large university hospitals or regional hospitals. At large university hospitals, we interviewed and mapped laboratory diagnostics units, which typically conducted NGS-based testing on solid and liquid biopsies. These units often employed multiple analytic methods (ie. microarrays plus sequencing) and were often involved in administering diagnostics for clinical trials or clinical research projects. Generally, these labs offered some combination of amplicon-based panel and exome sequencing, alongside supplemental methods like copy number variant (CNV) arrays or RNA-seq for structural variants. There was often a parallel pathology or molecular pathology unit at these health institutions which conducted routine sequencing for solid tumors, though these infrastructures were not generally well-connected. Bioinformatics was often comprised of custom-built analysis pipelines, and in many cases was offered on a high-performance computing cluster. These were usually run via hospital/university partnerships, and was also used for academic research. In many cases, commercial bioinformatics or interpretation software was also used, usually on local hardware, and often for later-stage variant analysis and reporting.

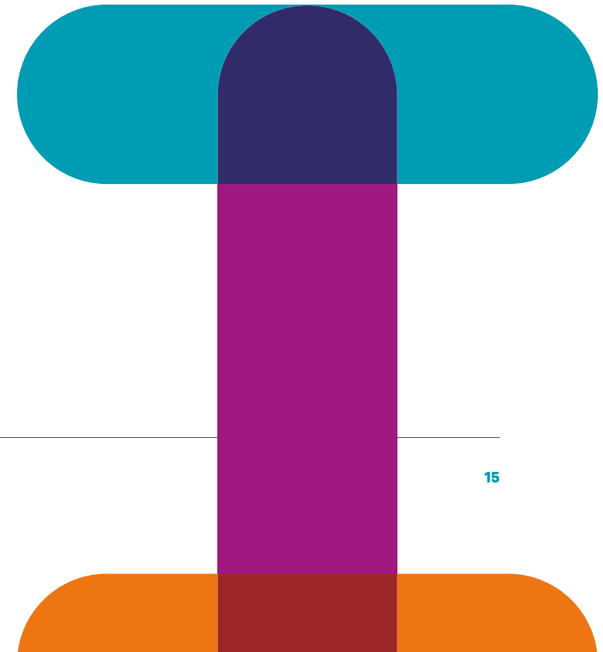
Regional hospitals typically used a low-throughput sequencing platform and often a single amplicon-based panel. NGS-based testing was usually housed within a pathology unit, and was well-integrated with existing histopathology workflows. These laboratories generally report variants from a smaller panel for routine use, as fewer biomarkers are relevant outside of a clinical trials setting. In all cases, these panels included content far beyond what was routinely reported and indicated in clinical guidelines and reimbursement rules, and labs indicated they could expand their test portfolio with reasonable ease. If these labs were not already participating in clinical trials, this was a high priority, with the rationale that participation had the potential to improve patient care. These units often collaborated closely with medical genetics or microbiology departments which also offered NGS-based diagnostics, going as far as sharing instruments or infrastructure in some cases, and often organizing de facto NGS-specific user groups within their institutions. Computing was usually performed on a local server used solely for NGS analysis, and both bioinformatics pipelines as well as interpretation tools were usually supplied from commercial vendors.

At both regional and university hospitals, data from NGS panels were interpreted alongside the results of histopathology or other molecular diagnostics, and often results were entered into a Laboratory Information Management System (LIMS). This was most often a pathology-oriented LIMS, where NGS results were summarized in a free text field, even if a NGS-specific LIMS was also used by the lab. Only one institution had an automated solution for transferring data between the LIMS and patient records within the hospital system, in most cases pathologists would enter a short free-text summary of findings in the patient journal, and in some cases would also attach a .pdf summary report.

Hospitals generally funded the implementation and development of these diagnostics with a combination of discretionary funding from the hospital budget and project-based research funding. Units performing routine sequencing were heavily influenced by national reimbursement and coding decisions when making technical decisions and when determining future requirements. The health institutions we visited aimed to maintain ISO 15189 certificates, which are required for medical laboratories, but did not generally pursue ISO 13485 (quality management systems for medical device manufacturers), ISO 27001 (information security management) or ISO 20916 (clinical performance studies). All laboratories developed and used lab-developed tests for NGS-based testing rather than approved CE-IVD tests, although some labs did use CE-marked diagnostics for other purposes, typically for more established biomarkers and indications.

Units performing diagnostics which support clinical trials focused on offering a broad range of biomarkers to attract new trials. Participation in trials funded testing at these units, although infrastructure, instruments, and other costs were often supported by a combination of trials and grants and hospital funding. Units performing routine diagnostics were heavily reliant on national guidelines and reimbursement rules for determining the biomarkers and tests offered, but in every case had the capacity to offer additional biomarkers should guidelines and reimbursement rules change.

Both regional and university hospitals generally employed staff with experience in molecular biology, pathology, and genetics. In addition, larger university hospitals employed groups of bioinformaticians and IT experts, which were needed for both the development, verification, and validation of analytic pipelines, the integration of tools and maintenance of infrastructure, and in some cases the development of new computational tools and analytics packages. Regional hospitals were less likely to employ an extensive team of bioinformaticians, and relied more on commercial software and the expertise of molecular biologists, geneticists, and clinicians when interpreting data. Both large and small units noted that providing these services requires a high degree of specialization, and that staff recruitment, training, and retention was critical for their operations. Many sites provided either formal or informal training and mentoring programs, and many were involved in joint educational activities with local medical schools. Educating clinicians on NGS testing was a topic raised by many of the units serving patients outside of a clinical trials setting, and represented an ongoing dialogue, often via regular user-group meetings, with the hospitals oncologists and other clinical staff.



Themes:

Landscape

Ground-up implementations show somatic sequencing is viable in a variety of settings, but leads to a heterogeneous landscape.

PAGE 18

Highlights

- Uptake of NGS-based diagnostics has occurred in a distributed, ground-up manner, and is viable in a broad range of healthcare settings.
- This distributed approach develops local competence, but leads to heterogeneous diagnostics where labs individually develop assays, systems, and practices.
- Mechanisms to coordinate activities, harmonize diagnostics, and address topics of national relevance are needed, especially on topics too large in scope for any single lab to address.

Organization

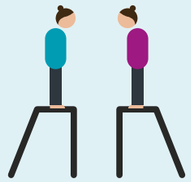
Broad change is hindered by complex mandates, silos, and unclear governance.

PAGE 20

Highlights

- Many of the barriers to implementing NGS-based cancer diagnostics are related to organizational factors within hospitals, not technological limitations.
- Future policies and initiatives should consider both pre-existing infrastructure as well as organizational and cultural factors that may serve as barriers or accelerators.

Innovation



Implementation gaps hinder the uptake of new technologies.

PAGE 22

Highlights

- NGS-based tests for routine clinical use have robust requirements for safety, clinical validity, and technical performance, however specific funding to support these requirements is missing.
- Proof-of-concept work or pilots in a research setting often do not adequately address these requirements, and diagnostics labs must prioritize this work against routine operations.
- The end of the IVDR transition period in May 2022 substantially increases the responsibilities of these labs, however few were directly involved in their hospitals IVDR transition plan.

Guidelines

A need for systematic approaches to the development and use of standards and guidelines.

PAGE 24

Highlights

- Clinical guidelines for testing are critically important, and need a standardized process to include molecular indications.
- Many recommendations and guidelines are available for parts of the NGS process, however generally do not offer concrete or specific recommendations. Interpretation of these guidelines could be addressed by national working groups.
- Existing quality management certifications are valuable, but may not address the technical aspects of NGS testing adequately.

Integration

NGS-specific infrastructure is poorly integrated with other clinical systems, and this tech debt introduces risks and impacts the effectiveness of staff.

PAGE 26

Highlights

- Stakeholders must recognize that NGS-based diagnostics have significant IT requirements, and that inadequately addressing integration leads in scalability issues and inefficient workflows for clinical staff.
- Sufficient resources must be made available when implementing these diagnostics, and provisions made to ensure these systems are kept up to date.
- Integration between NGS-specific systems and EHR systems is a particular challenge for many hospitals.

Reimbursement

Diagnostics units lack appropriate reimbursement and funding mechanisms.

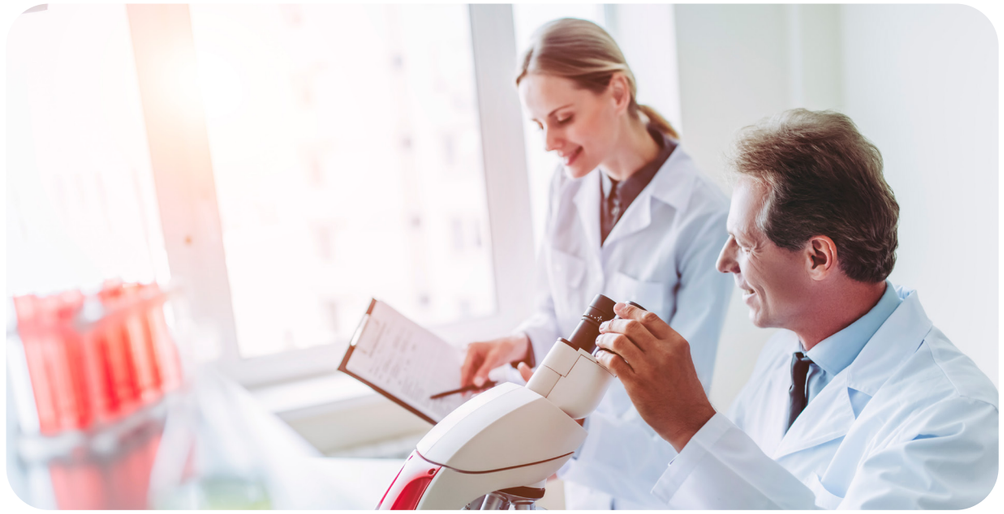
PAGE 28

Highlights

- Reimbursement decisions are a prerequisite for NGS-based cancer diagnostics outside of clinical trials, and dictate the design decisions and diagnostic offerings of hospitals.
- The breadth of NGS-based methods with clinical utility is expanding rapidly, and these methods may challenge pre-existing reimbursement models.

Landscape

Ground-up implementations show somatic sequencing is viable in a variety of settings, but leads to a heterogeneous landscape



We observed health institutions conducting NGS-based molecular diagnostics (MDx) at a variety of scales, ranging from regional hospitals processing small amplicon panels manually, through to clinical trials units conducting a broad array of diagnostics with high degrees of customization and automation. The prevalence of NGS-based testing suggests that the minimum investment in infrastructure, laboratory, and people required to implement simple NGS-based diagnostics is achievable in a broad range of health settings.

A common theme when discussing the environment and factors laboratories were faced with when initially deciding to offer NGS-based diagnostics was that, in almost all cases, these decisions were driven by acute clinical needs. In most instances, decisions to develop these capabilities were bottom-up, not driven from hospital or health system administration, and in many cases these decisions were taken in the absence of applicable funding instruments, reimbursement, or infrastructure. Both regional and university hospitals reported that their first NGS instruments or bioinformatics clusters were purchased with a mixture of hospital and research funding, or for a certain research project, after which they were used for a combination of diagnostics and clinical research. At many sites, infrastructure was shared between pathology and other diagnostics or research units, and in one instance two diagnostics units 'cross-validated' protocols on each other's instruments to reduce down-time due to instrument maintenance and upgrades. At other hospitals, expensive bioinformatic, automation, and sequencing infrastructure was often shared through core facilities with research or other clinical groups.

While the availability of these diagnostics despite a lack of supporting mechanisms highlights their importance to modern cancer treatment, this bottom-up approach has both positive and negative implications.

On one hand, sites with strong bottom-up initiatives generally display a high level of integration between histopathology and NGS-based diagnostics, often using the same staff, IT systems, and tightly integrating multiple modalities of MDx. The diagnostic workflows and design decisions made tend to be well-suited for local conditions. Pathology reports include relevant clinical findings from many types of molecular diagnostics, and close ties between molecular pathology units and treating physicians lead to a high level of clinical utility for these services. As an example, multiple sites described how the contents of their reports were tailored for actionable information requested by oncologists in their hospitals, and described an iterative process for developing these reports, especially when first introducing NGS-based MDx.

One drawback to this bottom-up approach, however, is that it leads to a very heterogeneous implementation in a national context. Units use different reagents, sequencers, panels, and analysis pipelines, and develop different practices for interpreting data and reporting variants. These differences have the potential to lead to undesired heterogeneity in diagnostic quality, in the quality of data for real-world evidence, in system robustness and reproducibility, and ultimately in patient care.

Many technical and practical questions need to be investigated independently at each site, and some topics, such as IVDR compliance, may be out-of-scope for the expertise of these units and could be better addressed at a national or regional level.

One further note is that this multi-site ground-up approach places a collectively higher total cost on the healthcare system, as diagnostics, sequencers, software, reports, and other systems are designed for each site. This is, however not necessarily a wholly negative point. Interview subjects repeatedly pointed to the need for greater education and understanding of molecular diagnostics for both clinical and laboratory staff, and the distributed approach develops both local competence and technology familiarity. One lab pointed out that even with approved turnkey CE-marked diagnostics, expertise is needed both to inform diagnostic choice and to identify potential issues during use.

In multiple countries, laboratory staff highlighted a greater need for national forums or mechanisms to coordinate these distributed efforts. Interview subjects noted various associations and stakeholders with the potential to take this role, but cited both a lack of mandate or leadership as well as local barriers to participation as reasons that these coordination groups were not in place.

HIGHLIGHTS

- Uptake of NGS-based diagnostics has occurred in a distributed, ground-up manner, and is viable in a broad range of healthcare settings.
- This distributed approach develops local competence, but leads to heterogeneous diagnostics where labs individually develop assays, systems, and practices.
- Mechanisms to coordinate activities, harmonize diagnostics, and address topics of national relevance are needed, especially on topics too large in scope for any single lab to address.

Organization

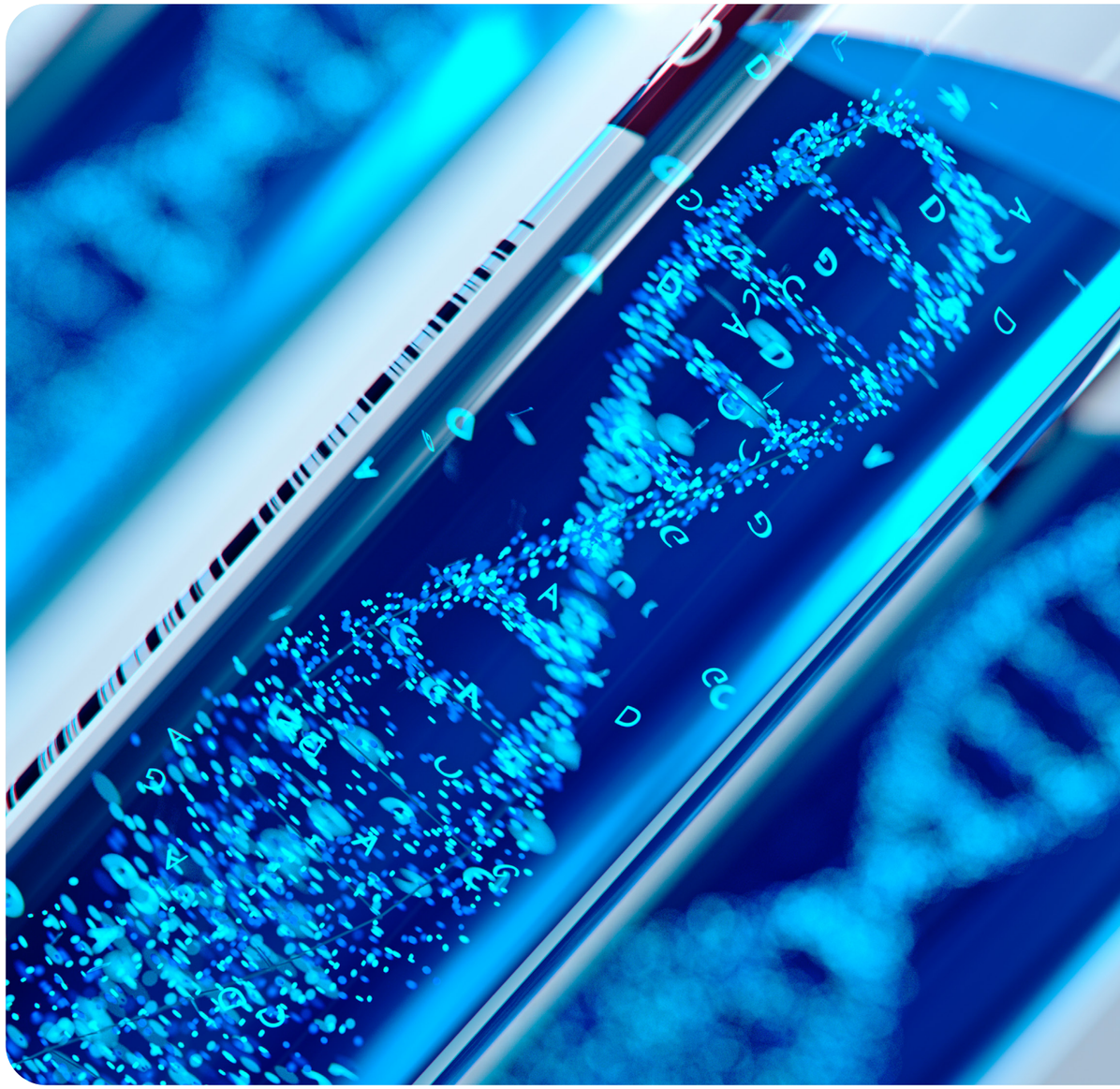
Broad change is hindered by complex mandates, silos, and unclear governance

Two patterns were apparent when labs discussed how they first began sequencing. Amongst smaller institutions, discussions were centered around the difficulties of obtaining necessary start-up funds, and around the technical challenges of choosing molecular targets, library prep reagents, NGS instruments, bioinformatics software, and validating research-grade tools for use in a diagnostic setting. In contrast, larger institutions often discussed political and organizational roadblocks, such as competition between units for resources or mandates. In several university hospitals, these organizational barriers have led to multiple, parallel units with overlapping activities, often with mixed and unclear responsibilities. In some instances, clinicians described how it was difficult to obtain desired diagnostics, since certain prerequisite technologies or clinical areas were viewed as outside of scope by multiple labs within the hospital. At some institutions, it was difficult to identify the correct stakeholders to interview for this work, even with the aid of hospital management, and in some instances the internal stakeholders identified initially by management were not in fact involved in NGS-based diagnostics.

The intricacies and history of particular organizational structures within health institutions was not a central focus for this work, however it is apparent that these factors impact both daily operations at a hospital level and larger, systemic initiatives. Larger institutions were more silo-bound, but often had access to advanced computational and sequencing infrastructure. In contrast, smaller hospitals often had greater levels of coordination between diagnostic units, but often lack the infrastructure required for extreme sample throughput or computationally and sequencing-intensive applications. While complex, the impact of organizational factors should not be underestimated when planning future initiatives, developing policies, or implementing new technologies.

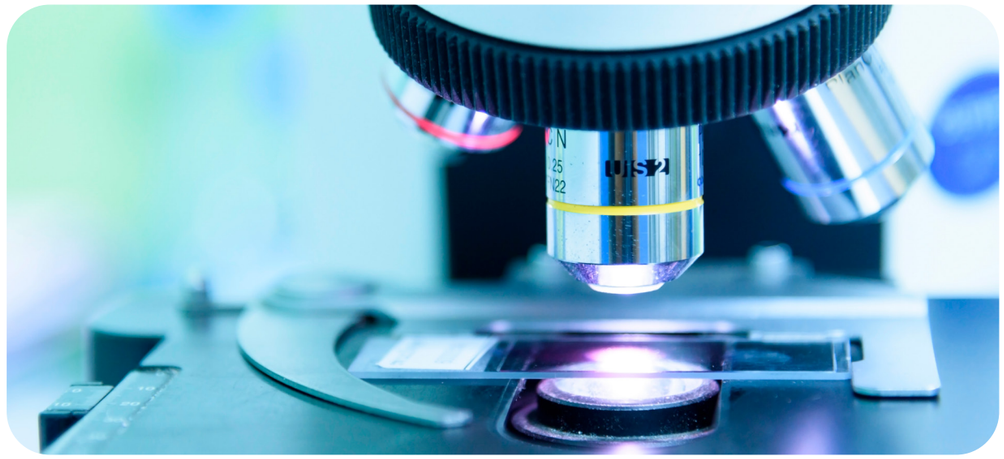
HIGHLIGHTS

- Many of the barriers to implementing NGS-based cancer diagnostics are related to organizational factors within hospitals, not technological limitations.
- Future policies and initiatives should consider both pre-existing infrastructure as well as organizational and cultural factors that may serve as barriers or accelerators.



Innovation

Implementation gaps hinder the uptake of new technologies



In settings where it would be logical for diagnostics to flow from initial development in a research context, through piloting, verification, and validation in a clinical context, and finally into a production diagnostics setting, several gaps exist where research and clinical stakeholders collaborate.

Particularly at larger university hospitals, there were significant gaps between research and clinical activities preventing the uptake of technologies developed in a research context. These tech-transfer gaps also contributed to organizational tensions. As a concrete example, at several sites, groups had access to the instruments, workflows, and bioinformaticians to perform broader or more sophisticated testing, however these resources were primarily used for research. Where they were used clinically, it was usually to support clinical trials, and routine Tumor sequencing was often performed by a wholly separate unit within the organization with its own goals and development pipeline.

The funding structures and end goals of these two types of activities are different. Researchers are incentivized to publish, preferably in high-impact journals, to obtain funding through competitive grants for their institutions, and to produce graduate students and teach to some level. In contrast, hospitals are tasked with providing robust, quality-assured diagnostic tests based on strong or overwhelming scientific consensus, often at scale and with quick turnaround time, and within a given reimbursement framework according to relevant regulatory standards.

The technical requirements for research and for diagnostics pipelines also differ greatly. In contrast to the open-ended methods required for scientific exploration, routine diagnostics and patient treatment rely on a relatively limited set of biomarkers and well-established scientific knowledge that require high quality and safety standards. The standards for scientific evidence required before a diagnostic pipeline can be implemented are significant. Biomarkers must be linked with clinical outcomes, and clinical utility must be demonstrated, meaning that in the absence of effective, available, and reimbursable treatments or without demonstrable prognostic value, particular targets are generally not used outside of clinical trials.

In addition to the need for strong scientific consensus and clinical utility in a diagnostic setting, bioinformatics tools and test protocols developed and published in academic research generally do not fulfill the quality requirements needed by diagnostics units. Conversely, the diagnostic units interviewed in this work have a core focus on test delivery, leaving a gap where technologies are conceptually viable, but lack the robust safety and performance controls needed for use in a healthcare setting. Incentives and policies that address the operationalization of research protocols in a diagnostic setting would help these labs develop additional capabilities. In settings where research and clinical teams worked in closer collaboration, these gaps were less severe.

As a concrete example, we spoke with research groups that routinely tested and developed methods and software for cancer testing in an academic setting. Clinicians we spoke with described a clear use-case and clinical value, however no clear trajectory for post-research, pre-clinical development and validation efforts was visible. While both researchers and clinicians want these tools to be adopted, incentives to validate and verify these tools are missing: this work would not generate high impact-factor publications, nor would it be reimbursable under current coding schemes. Hospital stakeholders we spoke to noted that developing and testing new assays or technologies was desirable to offer better care to patients, but that this work could only be conducted if case load and high-priority routine work allowed. Many hospitals also indicated that some work, such as the re-writing of academic or research-use-only packages to the standards of medical device software, was out of scope for both their diagnostic units and their supporting hospital IT staff.

The 2017 IVDR placed new requirements on both IVD manufacturers and health institutions that develop and use lab-developed tests. In brief, hospitals face some of the same requirements for demonstrating performance, safety, and quality as IVD manufacturers. The labs we interviewed were sometimes aware of the IVDR, but were not involved in their hospitals IVDR compliance strategy and in some cases believed their diagnostics fell outside of the regulation. There is significant overlap between the new requirements for hospitals and the topics described above which are currently poorly incentivized between academic and clinical units. While there is significant uncertainty amongst stakeholders regarding IVDR implementation, the new regulations provide specific action points which could serve to focus initiatives to address implementation gaps.

HIGHLIGHTS

- NGS-based tests for routine clinical use have robust requirements for safety, clinical validity, and technical performance, however specific funding to support these requirements is missing.
- Proof-of-concept work or pilots in a research setting often do not adequately address these requirements, and diagnostics labs must prioritize this work against routine operations.
- The end of the IVDR transition period in May 2022 substantially increases the responsibilities of these labs, however few were directly involved in their hospitals IVDR transition plan.

Guidelines

A need for systematic approaches to the development and use of standards and guidelines

Many of the institutions we interviewed highlighted the need for NGS guidelines. These could encompass clinical guidelines for when to provide testing, for which biomarkers to test or how to test for certain variants, technical guidelines for pipeline and panel design and quality control, or guidelines for interpreting or reporting variants.

Of key importance for testing were the national clinical guidelines. Typically published by national health infrastructures or professional societies, these include indications for testing, biomarkers, and clinical utility, and guide laboratories when determining which tests to offer and how to deliver these services. In areas where national clinical guidelines included relevant molecular biomarkers and guidance on testing, units generally reported that these were central to their lab's activities, and noted that due to the time required to develop appropriate assays, it would be desirable to have testing requirements well in advance of updates to guidelines for molecular testing. Both diagnostic and clinical stakeholders in all countries identified treatment areas where they believed national guidelines included sufficient guidelines for molecular testing, but could also identify areas where NGS guidelines were not available or did not address the topic of precision medicine and molecular diagnostics altogether.

We asked lab managers, clinicians, pathologists, and bioinformaticians about the process for developing these guidelines. Stakeholders reported differences in the adoption of MDx into guidelines between cancer types. For clinical areas where guidelines include molecular indications and information on molecular diagnostics, this was because at least one specialist on the drafting committee had substantial experience with molecular diagnostics, and was informed on recent state-of-the-art from international clinical consensus and scientific proceedings. Treatment areas without molecular guidelines were due to two primary reasons: either there was insufficient clinical evidence for prognostic or diagnostic value for molecular testing, or the committee drafting these guidelines lacked experts in molecular diagnostics, next-generation sequencing, bioinformatics, or similar relevant specializations.

While we have not conducted an in-depth study on the subject, interviewees gave two relevant pieces on information. Firstly, stakeholders reported that the governance and procedures for organizing committees, reviewing evidence, and drafting guidelines were in many cases not systematic, but rather ad hoc. As an example, we heard from one source involved in maintaining these guidelines that the committee would consider only therapies/diagnostics which had received positive reimbursement decisions from the national HTA process (Beslutningsforum in Norway). Another stakeholder involved with a different committee indicated they would consider therapies/diagnostics authorized by the European Commission if they felt these were appropriate for the patient, regardless of the reimbursement status in Norway. An organized framework for ensuring relevant expertise and processes are in place could help ensure molecular diagnostics are evaluated. Secondly, molecular pathologists who did participate in drafting these guidelines indicated that this was not an incentivized activity and participation in the groups depended on voluntary unpaid work, and directly competed with other clinical duties. Due to the central importance of these documents on a national stage, establishing a structured framework and process for developing these guidelines, ensuring representation from relevant fields in this process, and introducing incentives for securing engagement, all appear to be valuable actions to ensure clinical guidelines incorporate appropriate molecular testing and treatments. A joint MDx committee that consults other clinical groups on these topics could make the approach more harmonized across disease groups.

In addition to national clinical guidelines, labs make use of a variety of other professional guidelines for specific parts of the diagnostic workflow, such as sample preparation, read quality control, variant calling, interpreting pathogenicity, and reporting molecular findings. Units we interviewed knew about these guidelines and often reviewed them when determining what their own policies and protocols should be. It was rare that units would follow even widely adopted guidelines, such as the AMP/CAP guidelines on variant pathogenicity classification, often for various operational reasons (ie. in-house variant classification system was already established). In other cases, guidelines were described as 'too general' or 'too poorly defined' for use: recommendations were described as too high-level or generalized to be useful to these laboratories. As an example, guidelines from the scientific literature would recommend that laboratories use "appropriate" quality control measures, but would not provide more actionable guidance. This non-specificity was identified as a problem for both national and international publications.

Most of the units we mapped held ISO 15189 certificates. This ISO describes quality management system requirements for laboratories offering medical diagnostic tests, and is in fact a strict requirement for health institutions offering lab-developed tests under the upcoming IVDR. The units we talked to were accredited by different national bodies, and each looked at different aspects of the QMS and diagnostic pipeline to different degrees. All units we interviewed highlighted that the ISO process was valuable from a quality perspective, and that the act of codifying procedures and protocols, preparing for, and undergoing audits triggered a different way of thinking about lab procedures and quality that they felt contributed to the safety and validity of the services they provide. Several units reported that accrediting bodies failed to have sufficient background knowledge about genetic testing, NGS, and especially bioinformatics to be able to contribute to quality and safety discussions, and identified the lack of attention to aspects of the diagnostic workflow outside of the wet-lab as a concern. One unit noted that their accrediting body continually sent different staff to the lab, and that while this led to more superficial audits, the broad range of different backgrounds these auditors had was valuable.

When we asked units about their strategy regarding the use of lab-developed tests instead of CE-marked IVDs or software and compliance to the upcoming IVDR, many stated that they used lab-developed tests exclusively, and would continue to do so under the new regulations. At the time that some units first started NGS testing, approved IVDs were not available, and technology lock-in and re-verification and validation costs have prevented many units from transitioning to approved solutions. In other cases, CE-marked platforms were available, but were not chosen. Units conducting diagnostics for clinical trials noted that some trials required additional biomarkers for which no tests were available, and some units noted that approved medical tests cost more than research-use-only reagents or systems. When asked about IVDR and its impact on their units, many interviewees were aware that upcoming regulatory changes would impact their hospitals, but were not aware about specific implications for their labs or their institutions IVDR compliance strategy.

HIGHLIGHTS

- Clinical guidelines for testing are critically important, and need a standardized process to include molecular indications.
- Many recommendations and guidelines are available for parts of the NGS process, however generally do not offer concrete or specific recommendations. Interpretation of these guidelines could be addressed by national working groups.
- Existing quality management certifications are valuable, but may not address the technical aspects of NGS testing adequately.

Integration

NGS-specific infrastructure is poorly integrated with other clinical systems, and this tech debt introduces risks and impacts the effectiveness of staff

The bottom-up approach to implementing NGS-based diagnostics has advantages, particularly in ensuring a match between diagnostic capability and clinical need, however does not lend to a robust, long-term infrastructure strategy. The instruments, servers, software, databases, and APIs we mapped at these institutions tended to be poorly supported or integrated, and in many cases software issues directly impacted the clinics day-to-day work.

Infrastructure for analyzing NGS data tended to fall into one of two categories. Units performing diagnostics for clinical trials, typically at larger university hospitals, tended to use high-performance computing clusters for this work. These clusters were operated by separate parties but were usually within a joint academic or translational research setting, not within the standard hospital network. In these cases, diagnostics were supported by a team of bioinformaticians and software developers, and we observed several examples of fairly advanced in-house software solutions. As specific examples, one diagnostics unit, which provides services for a broad range of stakeholders, provided an advanced sample requisition system, which automatically assigned samples and pipelines, allocated and prioritized jobs, performed diagnostics and quality control checks, and integrated with laboratory NGS LIMS for sample tracking. In another instance, a unit performing clinical trials had access to a custom-built variant interpretation portal, that gathered information from testing along with a broad palette of scientific literature and contextual information to help clinicians interpret the impact of genetic findings for a specific patient.

For routine diagnostics, NGS data were generally analyzed on a local server dedicated for this purpose. These servers were within the hospital network, and often ran commercially available software. In some cases, software or clients for the interpretation or reporting of NGS data was installed on the PCs of laboratory staff. While units utilizing high-performance computing clusters and employing teams of bioinformaticians, architects, and developers relied heavily on this expertise, labs with isolated servers noted that maintaining software and databases was difficult, exacerbated in some instances by the inability to use software-as-a-service (SaaS) solutions. These labs also noted that updating these systems was difficult due to institutional access restrictions. In one example, a units running a local NGS server was using a population allele frequency database that was several years old, and was unable to update this due to the hospitals internet access restrictions. Both groups relying on standalone servers and high-performance computing felt their hospital IT providers were not capable of providing the necessary support, and as a result either brought these responsibilities in-house with their own, NGS-focused IT groups, or operated using largely unsupported turnkey solutions.

Labs usually had a long-standing histopathology-focused LIMS in place, supported by the hospital IT provider. In some cases, these LIMS were integrated with Electronic Health Record (EHR) systems. Many units also used one or more NGS-specific LIMS, but these were generally not integrated with other software. After finding clinically relevant results, many units would enter these into the histopathology LIMS as free text or in a comments field, to ensure that NGS findings were considered alongside histopathology findings when issuing a final report.

All units we interviewed used excel files to track various clinical information to different degrees, including sample and accession information, NGS findings, quality information and sample metadata, clinically relevant findings, classified variants, and variant classification rationale. Units recognized that the manual copying of data was both a burden on staff and a risk to patients, but hospital IT departments could generally not implement databases and commercial software tended to not support the exact data the lab wished to track. Units with bioinformatics staff, usually supporting clinical trials, would employ custom-built databases for tracking some information, but would still rely to some extent on excel for certain functions.

Findings from pathology were entered manually into hospital EHR systems, sometimes with standardized language, by laboratory staff. In one case, a large university hospital had an automated solution to transfer histopathology findings to the patient journal, but generally, IT systems used by the laboratory to assess and store findings were not connected with IT systems holding patient records. Findings from NGS-based diagnostics were usually included alongside histopathology and other molecular diagnostics conducted by the pathology lab in a single report, most commonly as a .pdf attachment or as a single free text field. Information that was included in patient journals was basic, and often excluded information about limitations of testing, methods, quality metrics, etc. This was intentional, and labs generally came to these decisions after discussions surrounding the value of additional information to the clinicians most likely to be reviewing these records, versus the potential of inducing information fatigue and missing key findings.

While hospitals have been able to implement basic systems to allow NGS testing, these efforts have accumulated significant technical debt. In many cases, software has not been integrated with other tools within the hospital, and data is not collected nor stored in structured formats or databases. Furthermore, hospital IT providers seem unable to deliver sufficient expertise in the field in many cases, forcing many units to bring these functions in-house. The failures to integrate and support these systems result in manual workflows that consume the time of laboratory staff, and in some cases generate quality and safety risks. The poor integration between NGS and EHR software furthermore splits diagnostic and clinical data, hindering the use of this data as real-world evidence for supporting clinical trials, internal quality assessment, or in pharmacovigilance or post-market surveillance efforts.

HIGHLIGHTS

- Stakeholders must recognize that NGS-based diagnostics have significant IT requirements, and that inadequately addressing integration leads in scalability issues and inefficient workflows for clinical staff.
- Sufficient resources must be made available when implementing these diagnostics, and provisions made to ensure these systems are kept up to date.
- Integration between NGS-specific systems and EHR systems is a particular challenge for many hospitals.

Reimbursement

Diagnostics units lack appropriate reimbursement and funding mechanisms

Funding and reimbursement is addressed differently in labs conducting clinical trials and those conducting routine cancer diagnostics. Units conducting diagnostics for clinical trials were generally funded by a combination of hospital or health system funding and through commercial partnerships and research grants. While these collaborations support a broad array of diagnostic activities, also over the long term, labs we interviewed highlighted several challenges. Firstly, developing clinical partnerships requires a strong clinical, patient-facing team (ie. the presence of a strong diagnostics unit is necessary, but not sufficient), and well-developed logistics systems. Diagnostics units rely on these pre-existing networks, and some of the labs we interviewed had a long-standing history of supplying diagnostics for trials, prior to offering NGS testing. Units also reported that a high degree of flexibility was required, as trials would often require specific targets, test methodologies, data formats, and software to be used to standardize across sites. Partially because of this, these units often offered a broader range of diagnostics, including exome, whole genome, RNA-seq, and microarrays. One topic that some units brought up were changes to the clinical trials paradigm: units are seeing more basket or umbrella trials, complicating the topic of funding, especially in instances where trials include re-purposed medications or indication expansion. While the labs we interviewed were generally well-supported, staff noted that getting new trials from partnerships or research grants was a continuing topic.

In contrast, units performing routine diagnostics were often initially supported through a combination of research grants and hospital discretionary funding, but were heavily dependent on national reimbursement rules to support their continued operations.

Until recently, some of the countries surveyed did not have NGS-specific reimbursement codes, and relied on adapting to systems designed for aCGH, sanger sequencing, or other molecular tests, often with unexpected results. For example, one unit reported that when they began NGS-based testing, reimbursement codes for BRAF testing were based on sanger sequencing, and charges were on a per-read basis. Due to the thousands of reads covering each variant, this could lead to an absurd reimbursement. In the absence of specific guidance on the topic, the lab chose a cost-based model to cover their operating expenses until rules were recently updated, however this example highlights the need for a systematic approach to reimbursement that reflects the rapidly advancing clinical knowledge and diagnostic capabilities available.

Multiple labs also reported differences in reimbursement for internal vs. external testing which led to apparently unintended consequences, such as clinicians triaging groups of patients to prioritize for external testing when assays were not available in-house, or electing to perform diagnostics in an outpatient rather than inpatient setting if possible. It is important to note that clinicians and diagnostic labs were focused on providing accurate, cost-effective diagnostics, and generally found solutions to address the quirks of their reimbursement systems.

Another topic these units addressed was the development, validation, and verification of new assays. These activities comprise a substantial ongoing workload for many units and have a higher importance than in more established diagnostic areas, where the focus may be on routinely performing a decades-old assay. Many of the labs we interviewed expressed an ongoing internal tension between spending time developing and validating new assays and conducting routine analyses, and noted that specific financial instruments for maintaining and updating diagnostic pipelines were not available. At larger institutions, differences in how units were incentivized seem to contribute to competition or tensions between units.

While funding and reimbursement varied across countries and between units, the topic is complex in all cases. Trials units need the means to attract international trials, and to support umbrella or basket trials which may encompass arms with established therapies, compassionate use, and experimental modalities. Units performing routine diagnostics need a reimbursement system that can adapt quickly to the state of the art, and that understands that these labs are responsible for assay development, validation, verification, and in some instances maintaining informatics and IT solutions.

HIGHLIGHTS

- Reimbursement decisions are a prerequisite for NGS-based cancer diagnostics outside of clinical trials, and dictate the design decisions and diagnostic offerings of hospitals.
- The breadth of NGS-based methods with clinical utility is expanding rapidly, and these methods may challenge pre-existing reimbursement models.

Conclusions

During the course of this work we interviewed dozens of clinicians, molecular biologists, pathologists, and bioinformaticians at both molecular pathology and clinical research laboratories. These different types of laboratories faced distinct but similar challenges, many of which were related to the broader organizations they exist in and other aspects of the health systems they interact with, rather than pure technology issues. These organizational factors differed between large university hospitals and regional hospitals.

To date, NGS-based diagnostics have been developed through parallel, ground-up initiatives, rather than centralized national efforts. This serves to provide close clinical integration and develops distributed, local competence, but has led to a heterogeneous landscape and in general fails to address topics which are out of scope for any single lab. Stakeholders in this work identified several topics for national collaboration, including addressing clinical guidelines, reimbursement issues, quality and regulatory topics, and several technical objectives as potential actions.

While the specific technologies and assays used by labs differed, there were consistent themes regarding infrastructure and bioinformatics support. Large university hospitals have generally used academic high-performance computing clusters to support their diagnostics, and have brought many IT and informatics functions in-house. Regional hospitals have tended to implement one-off, standalone servers with commercial software. While effectively solving immediate needs, these systems were generally not integrated with other IT systems, leading to inefficient workflows for staff, and were generally not maintained over time, potentially introducing safety or quality risks.

Many labs identified similar factors as critical for their success, such as their clinician outreach activities or education efforts. Labs also identified similar areas for development, such as national testing guidelines, funding for assay validation and verification, and the need for greater coordination between labs. Our hope is that this work can initiate further discussions surrounding these topics and lead to collaborative efforts to address these challenges in the future.

Labs also identified similar areas for development, such as national testing guidelines, funding for assay validation and verification, and the need for greater coordination between labs.

References

1. *Regulation (EU) 2017/746 of the European Parliament and of the Council of 5 April 2017 on in vitro diagnostic medical devices and repealing Directive 98/79/EC and Commission Decision 2010/227/EU. European Union. Official Journal of the European Union L117, 60 2017.*
2. *NGS diagnostikk innen kreft (somatiske forandringer)-Rapport fra kartlegging (AS-IS). Oslo University Hospital. 2020 [cited 2021-01-04]; Available from: https://spesialisthelsetjenesten.no/seksjon/persontilpasset-medisin/Documents/Prosjektrapport-NGS%20diagnostikk%20innen%20kreft_v1.0_offentlig.pdf*
3. *Cancer Today: Data visualization tools for exploring the global cancer burden in 2020. World Health Organization. 2020 [cited 2020-12-01]; Available from: https://gco.iarc.fr/today/home*
4. *Personalized Medicine at FDA: A progress & outlook report. Personalized Medicine Coalition. 2019 [cited 2020-08-10]; Available from: http://www.personalizedmedicinecoalition.org/Userfiles/PMC-Corporate/file/PM_at_FDA_A_Progress_and_Outlook_Report.pdf*
5. *Schwaederle, M., M. Zhao, J.Jack Lee, et al., Association of Biomarker-Based Treatment Strategies With Response Rates and Progression-Free Survival in Refractory Malignant Neoplasms: A Meta-analysis. JAMA Oncol., 2016. 2(11): p1452-1459.*
6. *National Comprehensive Cancer Network Guidelines. 2021 [cited 2020-07-10]; Available from: https://www.nccn.org/professionals/physician_gls/default.aspx*
7. *European Society for Medical Oncology Guidelines. 2021 [cited 2020-07-10]; Available from: https://www.esmo.org/guidelines*
8. *Cochrane Cancer. 2021 [cited 2020-07-10]; Available from: https://cancer.cochrane.org/*
9. *Summary of The Norwegian Strategy for Personalized Medicine in Healthcare: 2017-2021. 2016 [cited 2020-07-10]; Available from: https://www.helsedirektoratet.no/rapporter/strategi-for-persontilpasset-medisin-i-helsetjenesten/Summary%20of%20the%20Norwegian%20Strategy%20for%20Personalised%20Medicine%20in%20Health%20Care.pdf/_/attachment/inline/5a6c511c-b245-4546-8dfa-daa057f275dc:f0a88b9e56dddd83901639bea4de5c04919bf407/Summary%20of%20the%20Norwegian%20Strategy%20for%20Personalised%20Medicine%20in%20Health%20Care.pdf*
10. *A Randomized Phase II Trial Comparing Therapy Based on Tumor Molecular Profiling Versus Conventional Therapy in Patients With Refractory Cancer (SHIVA). 2013 [cited 2021-01-04]; Available from: https://clinicaltrials.gov/ct2/show/NCT01771458*
11. *Targeted Therapy Directed by Genetic Testing in Treating Patients With Advanced Refractory Solid Tumors, Lymphomas, or Multiple Myeloma (The MATCH Screening Trial). 2015 [cited 2021-01-04]; Available from: https://www.clinicaltrials.gov/ct2/show/NCT02465060*
12. *FOCUS4: A molecularly stratified trial programme in colorectal cancer. 2020 [cited 2021-01-04]; Available from: http://www.focus4trial.org/*
13. *Nordic Alliance for Clinical Genomics: 8th Clinical Workshop Report. 2019 [cited 2020-05-05]; Available from: https://nordicclinicalgenomics.org/assets/resources/nacg-8th-workshop-and-symposium-report---2019.pdf*
14. *Nakken, S., G. Fournous, D. Vodák et al., Personal Cancer Genome Reporter: variant interpretation report for precision oncology. Bioinformatics, 2018. 34(10): p1778-1780.*

Acknowledgements

We would like to thank the clinical professionals that opened their laboratories to us to make this work possible. In spite of tight schedules and conflicting priorities, you took the time to share your knowledge and perspectives, and to help us understand.

University Hospital North Norway

Universitetssykehuset Nord-Norge (UNN)
Department for Clinical Pathology

ORGANIZATION

The University Hospital of Northern Norway (Universitetssykehuset Nord-Norge, UNN) consists of several hospitals, including the University Hospital in Tromsø. Of the 4 departments there that conduct laboratory testing in clinical setting, 3 have NGS capabilities: Clinical Pathology, Medical Microbiology, and Clinical Genomics. Within Clinical Pathology, there are neighbouring sections for General Histology and Autopsy, which conducts IHC and histology analyses, and for Special Analysis, which is responsible for molecular pathology including NGS, electron microscopy, and cytometry. Routine NGS testing in molecular pathology is conducted on a fixed weekly schedule

DIAGNOSTIC OFFERING

The diagnostics the lab chooses to offer are based on testing recommendations in the Norwegian National Guidelines for Cancer. Members of the lab also participate in developing these guidelines. The degree to which guidelines include NGS testing or relevant biomarkers is driven by clinicians, and influenced by the composition of the panels writing these guidelines. The assays used by the lab cover clinically actionable biomarkers not included in the Norwegian National Guidelines, so the lab can relatively easily expand its reporting portfolio. Treatment pathways include reflex testing via NGS for a small number of cancer types, including certain lung cancers and metastatic colorectal cancer in patients <60 years. The specific technologies used for testing are informed by the expertise within the unit.

GUIDELINES FOR TESTING

The lab includes results which are indicated in the Norwegian National Guidelines for Cancer. The panel the lab uses includes a broader set of clinically relevant genes which are not included in these guidelines, but reporting is based on the national guidelines.

SAMPLES AND LOGISTICS

Clinical Pathology receives approximately 60 000 samples per year. Approximately half are solid cancer samples, and half are cytology samples for a cervical cancer screening program. Most solid samples are delivered on formalin, and encompass a broad range of surgical samples, biopsies, and from hospital departments and local practices across Troms and Finnmark. Additionally, a research biobank collects fresh-frozen samples, and some blood samples. All solid samples are analyzed by the unit for General Histology, and approximately 800-900 samples per year are referred to Molecular Pathology for NGS.

PREANALYTICS

Resection, fixation, paraffin embedding, tissue sectioning, IHC , and histological work-up are all performed by General Histology prior to any molecular testing. Sectioning for molecular diagnostics is performed within Molecular Pathology, and is based on pathologist Tumor load estimates. Molecular Pathology will macro dissect from sections if estimated Tumor load is low. Generally, the lab does not run analyses if estimated Tumor load is <10%. In cases where Tumor load is uncertain, the lab will run the samples but place caveats in the report regarding uncertain results. The lab does not generally analyze paired Tumor/normal samples

ASSAYS

The lab uses the TruSight Tumor 15 panel, which targets 15 genes commonly mutated in solid Tumors which have either FDA or EMA-approved therapies, strong evidence for clinical utility supported by the NCCN or ESMO guidelines, or are relevant for clinical research. The panel detects indels and somatic variants. DNA extraction is using Qiagen FFPE kits. Library preparation is done manually. Libraries are quantified via fluorometric assay prior to pooling, and while libraries are ran on a gel for QC, libraries are sequenced regardless of quality or adapter-adapter quantity. If samples fail QC after sequencing, the lab has developed a length assay for testing FFPE DNA degradation, to help exclude systemic library preparation issues.

SEQUENCING

Libraries are sequenced on an Illumina MiSeq, which generates 22-25 M paired-end 150 nt reads. The lab generally multiplexes <10 samples per run. Throughput is primarily limited by uneven coverage: coverage maximums can be as high as 100 000 with TruSight Tumor 15 chemistry. Sequencing is conducted weekly on a fixed schedule: sample delivery cutoff is Monday 13:00, samples are sectioned Monday PM, de-paraffinized overnight, DNA extraction and library preparation is performed on Tuesday, and libraries are pooled and sequenced on Wednesday. The lab aims for a maximum turnaround time of 10 days, and usually returns samples within 5-6 days, depending on submission date vs. the Monday cutoff.

DATA ANALYSIS

Data are analyzed on-site on a local server in the hospital network running Illumina Local Run Manager (LRM) and the TruSight Tumor 15 Analysis Package. Quality control measures are tracked for the run and for each sample, and include target coverage (>500 for all regions), per-sample coverage, cluster density, fraction of aligned reads, error rate, per-base quality score, and PhiX error rate (<1%). These metrics are tracked in separate excel sheets for trend analysis, to help the lab detect drift and systematic error. Failure to meet minimum per-base read depth or per-base error requirements will lead the lab to repeat the run.

INTERPRETATION

Variants are interpreted in Illumina Variant Studio, installed locally on hospital PCs. The lab imports data from LRM, and examines the clinical indication in the Symphony LIMS used by General Histology to document their findings and request molecular testing. The lab filters out variants and only examines variants in genes indicated in the Norwegian Guidelines (NRAS, BRAF, EGFR, and KRAS for lung and colorectal cancer), and examines variants located in exons or <20 bp into introns. Variants >1% population frequency in ExAC, or specific populations within the 1000 genomes project, are excluded. Variants are compared with variants the lab has detected previously, which are stored in excel. Novel variants are interpreted according to AMP/CAP guidelines, with the aid of COSMIC and CiViC. A brief summary of molecular findings is written in the Sympathy LIMS, and the final report is a synthesis of molecular and histological findings. Positive NGS findings will include the variant, classification, population frequency, and other relevant information, but are kept brief. Classification of all variants are stored in the Variant Studio database. At present only one clinician classifies all variants.

CLINICAL ACTION

Weekly MDT meetings are held for each type of cancer, and are attended by participants from oncology, radiology, surgery, pathology, and other members of the treatment team as needed. Different pathologists are assigned to each cancer specialty, so represent Clinical Pathology in the same meeting consistently. During these meetings, the team discusses each case and the clinical information available, including available trials, and will request new biopsies or tests if required. Molecular biologist or bioinformaticians do not attend this meeting, since the pathology reports are finalized within Clinical Pathology prior to this.

INFRASTRUCTURE

Data from the MiSeq is analyzed with Illumina Local Run Manager and then transferred to a server within the local hospital network, which can be accessed from client terminals within the hospital security and authentication systems. These clients run Illumina Variant Studio locally, which is used for variant interpretation. The Sympathy LIMS is used by to document histopathology findings, and Molecular Pathology manually summarizes the results of molecular testing as free text within that system. Requests for molecular testing and other important information about the sample, such as estimated Tumor load and TMN staging, is communicated through Sympathy. While the integration of Sympathy with the hospital's DIPS EHR system is limited, oncologists have access to both systems.

QUALITY AND VALIDATION

The lab maintains an ISO 15189 certificate from Norsk Akkreditering. NGS testing is conducting exclusively with RUO reagents, platforms, and software, and assays are validated and verified in-house as LDTs. Assays are validated on clinical samples and commercial reference materials. Cut off for variants are 1-2.5% . The lab re-validates with software updates, new reagents or lots, or other changes to the pipeline. The lab participates in an external quality program, which distributes actual tissue samples.

FUNDING

The lab is partially reimbursed for the cost of testing for outpatients through Helfo. Inpatient testing is included in fixed per-patient rates recovered by the hospital. The lab notes that the systems for calculating reimbursement was updated 01.01.2020 to also include NGS, and that it aims to cover operating costs while minimizing per-test rates to be able to operate and expand testing within the hospital.

FUTURE NEEDS

Practical workflows in the lab include many manual steps due to a lack of LIMS integration. Currently, the LIMS used in Pathology is not fit to include NGS analysis, and does not integrate with NGS software or the hospital EHR. Implementation of larger gene panels rely increasingly on access to remote servers for software updates or for cloud access for data analysis, and limited connectivity from the hospital ecosystem is a major obstacle that needs to be solved. Coverage uniformity is a key quality and throughput challenge, and the lab is evaluating alternative panels. Note that while all these panels include additional clinically relevant molecular markers, the lab only reports findings included in the Norwegian Guidelines for Cancer. The lab has the ability to increase capacity by at least 2-3 fold, which will also decrease turnaround time. Obtaining resources to develop molecular expertise within the unit is a priority.

Rigshospitalet
Center for Genomic Medicine (CGM)

ORGANIZATION

Rigshospitalet is one of the largest specialist hospitals in Denmark, and serves over 350 000 unique patients yearly. Three units within the diagnostics division of Rigshospitalet conduct routine NGS-based diagnostics. Clinical Genetics conducts rare and heritable disease testing, Pathology performs routine histology, IHC, and cancer panels, and the Center for Genomic Medicine (CGM), documented here, functions as both an NGS core for clinical units and provides diagnostics for clinical trials. Within CGM, there are units for Molecular Genetic Analysis (logistics, pre-analytics functions and PCR and MLPA testing), Arrays, NGS, and Bioinformatics. Laboratory units are housed at the Kennedy Center in Copenhagen, while bioinformatics is located at Rigshospitalet. In this work, we focused specifically on workflows supporting cancer trials, and not the work CGM performs for research or for Clinical Genetics. In this context, the CGM has delivered diagnostics since 2014 to the Phase I unit within the Department of Oncology, which enrolls patients for both clinical trials and off-label use. Approximately 30% of patients in this clinical pathway are enrolled in a trial.

DIAGNOSTIC OFFERING

The diagnostics the lab decides to implement are determined primarily by CGM staff, and are informed by the requirements of current and upcoming trials as well as by what is technically possible. CGM takes a broad approach and has the potential to analyze thousands of biomarkers, but the specific inclusion requirements set by specific clinical trials impacts what they focus on.

GUIDELINES FOR TESTING

The lab reports any results which may be relevant for cancer, including specific biomarkers as defined by available trials or potential off-label use provided by the phase I unit. The discussion of what is clinically relevant is conducted in weekly molecular Tumor board meetings with the phase I unit clinicians. Classification of germline variants is conducted according to ACMG guidelines, while somatic variants are classified with a custom system.

SAMPLES AND LOGISTICS

CGM receives approximately 400 patients from the phase I unit yearly. The unit receives multiple samples per patient, including multiple blood samples in EDTA and Streck tubes and duplicate biopsies in RNAlater. CGM conducts ctDNA monitoring for treatment response and MRD for approximately 20 patients per year. Samples are processed with multiple diagnostics, including NGS, as required by the potential trials the patient may be enrolled in. CGM also conducts routine analysis for approximately 3000 breast cancer samples per year. CGM and the phase I unit have a shared location within the hospital network to coordinate logistics, and CGM has its own requisitioning system for samples coming from other sites. Communication is handled through shared e-mail addresses, and most information at this stage is held in excel documents.

PREANALYTICS

Histological testing is not performed in CGM, but is coordinated by the phase I unit prior to requisitioning. CGM typically analyses paired Tumor/normal samples.

ASSAYS

The lab uses the Illumina TruSight Oncology 500 DNA panel for ctDNA samples, which assesses indels, SNVs, TMB, and MSI, and includes UMIs for more accurate quantification. For RNA from solid biopsies, the lab uses the Thermo Fisher GeneChip U133 Plus 2.0 expression array, which quantifies approximately 47 000 reference transcripts from 38 500 genes. For DNA from solid biopsies, lab uses the Thermo Fisher CytoScan HD SNP array, which detects SNPs, chromosomal, and copy number changes. The lab uses the Agilent SureSelect whole exome kit for DNA from both solid biopsies and EDTA blood.

DNA and RNA extraction from biopsies is automated with the Qiagen AllPrep kit on a QIAcube. DNA extraction for germline samples from EDTA blood is automated on a Tecan Evo. DNA extraction for ctDNA analysis from Streck blood is automated on a Qiagen QIAsymphony. DNA is quantified using a fluorometric assay. RNA quantity and quality are determined with a Nanodrop and Bioanalyzer, and the lab aims for RIN >7 for RNA-seq. DNA is sheared using a Covaris, and quality controlled with a fluorometric assay and Tapestation prior to hybrid capture for exome sequencing. Prior to sequencing, libraries are quality-controlled with a fluorometric assay and on a Tapestation, and Tumor/normals are pooled to remove run-to-run variability.

SEQUENCING

Libraries are sequenced on an Illumina NextSeq 550 paired-end 150 nt reads. The lab currently has additional Illumina sequencers in-house, including NovaSeq which is currently used for research and other clinical indications, and has the capacity to rapidly expand throughput for molecular pathology. CGM stores .bam files for 6 months, and archives .fastq, .cel, .vcf, and some .cram files on tape off-site.

DATA ANALYSIS

An on-site jump server encrypts and transfers data from the sequencer to a dedicated bioinformatics area within the DTU Computerome via direct fiberoptic line. CGM has 6 dedicated 28 CPU nodes, and clinical samples are prioritized over the normal queue if additional capacity is needed. Tasks are split into smaller jobs and parallelized when possible, orchestrated via snakemake. A file hierarchy is built, samples are demultiplexed, and per-run QC metrics (including Q30, number of reads per sample), and results are sent to pre-specified internal e-mail accounts based on IDs in the Illumina sample sheet. The end of this process triggers assay-specific bioinformatics pipelines. For exomes or whole genomes, reads are aligned and duplicates removed prior to parallelization, where 9 chromosome groups are processed in parallel with GATK. This pipeline will call, annotate variants, and filter out variants based on population frequency. After the bioinformatics group has finished, the NGS unit interprets data from the .vcf. The bioinformatics group maintains separate production and development environments, and a local git installation is used for version control.

INTERPRETATION

Clinicians at CGM interpret the results of molecular testing once all diagnostics are finished, and issue a single report prior to the patient's next appointment. Cytogenetics array data is analyzed with the Thermo Fisher Chromosome Analysis Suite, NGS data is analyzed with Qiagen Ingenuity Variant Analysis (IVA) software, and expression array data is analyzed with Thermo Fisher Microarray Suite software. CGM does not limit analyses to particular genes or biomarkers, and reports any relevant cancer-related findings. The unit follows ACMG guidelines for germline variant classification, and uses a custom scheme for somatic variants. The lab uses clinical knowledge bases including the Qiagen Knowledge base and Jackson CKB, among other sources, when classifying variants. CGM uses classifications from ENIGMA for breast cancer and InSiGHT for gastrointestinal cancer. The lab will reference ClinVar, but does not submit variants and does not explicitly trust ClinVar classifications without additional evidence. The lab reports VUS/VUCS and pathogenic variants, and does not include a likely pathogenic classification. The lab considers synthesizes the results of all molecular tests and issues a single report with relevant findings. This is issued as a .pdf, and clinicians responsible for the patient enter relevant information into their hospital's EHR.'

CLINICAL ACTION

A weekly, virtual molecular Tumor board is held with approximately 20 representatives from hospitals across Denmark involved with clinical trials. This meeting is attended by clinicians from the phase I unit, CGM, Clinical Genetics (if germline variants were identified), Pathology, and typically molecular biologists and clinicians from other Danish sites. Bioinformaticians from CGM do not attend, as this meeting focuses on clinical implications, not technical validity. The goal of this meeting is to review findings and assign patients to appropriate trials.

INFRASTRUCTURE

A local server at the Kennedy Center physically encrypts data and transfers it over a dedicated fiber line to Computerome, Denmark's life science computing cluster, where the bioinformatics unit maintains analysis pipelines. CGM has dedicated CPUs in Computerome, but does not have access to DRAGEN FPGAs. In 2018, analyses required approximately 6 million CPU hours and peak storage was 500 TB. Cold storage is handled off-site on tape. Clinical data are pseudonymized prior to transfer, and Computerome has its own firewall. Within the hospital network, a separate IT infrastructure includes EHR and other LIMS.

QUALITY AND VALIDATION

CGM maintains an ISO 15190 certificate from DANAK. NGS testing is conducted exclusively with RUO reagents, instruments, and software, and assays and bioinformatics pipelines are validated and verified in-house as LDTs. The lab validates assays on clinical samples and in-house titrated clinical samples, and will compare results between assays and technologies. The lab shares validation samples with other hospitals, and participates in external quality control programs. CGM has a structured approach to staff accreditation, and has built an internal certification system for this. The bioinformatics unit also conducts regular in silico validation, using GIAB and the Platinum Genome dataset.

FUNDING

Initial funding was provided by Rigshospitalet for a 500-patient research pilot, and CGM secured more research funds with the goal of attracting more clinical trials. Now, CGM is funded through a combination of hospital budget and innovation funding. Reimbursement is based on the number of tests performed.

FUTURE NEEDS

The lab aims to replace it's expression array, which quantifies transcript expression, calculates a proliferation index, and includes markers for several molecular sub-types, with RNA-seq in the near future. The lab also aims to replace it's exome diagnostics with whole genome sequencing, and to introduce low-pass WGS for chromosomal abnormalities once dual-index UMIs are available. The lab wants to replace the NextSeq with the NovaSeq for exome diagnostics, which would increase efficiency and potential throughput. On the bioinformatics front, the group wants to move more of the sequencing infrastructure to within Computerome, extending it to within the Kennedy Center.

Karolinska University Hospital and SciLifeLab

Karolinska Universitetssjukhuset (KUS/SLL)

ORGANIZATION

The Karolinska University Hospital (KUS) Laboratory employs 2300 staff across 8 clinical divisions, and process approximately 20 million clinical tests per year, delivered from several hospitals and clinics in the Stockholm area. SciLifeLab (SLL) is an academic research center with expertise in molecular biology, jointly supported by Swedish Universities. This work focused on diagnostics for hematological malignancies provided by the Hematology Center, utilising the labs for clinical pathology, clinical genetics, and SciLifeLab.

DIAGNOSTIC OFFERING

The diagnostics which KUS decides to deploy are based primarily on clinical need as determined by clinical staff and informed by scientific opinion, national, and international guidelines. Together with SLL, KUS offers a broad range of assays for both diagnostic and research purposes, and can tailor assays on a variety of platforms for specific clinical applications.

GUIDELINES FOR TESTING

Several sets of guidelines are relevant to molecular diagnostics in Sweden. National guidelines for various diseases including several (but not all) types of cancers are published by the National Board of Health and Welfare. These may contain some information about histological testing, but generally lack up-to-date molecular recommendations, and fail to include treatments with molecular indications. The New Therapies Council is a network of clinical experts with representatives from every healthcare region that publish recommendations about the use of new drugs. Finally, the regional cancer centers (RCC) cooperation maintains guidelines for most cancer types, although these may lack molecular guidelines.

SAMPLES AND LOGISTICS

The hematology center typically collects blood or bone marrow aspirates, and delivers around 150 samples per year to clinical genetics for a comprehensive molecular workup including MLPA, CNV arrays, FISH, karyotyping, and a small NGS panel, and around 30 samples per year to clinical pathology for histology and morphological analysis. The hematology center plans to implement a large gene panel with SciLifeLab in the near future.

PREANALYTICS

Histological testing is conducted at clinical pathology/cytology through a separate process. NGS tests from SLL are ordered through an online portal. This portal automatically checks requisition information and transfers data to the Illumina Clarity LIMS and the custom SLL customer support portal.

ASSAYS

Samples are sent to clinical genetics for molecular analysis and to clinical pathology/cytology for morphology. Clinical genetics uses an Illumina Trusight Custom Amplicon panel (TSCA) that includes 43 cancer-relevant genes, and also runs FISH analysis, a CNV array, karyotyping, and MLPA. In parallel to molecular analysis, clinical pathology/cytology conducts morphological assessment of parallel samples. Under the proposed SLL assay, DNA would be extracted at the hematology center and shipped to SLL. Custom, nationally-developed gene panels (200-250 genes) using Twist Biosciences hybrid capture technology will be used to generate libraries.

SEQUENCING

TSCA libraries are currently pooled and sequenced on an Illumina MiSeq. Up to 7 samples are pooled with one control, and runs are scheduled weekly. The proposed SLL panel will use an Illumina Novaseq 6000. Clinical samples will be pooled with panels, whole genome samples, metagenomics samples, and other libraries to consume capacity, and runs occur 1-3 times per week, as needed. SLL will aim for >1000x mean coverage.

DATA ANALYSIS

TSCA data are analyzed using Illumina software for alignment, quality control, and variant calling. Quality control measures include a Q30 threshold, minimum coverage of 500x, and presence of variant reads from both strands, from multiple amplicons covering the same position. Panel data from SLL will be analysed using a custom-developed workflow. QC metrics are summarised using MultiQC. Multiple callers are used for SNVs, Indels, and structural variants. Variants are annotated with VEP, COSMIC, GnomAD, and data from other databases, and are tagged as likely germline based on Tumor/normal variants if available. Annotated variants and alignment files are transferred from clinical genetics or SLL to hematology for interpretation and reporting

INTERPRETATION

TSCA .vcf are analyzed by molecular biologists and clinicians from the center for hematology with Illumina Basespace Variant Interpreter and Sophia Genetics Alamut Visual. First the unit filters out genes that are included in the panel but were not requested. The unit examines both coding and non-coding variants, and compares these to a database of previously-seen variants as well as a set of variants that are commonly-seen sequencing artifacts. The unit reports variants with VAF >10%, and will repeat the assay from the same DNA sample if VAF is 5-10%. The unit will request a normal sample if high-frequency, suspected germline variants are found. The unit issues a single report summarizing NGS, aCGH, and FISH assays (if available). This is printed as a .pdf and imported manually into the clinical genetics LIMS, Starlims, which automatically syncs with the Takecare EHR used in the wider hospital.

Data from the proposed large panel assay will be interpreted by molecular biologists and clinicians from the center for hematology. Interpretation will be performed using a combination of custom-developed tools and Sophia Genetics Alamut Visual. Users generally filter out variants in genes not included in an appropriate in silico panel, and filter on quality, depth of sequencing, population frequency, and other annotations and in silico pathogenicity predictions. Relevant findings are exported from the custom tool as a .pdf and transferred manually into Starlims, as above.

CLINICAL ACTION

Tumor boards are a recent addition to the diagnostic pathway, and are held twice a month. During these meetings, geneticists, hematologists, and pathologists discuss the most difficult cases with the aim of determining appropriate clinical course. Bioinformaticians have occasionally attended these meetings, but primarily to gain insight into user needs. Genetic variants are presented from Starlims and from excel files, and pathology data is presented from a separate LIMS. Annotations are made directly in the patient record in Takecare.

INFRASTRUCTURE

Data are analyzed on a local server using custom-built, dockerized bioinformatics pipelines. Sample ordering, data delivery, and other administrative tasks are handled through a central portal at SLL. After analyzing data, the center for hematology prints .pdf reports and imports these into KUS IT systems, including Starlims, which is integrated with and automatically downloads reports to the hospital EHR system, Takecare.

QUALITY AND VALIDATION

SLL holds an ISO 17025 accreditation from Swedac for selected assays. NGS is conducted exclusively with RUO reagents, platforms, and software, and assays are validated and verified in-house as LDTs. SLL invests significantly in the development of new bioinformatics software. Assays are validated on clinical samples and commercial reference materials. SLL participates in external quality programs. SLL develops integration and unit tests for both minor and major software versions, and has tests in place for the majority of it's code base.

FUNDING

Healthcare in the Swedish system is decentralized and organized primarily at a regional level, and the center for hematology at KUS is funded by the Stockholm Regional Council. Diagnostics provided to the center from laboratories or other units are reimbursed through transfer payments. SLL invests a substantial amount of research funding into platform development, and is reimbursed by both clinical and research users for services.

FUTURE NEEDS

The hematology center aims to begin using the SLL pipeline in the near future. SLL itself has substantial sequencing and bioinformatics capacity, and aims to deliver additional clinical pipelines. One challenge has been harmonising test offerings to the entire country, and work is ongoing at the national level on harmonizing the panel contents. More explicit guidance on molecular testing in the national treatment guidelines would aid adoption of molecular tests.

VESTFOLD HOSPITAL

Vestfold Hospital
Sykehuset i Vestfold (SiV)
Pathology Unit

ORGANIZATION

Tønsberg Hospital (Sykehuset i Vestfold, SiV) serves a population of approximately 230 000. Diagnostics are performed by 4 units within a Clinic for Medical Diagnostics: Radiology, Medical Biochemistry, Microbiology, and Pathology. While clinical pathologists, histopathology, immunohistochemistry, and silver in-situ hybridization are all within the Pathology Unit, NGS equipment is housed within the adjoining Microbiology Unit, which offers PCR and NGS-based microbial and viral testing in addition to culture-based assays. Routine NGS testing in pathology was initiated May 2019, and is conducted on a fixed weekly schedule. The 4 diagnostics units hold bi-weekly alignment meetings, and the 3 laboratory units also hold informal, joint meetings for the broader medical community quarterly.

DIAGNOSTIC OFFERING

The diagnostics the lab decides to implement are determined primarily by lab staff, and are based on testing recommendations included in the Norwegian National Guidelines for Cancer. Since these guidelines address molecular testing at only a basic level, the assays used by the lab include other clinically relevant biomarkers, which are not reported but could be if recommendations change. In general, colorectal and melanoma samples are tested on oncologist or surgeons request. CRC relapse patients <60 years of age reflex to lynch syndrome screening via MMR/MSI. Certain types of lung cancers, such as adenocarcinoma, reflex to NGS testing as well

GUIDELINES FOR TESTING

The lab reports results which are included in the Norwegian National Guidelines for Cancer, and additional results as coordinated with Oslo University Hospital or if there are clinical trials available. While there is no direct guideline for molecular or NGS testing, some of the relevant national guidelines include information on a limited set of genes to be included. The assays the lab uses detect a broader range of clinically actionable variants, so test offering could be expanded.

SAMPLES AND LOGISTICS

Pathology receives approximately 50 000 samples per year. Approximately half are solid biopsies or surgical samples, and half are cytological specimens, including cervical smears, fluids, and aspirations from lungs or lymph nodes. Solid samples are usually delivered on formalin, and are a combination of surgical samples, biopsies (most often lung or colorectal), and suspected skin cancers (often from local practices). Approximately 200 samples per year are tested using NGS. Samples are occasionally received from outside the hospital, usually from other hospitals without NGS testing capabilities. Samples from other hospitals may be already fixed and embedded.

PREANALYTICS

Prior to molecular testing, samples are paraffin embedded with a highly automated workflow prior to histological work-up. Samples are sectioned, stained, and reviewed by pathologists before any molecular testing. Throughout this process, 2D barcodes printed on frames and slides are used to track samples, and this system is integrated with the pathology LIMS. Pathologists analyze approximate Tumor load and tissue size prior to sectioning for NGS, and aim for >10% Tumor cells if possible. Block macro-dissection may be needed if Tumor load is low, however the lab will run samples even with 5-10% estimated Tumor load, with caveats in the final report regarding false negatives. The lab does not routinely analyze paired Tumor/normal samples.

ASSAYS

The lab uses the Oncomine Focus Panel, which covers hotspots in 52 cancer-relevant genes and includes CNVs, SNVs, indels, and selected fusions. All targets have either FDA or EMA-approved therapies, strong evidence for clinical utility supported by NCCN or ESMO guidelines, or are important for inclusion in registered clinical trials. The panel includes both DNA and RNA-based biomarkers. DNA extraction is automated on a PSS MagLead 12gC, while RNA extraction is done manually using a Zymo RNA FFPE kit. Libraries are prepared for DNA and RNA separately, but can be automated if the lab has greater throughput. Libraries are quantified via qPCR prior to template preparation and chip loading, however the lab does not use any automated electrophoresis assay for QC, since these are patient samples that would be sequenced even if low-quality. In addition to NGS, the lab conducts IHC, SISH, and PCR testing.

SEQUENCING

Libraries are sequenced on an Ion Torrent S5 XL using 510 or 520 chips and 400 bp reads. This delivers 2-3M reads (0.6-1 Gb) or 4-6M reads (1.2-2 Gb), respectively. The manufacturer recommends at least 500 000 reads per sample for this panel, and the lab multiplexes 1-4 samples on the 510 chip or 5-7 samples on the 520 chip, consistent with the labs weekly throughput. Higher capacity chips are available (<32 samples per run with a 530 chip), and will be considered should sample volume increase substantially. The lab maintains sequencing data for 1 year, but does not archive raw data or store libraries long-term. Isolated DNA and RNA, along with FFPE blocks, are archived.

DATA ANALYSIS

Data are analyzed on-site with local servers running the Torrent Suite and Ion Reporter, both software packages developed by the NGS platform manufacturer. Quality control standards are documented, and chip loading, clonality, read depth, coverage uniformity, amplicon coverage, strand-bias, VAF, and read quality are all examined. Data are then transferred to Ion Reporter, which is used to filter out low-quality variants. RNA samples must exceed minimum relative expression levels for 3 of 5 housekeeping genes and have a minimum 20 000 mapped reads to pass quality control. As additional layers of quality control, the lab examines allele frequency vs. estimated Tumor load, clinical data, the frequency of that variant in the type of cancer, and if multiple samples present the same variant in the same run, the assay would be repeated. Molecular biologists and pathologists evaluate NGS results together, and examine quality metrics in the Ion Reporter software. The results are also controlled by a bioinformatician if needed.

INTERPRETATION

Interpretation and clinical evaluation are conducted with the aid of Oncomine Reporter, which summarizes relevant biomarkers, clinical trials, guidelines, and targeted therapies. The lab does not report all targets included in the Oncomine panel, only variants in the genes included in the Norwegian national guidelines (for example: ROS1, KRAS, ALK, EGFR, and BRAF by NGS, and PD-L1 by IHC in lung cancer). The lab does not use the AMP/CAP variant classification guidelines, but rather discusses the significance of variants with clinicians. During interpretation, the lab examines data in ClinVar (though it does not submit classifications), and looks for potential clinical trials in Oncomine Reporter and MyCancerGenome. Findings are appended to the initial report in the pathology LIMS.

CLINICAL ACTION

A weekly molecular MDT meeting is held to discuss the results of the weeks testing prior to releasing reports. This meeting is open to clinicians and other laboratory representatives, some of which regularly attend, but includes minimally one representative technician, pathologist, bioinformatician, and unit leaders. In this meeting, the team review each case, evaluate the results of molecular and immunohistochemistry testing, and determine what findings should be documented in the report.

INFRASTRUCTURE

Data are analyzed on a local server running the Torrent Suite that is co-located in a secure location with the sequencing platform. The Torrent Suite is used for quality control, read filtering, alignment, and variant calling. A separate server within the hospital IT infrastructure, including standard user authentication and security protocols, runs Ion Reporter, used for primary analysis, and Oncomine Reporter, used for clinical analysis. A report from Oncomine Reporter is submitted to the pathology LIMS, where a common histopathology/NGS report is issued.

QUALITY AND VALIDATION

The lab maintains an ISO 15189 certificate from Norsk Akkreditering. NGS testing is conducted exclusively with RUO reagents, platforms, and software, and assays are validated and verified in-house as LDTs. Assays are validated on clinical samples and commercial reference materials, which are critical for fusions and uncommon variants. The lab shares samples with other hospitals for cross-validation, and participates in an external quality program. New reagent lots are verified using reference controls and previously-tested clinical samples.

FUNDING

The initial investment for the purchase of NGS equipment was shared between research and hospital funding. For outpatients, the lab is partially reimbursed for the cost of testing through Helfo, which administers payments on behalf of the Norwegian national insurance scheme to providers. Inpatient testing is included in fixed per-patient rates recovered by the hospital.

FUTURE NEEDS

Workflows are currently inefficient due to a lack of LIMS integration. Currently each of the 4 diagnostic units runs a different LIMS, and certain lab equipment also run LIMS, none of which are interoperable or integrated. As a practical example, after interpreting test results, a .pdf is appended to the sample entry in the pathology LIMS, which does not accept NGS data. In a free text field, staff manually copy-paste standardized information about the test from a word document, and write notes about the main findings. The lab has sufficient sequencing capacity to increase testing 4-fold, with only minor additional equipment requirements. The lab aims to implement a cfDNA test in the near future. Developing the competence of technicians and retaining bioinformatics expertise is a key priority for delivering robust service, and additional funding for technicians is needed. The lab has the capacity to include additional molecular markers, but requires the Norwegian Guidelines for Cancer to be updated to include these markers. Finally, the lab has the goal of participating in more clinical trials.

Haukeland University Hospital

Haukeland Universitetssykehus (HUS)
Section for Cancer Genetics

ORGANIZATION

Haukeland University Hospital (HUS) is the largest health institution in Norway's western regional health trust with ties to the University of Bergen, and conducts around 1 million patient consultations annually. A recent reorganization within the laboratory clinic has consolidated several groups conducting cancer diagnostics into a single entity for cancer genetics (Kreftgenetikk, KG), which contains units for hematological malignancies and for solid tumors . In addition to KG, the HUS laboratory clinic also houses departments for Immunology and Transfusion Medicine, Medical Biochemistry and Pharmacology, Medical Genetics, Pathology, Microbiology, Bioinformatics, and Biobanking. Medical genetics and microbiology also offer NGS-based diagnostics, and share some laboratory space and equipment with cancer genetics. The tumor genomics unit within KG works as part of the Pathology department within the laboratory clinic. Within the pathology department, there are neighbouring sections for general histology, cytology and autopsy (which conducts IHC and histology analyses), and for special analysis, which is responsible for molecular pathology including NGS and electronc microscopy that KG delivers. KG already performs routine NGS panels for solid tumors, and aims to add panels for hematological malignancies in the near future.

DIAGNOSTIC OFFERING

The diagnostics the lab offers are determined by staff in consultation with hospital clinicians, and are based on the Norwegian national guidelines as a minimum. KG coordinates NGS-based diagnostics with other modalities such as FISH, immunohistochemistry, G-banding, sanger sequencing, fragment analysis, and qPCR to offer appropriate tests for each biomarker.

GUIDELINES FOR TESTING

The lab relies on Norwegian national guidelines for determining the minimum test offering, and several pathologists participate in the committees that draft these guidelines. There are several biomarkers currently analyzed with other technologies that the lab believes are good candidates for NGS-based testing, including both cancer hotspots, gene fusions, and copy number variations.

SAMPLES AND LOGISTICS

The lab has a well-established pipeline for solid tumor testing and is in the process of in-sourcing NGS-based diagnostics for hematological malignancies, which are currently sent to other labs for analysis. The lab processes approximately 7000 solid tumor analyses per year, of which approximately 500 are analyzed with NGS. Approximately 1400 leukemia patients are treated each year. Often multiple samples are delivered, usually bone marrow aspirates or blood. Approximately 1800 analyses are performed, including for minimum residual disease, and around 300 of these are NGS tests. The lab performs diagnostics for other hospitals in the health region, who often submit samples via overnight post.

PREANALYTICS

Resection, fixation, paraffin embedding, tissue sectioning, and histological work-up are all performed by pathology prior to molecular testing. Most solid samples are delivered formalin-fixed. Bone marrow and blood samples are usually delivered in EDTA, heparin, or Pax tubes. DNA extraction from solid Tumors is conducted with the Omega EZNA tissue kit, either manually or on a Hamilton platform. DNA extraction from hematology samples is conducted with the Qiagen Blood and Tissue kit, either manually or on a QIAsymphony. DNA is quality controlled after extraction with a Qubit. RNA is extracted from PAXgene blood tubes with PAX RNA columns and quality controlled via Tapestation.

ASSAYS

For solid biopsies, the lab uses the TruSight Tumor 15 panel, which targets 15 genes commonly mutated in solid Tumors which have either FDA or EMA-approved therapies, strong evidence for clinical utility supported by the NCCN or ESMO guidelines, or are relevant for clinical research. The panel detects indels and single nucleotide variants. The lab is currently validating the AmpliSeq Focus Panel for Illumina as a replacement. For hematology samples, the lab is currently implementing the AmpliSeq Myeloid panel, which is a combined DNA/RNA panel covering DNA variants, common fusions, and gene expression levels.

SEQUENCING

Libraries are quality controlled with a Qubit and Tapestation prior to pooling and sequencing. Libraries are sequenced weekly on an Illumina MiniSeq using V3 chemistry and paired-end 151bp reads. 8-10 TST15 libraries are multiplexed and sequencing is conducted over the weekend, with results delivered early the following week. The lab aims for a turnaround time of one week after biopsy, however this can be difficult if samples are sent from other hospitals, are lacking sufficient requisition information, and miss the weekly submission cut-off.

DATA ANALYSIS

Concurrent with sequencing, the MiniSeq runs Illumina Local Run Manager to filter, trim, base-correct, and align reads. Due to the small panel size, results are available as sequencing finishes. The lab uses a quality control model that examines various sequencing parameters including cluster density, total reads, demultiplexed read count per sample, phix error rate, and coverage. Per-base coverage is >500x for all genes in the panel. After primary data analysis, samples are manually uploaded to the Agilent Alissa Clinical Informatics Platform, running on local hospital server.

INTERPRETATION

Lab staff pass samples through an automated filtering pipeline in Alissa. This filters reads to annotated exons or coding regions, calculates variant frequency, and annotates variants with data and variant interpretations from external databases including civic, clinvar, and COSMIC. Variants with interpretations in any one of those databases with classifications of pathogenic or likely pathogenic are considered. The variant cut-off is >3% variant allele frequency.

CLINICAL ACTION

Molecular diagnostics and pathology results are discussed within the lab before being presented in multidisciplinary team meetings. The lab does not currently participate in a molecular Tumor board, however pathologists do.

INFRASTRUCTURE

Data is transferred automatically from the sequencer to a hospital server, where Illumina Local Run Manager is used for primary data analysis. The MiniSeq, MiSeq and analysis server are all located within the hospital network, and authentication, security, and maintenance is handled by Helse Vest IKT. The lab reports that setting up the analysis server was relatively straight forward, although storage space is becoming an issue. Processing speed does not bottleneck diagnostic delivery, however this might becoming a limiting factor with larger panels in the future. The lab relies on the Unilab LIMS, which automatically transfers molecular pathology reports to the hospital DIPS EHR system.

QUALITY AND VALIDATION

The lab maintains an ISO 15189 certificate from Norsk Akkreditering for many diagnostics, and will expand the scope of this to NGS in the future. NGS testing is performed with RUO reagents, platforms, and software, and assays are validated and verified in-house as LDTs. Assays are validated on clinical samples and commercial reference materials. NGS tests are validated against FISH, qPCR, or sanger sequencing as appropriate. Updates to the bioinformatics pipeline trigger re-validation. The lab participates in external quality assessments.

FUNDING

The lab is partially reimbursed for the cost of testing for outpatients through Helfo. Inpatient testing is included in fixed per-patient rates recovered by the hospital. The lab notes that the systems for calculating reimbursement was updated 01.01.2020 to also include NGS, and that it aims to cover operating costs while minimizing per-test rates to be able to operate and expand testing within the hospital.

FUTURE NEEDS

The lab is immediately occupied with organizational changes and the introduction of the AmpliSeq Myeloid, Childhood Cancer, and Focus Panels. The AmpliSeq Focus panel will replace the TruSight Tumor 15 (TST15). Future developments for the lab have a large focus on bioinformatics and analysis pipelines, developing a high degree of cross-training in molecular staff, and an effective LIMS system.

St. Olavs University Hospital
Universitetssykehuset i Trondheim
Department for Pathology

ORGANIZATION

Trondheim University Hospital in the Mid-Norway health region operates several hospitals and clinics in Trondheim and Trøndelag including St. Olavs hospital. Within the St. Olavs Laboratory Medicine Clinic, departments for Medical Microbiology and Medical Genetics, and Pathology all have some NGS testing capabilities. The Pathology unit conducts routine histopathology and molecular testing for cancer samples, and accepts samples from clinics and hospitals across Trøndelag and parts of Møre and Romsdal. The pathology unit houses approximately 100 employees, including 20 pathologists which refer samples for molecular testing and 6 staff in molecular pathology which conduct molecular analyses, including NGS.

DIAGNOSTIC OFFERING

Whether a certain test or molecular marker is included depends on inclusion in guidelines or user demand, but also indicators of cost effectiveness within the lab, reimbursement, and the foreseeable sample throughput and outsourcing possibilities. Part of the initial reason for moving to NGS testing was the recognition that parallel sequencing of many genes in a small panel was more time- and cost-efficient than running 10-15 PCR or sanger sequencing assays. The lab recognizes that assay development takes time, and makes efforts to continually survey current research and upcoming drug approvals to avoid situations where a certain therapy is available but an appropriate companion diagnostic is not, however the reimbursement of therapies/biomarkers by the national health technology assessment body (Beslutningsforum) is the primary driver for offering a test.

GUIDELINES FOR TESTING

The lab offers diagnostics covering minimally the Norwegian guidelines for molecular testing for various cancers, but also relies on international or European consensus guidelines from the World Health Organization or international disease organizations such as the International Association for the Study of Lung Cancer. In addition to testing guidelines from various organizations, the lab includes biomarkers requested from local pathologists and oncologists.

SAMPLES AND LOGISTICS

Samples from St. Olavs and other hospitals are received at medical biochemistry before being forwarded to pathology. The pathology unit receives approximately 50 000 histology cases per year. Most samples are solid biopsies from colorectal and breast cancer patients. Around 6000 samples per year require molecular testing, which is an increase from approximately 3000 samples in 2010. Of these 6000 samples, around 550 are processed with NGS-based diagnostics.

PREANALYTICS

Samples are usually provided already formalin fixed with a variety of protocols, but occasionally fresh-frozen cytogenetic samples are received. Histology then registers samples and conducts macroscopic assessment and dissection. Tissue samples are paraffin embedded with either a fully-automated or one of several manual instruments, and blocks are barcoded for sample tracking. Block are sectioned with one of 15-20 microtomes, and Haemotoxylin/Eosin staining and cover-slipping with film is performed on an automated platform. Slides are scanned with Philips or Hamamatsu scanners, which can also handle large-format and fluorescent slides, into Philips IMS software. Approximately 600-700 slides are processed per day. Pathologists review slides digitally, but also analyze slides with microscopes manually, and classify Tumors and decide if molecular diagnostics or additional testing is required. Immunohistochemistry and in-situ hybridization-based tests are conducted with 5 Roche Benchmark Ultra instruments, and sanger sequencing, PCR testing, and a Nanostring platform are also available.

Prior to molecular testing, blocks are dissected based on instructions from the requisitioning pathologists, who are co-located with the histology and molecular labs. Pathologists estimate a number of sections required based on Tumor size and estimated Tumor load, which are sectioned into barcoded 2 ml microfuge tubes.

ASSAYS

The lab uses the Oncomine Focus Panel, which covers hotspots in 52 cancer-relevant genes and includes CNVs, SNVs, indels, and selected fusions. All targets have either FDA or EMA-approved therapies, strong evidence for clinical utility supported by NCCN or ESMO guidelines, or are important for inclusion in registered clinical trials. The panel includes both DNA and RNA-based biomarkers. DNA is extracted with the Qiagen FFPE DNA kit on a QIAcube, RNA is extracted manually with a Qiagen RNEasy FFPE kit if needed. Nucleic acid is eluted into 100-200 ul elution buffer depending on the kit, and DNA or RNA concentration is measured via qubit. The lab has developed qPCR assays to test for fragmentation, either by amplifying a 306nt segment of FCGR3 for DNA or ACTB for RNA. If samples fail, additional sections will be taken if possible. If insufficient sample is available, the lab will still run analyses, but will note this when interpreting data. In addition to NGS, the lab conducts sanger sequencing, qPCR, IHC, ISH/FISH, and ddPCR via Prosigna/Nanostring.

SEQUENCING

The lab uses an Ion Chef for library preparation. Libraries are sequenced on an Ion Torrent S5 using 520 chips, which typically yield 5.5-6 million usable reads per chip. The lab runs barcoded sets of 7 test samples plus one no template control per run, and usually performs two sequencing runs per week. The lab cycles through a total of 32 unique barcodes to help avoid sample cross-talk. Sample throughput could be increased with higher capacity chips, provided staffing and library preparation bottlenecks are not reached. In addition to excess DNA/RNA and FFPE blocks, the lab archives un-aligned .bam files, and will likely have to expand storage soon.

ST. OLAVS HOSPITAL
UNIVERSITETSSYKEHUSET I TRONDHEIM

DATA ANALYSIS

Data are analyzed on-site with a local server running Thermo Fisher Ion Torrent Suite and Ion Reporter. Quality control measures are tracked per run and per sample, and include average and minimum coverage, read quality, per-sample sequencing depth, alignment rate, and per-amplicon read depth and coverage. Quality metrics are examined in the Ion Torrent software, Integrated Genome Viewer, and excel. Variant allele frequency should roughly match pathologist Tumor load estimates. The lab cycles through 32 sample barcodes every 2 weeks to reduce sample crosstalk, analyzes an NTC with each run, and checks a known common SNP in EGFR exon 20. Many of these quality metrics, such as coverage, depth, VAF, uniformity, % of target amplicons with >500 reads, are entered into the Sympathy LIMS.

INTERPRETATION

The lab examines quality metrics and variants in the Ion Reporter software, which connects to the proprietary Oncomine database and other sources of data including clinvar and OMIM. Variants pre-classified as benign, likely benign, or of unknown clinical significance are filtered out by default, and the Oncomine Focus panel by design contains only targets with robust evidence for clinical relevance. Pathologists request specific genes, and the lab will report any variants classified as pathogenic or likely pathogenic in the Oncomine database. Additional findings from the Focus panel, such as variants not requested but relevant for that particular cancer, or variants which are pathogenic in other cancers, are sometimes included in a secondary findings section in the final report, and other times a note is added for clinicians to contact the lab directly to discuss secondary findings. Findings are entered as templated free text in Sympathy, and additional information such as the Ion Torrent Report can be appended as a .pdf.

CLINICAL ACTION

The lab participates in weekly MDT meetings for each type of cancer treated at the hospital. These meetings bring together clinicians and pathologists to discuss relevant histology and molecular test results. Molecular biologists and staff with bioinformatics expertise do not join these meetings. For specific clinical specializations, these meetings are held at the pathology unit so that clinicians can directly examine slides.

INFRASTRUCTURE

Data from the S5 is analyzed with Ion Torrent suite and Ion Reporter on a local server. Pathologists document histopathology findings in the Sympathy LIMS on hospital servers, which supports a digital pathology workflow. There are no integrations between Ion Reporter and Sympathy. Pathologists request molecular testing through Sympathy, and findings from molecular testing are entered into pre-filled fields in Sympathy as non-structured text after being verified by the responsible molecular biologist. Clinicians access reports from pathology within Sympathy, and determine what information should be entered into the patient journal, which is entered manually.

QUALITY AND VALIDATION

The lab is pursuing ISO 15189 accreditation from Norsk Akkreditering for some diagnostics, but not NGS testing. NGS tests are performed exclusively with RUO reagents, platforms, and software, and tests are validated and verified as in-house LDTs. The unit was aware of the upcoming IVDR, and expressed concerns that manufacturers would not make sufficient performance data available, that CE-marked tests would not reach adequate safety and performance, and that some labs will trust the CE-mark and deploy diagnostics without adequate in-house validation.

New NGS panels are validated on 60-70 previous clinical samples, with concordance to prior molecular diagnostic and additional variants being two key outcomes. The lab also uses commercial reference materials to help define sensitivity, limit of detection, and minimum Tumor load down to 5% VAF. The lab participates in external quality assessments, but notes that often sample quality is higher than what can be reasonably expected from routine use, and thus these exercises describe best-case scenarios.

FUNDING

St. Olavs has a yearly budget for new and replacement equipment, and has a highly competitive process to prioritize funding decisions. The pathology lab purchased their NGS instrument by selling one of their digital pathology scanners to another hospital. The lab is reimbursed for outpatient diagnostics through Helfo, and inpatient testing is recovered through the hospital. The lab notes that while the national reimbursement rates for NGS panels provide a standardized fee, the underlying cost structures of laboratories may differ widely. The lab faces challenges when developing and validating new tests: while regular samples are reimbursed, there is no budget for testing new assays.

FUTURE NEEDS

The lab currently has sufficient sequencing capacity, but is continually evaluating the need for new panels based on clinician requests, guidelines, national reimbursement decisions, and upcoming clinical trials. In the future the lab will establish Oncomine childhood cancer, BRCA, and myeloid panels. New technologies require sufficient demand, clinical utility, and some level of automation as to balance increased workload. The lab has benefitted substantially from sabbaticals and training at other institutions, and would like to promote these and other education initiatives for both clinical and pathology staff. The existing digital pathology LIMS works well, but the lab would like to log structured data into both their LIMS and the hospital EHR in the future. The lab notes the positive trends in cancer trials initiatives in Norway, but also points out the need for an established, national forum for labs to collaborate and coordinate.

Oslo University Hospital

Oslo Universitetssykehus (OUS)
Experimental Pathology

ORGANIZATION

Oslo University Hospital (Oslo Universitetssykehus, OUS) is the largest hospital system in Norway. Significant re-structuring during the late 2000s brought several hospitals in Oslo under a common administrative structure. OUS is structured into 15 divisions, of which the clinics for Cancer and for Laboratory Medicine (KLM) are relevant for cancer diagnostics. KLM includes 6 pathology units, including a unit for Molecular Pathology which has implemented NGS panels in routine diagnostics for cancer, and a unit for Experimental Pathology, which provides diagnostics for clinical trials, and is the sponsor for the upcoming IMPRESS cancer trial. KLM also houses a unit for medical genetics, which conducts medical diagnostics for rare disorders and germline variants. Within the cancer clinic, the institute for cancer research (IKF) performs translational research and directly supports other units, and houses both genomics and bioinformatics cores. Also within the cancer clinic, the department for cancer treatment (AKB) is responsible for patient treatment, and includes a unit for clinical trials and research. OUS has close ties to the University of Oslo (UiO), including may joint appointments, and shares both computing and sequencing infrastructure. This work focuses on the Experimental Pathology unit within KLM.

DIAGNOSTIC OFFERING

The lab offers tests as needed based on the clinical trials in progress, either in-house, or through the genomics core within the OUS IKF. A variety of platforms are available in different units, including low- and high-throughput Illumina sequencers, Ion Torrent, and Nanostring.

GUIDELINES FOR TESTING

The lab is primarily involved in conducting clinical trials and performing diagnostics to support treatment decisions within this context, and as such aims to perform diagnostics beyond what is recommended in national or international clinical guidelines. Other units under KLM, such as the unit for molecular pathology, are more reliant on national reimbursement decisions and testing guidelines when developing their test portfolio.

SAMPLES AND LOGISTICS

Patients are referred to the unit through clinical trials, including IMPRESS, and can be recruited from hospitals around Norway. Samples include solid biopsies, surgical samples, and blood from a variety of cancers including sarcoma, lung, and prostate, and are taken from both primary and metastasises, as appropriate. Approximately 250-500 patients per year are expected to take part.

PREANALYTICS

The lab accepts both solid biopsies and blood samples, depending on clinical indication, and has study protocols in place defining how these samples are processed and quality controlled.

ASSAYS

The unit has access to a variety of tests, either in-house, or at the IKF genomic core. Genomic work-up relies primarily on the Illumina TruSight Oncology 500 (TSO500) panel, which assesses indels, SNVs, TMB, and MSI, and includes UMIs for more accurate quantification. TSO500 is a combined DNA/RNA assay, and covers 523 genes (1.94 Mb) for DNA variants and 55 genes (358kb) for RNA variants. The Nanostring ProSigna assay is also available, and may be used as needed for confirmatory testing or in specific arms of the trial.

SEQUENCING

TSO500 libraries are sequenced on Illumina instruments at the cancer genetics core facility. Within other contexts, various Illumina and Ion Torrent instruments are also available.

DATA ANALYSIS

Sequencing data is processed with the Illumina TSO500 data analysis pipeline, which includes base calling, alignment, quality control metrics, variant detection, and MSI and TMB calculation. Currently this is conducted via a dockerized workflow on a local server within the hospital network, however there is the possibility to mirror this analysis on the UiO TSD should higher throughput or more complex analysis be required.

INTERPRETATION

Variants detected with the TSO500 pipeline are imported into the Personal Cancer Genome Reporter (PCGR), an open-source software package for interpreting individual cancer genomes. PCGR collects data from numerous external classification and frequency databases such as CIVIC, ClinVar, COSMIC, and GnomAD, provides in-silico predictions, identifies relevant trials, and provides users an interactive, GUI-based platform to explore variants and their clinical significance. Data can be exported from PCGR as either .pdf or interactive .html for analysis in a molecular Tumor board or MDT meeting.

Oslo University Hospital

CLINICAL ACTION

After sequencing and interpretation, results are interpreted by a molecular Tumor board set up within IMPRESS. This flexible style of trial allows the sponsors to open new arms with small cohorts, and to expand these should treatment modalities prove effective.

INFRASTRUCTURE

To date, the lab is running a dockerized Illumina TSO500 pipeline running on a local server with 30TB storage to process NGS data. As trial throughput increases, it is possible that the lab will face storage or processing time limitations and will require another solution. For this, the lab has the option to transfer data to the University of Oslo service for sensitive data (TSD), a high-performance computing infrastructure which is already used for both research and clinical samples by the OUS cancer and medical genetics units. Other IT systems in place are DocuLive, which is a pathology-focused LIMS system, Viedoc, a clinical trials data capture tool, and DIPS Arena, the hospitals EHR system. Other units offering MDx at OUS, such as the genetics core at IKF, molecular pathology, and the medical genetics unit under KLM have different LIMS in place.

QUALITY AND VALIDATION

The sequencing assays and bioinformatics used in this context are mainly LDTs, and are validated, verified, and monitored by the diagnostics labs offering them. The exception to this is the Nanostring ProSigna assay, which is a CE-marked IVD. As part of the test validation pipeline, a control panel of 50-100 clinical samples covering many cancer types is sequenced and compared to previous results. Some of the units within KLM maintain ISO 15189 certificates, and aim to develop NGS-based assays under this quality management system in the future.

FUNDING

Various funding mechanisms are in place to support different aspects of these activities. The IMPRESS study is supported by the south-east Norway health region, which also supplies funding to the core facilities at OUS. Many staff have joint appointments from both OUS and University of Oslo (UiO). A mixture of research grants, hospital funding, HELFO reimbursement, and health region funding is provided towards various laboratories at OUS, resulting in a complex system of incentives and support for various diagnostic pipelines or parts of diagnostics pipelines.

FUTURE NEEDS

One of the priorities for the unit is ensuring other hospitals in Norway have access to IMPRESS so that patients can participate regardless of their location. The unit is also invested in developing further clinical trials and in promoting the use of molecular diagnostics and precision medicine approaches for cancer treatment.

SAFER, SMARTER, GREENER

DNV GL AS
NO-1322 Høvik, Norway
Tel: +47 67 57 99 00
www.dnvgl.com

Courtney Nadeau
Principal Researcher,
Precision Medicine Program,
Group Technology and
Research (GTR)

E-mail: courtney.david.
nadeau@dnvgl.com
Mobile +47 45 84 73 25

DNV GL
DNV GL is a global quality assurance and risk management company. Driven by our purpose of safeguarding life, property and the environment, we enable our customers to advance the safety and sustainability of their business. We provide classification, technical assurance, software and independent expert advisory services to the maritime, oil & gas, power and renewables industries. We also provide certification, supply chain and data management services to customers across a wide range of industries. Operating in more than 100 countries, our experts are dedicated to helping customers make the world safer, smarter and greener.

Group Technology & Research
At Group Technology & Research our focus is on new knowledge and technology areas that have a long term impact on the industries we serve. Our White Papers highlight findings from our research programmes, and are intended to advance knowledge and to help prepare our customers, their stakeholders and our own organization for the future. Find out more at www.dnvgl.com/research