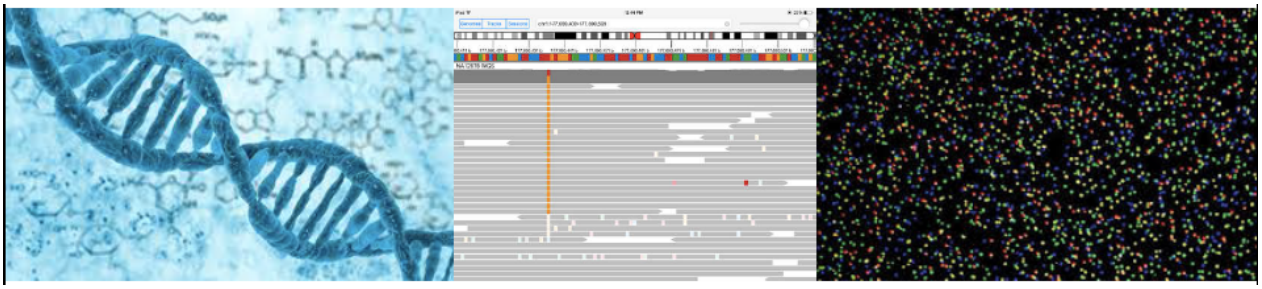
Personalised Cancer Medicine Program
at Karolinska Institutet



Molecular pathology investigation

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Content

1. General introduction

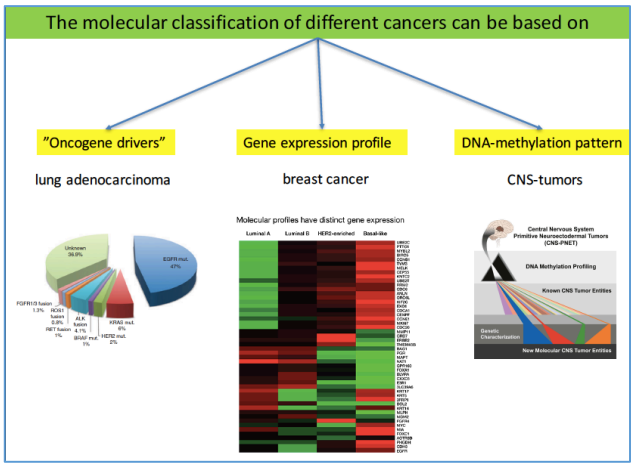
- Molecular pathology is a branch of the biomedical sciences which focuses on the progress, development, and evolution of diseases on the molecular level. It can be applied practically to patients in addition to being utilized in biomedical research to learn more about specific diseases. Usually, molecular pathology is treated as a subspeciality of the field of pathology, but it also involves genetics, immunology, and many other aspects of the medical field, and people can approach it from a number of perspectives.

- Precision medicine, also called "personalised medicine," is defined by the National Cancer Institute as "a form of medicine that uses information about a person's genes, proteins, and environment to prevent, diagnose, and treat disease."

- Personalised medicine in oncology emerged with the advent of molecularly targeted agents almost two decades ago and is mainly based today on the DNA molecular information of the patients' tumors.

- Molecular characterization of the cancer is a necessity for the diagnosis, treatment and

follow up of patient with cancer in the modern era of oncology. For certain cancer types molecular characterization of driver mutation can lead to molecularly-targeted therapies (as illustrated in Figure 1). For other cancer forms identification of different subtypes based on gene expression profiling or DNA methylation pattern can have prognostic and/or therapeutic implications.



- Experience from cancer centers that implemented a PCM approach to cancer treatment (Memorial Sloan Kettering Cancer Center, German Cancer Research Center (DKFZ) and many others) showed that this multidisciplinary effort has required a close collaboration between hospital leadership, oncology, pathology, molecular diagnostics, computational biology, clinical and translational researchers and pharmaceutical partners to meet the needs of the patients in a rapidly changing environment (1).

- Furthermore, to accelerate the development of PCM, molecular screening programs for identification of potentially targetable genetic alterations and participation in clinical trials are needed.

2. Aims of the investigation

a) Present the strengths and weeknesses of the current organization/infrastructure
b) Identify possible obstacles, problem areas within the workflow
c) Give suggestions for measures - ”action plan”

The results presented in this investigation are based on mettings/interviews with key leaders at Karolinska Institutet (KI), Karolinska University Hospital (KUH), Stockholm County Council (SLL), scientific literature review, personal experience in clinical molecular pathology, and visit at Wellcome Trust Sanger Institute at Hinxton, UK and at the German Cancer Research Center (DKFZ) in Heidelberg, Germany.

3. Reflections on the availability of tumor material

A separate PCM investigation analysis the issues of biobanking for PCM research and the reader is referred for more details to that report. ⇒

The workflow of inclusion of patients in molecularly-targeted clinical trials starts with the aquisition of cancer tissue for diagnosis and additional analysis.

- As a general comment one should note that the majority of clinical trials that run today require, in the study inclusion criteria, biopsy material. Therefore in this patient group the acquisition of tissue material by at least core needle biopsy should be advocated. A weekness of the analysis of cytological material is that it does not allow for studies on the tumor microenvironment where the histological context is a prerequisite. Furthermore, preservation of formalin-fixed paraffin-embedded (FFPE) biopsy material would also give the possibility for retrospective analysis of the tumor tissue in future research projects. An optimal tissue acquisition strategy would need both the fine needle aspiration and core needle biopsy components.

- As the small biopsies contain limited amount of cancer tissue, medical community

working with this material must be aware of the importance of quality and quantity issues of the aquired cancer tissue in order to optimise the conditions for clinical diagnostics, research and biobanking. Blood samples should also be biobanked in parallel with the tissue samples from all patients (preferably before and after the treatment) for future germline and biomarker analysis.

- Awareness should be raised at all levels though the medical community (from endoscopists to interventional radiologist to laboratory technician and pathologists) with regard to the optimal handling of the cancerous tissues paying attention not to exhaust the material only for diagnostic pursoses. All involved parties has to understand that todays cancer diagnostics is followed up by a multitude of molecular analyses with the aim to provide information for the treatment planning of the patient. This could be done through the education of the personnel, clearly written guidelines (SOPs) for the handling of the biopsy specimens and through closer multidisciplinary collaboration between the involved parties. It is also important to monitor the cold ischemic time by recording the time when the biopsy was taken. The routine material handling has to be adapted to the research methods in question paying attention for special requirements of tissue handling e.g. proteomic analysis or live cell culture experiments (for further reading see reference 2). These practice changes will add additional costs and workload demands on the pathology departments involved in such PCM projects.

- The preferred material for multiomics analysis is fresh tumor material. Unfortunately this is available only in a minority of the cases as the standard material in the pathology departments is still the FFPE tumor material. A further dimension to the molecular characterisation of the cancer is that altough the primary tumor is removed by surgery and offers plenty of tumor material for both diagnostic and research purposes, in the metastatic setting when the molecular characterization of the cancer cells needs to be reinvestigated to tailor further treatment, the lack of available tumor tissue becomes a limiting factor.

A necessary measure to address the aforementioned problem is to increase the number of biopsies

taken from metastases. This is fundamental for PCM development and because of this need the radiology units, responsible for guided biopsies, need to develop efficient centras for advanced tissue sampling. (for details on radiology for PCM research please refer to the separate PCM investigation on that topic ⇒).

- An additional neglected source of biobanking is the extracted DNA used for clinical diagnostic analyses. In line with this even unused sequencing libaries could be preserved for future research applications.

4. How much should one sequence?

The number of genes with validated clinical impact is relatively small (Figure 2). However, when it comes to research the demands are much greater.

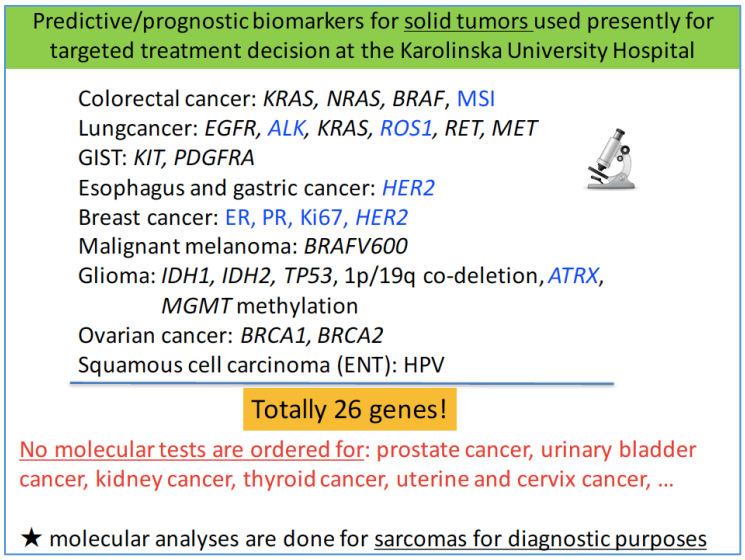


Figure 2: Molecular analyses performed in clinical routine at the Department of clinical pathology, Karolinska University Hospital

Recent advances in technology have resulted in relatively wide and inexpensive access to high-throughput sequencing methods (3). As a consequence, the number of raw data elements that need to be parsed and translated into clinically meaningful data via strategically designed bioinformatics pipelines increases in terms that we have never experienced before. Most commercially available sequencing platforms are accompanied by at least a basic form of bioinformatics software; however, data analysis and interpretation continue to pose a significant challenge, even in the setting of major academic institutions with custom designed bioinformatics pipelines. The lower limit of detection for most commercially available next-generation sequencing (NGS) platforms with adequate depth of coverage (at least 200 reads per base) is approximately 5% mutant allele in the background of non-mutated DNA. Whether known driver mutations with very low frequencies in an adequately cellular tumor sample should be used to guide clinical decision-making is a subject of debate (2).

For research purposes from a genomics perspective one of the aims is to combine whole genome

sequencing (WGS) or whole exome sequencing (WES) with transcriptomics (RNA-seq). Whole-exome sequencing allows simultaneous sequencing of ~95% of the genome coding region, which is composed of ~20,000 genes. Whole-genome sequencing looks at the mutational profile within the genomic regions that span not only the exomes but also the regions outside exome boundaries.

For biomarker discovery and basic cancer biology research the DNA- and RNA-sequencing should be complemented with proteomic studies, in vitro drug sensitivity assays, genome-wide DNA methylation/epigenetic analysis aiming at a multiomics characterization of the cancers.
For clinical applications, screening the whole genome or exome is challenging owing to the large genomic area to be sequenced, associated costs, complexity of data, and lack of known clinical significance of all genes (4).
To enable clinical decision based on genomic analysis and to also facilitate research in the cancer genomic field several institutions have opted for a targeted sequencing approach based on hybrid capture technology for a gene panel consisting of 300-500 cancer genes. Multiple commercial companies who provide cancer panel sequencing have also adopted a similar approach.

Broad genomic characterisation of cancer can inform on:

- SNV, CNV, fusion genes that could be targeted with small molecules
- identification of neo-epitops that might be tageted by immunotherapy
- microsatellite instability and homologous recombination defect status of the tumor
- mutational load that can serve as predictive biomarkers for treatment with checkpoint inhibitors
- predict toxicity to chemotherapeutical agents through the analysis of SNPs in drug-metabolizing enzymes
- mutational signatures
- intratumoral genetic heterogeneity

Related to the cost of sequencing a recent projection analysis showed that as sequencing costs continue to decrease over time, costs associated with analysis of data downstream of sequencing are expected to grow by ~50% between 2010 and 2020 (5).

On the KI-KUH campus the most advanced sequencing facilities are located at the Science for Life Laboratory. Because of this and because of its proteomic-, single-cell analysis plattforms and advanced microscopy facilities the SciLifeLab is a central partner for any PCM project in the Stockholm area.

Besides the SciLifeLab there are multiple core facilities located on the campus that provide genomic analysis (for instance the Mutation Analysis Facility www.maf.ki.se; KIgene- genetic analysis at CMM http://ki.se/en/research/kigene-genetic-analyses-at-cmm; Clinseq http://clinseq.org). Futhermore benchtop sequencers and other plattforms for molecular analysis are located at the Departments of clinical pathology and clinical genetics at the KUH.

5. Identified problem areas

1. Limited experience with whole genome or exome sequencing on FFPE material

2. Limited availability of bioinformaticians with expertise in cancer genomics on the KI/KUH campus.

3. Difficulties in developing and validating a custom made bioinformatics pipeline for cancer genomics.

4. No clear unified strategy on data storage and data sharing on the KI-KUH campus.
   For detailed discussion on this topic the reader is referred to the separate PCM report on IT and data sharing ⇒

5. Difficulties with the start up of genomic studies for smaller research groups that do not have experience with these types of analyses.

6. The question still remains how much of these multiomics analyses should be done as part of the clinical practice (health care system financed) and how much as part of research (research fund financed).

7. Even if a broad molecular characterisation is wished for all patients one has to keep in mind that there still will be certain patients (in the range of 20-30 percent when whole exome sequencing was used in different studies) that cannot be included in the broad panel approach because of minimal tumor content of the biopsies and other reasons. Because of this possible problem backup analysis methods have to be in place to give all patients the possibility for a basic molecular characterization of their tumor.

8. Germline variants are commonly found in individuals undergoing tumor-normal sequencing. Because of this patients have to be informed on the possibility of detection of a germline mutation and written informed consent for genetic profiling has to be aquired from each patient before such analyses are done. In line with this problem analysis of 1566 consecutive patients undergoing tumor-normal sequencing with a custom 341-gene panel at Memorial Sloan Kettering Cancer Center, nearly 16% of patients carried germline pathogenic or likely pathogenic variants in a gene linked to an inherited human disease (6).

9. A specific ethical issue relates to the finding of actionable mutation without a treatment arm in place at the local oncology hospital. Finding the actionable target and informing the patient on the possiblity of inclusion in a clinical trial with a new molecularly-targeted drug that is not available in Sweden/Europe might give rise to false hopes to treatment and is of questionable ethical conduct.

10. Several potentially useful proteomic and in situ methods for PCM research are less studied and there are no readily available core facilities for the interested researchers. Furthermore there are research areas with potential PCM interest that need more investment and development on the campus (circulating tumor cells (CTC), patients-derived tumor xenograft models (7), and immunogenomics, to name a few).

6. Suggested action plan

1) Develop a broad (300-400 genes) hybrid capture-based DNA sequencing pipeline using the available infrastructures and expertise in the KI-KUH-SciLifeLab with the aim to characterise the patients cancer tissue in parallel with germline analysis to enable participation of patients in molecularly-targeted clinical trials. In parallel with this an RNA-sequencing pipeline should be developed.

2) Recruite/employ bioinformaticians dedicated to develop and implement a cancer genomic bioinformatic pipeline for data analysis and reporting.

3) Initiate a molecular tumor board with participation of medical oncologists, radiation therapists, researchers, geneticists and pathologists to help translate the genomic findings into clinical actionable reports.

4) Start the inventory of the available plattforms/core facilities on the campus with easily available information on KI-KS homepage about the methods, contact persons and other relevant information.

5) Develop a core facility for the analysis of CTCs and cell free tumor DNA ("liquid biopsies").

6) Develop clear guidelines on the clinical versus research parts of the PCM projects in order to adress the medico-legal and financial issues.

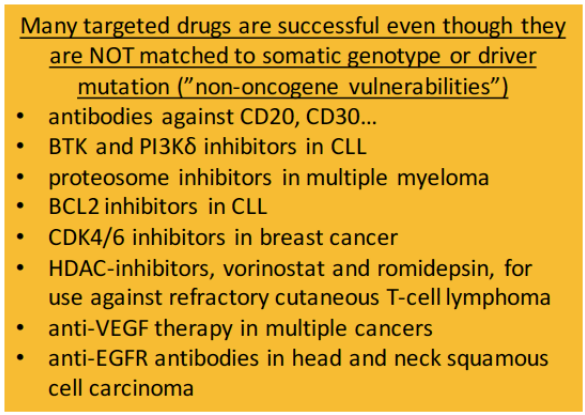
7) Develop pretest education tools to inform patients about the potential of identifying inherited susceptibility or previously undiagnosed genetic disease. Furthermore protocols have to be developed for appropriate communication ruotines of the posttest results.

8) Dedicated investments in infrastructure and clinical studies to integrate studies on immunotherapy, tumor immune microenvironment, immunogenomics and cellular therapy as parts of PCM projects.

7. Final remarks

Trials on targeted therapies have some limitations:
a). Most patients lack a target against which available biomarker-based therapies could take aim. In the National Cancer Institute's (NCI) Molecular Analysis for Therapy Choice (NCI-MATCH) Trial, which screened for mutations in 143 genes in tumors that did not respond to standard medication with available 10 trial treatment arms only about 10% of patients screened have been candidates for an arm of the trial (8).
b). Despite our increase in knowledge on the cancer genome it remains a struggle to differentiate 'passenger' aberrations that do not impact on cell function from significant 'driver' abnormalities to which the cancer cell is addicted for growth and survival.
c). Even those with an "actionable" biomarker might not be cured, because their tumor cells contains other abnormalities that counteract the effects of biomarker-driven therapy.

Despite the entusiasm for molecularly-targeted therapy based on tumor molecular profiling one should not forget that knowledge on basic tumor biology is at least as important. This is exemplified by the plasma-cell malignancy called multiple myeloma for which disease 16 new treatments have been approved in the past 12 years, including 7 new FDA-approved treatments in 2015 alone, without specific requirement for genomic testing (9).



Finally I would like to point out that everyone involved in the planning of the future KI PCM project has to understand that the key to the success will be to coordinate and link together all the aspects of the PCM project discussed in detail in the six investigations because all the different parts are vital and have to function in streamline.

8. References

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