International Journal of Latest Trends in Engineering and Technology
Special Issue - ICTIMESH-2019 pp. 005-009
e-ISSN:2278-621X

NEW DEVELOPMENTS IN DIAGNOSTIC INSTRUMENTATION AS A PREREQUISITE FOR QUICK TREATMENT DECISIONS SUPPORTING PERSONALIZED THERAPEUTIC APPROACHES IN ONCOLOGY

Tobias Preckel[1]

Abstract- Along with cardiovascular disease and neurodegenerative indications cancer is among the major causes of premature death in the western world. The growing understanding of the molecular causes of tumor growth shows that tumors are genetically heterogeneous between individuals as well as within an individual. Thus, a standard approach of treatment for certain tumors becomes questionable. On the one hand, standard pathology methods for tumor typing are mainly histology based and involve (immuno-) histochemical staining of biopsy-derived tissue sections. On the other hand, next-generation DNA Sequencing provides detailed genetic information about a tumor genome. Both methods generally require centralized facilities and statistics indicate that it can take up to several weeks until a result is reported. In contrast, current treatment schemes require detailed knowledge on specific tumor mutations for treatment selection. Here, new developments in diagnostic instrumentation are paving the path towards decentralized gene-based tumor diagnostics and provide data to allow faster treatment decisions.
Keywords – Tumor Typing, Precision Medicine, Biomarker, Microfluidic

1. INTRODUCTION

Medical diagnostics can be structured into three fundamental areas. The oldest physical methods concern Palpation which involves feeling the patient's body to assess organ size and shape. Another physical method is Percussion which means tapping to also assess organ size and shape but also the organ's boundaries. Auscultation involves the perception of sounds determined with stethoscope. Finally, determinations of blood pressure, temperature measurement, Electrocardiography, Electroencephalography are part of this family. Imaging techniques as the second group initially started out with projection radiography (X-ray) and have evolved through advancements in optical techniques, endoscopy, sensitive detectors & tracer development. They now comprise modern techniques such as Computer Tomography (CT), Magnetic Resonance Imaging (MRI) and Positron-Emission Tomography (PET). Molecular Diagnostics on the other hand involve molecule based tests with tissue or body-fluid samples to assess enzyme activity, minerals, metabolites, cellular activity, gene expression and genetic sequence. This group of methods is currently used in a multi-parametric environment in parallel with other techniques such as imaging. However, in recent years, technological advances in instrumentation and techniques that target genetic information are changing the playing field towards a stronger role of molecular methods in medical diagnostics.

2. BIOMARKER-BASED TESTING

The basic principle of molecular diagnostics relies on the detection and quantification of biological markers in a patient sample. Biological markers are defined as measurable indicators of a biological process, e.g. the presence or severity of a disease, e.g. LDL/HDL cholesterol is used as a risk indicator of coronary disease while certain variations of the BRCA1 gene are risk indicators for breast cancer. Diseases occur because of a faulty step in the process, either through a mutated DNA sequence or because of a changed pattern of RNAs (infections, toxins) leading to altered protein production [1]. Often the assessment of a single marker molecule is not sufficient for a therapeutic decision and thus several biomarkers are tested in panels or are used in combination with other parameters to assess the disease state. The key challenge in the development of any molecular diagnostics test is finding the right biomarker indicative of the disease.

In principle, all types of biomolecules can be used as biomarkers and significant advancements have allowed tapping into diagnostic information on a protein- (proteomics, immunoassays), mRNA- (RNA Sequencing, RT qPCR, DNA Microarrays) or DNA level (DNA sequencing). Novel methods have brought down the cost of whole genome sequencing to a level that is in the reimbursement window of health insurance companies. As a result, DNA sequencing is now clearly established as a diagnostic technique in medicine. The decline of sequencing costs is expected to continue. Additionally, diagnostic data from various instrumental platforms is increasingly digitized allowing measurement and diagnosis to happen in different geographic locations with fast turnaround time. Advances in artificial intelligence (AI) are providing ways to make sense of the vast

[1] Department of Medical Engineering, School of Engineering, Pforzheim University of Applied Sciences, Pforzheim, Baden Württemberg, Germany

diagnostic data from diverse sources with increased accuracy. Recently, the US Federal Drug Administration (FDA) has approved the first instrument platform for digital pathology, a first step to apply the potential of machine learning in cancer diagnostics [2, 3]. The next step has already been taken and involves AI in the analysis of genetic, protein and metabolite data [4].

The development of Biomarker-based tests is also moving up the pharmaceutical value chain. Pharmaceutical drug development is based on the hypothesis that the abnormal activity of molecules controlling metabolic processes cause disease. Subsequently, diseases can be cured by influencing these abnormal molecules, ideally by shutting them down through a drug molecule that binds to them. The Target Selection process deals with finding the relevant molecule that plays a pivotal role in causing the disease while the Drug Discovery process involves a shotgun approach where potential drug molecules are tested for binding against the target (Hits). In the Drug Development process hits are further refined and tested for efficacy, toxicity and are optimized for formulation and production. Clinical trials involve testing potential drugs for efficacy and safety with increasing number of participants to finally obtain regulatory approval to bring the drug to the market [5]. The final product is selected to curtail symptoms in a large patient group. This approach whereby a uniform standard therapy of medication & dosage is applied has accepted side effects as a necessary evil. However, in spite of a highly complex process and enormous investments one finds a high level of ineffectiveness of common drugs in a patient group [6]. Often, only a fraction of patients with the same diagnosis and treatment responds positively because small differences on a genetic level between individuals lead to varied responses to treatment.

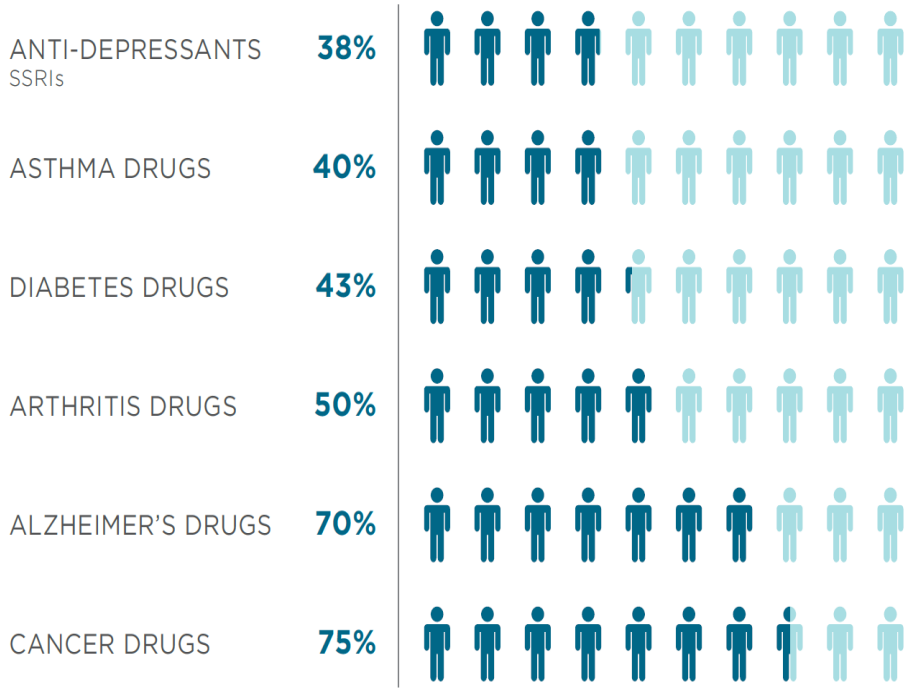

Fig.1: Average of patient population percentage for which a particular drug in a class is ineffective [Source: B. Spear, M. Heath-Chiozzi, J. Huff, "Clinical Trends in Molecular Medicine" Volume 7, Issue 5, pp.201-204, 2001]

This often does not become apparent in standard clinical trials with a homogenous and comparatively small group of tested subjects. As a result, drug manufacturers now use a co-development approach for drugs and diagnostic tests (so called Companion Diagnostics) whereby biomarkers are selected that can be used as an indicator of whether the drug will be effective in a patient. In the end, the pharma product is a drug plus a test that allows stratification of patient groups to select patients where the drug will have a beneficial effect and a minimum of unwanted side effects. Accordingly, this approach has been termed "Precision Medicine" or "Personalized Medicine". It promises the delivery of patient benefits, healthcare cost savings and revenue opportunities. The number of Companion diagnostics tests is on the rise, from 72 in 2011 to 115 in 2014 [7]. The tests assess genetic makeup, metabolite profile, enzymatic activity, etc. and play an important role in cancer diagnostics. In some cases, the tests may also allow a personal risk assessment prior to disease outbreak.

Disease research and drug discovery are aided by advances in cell culture techniques that support development 3D cell culture systems, organoids (Organ-on-chip and micro-tissues) and scaffold based systems. Combining cell reprogramming (CRISPR Cas and other methods) with stem cell technology allows generating new disease-in-a-dish systems for Drug Discovery and disease modeling, esp. for rare diseases where finding patients is a challenge [8].

Cancer diagnosis is typically achieved by the use of imaging techniques (optical endoscopic techniques, MRT, CT, PET-Scan, SPECT, etc.) either proactively or once symptoms occur. Here, in spite of the advancements made, imaging techniques require

a minimum tumor size or metabolic activity for diagnosis. Additionally, different tumors may show a similar pattern in an image. The acquisition of biopsies will allow tissue preparation by formalin fixation and paraffin-embedding (FFPE) and tumor typing by staining of tissue sections followed by microscopy. The time from obtaining consent for biopsy acquisition to discussion of pathology results may be up to several weeks. Indeed, statistics show that in the US results take up to an average of 4 weeks and as much as 25% of tumor-diagnosed patients take up therapy before results are in risking unwarranted side effects and ineffective therapies [8]. Because tumors acquire genetic mutations over time they represent moving targets which speaks to the importance of a prompt diagnosis. NCCN (National Comprehensive Cancer Network) a not-for-profit organization of close to 30 leading cancer centers regularly publishes treatment guidelines many of which rely on specific knowledge of genetic information of the tumor [10]. Information on specific tumor mutation can aid in selecting effective treatments as well as prevent unnecessary side effects. Also, the knowledge, that a diagnosed tumor carries mutations which imply a poor prognosis can be a trigger point to integrate patients into clinical studies for new drugs.

Also, because of their heterogenic nature by the time a tumor becomes detectable it likely consists of various cells with different mutations. A single treatment will usually only decimate a subset of tumor cells and over time the selective pressure of the treatment will likely benefit tumor cells with a specific genome. Thus, a single treatment will very likely not eliminate all tumor cells. Rather continuous monitoring of disease progression during treatment will be required [11]. Invasive tissue biopsy acquisition may not be the ideal technique to continuously sample tumor tissue. Modern approaches are targeting easily accessible blood-borne tumor-biomarkers (tumor-derived free DNA or circulating tumor cells). Such Liquid Biopsy assays are now being evaluated for diagnosis, treatment selection and monitoring of the disease [12]. Here, one of the issues is to overcome is the low concentration of these biomarkers in the bloodstream which poses a significant challenge for sample preparation techniques.

3. NEW INSTRUMENT DEVELOPMENTS

In principle, diagnostic systems can be placed in centralized labs or at the patient location (point of care, POC) or in the doctor's office. Centralized testing allows for more cost effective use and larger investments while decentralized testing ideally has the advantage of shorter time-to-result. It requires a higher degree of ease-of-use as expert personnel may not be as readily available as in a centralized facility. Also, in term of tumor diagnosis a decentralized system ideally works with a sample that is readily accessible, e.g. a liquid biopsy. In terms of a technological approach, a quantitative polymerase chain reaction (qPCR) is preferable over a next-gen DNA sequencing technique because of its lower complexity. The availability of a lab infrastructure may be limited in a hospital or doctor's office and therefore decentralized devices ideally combine a multitude of instrument functionalities and utilize consumables which hold all required infrastructure and reagents necessary for the test.

Ideally, as lab-space is limited instrument hardware is small and user interaction during the test is minimized to reduce the potential for user-caused mistakes, e.g. pipetting errors. Pre-formatted reagents and consumables can help to reduce sources of contamination. For the past two decades microfluidic technology has been utilized to map complex lab processes in a chip-architecture. Because of the small format it achieves a reduction of sample and reagent volume and short analysis time. Additionally, user interaction during the analysis is minimized leading to a reduction of errors. Data is obtained in a digital format lending itself to electronic lab notebooks and review by decentralized researchers. Applications such as cell-based assays and analysis of protein or nucleic acid molecules have been successfully implemented on commercial analysis systems. They are based on the controlled movement of liquids by pressure- or electroosmotic driven flow inside networks of microfluidic channels [13]. Next to planar chip-based formats CD-based centrifugal formats have received great deal of attention due to their potential use in biomedical point of care applications [14]. In general, one of the major challenges for all microfluidic devices lies in the development of fluid actuation schemes, i.e. implementation of valves. Here, recent trends indicate that technology is moving to bigger channel diameters ("millifluidics") to allow a larger footprint for valve implementation and to be less prone to clogging issues.

General requirements for instrumentation and reagent & chip-cartridge are:

Instrument requirements

- Universal (optical) detection system that allows different tests to be conducted on the same instrument
- Implementation of quick and precise temperature settings, pumps, valves, electrodes, sensors, techniques for sample disintegration & cell lysis (e.g. ultrasound)
- Easy-to-use Software and data analytics
- Barcode reader (to avoid sample exchange)
- Secure interfaces for data transfer
- Tolerant to changing environmental conditions
- Easy-to-use & cost-effective to produce

Reagent & Chip-Cartridge requirements

- Closed system to avoid contamination of user and sample mix up (all reagents, standards, markers and matrices are prefilled)

- A single cartridge per sample and test (can be multiplexed)
- (Optical) detection area
- Micro- or millifluidic architecture of cavities and channels to increase speed and minimize reagent consumption
- Applicability to (FFPE-) tissue and fluid samples
- qPCR-, sample extraction- and sample clean up capability
- Pneumatic or electrophoretic movement of liquids, implementation of valves
- Easy-to-use & cost-effective to produce
- Ideally shippable at ambient temperature
- Storable (shelf life ideally 6 months & longer which may require lyophilized reagents)

Several biomarker-based instrument platforms that showcase the implementation of these requirements have already been developed and commercialized [15-17]. In all cases, qPCR has been selected as the diagnostic assay. They have in common a universal detection system with a small footprint, a cartridge-based approach with preformatted reagents and a cartridge infrastructure with small channel dimensions. Compared to traditional lab-based or DNA Next-Gen-Sequencing approaches hands-on time in the biomarker-based systems has been reduced to minutes and total analysis time to 2-4 hours. All systems are scalable, allowing the user to adapt to growing sample numbers by adding more analysis instruments. With respect to different systems, a distinction is made with respect to the sample lysis step: it is either carried out within the cartridge [15, 16], or on a separate instrument system [17]. The reason for this may lie in the specific samples which present more or less of a challenge with respect to disintegration. While some of the products are initially applied to the identification of pathogens based on DNA signatures [15, 17] one system specifically focuses on cancer diagnostics [17]. Here, a study tested 43 archival DNA samples that had previously provided valid DNA Next-Gen-Sequencing results for EFFR mutations. In all 43 cases the system confirmed the EGFR mutational status [18]. However, it is conceivable that because of their capabilities all three systems can be used for typing of tumors.

4. CONCLUSION & OUTLOOK

Biomarker-based decentralized cancer diagnostics allow therapy selection based on specific cancer mutations (targeted therapies) or by genetics rather than location of the tumor. In the latter case, a single therapeutic approach may even target multiple cancer types (pan-cancer therapies). Additionally, biomarker-based tests for specific genetic signatures have applicability beyond therapy selection in that they provide an assessment of individual cancer risk as well as of prognosis. Thus, biomarker-based diagnostics allows for decentralized molecular testing to reduce the time required to actionable result. It can therefore play an important role in accelerating the access of patients to individual treatments and precision medicine.

Beyond their value in oncology decentralized gene-based molecular diagnostics tests have enormous value in the context of infectious disease testing [15, 17]. Antimicrobial resistance (lack of susceptibility of bacteria to antibiotics, AMR) is caused by excessive use of antibiotics in medicine and especially livestock production. Antibiotic resistance of common bacterial strains and the advent of multi-resistant bacteria are some of the biggest issues in clinics. The continuous presence of low doses of antibiotics encourages growth of resistant bacteria. New antibiotics and faster decentralized tests are needed to identify resistant bacteria, to reduce unnecessary prescription of antibiotics (adequate use of antibiotics) and to discriminate between viral and bacterial infections [19]. Testing is moving from culture based typing systems to a closed tube PCR-based genetic testing in light of reduced time-to-result.

Testing may already need to happen at the transition of agricultural products into the market. Fruits and vegetables are a reservoir for transferable antibiotic resistance genes. Bacteria associated with fruit and vegetables can carry various plasmids that might represent an important link between the environmental and human gut microbiomes [20]. This seems to be an important pathway for disseminating transferable antibiotic resistances and is particularly relevant for patients under antibiotic treatment.

5. REFERENCES

[1] M. Jackson, L. Marks, G. May, J. Wilson, “The genetic basis of disease”, Essays Biochem., 62, pp. 643–723, 2018

[2] M. Kent, T. Olsen, T. Feeser, K. Tesno, J. Moad, M. Conroy, M. Kendrick, S. Stephenson, M. Murchland, A. Khan, E. Peacock, A. Brumfiel, M. Bottomley, “Diagnostic Accuracy of Virtual Pathology vs Traditional Microscopy in a Large Dermatopathology Study”, JAMA, 153, pp. 1285-1291, 2017

[3] Press release “FDA allows marketing of first whole slide imaging system for digital pathology”, https://www.fda.gov/news-events/press-announcements/fda-allows-marketing-first-whole-slide-imaging-system-digital-pathology, accessed 28 November 2019

[4] K.-H. Yu, G. Berry, D. Rubin, C. Ré, R. Altman, M. Snyder., “Association of Omics Features with Histopathology Patterns in Lung Adenocarcinoma”, Cell Systems, 5, pp. 620-627, 2017

[5] J. Manthan,D.Sreedhar, L. Virendra, A. Pise, U. Nayanabhirama, "Drug Development Process : A review", Pharmaceutical Reviews, 5, 2007

[6] Interview “Glaxo chief: Our drugs do not work on most patients”, Independent, 8 December 2003, https://www.independent.co.uk/news/science/glaxo-chief-our-drugs-do-not-work-on-most-patients-5508670.html , accessed 28 November 2019

[7] Personalized Medicine Coalition, “The Case For Personalized Medicine”, 4th Edition, 2014

[8] D. Hockemeyer, R. Jaenisch, “Induced pluripotent stem cells meet genome editing”, Cell Stem Cell., 18, pp. 573–586, 2016

[9] M. Schwaederle, B. Parker, R. Schwab, P. Fanta, S. Boles, G. Daniels, L. Bazhenova, R. Subramanian, A. Coutinho, H. Ojeda-Fournier, B. Datnow, N. Webster, S. Lippman, R. Kurzrock, "Molecular Tumor Board: The University of California San Diego Moores Cancer Center Experience", The Oncologist, 19, pp. 631–636, 2014
[10] National Comprehensive Cancer Network, https://www.nccn.org, accessed 26 November 2019
[11] R. Leary, I. Kinde, F. Diehl, K. Schmidt, C. Clouser, C. Duncan, A. Antipova, C. Lee, K. McKernan, F. De La Vega, K. Kinzler, B. Vogelstein, L. Diaz, V. Velculescu, "Development of Personalized Tumor Biomarkers Using Massively Parallel Sequencing", Sci. Transl. Med., 2, pp., 2010
[12] G. Rossi, M. Ignatiadis, "Promises and Pitfalls of Using Liquid Biopsy for Precision Medicine", Cancer Res., 79, 2019
[13] S. Chan, G. Lüdke, M. Valer, C. Buhlmann, T. Preckel, "Cytometric analysis of protein expression and apoptosis in human primary cells with a novel microfluidic chip-based system", Cytometry,55A, pp. 119-125, 2003
[14] J. Gilmore, M. Islam, R. Martinez-Duarte, "Challenges in the Use of Compact Disc-Based Centrifugal Microfluidics for Healthcare Diagnostics at the Extreme Point of Care", Micromachines, 7, pp. 52-88, 2016
[15] Bosch, Vivalytik System, https://www.bosch-vivalytic.com/en/, accessed 27 November 2019
[16] Biocartis, Idylla System, https://www.biocartis.com/meet-idylla , accessed 27 November 2019
[17] Curetis, Unyvero 50 System, https://curetis.com/products/unyvero-a50-system/ , accessed 27 November 2019
[18] C. De Luca, A. Rappa, G. Gragnano, U. Malapelle, G. Troncone, M. Barberis, "Idylla assay and next generation sequencing: an integrated EGFR mutational testing algorithm", J. Clin. Pathol., 71, pp.745-750, 2018
[19] AMR report 2016, https://amr-review.org/ , accessed 26 November 2019
[20] K. Blau, A. Bettermann, S. Jechalke, E. Fornefeld, Y. Vanrobaeys, T. Stalder, E. Top, K. Smallaa "The Transferable Resistome of Produce", mBio, 9, pp. 1-15, 2018