Personalized Medicine:
from molecular methods to targeted therapy in cancer

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Abstract

Personalised medicine, according to the Advisory group for the H2020 Health, refers to a medical model using characterisation of individual phenotypes and genotypes for tailoring the right therapeutic strategy for the right person at the right time, and/or to determine the predisposition to disease and/or to deliver timely and targeted prevention. The development of genomic technologies methods leads to the identification of multiple mutations in a large variety of cancers. The data based on molecular technologies like molecular profiling, DNA, RNA expression methods, and also immunohistochemistry and proteomics aims to identify and evaluate molecular targets that may be candidates for drug discovery. In general the mechanism of targeted cancer therapies focuses on blocking growth, progression and spread of cancer by interfering with molecular targets that are involved in this process.

Small molecules like tyrosine-kinase inhibitors and serine/threonine kinase inhibitors or monoclonal antibodies are the examples of anticancer targeted therapy. The application of personalized medicine is still a work in progress. The development of targeted therapies makes cancer treatment more effective and reduce the cytotoxic effect of non-cancer cells.

In this review, the methods of identification targeted molecules like zebrafish cancer model and molecular profiling will be presented. This review will focus on the existing and future technologies that could improve the development of targeted therapies for treatment of resistant cancer in individual patients. Specifically, it will concentrate on reviewing the examples of current successful oncologic therapies include BRAF inhibitors (vemurafenib), RET inhibitors (sorafenib), and epidermal growth factor.

Introduction

WHO reported that cancer is the second leading cause of death globally, accounting for an estimated 9.6 million deaths, or one in six deaths, in 2018. Lung, prostate, colo-rectal, stomach and liver cancer are the most common types of cancer in men, while breast, colorectal, lung, cervical and thyroid cancer are the most common among women (WHO). Conventional treatment of cancer are hormonal and cytotoxic therapies.

The poor clinical outcome for most cancer types is caused by a diverse array of factors, including late diagnosis, tumor heterogeneity, metastasis, lack of targeted treatment options and resistance to therapy, tumor recurrence, and a failure to translate preclinical breakthroughs into meaningful patient benefit (Doll, 2019). Targeted cancer therapies are expected to be more effective than conventional treatment.

Personalized medicine refers to medical model using characterisation of individual phenotypes and genotypes (e.g. molecular profiling, medical imaging, lifestyle data) for tailoring the right therapeutic strategy for the right person at the right time. Moreover personalized medicine allowed to determine the predisposition to disease. Personalized medicine relates to the concept of patient-centred care.

Gene expression profiling and genome-wide sequencing have played significant roles in knowing a tumor's molecular sequence and allowed for creation of targeted therapies. Targeted therapy is one of the most developing therapy for cancer. Molecular targeted therapy blocks the growth of cancer cells by interfering with specific targeted mole-cules involved in carcinogenesis and tumor growth.

Targeted cancer therapies may be more therapeutically beneficial for many cancer types, including lung, colorectal, breast, lymphoma and leukemia. Moreover, recent advances made it possible to analyze and tailor treatments to an individual patient's tumor. The main types of targeted cancer therapies are monoclonal antibodies, small molecule inhibitors and immunotoxins (Baudino, 2015).

Latest advances in molecular methods such as next-generation sequencing (NGS), including DNA sequencing, RNA sequencing, whole-exome sequencing, copy number variation analysis, and DNA methylation arrays, have increased our understanding of cancer biology, and leads to the development of a new comprehensive molecular cancer classification (Sicklick, 2019).

In 1984, EGFR, as the first receptor, was associated with an oncogene, v-ERBB, which was known to induce sarcomas and leukemias in chickens (Downward, 1984).

The first clinical trial of molecularly targeted drugs for the off-label treatment of heavily

pretreated metastatic cancer was the SHIVA trial. The molecular profile of each patient's tumour was established with a mandatory biopsy of a metastatic tumour and large-scale genomic testing. Only patients with a molecular alteration in one of three molecular pathways (hormone receptor, PI3K/AKT/mTOR, RAF/MEK) were included. Patients were matched to one of ten regimens including 11 available mole-cularly targeted agents (erlotinib, lapatinib plus trastuzumab, sorafenib, imatinib, dasatinib, vemurafenib, everolimus, abiraterone, letrozole, tamoxifen). The results show that use of mole-cularly targeted agents outside their indications does not improve progression-free survival compared with treatment at physician's choice in heavily pretreated patients with cancer (Le Tourneu, 2015).

In the same time combination of everolimus with trastuzumab plus paclitaxel as first-line treatment for patients with HER2-positive advanced breast cancer (BOLERO-1: A Phase 3, Randomised, Double-Blind, Multicentre Trial) shown that progression-free survival was not significantly different between groups in the full analysis population (Hurvitz, 2015).

Disease progression in patients with HER2-positive breast cancer receiving trastuzumab might be associated with activation of the PI3K/Akt/mTOR intracellular signalling pathway. The addition of the mTOR inhibitor everolimus to trastuzumab might restore sensitivity to trastuzumab. In a randomised, double-blind, placebo-controlled phase 3 trial of everolimus for women with trastuzumab-resistant, HER2-positive advanced breast cancer (BOLERO-3), the addition of everolimus to trastuzumab plus vinorelbine significantly prolongs progression-free survival (PFS) in patients with trastuzumab-resistant and taxane-pretreated, HER2-positive, advanced breast cancer (Andre, 2015).

SEARCH STRATEGY AND SELECTION CRITERIA

In development of targeted therapies the first step is identification of marker and the next step is understanding the role of marker (prognostic, predictive).

In marker selection the following methods are developed:

GENOMICS

Understanding the molecular characteristics at a genomic level is critical to develop new treatment strategies. The identification of individual targetable alteration with a genomic methods might predict a therapeutic response to immune-checkpoint inhibitors or identify cancer-specific proteins. Based on that, personalized anticancer vaccines are designed. Clinical applications of cancer genomics include moni-to-ring treatment responses and characterizing mechanisms of resistance. Traditional appro-aches to the genetic characterization of clinical oncology specimens include cytogenetic analysis, fluorescence in situ hybridization (FISH), and molecular studies of single genes. These methodologies are complementary to each other and generate information of diagnostic and prognostic relevance. Next-generation sequencing (NGS) allows rapid analysis of multiple genes for clinically actionable somatic variants (Cottrell, 2014; Al.-Kateb, 2015). The application of massively parallel or next-generation sequencing (NGS) to large-scale cancer genomics discovery projects has revealed new information about the underlying genomic drivers of cancer development and progression across multiple anatomical locations. NGS and various analytical approaches are now being introduced into clinical practice to better inform the clinical care of patients with cancer (Berger, 2018). The application of NGS technologies to the characterization of human tumours has provided unprecedented opportunities to understand the biological basis of different cancer types, develop targeted therapies and intervenetions, discover genomic biomarkers of drug response and resistance, and to guide clinical decision-making regarding the treatment of patients (Garraway, 2013; Hyman, 2017). Increased levels of precision are being achieved in the clinical care by including cancer genomics in diagnostic medicine.

Therapeutic aplications of DNA sequencing was evaluated in I-PREDICT clinical study. This cross-institutional prospective study used tumor DNA sequencing and timely recommendations for individualized treatment with combination therapies. Administration of customized multi-drug regimens was feasible, with 49% of consented patients receiving personalized treatment. Targeting of a larger fraction of identified molecular alterations, yielding a higher 'matching score', was correlated with signifi-

cantly improved disease control rates, as well as longer progression-free and overall survival rates, compared to targeting of fewer somatic alterations (Sicklick, 2019).

Whole-genome, whole-exome, and whole-transcriptome sequencing pro vide the opportunity for discovery the full spectrum of oncogenicalterations in cancer tumours (12, Caldow Pilgrim, 2013). Cancer precision medicine in the clinical practice mainly focuses on the role of liquid biopsy, particularly circulating tumor DNA, as a potential tool for cancer screening, selection of an appropriate drugs, surveillance of minimal residual diseases, and early detection of recurrence (Low, 2019).

The development of NGS approaches in clinical laboratories need guidelines to ensure that NGS testing to direct patient care is performed to the same rigorous standards as other clinical tests focused on the analysis of nucleic acids, such as DNA sequence analysis by Sanger methodlogy, DNA copy number analysis by microarray analysis, and detection of chromosome aberrations by interphase FISH [8]. The guidelines for clinical NGS analysis (both the technical and bioinformatics components) was publi-shed by The College of American Pathologists (CAP), the U.S. Centers for Disease Control and Prevention (CDC) and the New York State Department of Health (Cottrell, 2013; El-Khoueiry, 2018). Several organizations formalized guidelines under which clinical NGS can be performed (Cottrell, 2013). The quality and use of molecular tests in medicine routine practice are regulated by Implementation of Guidelines on PG and PK, Good Genomic Practices, Guidelines on genomic BM and drugs co-development, PG methodology in PhVG ICH E18 genomic samples and data handling (Garcia, 2017).

The Cancer Genome Atlas (TCGA) and the International Cancer Genome Consortium (ICGC) have surpassed the 1000 Genomes Project by sequencing thousands of tumors across different cancer types. Comprehensive genetic profiling of tumor samples has uncovered novel oncogenes and tumor suppressor genes by comparing their mutation frequencies with the background mutation rate, by detecting mutation profiles with significant bias toward certain mutation types (Becquemont, 2009).

Methodology implications for drug clinical development of Next Generation Sequencing (NGS) for clinical use are analysis of a panel of genes, analysis of whole exome or genome and large unbiased sequencing.

PROTEOMICS, PHOSPHOPROTEOMICS, PROTEOGENOMICS

Analysis of the expressed proteins in a tumor and their modification states reveals if and how DNA mutations are translated to the functional level. Proteomic changes including posttranslational modifications are essential steps of oncogenesis. Proteomics technology has only recently become comparable in depth and accuracy to RNAseq and allow the analysis of formalin-fixed and paraffin-embedded biobank tissues, on both the proteome and phosphoproteome levels. Mass spectrometry-based proteomic studies is technology for large-scale and unbiased proteomic analyses. Proteomic with genomic and clinical data generate a personalized panomics profile for each patient for better treatment decisions (Doll, 2019) modification states reveals if and how these mutations are translated to the functional level.

Phosphoproteomics measures the identity and quantity of tens of thousands of phosphorylation sites, serving as an ultimate read-out of the activity of kinases to detect aberrant kinase activities and altered signaling pathways, which are the most important alterations in oncogenic transformation. Drugs targeting mitogen-activated protein kinase 1 (MAPK), PI3K, serine/threonine-protein kinase B-raf (BRAF), vascular endothelial growth factor (VEGF), ALK, EGFR inhibit their targets directly at the protein level – and not at the gene level (Yaffe, 2013).

Cancer proteogenomics promises new insights into cancer biology and treatment efficacy by integrating genomics, transcriptomics and protein profiling including modifications by mass spectrometry (Satpathy, 2020).

PHARMACOGENOMICS

Pharmacogenomics is the study of the role of the genome in drug response and allows to optimize drug therapy, based on the patients genotype, to ensure maximum efficiency with minimal adverse effects (Bequenmont, 2009). In cancer treatment, pharmacogenomics tests are used to identify which patients are most likely to respond to certain cancer drugs. Innovative

tumor profiling methodologies are utilized to elucidate the pharmacogenomic landscape of tumor cells in order to support the molecularly guided delivery of therapeutics. Personalized medicine in oncology link the data of genomic, transcriptomic, and proteomic analysis of tumor samples to aid in therapy decision. In a typical screening of such type, multiple genes and proteins impli-cated in tumor initiation, progression, and drug resistance are analyzed in tumor biopsies.

The success of the technology has already been demonstrated for various combinations of alte-red biomarkers and therapeutic molecules, such as epidermal growth factor receptor (EGFR) expression and EGFR tyrosine kinase inhi-bitors/antibodies, or the expression of program-med death ligand 1 (PD-L1) with anti-PD1 and anti-PD-L1 therapies (Astras, 2020).

MOLECULAR IMAGING

Medical imaging plays a central role in clinical oncology. The future of such imaging is mole-cularly targeted imaging agents. Molecular imaging differs from conventional anatomical imaging in that imaging probes are utilized to visualize target molecules-of-interest. Molecular imaging plays an important role in oncology and personalized medicine by allowing earlier diagnosis, assessing early response to treatment and by predicting treatment response. Molecular imaging has an impact on drug development by streamlining preclinical and clinical tests for new drug candidates. Molecular imaging allows not only localization of a tumor in the body but also allows imaging of the expression and activity of specific molecules (protein kinase) as well as biological processes like angiogenesis, hypoxia and apoptosis. This processes influence on tumor behavior and response to treatment (McDermott, 2016).

Several markers related to angiogenesis inclu-ding VEGF/VEGFR, amb3 integrins, hypoxia-inducible factor-1 or MMPs can be targeted for single-photon emission computed tomography (SPECT)/PET angiogenesis imaging. Preclinical

trials on probes currently used for imaging the VEGF and VEGFRs pathways, such as antibo-dies against VEGF and radiolabeled VEGF-A, have shown promising data for further imple-mentation in clinical practice (Fukumura, 2007). In other clinical study a method for conjugating a therapeutic antibody to a molecular magnetic resonance imaging was investigated. This study concluded that cet-PEG-dexSPION nanoparticle could be a promising nanomedicine for thera-peutic targeting of EGFR-expressing tumor cells. The therapeutic antibody cetuximab and non-invasive monitoring methods improved treatment efficacy (Tseng, 2015).

There are many advantages associated with the ability to measure receptor expression by ima-ging: its' non-invasiveness, the ability to assess sites, which are difficult to sample and avoids sampling error from biopsies when receptor expression is hetero-geneous. Tumor receptor imaging can measure the therapeutic target expression and could be used to direct patient selection for targeted therapy (Mc Dermott, 2016).

IMMUNOHISTOCHEMISTRY

The use of immunohistochemistry (IHC) for the determination of carcinoma biomarkers is a well-established and powerful technique. Immuno-histochemisty is readily available in pathology laboratories, is easy to perform, assess and can provide clinically meaningful results in relatively short time (Thunninsen, 2017). There is a significant correlation between the IHC marker expression and disease progression and the prognosis of drug effects (Barbalan, 2018).

Multiplex immunohistochemistry allows the demonstration of multiple protein antigens in individual histological sections of formalin-fixed paraffin-embedded tumors or other types

of tissue. Well-designed and optimized immu-nohistochemistry assays maximize the infor-mation available from limited tissues and demonstrating the histo-anatomical relationships among key cell types which express the included biomarkers (Steele, 2018).

The American Society of Clinical Oncology and the College of American Pathologists develop a guideline to improve the accuracy of immu-nohistochemical (IHC) estrogen receptor (ER) and progesterone receptor (PgR) testing in breast cancer and the utility of these receptors as predictive markers (Hammond, 2010).

ZEBRAFISH CANCER MODEL

The zebrafish (*Danio rerio*) has been established as one of the most important model organisms for cancer research. This model is particularly suitable for live cell imaging and high-throughput drug screening. The zebrafish represents a powerful platform for cancer research in the development of target therapies. The zebrafish cancer model was improved for drug discovery and toxicological and phenoltypical screening (Bootorabi, 2017).

The zebrafish is ideal for large-scale screening approaches and allows both chemical and genetic screening to identify genes and pathways underlying diseases, as well as phenotypic screening for the discovery of new drugs (Zhao, 2015). The compounds, drugs or small molecules, can be added directly to the water environment of the zebrafish (MacRae, 2015). Zebrafish allow unrivalled *in vivo* imaging of cellular behaviour thanks to optical clarity and a range of tissue specific transgenic lines (Brown, 2017). The noninvasive high-resolution imaging methods in transparent zebrafish embryos visualize cancer progression and reciprocal interaction with stroma in a living organism (Chen, 2017).

The zebrafish cancer models are link between *in vitro* cell culture and *in vivo* mammalian models for a rapid pre-clinical drug development. Moreover, given the high genetic and physiological similarities with humans, zebrafish can be used for anticancer drug screening. Transplanted human cancer cells are able to respond to zebrafish cytokines, modulate the zebrafish micro-environment, and take advantage of the zebrafish stroma during cancer progression. In addition to genetic and molecular studies, zebrafish model is also ideal for large-scale chemical screens to identify small molecules that influence different aspects of hematopoiesis (Gore, 2018).

A transgenic zebrafish melanoma model based on the zebrafish mitfa promoter coupled with the human oncogenic HRASG12V (Le, 2013). Transgenic zebrafish embryos developed melanocyte hyperplasia with the induction of RAS-RAF-MEK-ERK and RAS-PI3K-AKT-mTOR signaling pathways. The zebrafish model was useful for the screening of compounds directed against mitogen-activated protein kinases, extracellular signal-regulated kinases (MEK/ERK) and PI3K/mTORi pathways (Rapamycin), alone or in combination (Thomas, 2012). Clinical trials using rapamycin analogs combined with MEKi or PIK3K/mTORi are currently underway (Bootorabi, 2017).

The zebrafish model has been recently used to identify key molecules in skin cancer, which includes melanoma and squamous cell carcinoma (SCC), as well as compounds for SCC target therapy (Shin, 2016; Jun, 2011). Transgenic mitf-BRAFV600E; p53/zebrafish embryos have been created for the evaluation of early transcriptional activity within melanoma pathogenesis and to provide a model for chemical genetic screening in the context of melanoma therapy (White, 2011).

INNOVATIONS IN CLINICAL TRIAL DESIGN

New trial design uses gentic profiles to highlight biomarker differeces. In recent years, the therapeutic management of selected patients with cancer based on patient's mecha-nisms of tumorigenesis, DNA profiling using next-generation sequencing, proteomic and RNA analysis, and immune mechanisms after bioinformatic analysis is essential to optimize patient's treatment (Fountzilas, 2018).

The traditional, large phase II and phase III adjuvant trial models need to be replaced with smaller, shorter, and more focused trias that need to be more efficient and adaptive in order to quickly assess the efficacy of new agents. The shift from the traditional multiphase trial model to an increase in phase II adjuvant and neo-adjuvant trials in earlier-stage disease incorporating surrogate endpoints for long-term survival enables better efficacy of therapeutic agents in shorter time frazes (Wulfkuhle, 2017).

The National Cancer Institute–Molecular Analysis for Therapy Choice (NCI-MATCH) trial is a study that relies on genomic assays to screen and enroll patients with relapsed or refractory cancer after standard treatments. The analytical validation processes for the next-generation sequencing (NGS) assay that was tailored for regulatory compliant was used in the trial. Thousands of patients who have relapsed or refractory solid tumors and lymphomas after standard systemic treatment was recruited and screened. The patients were assign

to a treatment appropriately matched to their cancer genotype. Analytical validation involved testing cells and tumor tissues of multiple types in an effort to determine assay performance over a wide range of tumor specimens. The NCI-MATCH trial will provide an opportunity for cancer patients to be matched to treatments targeted to specific molecular defects based on the genomic analysis of their tumors (Lih, 2017).

The FOCUS4 (Molecular selection of therapy in colorectal cancer: a molecularly stratified randomized controlled trial program) trial evaluates patients with advanced, metastatic colorectal cancer whose disease is stable or responds to first-line chemo-therapy, who are assigned to one of five sub-studies for randomization to a targeted agent (vs. control) based on tumor biomarkers (Kaplan, 2013).

In "N-of-1" trial, the determination of the optimal treatment for each patient based on tumor characteristics were performer. In a modified "Nof-1" study design, the anti-tumor

activity of anticancer agents was matched to patients' genotype. Patients were treated according to their molecular profiling. The progression-free survival (PFS) was longer with the targeted treatment compared to PFS associated with their previous systemic treatment. In this study, tumor whole-genome sequencing and RNA expression analysis identified suggested targets for anticancer therapy in 13 tumor types (Von Hoff, 2010).

The use of vemurafenib and trametinib in BRAF V600E-mutated melanoma patients has led to substantial survival improvements (Sosman, 2012). Targeting EGFR mutations and the EML4-ALK fusion product in lung cancer with erlotinib and crizotinib, respectively, has led to remarkably improved outcomes (Shaw, 2013). Targeting the PI3K/AKT/mTOR pathway with cognate inhibitors used in combination (but not as single agents) resulted in stable disease for greater than 6 months and partial response rates of up to 45% in individuals with PIK3CA mutations (Janku, 2014)

REVIEW

TREATMENT OPTIONS

PI3K/AKT/MTOR PATHWAY GENES

Mutations in PIK3CA activate the AKT/mTOR pathway and have been described in breast, colon, gastrin, brain and biliary tract cancers (Holcombe, 2015). Somatic muta-tions are less common in biliary tract cancers; PTEN and PIK3CA mutations were observed in about 1

and 12-14% of GBCA, respectively (Ross, 2015). The tumors with these mutations are sensitive to PI3K specific inhibitors currently under investigation, as well as mTOR inhibitors, such as everolimus, temsorolimus, and rapamycin (Sicklicki, 2016).

HER2

The earliest targeted therapies block growth signals like trastuzumab (Herceptin), gefitinib (Iressa), imatinib (Gleevec), and cetuximab (Erbitux). Over the past 2 decades, there has been an extraordinary progress in the regimens developed for the treatment of human epidermal growth factor receptor 2 (HER2)-positive breast and stomach cancer. Trastuzumab, pertuzumab, lapatinib, and ado-trastuzumab emtansine (T-DM1) are commonly recommended anti-HER2 target agents by the U.S. Food and Drug Admi-nistration (FDA) (Wang, 2019). Studies on the HER2 gene develop pharmacological anti-HER2 agents to inhibit this pathway. In 1998 trastuzumab became a therapeutic for

breast cancer patients with HER2 overexpression (Balduzzi, 2015) Trastuzumab is a monoclonal IgG1 class humanized murine antibody, binding the ECD of HER2 transmembrane receptor (Pinto, 2013). The mechanism of action is binding to the the HER2 receptor and inhibit signal transduction pathways and angiogenesis. Moreover, trastuzumab is cell-cycle agrest, apoptosis and DNA rep air induction agent (Sakai, 2018).

Trastuzumab was the first target approved specifically for early stage HER2-positive breast cancer in combination with cytotoxic agents, such as taxane, after completion of doxorubicin therapy (Ferretti, 2006).

PHILADELHIA CHROMOSOME

The identification of Philadelphia chromosome [t(9;22)] led to the discovery of imatinib mesylate. Imatinib was approved by the FDA in

2002 for the treatment of newly diagnosed Philadelphia chromosome positive chronic myeloid leukemia (Druker, 2001).

BRAF PROTEIN MUTATION

The cell growth signaling protein BRAF is present in an altered form (BRAF V600E) in many melanomas. Vemurafenib targets this mutant form of the BRAF protein and is approved to treat patients with inoperable or metastatic melanoma that contains this altered BRAF protein (Janku, 2014).

MUC 16

MUC16 (CA125) has been extensively used as a biomarker for ovarian cancer, and its expression has been associated with disease progression. MUC 16 plays role in fundamental processes, including protection of the epithelium and human carcinogenesis. The expression of mucins in resting, normal polarized cells is intricately controlled, with expression restricted on the apical membranes of exposed epithelia. Loss of cell polarity during carcinogenesis lead to mucins expression all over the cell surface. The cell become available to interact with several growth factor receptors, that are typically restricted to the basolateral surface, and modulate their downstream signaling in various cancers (Joshi, 2016).

MUC16 overexpression has been observed in several human malignancies, including ovarian, pancreatic, breast, and lung (Haridas, 2011).

Moreover, MUC 16 and its ligands are potential targets for therapeutic intervention using monoclonal antibodies and immunotherapy (Aithal, 2018).

Current clinical study: Cyclophosphamide Followed by Intravenous and Intraperitoneal Infusion of Autologous T Cells Genetically Engineered to Secrete IL-12 and to Target the MUC16ecto Antigen in Patients With Recurrent MUC16ecto+ Solid Tumors is two phases trial. Screening phase to determine MUC16ecto tumor expression in ovarian, primary peritoneal or fallopian tube carcinoma. In intervention phase patients are eligible to receive the CAR+ T cells, the frozen leukapheresis product will be thawed and used to generate the 4H11-28z/fIL-12/EGFRt+ genetically-modified T cells to evidence ist safety and anti-tumor activity (Clinicaltrial.gov).

CURRENT CLINICAL TRIALS

To share the data on cancer medicine the new network was created. The Oncology Data Network (ODN) is a fully cooperative, collaborative data-sharing European network providing near real-time information on cancer medicine usage at scale. Data on cancer medicine use are collated through technology-enabled automation direct from participating hospitals' existing systems and translates from diverse sources into a common language enabling direct comparability via an automated regimen mapping algorithm (Kerr, 2020).

Integration of genomic data with drug screening from personalized in vitro and in vivo cancer models guide precision cancer care and fuel next generation research. The development of a robust precision cancer care platform, which integrates whole exome sequencing (WES) with a living biobank enables high throughput drug screens on patient-derived tumor organoids. 56 tumor-derived organoid cultures, and 19 patient-derived xenograft (PDX) models have been established from the 769 patients enrolled in an IRB approved clinical trial. To exetend the genomics for better identification therapeutic options for the majority of patients with advanced disease, high throughput drug screening effective strategies were used. Analysis

of tumor derived cells from four cases, two uterine malignancies and two colon cancers, identified effective drugs and drug combinations that were subsequently validated using 3D cultures and PDX models. This clinical trial creates the platform to provide personalized therapeutic options for individual patients and promotes the discovery of novel therapeutic approaches (Pauli, 2017).

Next-generation sequencing (NGS) of circulating tumor DNA (ctDNA) is not yet routinely implemented in the setting of a phase I clinical trials. To supports blood-based genomic profiling a new molecular profiling program TARGET was designed. The primary aim is to match patients with a broad range of advanced cancers to early phase clinical trials on the basis of analysis of both somatic mutations and copy number alterations (CNA) across a 641 cancer-associated-gene panel in a single ctDNA assay. For the first 100 TARGET patients, ctDNA data showed good concordance with matched tumor and results were turned round within a clinically acceptable timeframe for Molecular Tumor Board (MTB) review. When a 2.5% variant allele frequency (VAF) threshold was applied, actionable mutations were identified in 41 of 100 patients, and 11 of these patients

received a matched therapy. These data support the application of ctDNA in this early phase clinical trial. Genomic profiling of contemporaneous tumor material enhances patient verification to novel therapies and provides a practical template for bringing routinely applied blood-based analyses to the clinic (Rothwell, 2019).

The ESCAT trial defines clinical evidence-based criteria to prioritise genomic altera-tions as markers to select patients for targeted therapies. This classification system aims to offer a common language in cancer medicine and drug development. The Euro-pean Society for Medical Oncology (ESMO) Translational Research and Precision Medicine Working Group (TR and PM WG) propose a classification system for molecular aberrations as clinical targets. ESCAT defines six levels of clinical evidence for molecular targets according to the implications for patient management: 1. targets ready for implementation in routine clinical decisions, 2. investigational targets that likely define a patient population that benefits from a targeted drug but additional data are needed; 3. clinical benefit previously demonstrated in other tumour types or for similar molecular target, 4. preclinical evidence of actionability, 5. evidence supporting co-targeting approaches and the last – lack of evidence for actionability (Mateo, 2018).

DISCUSSION AND SHORT CONCLUSION

The promising model is to combine targeted therapy with other therapeutic strategies like chemotherapy, radiation, and immunotherapy to determine how they may combine to exert more efficacious therapeutic effects and improve the outcomes of cancer patients.

Historically, cancer has been studied, and therapeutic agents have been evaluated based on organ site, clinical staging, and histology. The development of molecular profiling methods has expanded knowledge of cancer at the molecular level. Numerous cancer subtypes are being described based on biomarker expression and genetic mutations rather than traditional classifications of cancer. The development of new molecular methods promotes the discovery of novel therapeutic approaches that can be assessed in clinical trias. Moreover, provides personalized therapeutic options for individual patients where standard clinical options have been exhausted.

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