Chapter 8
How Genome-Wide Analysis Contributes to Personalized Treatment in Cancer, Including Gynecologic Cancer?

Hisamitsu Takaya

Abstract Cancer omics analysis, which started with large-scale cancer genome data analysis, is becoming in this era multiomics analysis, which integrates multiple omics analyses with the development of analytical technologies. Omics analysis is being conducted in many parts of the world, the accumulated analysis data are rapidly growing, and new clinical trials are often conducted based on omics analysis. It is anticipated that future cancer treatments will require skills to search and analyze omics data more efficiently. In addition, different approaches are being used to validate data obtained from omics analyses in clinical practice compared to those used in the past. Master protocols are protocols designed with multiple subtrials within the framework of an overall trial structure, and they represent a paradigm shift in clinical trials, such as biomarker-driven clinical trials across cancer types or adaptive designs that allow for the interruption and addition of new subtrials. They are expected to play a role in the development of personalized treatment, which will become even more individualized in the future.

Keywords Cancer genome · Omics · Precision medicine · Master protocol · Umbrella trial · Basket trial · Platform trial

8.1 Introduction

Large-scale cancer genome analysis, which began with The Cancer Genome Atlas (TCGA), has spread widely as next-generation sequencers (NGS) have become more widely available, research costs have decreased, and research has been conducted into a variety of cancer types. As a result, not only genome analysis but also

H. Takaya (✉)
Department of Obstetrics and Gynecology, Kindai University, Osaka, Japan
e-mail: htakaya@med.kindai.ac.jp

M. Mandai (ed.), *Personalization in Gynecologic Oncology*, Comprehensive Gynecology and Obstetrics, https://doi.org/10.1007/978-981-19-4711-7_8

various omics analyses, such as gene expression, protein, and metabolite analyses, have been conducted, and knowledge of cancer omics has become indispensable to current cancer research. Drug discovery and clinical trials using these omics data are also being conducted, and knowledge in this field is becoming indispensable for clinicians.

In this chapter, we introduce the basic knowledge about omics data, databases required for actual handling of omics data, and analysis methods for actual cancer genome data using mainly TCGA data to understand personalized therapy using cancer genome data. Clinical trials using the new technology are also discussed, as well as the current status of clinical trials in gynecological oncology.

8.2 Omics Analysis

The word "genome" was coined by H. Winkler in 1920 [1] as "the set of chromosomes carried by gametes" and later redefined by Hitoshi Kihara [2] as "the minimum set of chromosomes essential to make an organism what it is." It was coined using the Greek suffix "-ome," meaning "all." Subsequently, as the analysis of genomes progressed, analysis to grasp the entire picture of mRNA and proteins, which are gene transcripts, as well as metabolic products, was promoted, giving rise to the terms "transcriptome," "proteome," and "metabolome," with "-ome" as a derivative of genome. In addition to molecular information, there are many other terms with "-ome," such as interactome, which is comprehensive information on interactions between molecules in living organisms, and microbiome, which is comprehensive information about bacterial flora. The term "omics" refers to the field of research that addresses these "-omes"; the comprehensive analysis of each is collectively called "omics analysis," and the information obtained by the analysis is called "omics information."

Cancer is a disease that encompasses an extremely complex system. As a system, cancer cells are intricately involved in interactions with surrounding tissues, such as the microenvironment and immune system, interactions between tumor cells, and factors such as transcriptional regulation, gene coexpression, signal transduction, metabolic pathways, and protein interactions within tumor cells; these layers of factors must be elucidated to understand the phenomenon of cancer as a disease [3, 4]. Therefore, a method called multiomics analysis, which integrates omics information involving cancer, is now being used to characterize the cancer system at a phenomenological level [5, 6]. The main omics analyses used in multiomics analysis include genomics, which addresses genomic sequences and their mutations, such as insertions, deletions, single nucleotide variations, and copy number variations; epigenomics, which analyzes DNA methylation, histone modifications, chromatin accessibility, and chromosomal 3D structure; transcriptomics, which analyzes quantitative gene expression and measures transcripts, such as microRNAs and long noncoding RNAs; proteomics, which analyzes protein expression and quantification, posttranslational modifications and protein–protein interactions; and

metabolomics, which analyzes the quantification of metabolites of small molecules, such as amino acids, fatty acids, and carbohydrates. NGS is mainly used for genomics, epigenomics, and transcriptomics, while various mass spectrometers are used for proteomics and metabolomics. With the spread and advancement of next-generation sequencing technologies, more high-throughput omics analyses can be performed at a lower cost, and statistical tools, such as machine learning, are becoming more widely used, making it possible to integrate multiple omics analyses.

In the case of solid tumors, it is necessary to collect tumor tissue itself by some method for omics analysis. Recently, however, a method called liquid biopsy has sometimes been used to extract the genome from cancer cell-derived DNA (cell-free DNA) [7–9] or circulating tumor cells [10, 11] in the blood for analysis, rather than extracting the cancer genome from the tumor tissue itself. With liquid biopsy, cancer genome information can be obtained only by blood sampling, even in cancer types for which tumor tissue is difficult to obtain, and changes over the course of treatment can also be analyzed because the test can be performed many times [12, 13]. Tissues collected by biopsy or surgery contain not only tumor cells but also stromal cells, lymphocytes, vascular endothelial cells, and many other types of cells, which sometimes interfere with accurate analysis by constituting noise in genome analyses [14–16]. Therefore, a method called single-cell analysis has been developed, in which the collected tumor tissue is separated into single cells, and genomics analysis is performed on each cell [17, 18]. This method makes it possible to analyze the genomic data of each cell, and the characteristics of tumor cells and the relationships between cells are being clarified [19, 20] (Fig. 8.1).

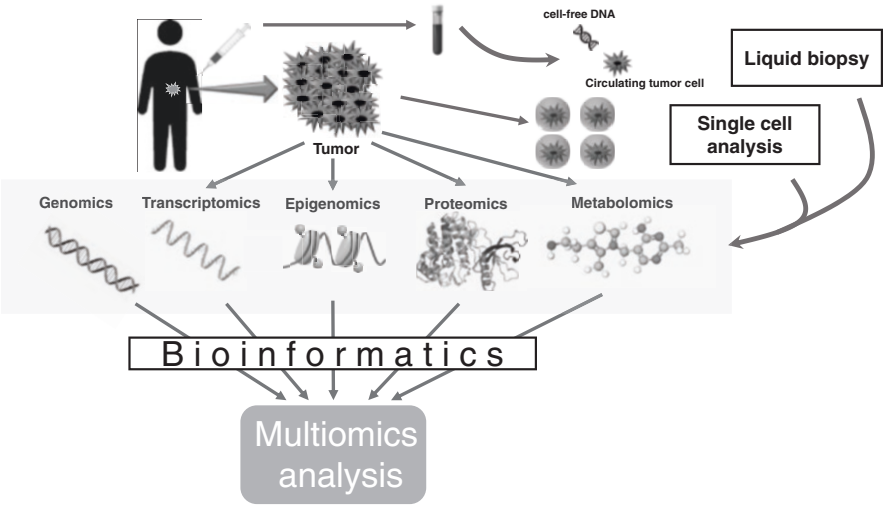

Fig. 8.1 Concept of omics analysis. Omics analysis is the analysis of the genome, transcriptome, epigenome, proteome, metabolome, and other information obtained from tumors. Bioinformatics is necessary for multiomics analysis that integrates these data. Liquid biopsy, which obtains omics information from circulating tumor cells in the blood, and single-cell analysis, which analyzes single cells constituting a tumor, have been developed, and their integrated analysis is also underway

8.3 Omics Databases and Analysis Tools

With the development of next-generation sequencing technology, sequencing has become faster and less expensive, and an enormous number of omics analyses are being conducted worldwide, resulting in an explosive increase in the amount of data accumulated. Conversely, the increase in the number of public databases around the world has made it difficult for users to obtain the data that they need for their research purposes. In Japan, the National Bioscience Database Center (NBDC) was established in 2011 to integrate various life science databases and promote data sharing and utilization. The database catalog [21] is available to the public, facilitating database searches. The main databases are listed below.

- Nucleotides
  GenBank [22, 23] is a nucleic acid sequence database maintained by the National Center for Biotechnology Information (NCBI). It is part of the International Nucleotide Sequence Database Collaboration (INSDC), which is operated by the NCBI, the Nucleotide Archive (ENA) [24], and the DNA Data Bank of Japan (DDBJ) [25], and data are exchanged between these organizations.
- Genomes
  The UCSC Genome Browser [26, 27] is a project of UCSC that automatically annotates eukaryotic organisms with genomes that have been decoded and publishes the results in a database. The genome information used is the same as that of NCBI and Ensembl, but the annotated information is diverse, including originally calculated information and information from NCBI and Ensembl. One of the characteristics of this system is that the annotated information itself is often newer because of the high-speed automatic annotation.

  Ensembl [28, 29] which is a joint project of the European Molecular Biology Laboratory (EMBL)-European Bioinformatics Institute (EBI) and the Sanger Centre, performs automated annotation of eukaryotic organisms with genomes that have been decoded and publishes the results in a database. The information provided by Ensembl is the same as the NCBI and UCSC browsers for genomes, but the annotations are predicted by Ensembl's own pipeline. Therefore, the information differs slightly from that of NCBI Mapviewer and others. The prediction pipeline focuses on predicting protein-coding genes as accurately as possible, so the prediction accuracy is high.

  NCBI Genome [30] is a database of genome information managed and operated by NCBI. In recent years, genome information about many new species of organisms has been registered, and one can quickly determine how much nucleotide sequence information has been revealed for the species in which one is interested. The Genome Data Viewer allows users to visualize molecular data in a genomic context and graphically display data about a given experiment or sample. Genome information about species commonly used in research can be organized for easy visual and understandable retrieval from a phylogenetic tree, or genomes can be compared [31].

- Epigenomes
  The International Human Epigenome Consortium (IHEC) is an international consortium that aims to map the human epigenome in relation to various diseases and life phenomena, and the IHEC-Data Portal [32] is populated with data from various databases. By selecting the species, tissue, assay method, and provider, one can view the available datasets in a grid view, track them in the UCSC Genome Browser, and download the data in batches [33].

  The NIH Roadmap Epigenomics Mapping Consortium aims to provide epigenomic maps of histone modifications and DNA methylation in various tissues and cell types related to human diseases. The Roadmap Epigenomics Project [34] allows users to browse data by adult, fetal, brain, stem cell, etc., and to view genomic information in the USCS Genome Browser. Protocols, tools, and project information are also provided [35].
- Gene Expression
  The NCBI Gene Expression Omnibus (GEO) [36, 37] is a database of gene expression information provided and maintained by NCBI. GEO mainly contains data obtained by microarrays, and the amount of registered data is very large. Not only can one search for gene expression datasets and gene profiles of interest among them, but one can also freely download the raw data.

  ArrayExpress [38, 39] is a database of gene expression information provided and maintained by EBI, and similar to NCBI-GEO, it mainly stores data obtained by microarrays and allows users to search for expression datasets and gene profiles and obtain raw data from them.
- Proteins
  The Universal Protein Resource (UniProt) [40, 41] operated and maintained by EMBL-EBI, the Swiss Institute of Bioinformatics (SIB), and the Protein Information Resource (PIR), is a database of protein UniProt consisting of UniProt Knowledgebase (UniProtKB), UniProt Reference Clusters (UniRef), and UniProt Archive (UniPrac). UniProtKB publishes SwissProt, which is manually annotated with high-quality annotations based on information from the literature, and TrEMBL (Translated EMBL Nucleotide Sequence Data Library), which is mechanically annotated. UniRef provides the results of preformed sequence homology searches, and UniPrac compiles information, such as IDs of other databases by sequence ID.

  InterPro [42, 43] is an integrated database that collects descriptions of protein family classifications, domains, and functional sites based on EBI. It brings together multiple databases that contain the characteristics of various proteins and provide protein characteristics at various levels. Using InterProScan, a database search tool, a single amino acid sequence can be searched in multiple databases integrated by InterPro to efficiently infer protein families and domain repeat structures that match the queried sequence.

  The PRIDE (Proteomics Identifications Archive) database [44, 45] is a public repository of proteomics data operated by EMBL-EBI.

- Other
  ENCODE [46] is a database that aims to compile a comprehensive list of functional factor parts of the human genome. It contains information about factors that function at the protein and RNA levels, as well as regulatory factors that control the environment in which cells and genes are activated, including methods of analysis, sample outlines, replicon types, experimental conditions, and analysis flow for the studies [47].

The Cancer Genome Atlas (TCGA) is the largest and most comprehensive cancer genome database launched by the National Institutes of Health (NIH) in 2006. The TCGA dataset contains more than 10,000 cases of 33 different cancer types, and omics information, such as cancer genomes, epigenomes, and transcriptomes, is publicly available. The level at which this omics information is used (raw data or data after being processed by multiple software) depends on the researcher's intended use, but software for multiomics analysis is needed to analyze the data in an integrated manner. Such software is developed by bioinformaticians around the world, and most of it is available as freeware, so it can be installed and used according to the purpose of use, but to use such software, some knowledge of programming languages such as R, Python, Perl, etc., is needed. Many cancer researchers have not mastered the art of bioinformatics analysis and find it difficult to explore TCGA data resources, but several web tools have been developed that allow them to analyze TCGA data. cBioPortal for Cancer Genomics [48–50] is a web tool developed at Memorial Sloan Kettering Cancer Center that integrates omics data from multiple public databases, including TCGA data, and integrates omics and clinical data for analysis and visualization. Broad GDAC Firehose [51] is a pipeline for processing and analyzing large datasets via dozens of quantitative algorithms developed at the Broad Institute and the results of these analyses, which can be explored and visualized using FireBrowse [52]. UCSC Xena [53, 54] is a web browser-based visualization and analysis tool for large public cancer genome datasets from TCGA, ICGC (International Cancer Genome Consortium), GDC (Genomic Data Commons), and other databases. It is possible to freely combine and analyze SNVs (single nucleotide variants), INDELs, large-scale structural variations, CNVs (copy number variations), gene expression, DNA methylation, ATAC-seq, and other data from each database. LinkedOmic [55, 56] includes multiomics data from 32 TCGA carcinomas, as well as proteomics data from breast, ovarian, and colorectal cancer TCGA was generated by the Clinical Proteomics Tumor Analysis Consortium (CPTAC). It is a web tool that analyzes, compares, and makes biological sense of data using three modules: LinkFinder, LinkInterpreter, and LinkCompare. In addition to the above, there are many web tools, including The Cancer Proteome Atlas Portal (TCPA) [57, 58], which is an integrated data portal for analyzing and visualizing TCGA proteomic data; MEXPRESS [59, 60], which enables visualization of TCGA clinical data, gene expression data, and DNA methylation data; and GEPIA2 [61, 62], which can analyze and visualize data from the GTEx (Genotype Tissue Expression) project, which examined gene expression in TCGA, human body

tissue, and genotypes. As described, there are a vast number of analysis tools in existence, and it is difficult to determine which tool to use. For major tools, there are often videos on how to use them and the features of the tools, which can be used as a reference.

8.4 Cancer Clinical Trials Using Omics Data

With technological advances in omics analysis, molecular markers that are useful for predicting therapeutic efficacy have been identified in various types of cancer. Molecularly targeted drugs that are expected to be effective against patients with such biomarkers have been developed, and their efficacy has been reported in multiple clinical trials. Representative examples include vemurafenib for metastatic malignant melanoma with the BRAF V600E mutation [63], gefitinib for *EGFR* mutation-positive non-small cell lung cancer [64], cetuximab [65], and panitumumab [66] for *KRAS* wild-type colon cancer, crizotinib [67], alectinib [68], and ceritinib [67] for *ALK* fusion gene-positive non-small cell lung cancer, and so on. In the development of such molecular-targeted drugs, which are expected to be effective against a specific biomarker, problems in terms of development cost and time have been considered, such as the need to conduct as many clinical trials as the number of drugs to be developed to verify their efficacy and the need to verify efficacy for each cancer type when biomarkers are detected across multiple cancer types. Therefore, a comprehensive clinical trial protocol called a master protocol has been proposed and implemented in recent years [69]. The draft guidance published by the US FDA in 2018 defined a master protocol as a single protocol designed with multiple subtrials that evaluate the effects of one or more investigational drugs on one or more disease subtypes with different objectives within the framework of the overall study structure and within the overall clinical trial framework. Each subtrial is often categorized by population based on cancer type, histology, and biomarkers, and by conducting each subtrial in parallel based on a comprehensive protocol, more hypotheses can be tested efficiently and in less time.

Master protocols are classified into three categories according to the characteristics of the target population and the type and number of study treatments: basket trials, umbrella trials, and platform trials. Basket trials are trials designed to validate a single investigational drug or drug combination in different populations defined by specific genetic/molecular biomarkers, rather than patient eligibility being limited to a specific cancer type. Thus, each subtrial (basket) is composed of different types of cancer, and each subtrial tests a different treatment (Fig. 8.2). The advantages of a basket trial include the potential to offer patients with a broad range of cancer types a treatment option with a molecular-targeted agent that might not have been tested in clinical trials for their disease, the short time from initial diagnosis and eligibility to subsequent cohort assignment and initiation of treatment, and the

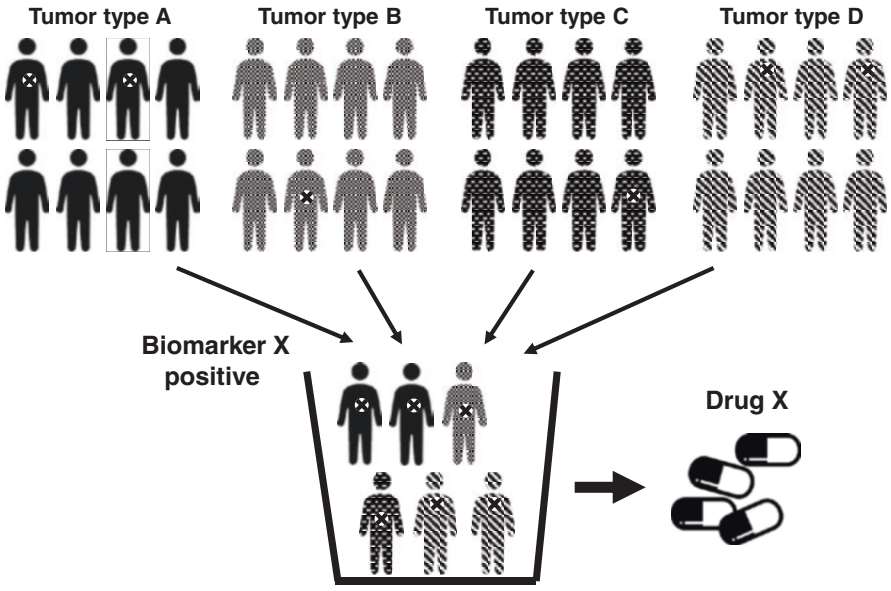

Fig. 8.2 Scheme of basket trials. Basket trials are master protocols for targeted therapy based on specific biomarkers from multiple cancer types. Each subtrial is often a single-arm exploratory study

often small number of patients in each cohort, resulting in a short time to results being reported. One problem is that the basket trial assumes that classification by molecular characteristics of the tumor can substitute for classification by tumor histology but that histology might be a stronger predictor of response to targeted therapy than biomarkers [70].

Umbrella trials evaluate targeted therapies in specific cancer types by assigning patients to one of a number of subtrials defined by genetic mutations or biomarkers. Subtrials are often single-arm or randomized subtrials for validation purposes, whereas basket trials are generally single-arm subtrials for exploratory purposes (Fig. 8.3). By fixing the cancer type of interest, umbrella trials are able to draw cancer-specific conclusions with less heterogeneity that might exist within a given cohort compared to basket trials. In addition, randomized trials of targeted and non-targeted therapies in subtrials can evaluate the presumed mechanism of action of a therapeutic agent and empirically distinguish between prognostic and efficacy-predicting markers. However, the feasibility of targeting a single cancer type creates problems. Particularly for rare diseases, allocation to subtrials by biomarker can slow enrollment within a cohort and thus slow trial progression. There is also the challenge that, if a large, long-term protocol design is needed, changes in treatment status, such as the emergence of a new standard of care during the period, might render the subtrial less clinically meaningful in its original setting, further lengthening the duration of the trial [70].

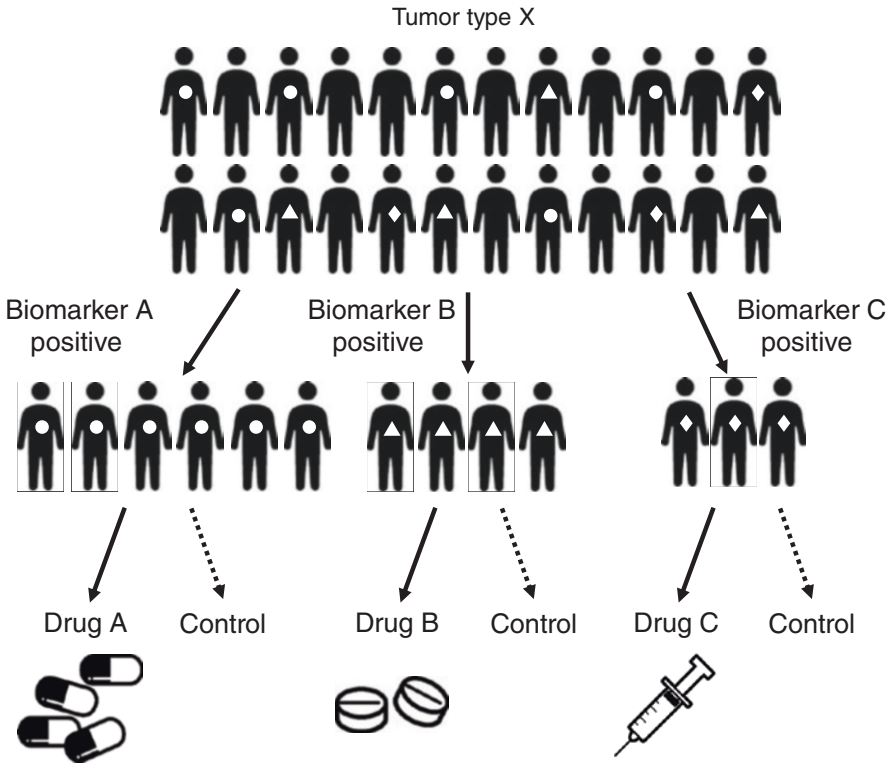

Fig. 8.3 Scheme of umbrella trials. Umbrella trials are master protocols in which targeted therapy is administered in each subtest defined by multiple biomarkers for a specific cancer type. Each subtrial is a single-arm or randomized trial and is often a validation trial

Platform trials are a generic term for randomized designs with a common control group and several different targeted treatment groups and trials that allow for the addition or exclusion of new treatments or eligible patients during the trial (Fig. 8.4). Platform trials evaluate the efficacy and futility of each targeted therapy in an interim analysis, and the treatment effect is often modeled as an independent parameter across biomarker-defined subtypes according to a Bayesian hierarchical model. Platform trials are often long-term trials because new trials can be added, and as with umbrella trials, the standard of care can change during the trial period due to the emergence of new treatments (and possibly the trial itself, which was originally conducted) [71]. In such cases, the protocol, statistical analysis plan, informed consent document, etc., might need to be modified, and the trial might have to be suspended [72].

Examples of these master protocol trials are listed below:

- Basket Trials
  The NCI-MATCH (NCI Molecular Analysis for Therapeutic Choice) trial consists of 24 substudies evaluating the efficacy of at least 17 targeted therapies in patients with solid tumors and lymphomas who have received at least one regi-

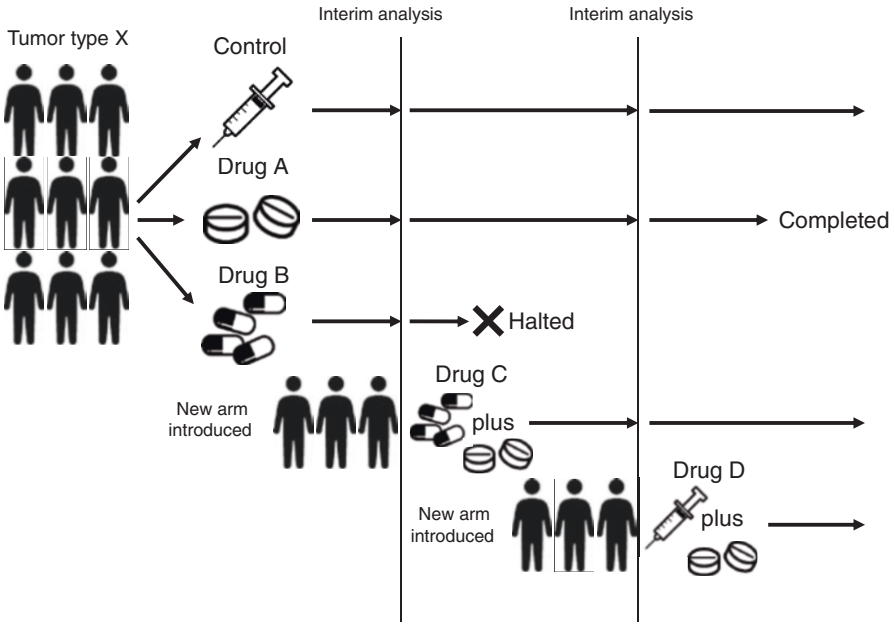

Fig. 8.4 Scheme of platform trials. Platform trials are randomized designs, in which several different targeted therapy arms share a common control group. New target treatment groups can be added during the trial, and existing treatment groups can be excluded

men of therapy. The primary endpoint of each subtrial was tumor shrinkage, with a single-arm design based on a binomial distribution to enroll 35 patients and an important secondary endpoint of 6-month progression-free survival [73]. The NCI-MATCH study can be interpreted as a basket study because it evaluates the efficacy and safety of targeted agents across cancer types in a molecular marker-positive population. In contrast, subtrials might be conducted to evaluate the efficacy and safety of multiple targeted agents against a molecular marker of interest in a specific cancer type, which can be interpreted as an umbrella study. Thus, the NCI-MATCH study can be described as a study with the characteristics of both a basket study and an umbrella study.

The AcSé study is a phase II study of various solid tumors (e.g., gastrointestinal, breast, kidney, ovarian, and thyroid cancers), consisting of 23 substudies evaluating the efficacy and safety of crizotinib alone in patients with at least one *ALK*, *MET*, *RON*, or *ROS-1* mutation [74]. Each substudy is defined by mutation and pathology and is designed according to a two-stage design. NSCLC with *ROS-1* translocation and esophageal/gastric cancer with *MET* amplification has been reported thus far [75, 76].

The KEYNOTE-158 trial is a phase II study of solid tumors refractory to standard chemotherapy with MSI-high—a condition in which microsatellite instability (MSI) due to abnormal DNA mismatch repair (dMMR) is frequently observed, except for unresectable or metastatic colorectal cancer—which evalu-

ated the efficacy and safety of pembrolizumab, an anti-PD-1 antibody. The primary endpoint was the objective response rate (ORR), with a median ORR of 34.3% [77].

- Umbrella Studies
  The ALCHEMIST (The Adjuvant Lung Cancer Enrichment Marker Identification and Sequencing Trial) trial is a randomized umbrella trial for patients with *ALK*- or *EGFR*-positive high-risk lung adenocarcinoma. Patients with *ALK*- or *EGFR*-positive disease will be enrolled in a randomized phase III subtrial of crizotinib versus placebo or erlotinib versus placebo. The primary endpoint of each trial will be overall survival, and interim analyses are planned; if both *ALK* and *EGFR* are negative, PD-L1 expression will be measured, and enrollment in a randomized subtrial of nivolumab plus observation will be considered. The primary endpoints of this subtrial are overall survival and disease-free survival [78].

  The Lung-MAP trial was initiated as an umbrella study to test the efficacy of multiple targeted therapies in advanced or recurrent squamous non-small cell lung cancer. All subtrials were designed as randomized phase II/III or single-arm phase II trials, with biomarker screening resulting in taselisib being assigned for *PIK3CA*-positive patients, palbociclib for patients positive for cell cycle gene mutations, rilotumumab plus erlotinib for patients positive for *c-MET,* and ADZ4547 for *FGFR*-positive patients; patients with positive homologous recombination repair abnormalities were assigned to tarazoparib and a control group. Biomarker-negative patients were randomized to durvalumab plus docetaxel, nivolumab plus ipilimumab or nivolumab alone for anti-PD-(L)1 therapy-naive patients as an unmatched subtrial and durvalumab plus tremelimumab for patients relapsing after anti-PD-(L)1 therapy. The primary endpoint of the substudy was progression-free survival or overall survival [79].

  The plasmaMATCH trial is a nonrandomized, phase IIa trial to test the efficacy of targeted therapy in advanced recurrent breast cancer by detecting targeted gene mutations and testing circulating tumor DNA (ctDNA). ctDNA testing identified *ESR1*, *HER2*, *AKT1*, and *PTEN* mutations. Patients were classified into four cohorts according to mutations and tumor estrogen receptor status and were treated with fulvestrant, neratinib, and capibasertib as single agents or in combination. The primary endpoint was the objective response rate [80].
- Platform Trials
  The FOCUS4 trial is a placebo-controlled, multiarm, multistage, randomized trial testing the efficacy of multiple targeted therapies for untreated colorectal cancer. In a population of patients with specific molecular markers, safety is evaluated in the first stage, proof of concept is confirmed in the second stage, short-term efficacy is evaluated in the third stage, and long-term efficacy is evaluated in the fourth stage. The efficacy endpoints are progression-free survival and overall survival. In such a multiarm, multistage trial, new treatments can be added during the trial, or treatments that prove to be futile can be excluded before the third or fourth stage, corresponding to a Phase III trial. All FOCUS4 trials are open to patients with negative molecular markers [81].

The STAMPEDE trial is a randomized platform trial with a multiarm, multistage design in high-risk prostate cancer patients. The trial was originally initiated as a five-arm study comparing a control group with a single agent or a combination of zoledronic acid, docetaxel, and celecoxib in patients initiating hormone therapy [82]. It was subsequently modified multiple times, with the addition of treatment groups with abiraterone and enzalutamide administered as single agents or in combination with radiation therapy [83, 84]. The current protocol continues to study metformin and transdermal estradiol.

The I-SPY2 trial is a phase II, adaptive, randomized, controlled trial evaluating the efficacy of a new investigational agent in combination with standard neoadjuvant chemotherapy for stage II/III high-risk breast cancer [85]. In this trial, enrolled patients will be classified into ten molecular subtypes and assigned to a study arm according to subtype based on an adaptive randomization engine using predictive probability. The primary endpoint is pathologic complete response (pCR) or a residual cancer burden (RCB) of 0. A Bayesian design is used, in which the predictive probabilities are updated as needed based on treatment results, and new predictive probabilities are assigned. When the predicted probability of a study drug reaches a predefined level of efficacy in one or more subtypes, the drug is "graduated" and proceeds to Phase III trials. Up to five study drugs can be evaluated in parallel at the same time, including combinations. To date, graduation has occurred for neratinib [86], veliparib with carboplatin [87], MK-2206 [88], and pembrolizumab [89].

There are several other issues that have been discussed regarding cancer clinical trials with master protocols, in addition to those listed in the brief description of each trial. Ethical issues include that the complexity and duration of the trials cause the informed consent documents to be more complex, so patients might not be able to understand the documents or the trial concept itself to the degree necessary to provide correct informed consent. In addition, in trials in which the concept of a final dose has not yet been fully established, the adaptive plan could lead to the abandonment of suboptimal dose regimens as the trial progresses, and the benefit-risk ratio might change during the dose optimization process [72]. A master protocol is a single clinical trial that encompasses multiple subtrials, but each subtrial is independently validated, requiring very sophisticated and complex statistics. For example, statistical power could be lost if a single master protocol is hypothesized to be accepted by the results of the subtrials, resulting in the closure or opening of study groups even though no adjustments are specified in the protocol. Another complication noted for the control of Type I error is that multiplicity adjustment might be required for one treatment comparison but not for another [71, 90]. However, master protocols offer tremendous advantages in flexibility and efficiency in drug development, and it is anticipated that many trials will be designed in personalized therapy using cancer genomics data. Trial designs are also expected to increase the sensitive allocation of patients to matched therapies, including combination therapies according to multiple driver mutations, biomarkers, and pathways. Master protocols could also provide insights into the molecular mechanisms of

exceptional responders, in whom drugs that are not effective in other patients are found to be significantly effective, which could be useful in designing future master protocols for specific disease types.

8.5 Genomic Analysis and Personalized Treatment in Gynecologic Cancer

There are four types of gynecological cancers for which integrated genome analysis was performed by TCGA—ovarian cancer (high-grade serous carcinoma; HGSC), endometrial cancer, cervical cancer, and uterine sarcoma—and analysis results have been reported for HGSC [91], endometrial cancer [92], and cervical cancer [93].

- HGSC
  Whole-exome sequencing analysis of 316 HGSCs identified *TP53* somatic mutations in 96% of HGSCs. In addition, mutations in the *BRCA1/2* gene were found in approximately 20% of both germline and somatic cases. BRCA1/2 is involved in the DNA homologous recombination repair pathway, and mutations in genes encoding proteins involved in homologous recombination repair other than BRCA1/2 were also observed in HGSCs, suggesting that approximately 50% of HGSCs have abnormal homologous recombination repair (HRD).
- Endometrial Cancer
  Genomic analysis of 373 cases of endometrial cancer classified cancers into the following four categories: (1) POLE type (ultramutated) with a very high frequency of gene mutations; (2) MSI type (hypermutated) with a high frequency of gene mutations and methylation of the *MLH1* promoter region in many of them; (3) copy number low type (endometrioid), in which the frequency of mutations is low and microsatellite stable; and (4) copy number high type (serous-like), consisting mainly of serous-like tumors with significant copy number changes and low frequency of genetic mutations.
- Cervical Cancer
  Integrated genomic analysis of 228 cervical cancer cases revealed genomic alterations in either or both the PI3K-MAPK and TGFβ signaling pathways in more than 70% of cases. In addition, amplification of the *CD274* gene encoding PD-L1 and the *PDCD1LG2* gene encoding PD-L2 was observed in approximately 20% of the cases.

Although the integrated genome analysis of these three gynecological cancers has revealed new cancer genome features, there are only two targeted therapies in practical use in the field of gynecological cancer: PARP inhibitors (olaparib, niraparib, etc.) for *BRCA1/2* mutations or HRD-positive ovarian cancer [94–96]; and PD-1 inhibitors (pembrolizumab) for endometrial cancer with dMMR [97]. Compared to other types of cancer, personalized treatment in gynecological cancer has not progressed very much. However, efficient clinical trial designs for new

therapies based on master protocols have recently become possible, and new clinical trials based on master protocols are being conducted in gynecological cancers.

The AMBITION trial is an umbrella study of platinum-resistant recurrent ovarian cancer that uses HRD and PD-L1 biomarkers to allocate treatment groups, with HRD-positive patients receiving olaparib plus cediranib or durvalumab and HRD-negative patients receiving durvalumab plus chemotherapy or durvalumab plus tremelimumab plus chemotherapy based on PD-L1 expression; the primary endpoint is the objective response rate [98]. The BOUQUET trial (NCT04931342) [99] is a biomarker-driven phase II study of recurrent epithelial ovarian cancer in patients with non-high-grade serous carcinoma and non-high-grade endometrial carcinoma, including ipatasertib plus paclitaxel for patients with *PIK3CA/AKT1/PTEN* mutations, obimetinib for patients with *BRAF/NRAS/KRAS/NF-1* mutations, trastuzumab emtansine for patients with *ERBB2* amplification or mutations, and atezolizumab plus bevacizumab for the unmatched group. The primary endpoint is the objective response rate, and the study is designed as a platform trial. In addition, a project to develop a new adaptive platform trial called Ovarian CanceRx was announced in 2021 [100], and master protocol trials for recurrent ovarian cancer are expected to increase in the future. In endometrial cancer, a phase II umbrella study is underway of retifanlimab alone or in combination with epacadostat or pemigatinib in patients with advanced or metastatic endometrial cancer that has progressed on or after platinum-based chemotherapy (NCT04463771) [101], and more clinical trials based on similar master protocols are expected to follow.

8.6 Conclusion

Cancer genomics (omics) analysis is expected to become more comprehensive and detailed in the future, and more personalized medicine based on cancer characteristics is being sought. As cancer research progresses, a new clinical trial framework called “master protocols” has been proposed and implemented to promote more efficient and flexible clinical trials. With the rapid evolution of cancer omics analysis from bulk tumor analysis to single-cell analysis and from single-omics analysis to multiomics analysis, it is highly likely that such omics analysis will be applied in clinical practice. Although it is impossible for clinicians to examine each individual datum, it is necessary to accumulate knowledge to prepare for the advent of omics medicine in the trend of genomic medicine.

References

1. Winkler H. Verbreitung und Ursache der Parthenogenesis im Pflanzen und Tierreiche. Jena: Fisher; 1920.
2. Kihara H, Lilienfelt F. Genomanalyse bei *Triticum* und *Aegilops*. IV. Untersuchungen as *Aegilops* x *Triticum*- und *Aegilops* x *Aegilops*-Bastarden. Cytologia (Tokyo). 1932;3:384–456.

3. de Anda-Jáuregui G, Hernández-Lemus E. Computational oncology in the multi-omics era: state of the art. Front Oncol. 2020;10:423.
4. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011;144:646–74.
5. Chakraborty S, Hosen MI, Ahmed M, Shekhar HU. Onco-multi-OMICS approach: a new frontier in cancer research. Biomed Res Int. 2018;2018:1–14.
6. Hasin Y, Seldin M, Lusis A. Multi-omics approaches to disease. Genome Biol. 2017;18:83.
7. Jahr S, Hentze H, Englisch S, Hardt D, Fackelmayer FO, Hesch RD, Knippers R. DNA fragments in the blood plasma of cancer patients: quantitations and evidence for their origin from apoptotic and necrotic cells. Cancer Res. 2001;61:1659–65.
8. Schwarzenbach H, Hoon DSB, Pantel K. Cell-free nucleic acids as biomarkers in cancer patients. Nat Rev Cancer. 2011;116(11):426–37.
9. Alix-Panabières C, Pantel K. Liquid biopsy: from discovery to clinical applicationliquid biopsy: from discovery to clinical application. Cancer Discov. 2021;11:858–73.
10. Cristofanilli M, Budd GT, Ellis MJ, et al. Circulating tumor cells, disease progression, and survival in metastatic breast cancer. N Engl J Med. 2009;351:781–91. https://doi.org/10.1056/NEJMoa040766.
11. Pantel K, Alix-Panabières C. Circulating tumour cells in cancer patients: challenges and perspectives. Trends Mol Med. 2010;16:398–406.
12. Haber DA, Velculescu VE. Blood-based analyses of cancer: circulating tumor cells and circulating tumor DNA. Cancer Discov. 2014;4:650–61.
13. Sparano J, O'Neill A, Alpaugh K, Wolff AC, Northfelt DW, Dang CT, Sledge GW, Miller KD. Association of circulating tumor cells with late recurrence of estrogen receptor–positive breast cancer: a secondary analysis of a randomized clinical trial. JAMA Oncol. 2018;4:1700–6.
14. Heng HHQ, Stevens JB, Bremer SW, Liu G, Abdallah BY, Ye CJ. Evolutionary mechanisms and diversity in cancer. Adv Cancer Res. 2011;112:217–53.
15. Lee JH, Daugharthy ER, Scheiman J, et al. Highly multiplexed subcellular RNA sequencing in situ. Science. 2014;343:1360–3.
16. Ahmed R, Zaman T, Chowdhury F, Mraiche F, Tariq M, Ahmad IS, Hasan A. Single-cell RNA sequencing with spatial transcriptomics of cancer tissues. Int J Mol Sci. 2022;23:3042.
17. Shalek AK, Satija R, Adiconis X, et al. Single-cell transcriptomics reveals bimodality in expression and splicing in immune cells. Nature. 2013;498:236–40. https://doi.org/10.1038/NATURE12172.
18. Patel AP, Tirosh I, Trombetta JJ, et al. Single-cell RNA-seq highlights intratumoral heterogeneity in primary glioblastoma. Science. 2014;344:1396–401.
19. Azizi E, Carr AJ, Plitas G, et al. Single-cell map of diverse immune phenotypes in the breast tumor microenvironment. Cell. 2018;174:1293–1308.e36.
20. Lambrechts D, Wauters E, Boeckx B, et al. Phenotype molding of stromal cells in the lung tumor microenvironment. Nat Med. 2018;248(24):1277–89.
21. Home. Integbio database catalog. https://integbio.jp/dbcatalog/?lang=en. Accessed 31 Mar 2022.
22. Sayers EW, Cavanaugh M, Clark K, Ostell J, Pruitt KD, Karsch-Mizrachi I. GenBank. Nucleic Acids Res. 2020;48:D84–6.
23. GenBank overview. https://www.ncbi.nlm.nih.gov/genbank/. Accessed 31 Mar 2022.
24. Harrison PW, Ahamed A, Aslam R, et al. The European nucleotide archive in 2020. Nucleic Acids Res. 2021;49:D82–5.
25. Okido T, Kodama Y, Mashima J, Kosuge T, Fujisawa T, Ogasawara O. DNA Data Bank of Japan (DDBJ) update report 2021. Nucleic Acids Res. 2022;50:D102–5.
26. Gonzalez JN, Zweig AS, Speir ML, et al. The UCSC Genome Browser database: 2021 update. Nucleic Acids Res. 2021;49:D1046–57.
27. UCSC Genome browser home. https://genome.ucsc.edu/. Accessed 31 Mar 2022.
28. Howe KL, Achuthan P, Allen J, et al. Ensembl 2021. Nucleic Acids Res. 2021;49:D884–91.
29. Ensembl genome browser 105. https://www.ensembl.org/index.html. Accessed 31 Mar 2022.

30. Home. Genome. NCBI. https://www.ncbi.nlm.nih.gov/genome/. Accessed 31 Mar 2022.
31. Rangwala SH, Kuznetsov A, Ananiev V, et al. Accessing NCBI data using the NCBI Sequence Viewer and Genome Data Viewer (GDV). Genome Res. 2021;31:159–69.
32. Data Portal. IHEC. https://epigenomesportal.ca/ihec/. Accessed 31 Mar 2022.
33. Bujold D, de Lima Morais DA, Gauthier C, et al. The international human epigenome consortium data portal. Cell Syst. 2016;3:496–499.e2.
34. Roadmap Epigenomics Project. Home. http://www.roadmapepigenomics.org/. Accessed 31 Mar 2022.
35. Bernstein BE, Stamatoyannopoulos JA, Costello JF, et al. The NIH roadmap epigenomics mapping consortium. Nat Biotechnol. 2010;28:1045–8.
36. Home. GEO. NCBI. https://www.ncbi.nlm.nih.gov/geo/. Accessed 31 Mar 2022.
37. Barrett T, Wilhite SE, Ledoux P, et al. NCBI GEO: archive for functional genomics data sets—update. Nucleic Acids Res. 2013; https://doi.org/10.1093/NAR/GKS1193.
38. ArrayExpress < EMBL-EBI. https://www.ebi.ac.uk/arrayexpress/. Accessed 31 Mar 2022.
39. Athar A, Füllgrabe A, George N, et al. ArrayExpress update—from bulk to single-cell expression data. Nucleic Acids Res. 2019;47:D711–5.
40. UniProt. https://www.uniprot.org/. Accessed 31 Mar 2022.
41. Bateman A, Martin MJ, Orchard S, et al. UniProt: the universal protein knowledgebase in 2021. Nucleic Acids Res. 2021;49:D480–9.
42. InterPro. https://www.ebi.ac.uk/interpro/. Accessed 31 Mar 2022.
43. Marabelle A, Le DT, Ascierto PA, et al. The InterPro protein families and domains database: 20 years on. Nucleic Acids Res. 2021;49:1274–84.
44. PRIDE. Proteomics identification database. https://www.ebi.ac.uk/pride/. Accessed 31 Mar 2022.
45. Perez-Riverol Y, Bai J, Bandla C, et al. The PRIDE database resources in 2022: a hub for mass spectrometry-based proteomics evidences. Nucleic Acids Res. 2022;50:D543–52.
46. ENCODE. https://www.encodeproject.org/. Accessed 31 Mar 2022.
47. Abascal F, Acosta R, Addleman NJ, et al. Perspectives on ENCODE. Nature. 2020;583:693–8.
48. Cerami E, Gao J, Dogrusoz U, et al. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data: figure 1. Cancer Discov. 2012;2:401–4.
49. Gao J, Aksoy BA, Dogrusoz U, et al. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. Sci Signal. 2013;6:pl1.
50. cBioPortal for Cancer Genomics. https://www.cbioportal.org/. Accessed 31 Mar 2022.
51. Broad GDAC Firehose. https://gdac.broadinstitute.org/. Accessed 31 Mar 2022.
52. FireBrowse. http://firebrowse.org/. Accessed 31 Mar 2022.
53. Goldman MJ, Craft B, Hastie M, et al. Visualizing and interpreting cancer genomics data via the Xena platform. Nat Biotechnol. 2020;38:1–4.
54. UCSC Xena. http://xena.ucsc.edu/. Accessed 31 Mar 2022.
55. LinkedOmics. Login. http://www.linkedomics.org/login.php. Accessed 31 Mar 2022.
56. Vasaikar SV, Straub P, Wang J, Zhang B. LinkedOmics: analyzing multi-omics data within and across 32 cancer types. Nucleic Acids Res. 2018; https://doi.org/10.1093/NAR/GKX1090.
57. TCPA. Home. https://tcpaportal.org/tcpa/. Accessed 31 Mar 2022.
58. Li J, Lu Y, Akbani R, et al. TCPA: a resource for cancer functional proteomics data. Nat Methods. 2013;10:1046–7.
59. MEXPRESS. https://mexpress.be/. Accessed 31 Mar 2022.
60. Koch A, Jeschke J, Van Criekinge W, van Engeland M, De Meyer T. MEXPRESS update 2019. Nucleic Acids Res. 2019;47:W561–5.
61. GEPIA 2. http://gepia2.cancer-pku.cn/#index. Accessed 31 Mar 2022.
62. Tang Z, Kang B, Li C, Chen T, Zhang Z. GEPIA2: an enhanced web server for large-scale expression profiling and interactive analysis. Nucleic Acids Res. 2019;47:W556–60.
63. Chapman PB, Hauschild A, Robert C, et al. Improved survival with vemurafenib in melanoma with BRAF V600E mutation. N Engl J Med. 2011;364:2507–16.
64. Mok TS, Wu Y-L, Thongprasert S, et al. Gefitinib or carboplatin–paclitaxel in pulmonary adenocarcinoma. N Engl J Med. 2009;361:947–57.

65. Jonker DJ, O'Callaghan CJ, Karapetis CS, et al. Cetuximab for the treatment of colorectal cancer. N Engl J Med. 2007;357:2040–8.
66. Amado RG, Wolf M, Peeters M, et al. Wild-type *KRAS* is required for panitumumab efficacy in patients with metastatic colorectal cancer. J Clin Oncol. 2008;26:1626–34.
67. Shaw AT, Kim D-W, Mehra R, et al. Ceritinib in *ALK* -rearranged Non–small-cell lung cancer. N Engl J Med. 2014;370:1189–97.
68. Shaw AT, Gandhi L, Gadgeel S, et al. Alectinib in *ALK*-positive, crizotinib-resistant, non-small-cell lung cancer: a single-group, multicentre, phase 2 trial. Lancet Oncol. 2016;17:234–42.
69. Woodcock J, LaVange LM. Master protocols to study multiple therapies, multiple diseases, or both. N Engl J Med. 2017;377:62–70.
70. Renfro LA, Sargent DJ. Statistical controversies in clinical research: basket trials, umbrella trials, and other master protocols: a review and examples. Ann Oncol. 2017;28:34–43.
71. Lu CC, Li XN, Broglio K, Bycott P, Jiang Q, Li X, McGlothlin A, Tian H, Ye J. Practical considerations and recommendations for master protocol framework: basket, umbrella and platform trials. Ther Innov Regul Sci. 2021;55:1145.
72. Sudhop T, Brun NC, Riedel C, Rosso A, Broich K, Senderovitz T. Master protocols in clinical trials: a universal Swiss Army knife? Lancet Oncol. 2019;20:e336–42.
73. Flaherty KT, Gray RJ, Chen AP, et al. Molecular landscape and actionable alterations in a genomically guided cancer clinical trial: national cancer institute molecular analysis for therapy choice (NCI-MATCH). J Clin Oncol. 2020;38:3883–94.
74. Buzyn A, Blay JY, Hoog-Labouret N, Jimenez M, Nowak F, Le Deley MC, Pérol D, Cailliot C, Raynaud J, Vassal G. Equal access to innovative therapies and precision cancer care. Nat Rev Clin Oncol. 2016;13:385–93.
75. Moro-Sibilot D, Cozic N, Pérol M, et al. Crizotinib in c-MET- or ROS1-positive NSCLC: results of the AcSé phase II trial. Ann Oncol. 2019;30:1985–91.
76. Aparicio T, Cozic N, de la Fouchardière C, et al. The activity of crizotinib in chemo-refractory MET-amplified esophageal and gastric adenocarcinomas: results from the AcSé-Crizotinib program. Target Oncol. 2021;16:381–8.
77. Marabelle A, Le DT, Ascierto PA, et al. Efficacy of pembrolizumab in patients with noncolorectal high microsatellite instability/mismatch repair–deficient cancer: results from the phase II KEYNOTE-158 study. J Clin Oncol. 2020;38:1–10.
78. Govindan R, Mandrekar SJ, Gerber DE, et al. ALCHEMIST trials: a golden opportunity to transform outcomes in early stage non–small cell lung cancer. Clin Cancer Res. 2015;21:5439.
79. Herbst RS, Gandara DR, Hirsch FR, et al. Lung master protocol (Lung-MAP)—a biomarker-driven protocol for accelerating development of therapies for squamous cell lung cancer: SWOG S1400. Clin Cancer Res. 2015;21:1514–24.
80. Turner NC, Kingston B, Kilburn LS, et al. Circulating tumour DNA analysis to direct therapy in advanced breast cancer (plasmaMATCH): a multicentre, multicohort, phase 2a, platform trial. Lancet Oncol. 2020;21:1296–308.
81. Kaplan R, Maughan T, Crook A, Fisher D, Wilson R, Brown L, Parmar M. Evaluating many treatments and biomarkers in oncology: a new design. J Clin Oncol. 2013;31:4562–8.
82. James ND, Sydes MR, Clarke NW, et al. Systemic therapy for advancing or metastatic prostate cancer (STAMPEDE): a multi-arm, multistage randomized controlled trial. BJU Int. 2009;103:464–9.
83. James ND, Sydes MR, Clarke NW, et al. Addition of docetaxel, zoledronic acid, or both to first-line long-term hormone therapy in prostate cancer (STAMPEDE): survival results from an adaptive, multiarm, multistage, platform randomised controlled trial. Lancet. 2016;387:1163–77.
84. James ND, de Bono JS, Spears MR, et al. Abiraterone for prostate cancer not previously treated with hormone therapy. N Engl J Med. 2017;377:338–51.
85. Barker AD, Sigman CC, Kelloff GJ, Hylton NM, Berry DA, Esserman LJ. I-SPY 2: an adaptive breast cancer trial design in the setting of neoadjuvant chemotherapy. Clin Pharmacol Ther. 2009;86:97–100.

86. Park JW, Liu MC, Yee D, et al. Adaptive randomization of neratinib in early breast cancer. N Engl J Med. 2016;375:11–22.
87. Rugo HS, Olopade OI, DeMichele A, et al. Adaptive randomization of veliparib–carboplatin treatment in breast cancer. N Engl J Med. 2016;375:23.
88. Chien AJ, Tripathy D, Albain KS, et al. MK-2206 and standard neoadjuvant chemotherapy improves response in patients with human epidermal growth factor receptor 2-positive and/or hormone receptor-negative breast cancers in the I-SPY 2 trial. J Clin Oncol. 2020;38:1059–69.
89. Nanda R, Liu MC, Yau C, et al. Effect of pembrolizumab plus neoadjuvant chemotherapy on pathologic complete response in women with early-stage breast cancer: an analysis of the ongoing phase 2 adaptively randomized I-SPY2 trial. JAMA Oncol. 2020;6:676–84.
90. Di Liello R, Piccirillo MC, Arenare L, Gargiulo P, Schettino C, Gravina A, Perrone F. Master protocols for precision medicine in oncology: overcoming methodology of randomized clinical trials. Life (Basel, Switzerland); 2021. https://doi.org/10.3390/LIFE11111253.
91. Cancer Genome Atlas Research Network. Integrated genomic analyses of ovarian carcinoma. Nature. 2011;474:609–15.
92. Getz G, Gabriel SB, Cibulskis K, et al. Integrated genomic characterization of endometrial carcinoma. Nature. 2013;4977447(497):67–73.
93. Burk RD, Chen Z, Saller C, et al. Integrated genomic and molecular characterization of cervical cancer. Nature. 2017;5437645(543):378–84.
94. Pujade-Lauraine E, Ledermann JA, Selle F, et al. Olaparib tablets as maintenance therapy in patients with platinum-sensitive, relapsed ovarian cancer and a BRCA1/2 mutation (SOLO2/ENGOT-Ov21): a double-blind, randomised, placebo-controlled, phase 3 trial. Lancet Oncol. 2017;18:1274–84.
95. Moore K, Colombo N, Scambia G, et al. Maintenance olaparib in patients with newly diagnosed advanced ovarian cancer. N Engl J Med. 2018;379:2495–505.
96. González-Martín A, Pothuri B, Vergote I, et al. Niraparib in patients with newly diagnosed advanced ovarian cancer. N Engl J Med. 2019;381:2391–402.
97. O'Malley DM, Bariani GM, Cassier PA, et al. Pembrolizumab in patients with microsatellite instability-high advanced endometrial cancer: results from the KEYNOTE-158 study. J Clin Oncol. 2022;40:752–61.
98. Lee JY, Yi JY, Kim HS, Lim J, Kim S, Nam BH, Kim HS, Kim JW, Choi CH, Kim BG. An umbrella study of biomarker-driven targeted therapy in patients with platinum-resistant recurrent ovarian cancer: a Korean Gynecologic Oncology Group study (KGOG 3045), AMBITION. Jpn J Clin Oncol. 2019;49:789–92.
99. A study evaluating the efficacy and safety of biomarker-driven therapies in patients with persistent or recurrent rare epithelial ovarian tumors. Full text view. ClinicalTrials.gov. https://clinicaltrials.gov/ct2/show/NCT04931342. Accessed 31 Mar 2022.
100. Ovarian CanceRx. Global Coalition for Adaptive Research. https://www.gcaresearch.org/ovariancancerx/. Accessed 31 Mar 2022.
101. Safety and efficacy of retifanlimab (INCMGA00012) alone or in combination with other therapies in participants with advanced or metastatic endometrial cancer who have progressed on or after platinum-based chemotherapy. Full text view. ClinicalTrials.gov. https://clinicaltrials.gov/ct2/show/NCT04463771. Accessed 31 Mar 2022.