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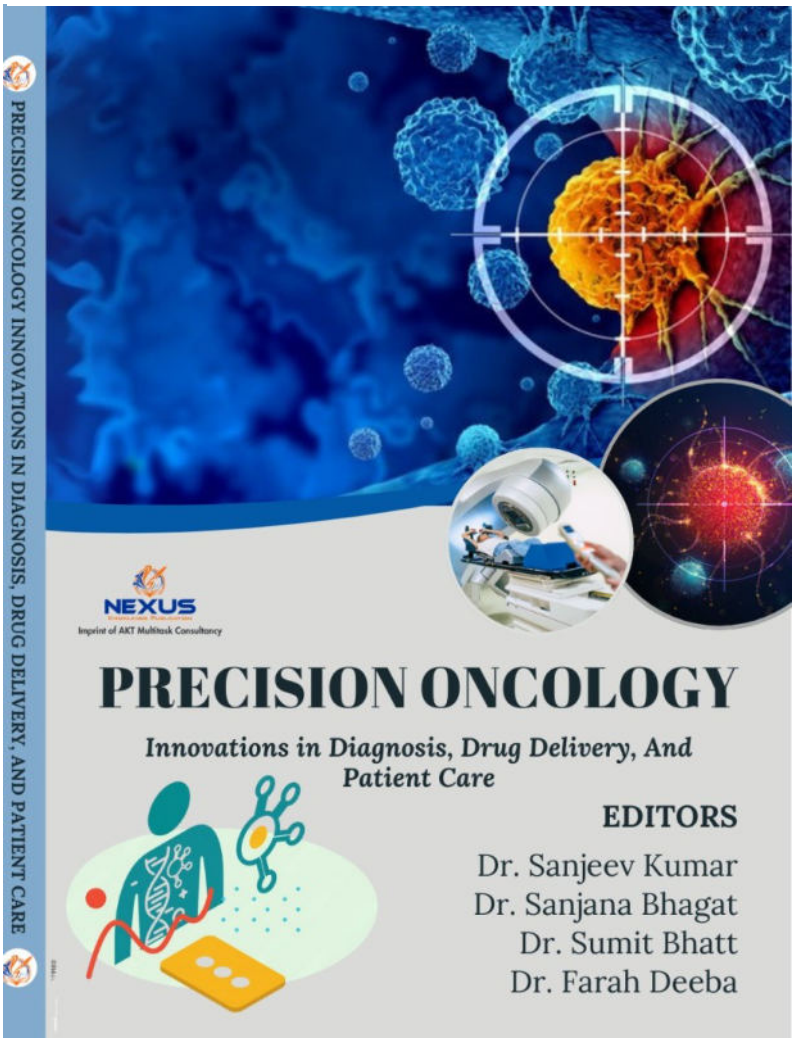
Precision Oncology: Innovations in Diagnosis, Drug Delivery, And Patient Care



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Chapter- 2

GENOMIC AND MOLECULAR PROFILING IN CANCER

RASHMI CHANDA
Assistant Professor
Faculty of Pharmaceutical Sciences,
ShriBalaji Institute of Pharmacy and Research Centre,
Raipur, Chhattisgarh, 492001
Email: Rashmichanda722505@gmail.com

SHIVANGI SHARMA
Assistant Professor
Shaheed Bhagat Singh College of Pharmacy,
Palampur, HP, 176061
Email: ssharma09@gmail.com

AYUSHI SAXENA
Assistant Professor
Varun Arjun College of Pharmacy
Pin - 242001
Email: ayushisaxena401@gmail.com

MONIKA KASHYAP
Assistant Professor
Institute of Pharmaceutical Sciences, IET Bhaddal
Pin - 140108
Email: mk32ei@gmail.com

Mr. PRATIK TERSE
Designation- Assistant Professor
Shree Pushpasen Sawant College of Pharmacy,
Kudal. Maharashtra.
Pin- 416521
Email: pratikterse@gmail.com

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Chapter 2…

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RASHMI CHANDA
Assistant Professor
Faculty of Pharmaceutical sciences,
ShriBalaji Institute of Pharmacy and Research Centre,
Raipur, Chhattisgarh, 492001
Email: Rashmichanda722505@gmail.com

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Assistant Professor
Shaheed Bhagat Singh College of Pharmacy, Palampur, HP, 176061
Email: ssharma09@gmail.com

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Pin - 242001
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Pin - 140108
Email: mk32ei@gmail.com

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Shree Pushpasen Sawant College of Pharmacy, Kudal. Maharashtra.
Pin- 416521
Email: pratikterse@gmail.com

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The core of the practice of precision oncology is genomic and molecular profiling, which gives a more precise idea on the specifics of each tumor. Through the DNA, RNA, protein expression, and epigenetic alterations in cancer cells, clinicians will be in a position to recognize actionable mutations, gene amplifications, or pathway dysregulations that promote tumor development. The resulting molecular understanding can be used to design individualized treatment regimens, and designed therapies can be tailored to the tumor profile as opposed to depending on the tissue of origin or the histological classification.

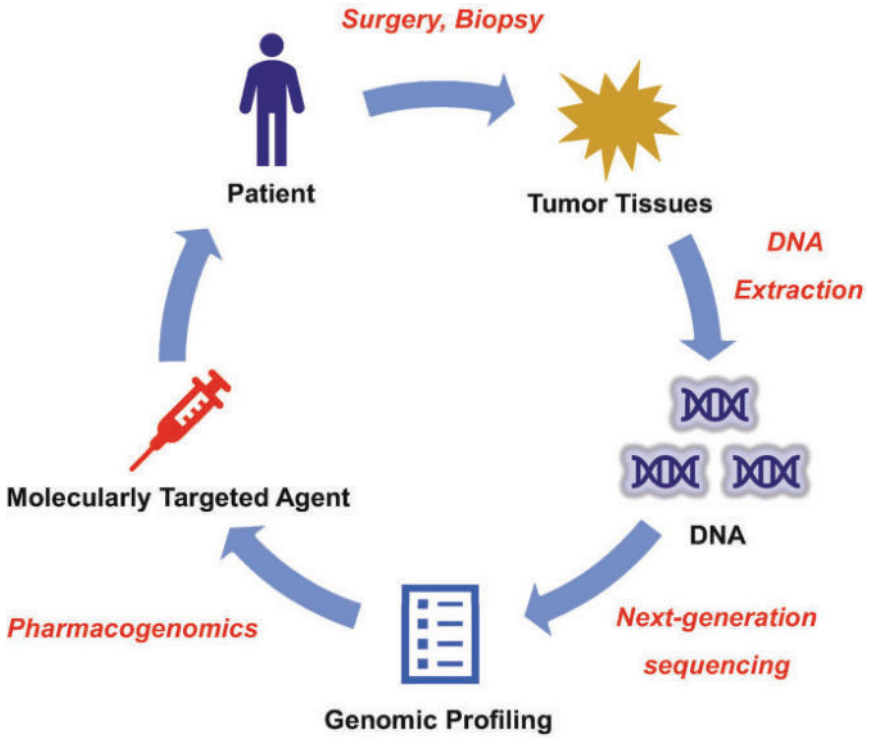

Figure 1: Genomic Profiling

Source: (https://link.springer.com/protocol/10.1007/978-1-4939-8639-2_14)

State-of-art technologies, including next-generation sequencing (NGS), whole-exome sequencing, RNA sequencing, and proteomic analyses, enable profiling of tumors on a scale that has never been reached before with unprecedented depth and resolution. Such tools not only identify somatic mutations, copy number variations, but also show fusions of genes, epigenetic alterations, and tumor mutational burden, which can also be used to affect treatment choices. The combination of multi-omics techniques can further be used to improve the knowledge of tumor heterogeneity, resistance mechanisms, and dynamic development of cancer cells and design more effective and adaptive regimes.

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Genomic and molecular profiling has a clinical role in decision-making in different phases of cancer management, such as early disease detection and planning on how to handle advanced cancer. It allows identifying patients with a high probability of response to specific therapies, immunotherapies or a combination of the two and tracking response using biomarkers or circulating tumor DNA. Also, the profiling strategies can offer essential knowledge to clinical trial recruitment to guide patients to precision-mediated interventions and lead to the overall advancement of novel therapies. Genomic and molecular profiling is the future of individualized, outcome-oriented cancer treatment by blending technology and clinical application.

2.1. NEXT-GENERATION SEQUENCING (NGS) TECHNOLOGIES

Next-Generation Sequencing (NGS) is an open-ended innovation in genomic technologies where the analysis of millions of DNA fragments in a single step has become possible. Contrary to conventional Sanger sequencing, where DNA is read sequentially, NGS can be massively paralleled, with significant data output and at a significantly lower price/base. The technology enables profiling of the genome, transcriptome, epigenome on a large scale, and it gives a multidimensional picture of tumor biology. NGS has formed the basis of precision oncology by identifying actionable mutations, gene fusions, copy number changes and other molecular abnormalities, enabling the discovery of molecular drivers that can be directly targeted with precision immune-based therapy.

NGS gives oncologists an opportunity to develop personalized treatment plans in clinical practice, identifying patient-specific genetic alterations and anticipating patient responses to therapy. It can also be used in longitudinal disease surveillance, identifying any resistance mutation emerging, or any form of clonal descent with time. In addition to informing the choice of targeted therapies, NGS is used to inform enrollment in the appropriate clinical trial, assists in risk stratification, and improves early diagnosis of complex or low-frequency cancer. NGS has transformed the practice of cancer management, which was in an interim between molecular understanding and the need to make tailored therapeutic decisions.

Types of NGS in Cancer

1. **Targeted Gene Panels**

Gene panels are specially created to examine a specific set of genes that are known to be of key importance in cancer formation and growth. These panels normally consist of 50 to 500

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genes, which have been chosen on a robust basis of evidence showing their association with tumorigenesis, response of treatment or prognosis. They target identifying important genomic changes including single nucleotide changes (SNVs), small insertions and deletions (indels), copy number changes (CNVs), and particular rearrangements of structures that are clinically relevant. Technology The targeted panels, by focusing on a selected group of genes, obtain a high depth of sequencing and thereby is sensitive and can identify low-frequency mutations that would otherwise be missed with more genomic techniques. The combination of such a high coverage makes them especially useful in clinical decision-making, where even rare but therapeutically interesting mutations can be with high confidence detected.

The adoption of targeted gene panels has become a common practice in regular oncology practice because these methods have unique practical benefits as compared to larger genomic profiling methods. They are cheaper and their turnaround times are quicker and this is necessary in clinical setting because of quick treatment decisions. The data produced by these panels could be directly used to inform therapy choice, such as the use of targeted or immunotherapies or combination therapy based on the tumor profile of an individual. Since the panels concern well-characterized, actionable mutations, the outputs are very useful in the care of patients and aid oncologists prioritize interventions meeting a proven clinical benefit. Also, they are designed with a narrow focus so as to reduce the chances of identifying variants of uncertain value that may complicate interpretation and postpone treatment. Altogether, the targeted gene panels represent a trade-off of precision, speed, and clinical relevance, so they are a staple of individualized cancer treatment.

2. Whole-Exome Sequencing (WES)

Whole-exome sequencing (WES) is sequencing of the entire set of protein-coding sequences of the genome, the exome. Though these regions constitute only approximately 12 percent of the human genome, they are home to most mutations identified to cause disease, including most cancer-promoting mutations. WES can give a holistic picture of the possible coding variants, and allows the detection of both common and rare mutations, which can be overlooked with targeted gene panels. This renders it especially useful in the discovery of new or unusual mutations, broadening the knowledge base on tumor biology, and the possible genetic factors behind the development of cancer. WES enables researchers and clinicians to produce a more comprehensive mutational picture of a tumor by analyzing the entire exome, which can provide insight into complicated genomic changes, and their functional impacts.

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WES is finding application in precision oncology to guide research and clinical-decision-making. It may be used to measure tumor mutational burden (TMB), a valuable biomarker of response to immunotherapies, and can identify neoantigens that could be used as the personalized cancer vaccine. Also, WES has the ability to reveal new treatment targets, which are out of the range of pre-defined panels, and offer prospects of new treatment approaches. Nevertheless, this increased scope has its costs: WES necessitates more complex bioinformatics treatment, more computing power, and interpretation to differentiate between clinically important and harmless mutations. Moreover, since WES mainly focuses on coding areas, it is not able to identify significant noncoding regulatory factors that affect the expression and behavior of tumors. WES may be considered as a potent resource to global genomic profiling of cancer and clinical car despite these constraints.

3. Whole-Genome Sequencing (WGS)

Whole-genome sequencing (WGS) constitutes an in-depth examination of the complete genomic environment, including more than just the protein-coding region of the genome, but the noncoding sequences, regulatory components, repetitive sections, and structure. This broad-based strategy allows identifying intricate genome changes, such as chromosomal rearrangements, large-scale insertions or deletions, copy number changes, and noncoding driver mutation that can contribute to the emergence and development of tumors. WGS enables researchers and clinicians to identify the full range of genetic changes that would otherwise go undetected by a specific set of gene panels or whole-exome sequencing and may inform understanding of the pathways that promote cancer progression, metastasis, and treatment resistance.

WGS has found extensive application in both research and clinical contexts in which common, aggressive, or treatment-resistant malignancies cannot be understood using the standard methods of investigation. Its comprehensive view helps to discover new therapeutic targets, noncoding mutations of regulatory relevance, and multifaceted structural changes, which could constitute a personalized approach to treatment. Nevertheless, WGS has high coverage coverage, but this has a trade-off: it is more expensive, it produces large volumes of intricate data and its correct interpretation requires more sophisticated bioinformatics infrastructure and computing resources. In spite of these hurdles, WGS is the most detailed technique of genomic profiling, which provides unmatched information about the coding and noncoding areas of the genome and becomes an indispensable instrument in precision oncology and cancer research.

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4. RNA Sequencing (RNA-seq)

RNA sequencing (RNA-seq) offers a high-resolution picture of the tumor transcriptome, including both the dynamic expression patterns of genes and alternative splicing forms of transcripts, as well as fusion transcripts that can lead to cancer progression. In contrast to DNA-based technologies, which mainly provide an insight into the presence of mutations or structural changes, RNA-seq demonstrates the functional implications of such modifications in the genome, which genes and pathways are deregulated in the tumor. RNA-seq can reveal how tumors respond to their microenvironment at the molecular scale, aiding researchers and clinicians to gain vital insights into oncogenic signaling, pathway activation and cellular responses to the tumor microenvironment.

RNA-seq has become a workhorse in clinical oncology to identify actionable targets, especially the fusion genes most likely to respond to targeted therapies, including ALK and ROS1 rearrangements in lung cancer. In addition to identifying targets, RNA-seq allows tracking of tumor dynamics, such as gene expression following treatment, to identify processes that may mediate therapy resistance or sensitivity. This dynamic profiling may inform the changes in treatment strategies, inform the combination therapies, and can even lead to the discovery of new therapeutic approaches. Through the combination of the RNA-seq with the DNA-based analysis, the clinicians can have a better insight into the tumor biology as they can connect the genomic alterations with the functional consequences as well as enhance the accuracy of cancer treatment.

Key Advantages of NGS

1. High Sensitivity and Specificity

Next-Generation Sequencing has an impressive sensitivity and specificity capability, and can be used to identify low-frequency mutations found exclusively in a small fraction of heterogeneous tumor cells. This is particularly essential in cancers that exhibit high intratumoral heterogeneity, i.e. that different subclones might be in possession of different mutations. The NGS can be used to characterize oncogenic drivers and potential mechanisms of therapy resistance by obtaining a more detailed molecular profile of the tumor by revealing these rare variants. Narrow specificity will restrict the number of mutations, one of them is a true positive, and the chance of false-positive findings is low, which enhances the reliability of clinical decision-making.

2. Multiplexing Capability

The possibility of identifying many genetic changes at a single time per assay is one of the key benefits of NGS. Conventional techniques usually need individual tests on each mutation or gene, which is costly and time-consuming and may need more samples. Conversely, NGS is able to simultaneously analyze and identify single nucleotide variants (SNVs), induced and deleted (indels), copy number variations (CNVs), and structural rearrangements in hundreds to thousands of genes or genomic regions. Such high-throughput multiplexing method not only conserves time but also enables clinicians to acquire a detailed molecular portrait directing the choice of targeted therapy, immunotherapy eligibility and clinical trial enrollment.

3. Longitudinal Monitoring

NGS is now used in liquid biopsies, including circulating tumor DNA (ctDNA), circulating tumor cells (CTCs) or exosomes, to allow time-dependent monitoring of cancer progression. Such a possibility enables clinicians to identify small residual disease (MRD) post-treatment, see emergent resistance mutations, and real-time therapeutic response without repeated invasive tissue biopsies. NGS enables adaptive treatment and early intervention in the event of relapse, as well as overall better patient management by offering a dynamic observation of tumor evolution.

Challenges

1. Data Interpretation Complexity

The large scale and quantity of NGS produced data demand advanced bioinformatics pipelines and expert skills to handle, process and interpret. Clinically relevant mutations can be accurately identified by differentiating between driver and passenger mutations; the former causes the progression of cancer and the latter are accidental. Combining sequencing data with clinical and pathological data is necessary, but difficult, and resoften demands multidisciplinary cooperation among molecular pathologists, oncologists, and bioinformaticians.

2. Variants of Unknown Significance (VUS)

NGS often reveals variants of unknown significance (VUS) - mutations the influence on which remains not yet defined. VUS existence may make it complex when it comes to making therapeutic choices, because it is not always clear whether a variant will cause development of the tumor or will influence responses to a drug. The clinicians should be careful in the interpretation of VUSs which may involve functional studies, databases and emerging literature to establish its pertinence.

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3. Cost and Accessibility

Despite a reduction in the price of NGS in the last 10 years, it is still comparatively expensive in contrast to the traditional single-gene tests. Accessibility can be constrained by high costs, complicated infrastructure demands and slower turnaround times especially in resource-constrained environments. Moreover, the requirement of highly skilled staff and bioinformatics assistance may be further obstacles to the extensive clinical adoption, slowing down the integration of precision oncology in some areas.

2.2. SOMATIC VS GERMLINE MUTATIONS IN CANCER

Somatic mutations refer to genetic changes that are acquired throughout the lifetime of an individual and that can be found in non-germline cells and as such limited to the tumor tissue. Such mutations occur due to environmental exposures, mistakes of DNA replication, chronic inflammation, or spontaneous mutational occurrences, and they are central in the initiation, progression, metastasis, and resistance to therapy. Somatic mutations, such as EGFR mutations with non-small cell lung cancer or KRAS mutations with colorectal cancer, are clinically viable actionable targets of precision oncology, and therapies such as tyrosine kinase inhibitors or monoclonal antibodies can selectively target tumor cells and spare normal tissue. Clinicians can optimize treatment efficacy and reduce systemic toxicity by focusing on the changes attained.

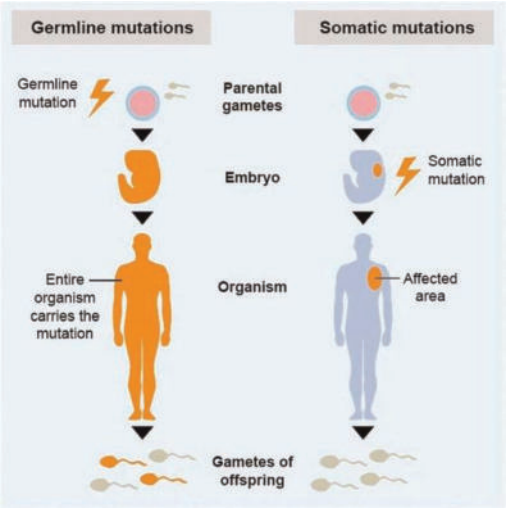

Figure 2: Somatic Mutations and Germline Mutations

Source: (https://www.researchgate.net/figure/Fundamental-difference-between-germline-and-somatic-mutations-wwwlearncolontownorg_fig3_380066207)

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Germline mutations, by contrast, are genetic alterations passed along to all cells including germ cells and that make individuals susceptible to inherited cancer syndromes. Examples of these are BRCA1/BRCA2 defects that predispose to breast and ovarian malignancies and TP53 defects that cause Li-Fraumeni syndrome. Germline profiling plays a pivotal role in risk assessment, early diagnosis, preventive intervention and family counseling. Contemporary oncology is becoming more and more unified in its combination of somatic and germline studies to offer a complete genetic focus. This two-fold approach will direct individualized treatment, determine prevention measures, and educate the relatives about a possible hereditary predisposition, so that cancer treatment will focus on the molecular nature of the tumor and on the inherited tendency of the patient.

1. *Somatic Mutations*

Somatic mutations are genetic changes in non-germline cells and hence, acquired within the lifetime of a person and not inherited. Such mutations do not affect the offspring and appear exclusively in tumor cells. The cause of somatic mutations is varied and can be characterized as follow: environmental exposures (ultraviolet radiation, tobacco smoke, or chemical carcinogens), malfunctions during the DNA replication, chronic inflammation, or spontaneous mutational processes. They are key in tumorigenesis, they play a part in tumor initiation, progression, metastasis, and therapy resistance. As an example, in non-small cell lung cancer (NSCLC) EGFR mutations cause constitutive activation of growth signaling pathways to promote tumor growth, whereas KRAS mutations in colorectal cancer confer a proliferative benefit and depend on response to selected targeted therapies. In treatment, cancer cells can be selectively killed through clinical therapies based on somatic mutations, including tyrosine kinase inhibitors (TKIs) or monoclonal antibodies, which can spare normal tissues, providing a basis of precision oncology.

2. *Germline Mutations*

Germline mutations are inherited inherited changes in genes that are found in all the cells of the body including the germ cells (sperm and egg) as well. The mutations may predispose the individuals to some of the cancers and hereditary cancer syndromes. Cases in point are BRCA1 and BRCA2 mutations that substantially raise the risk of breast and ovarian cancers and TP53 mutations that are linked to Li-Fraumeni syndrome where carriers are more prone to various cancers at an early age. The germline mutations are important in risk assessment, early diagnosis and prophylaxis, which include increased surveillance, prophylactic surgeries or

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changes in lifestyles. In addition, the therapeutic implications of certain germline mutations are also therapeutically relevant; tumors bearing BRCA1/BRCA2 mutations are also sensitive to PARP inhibitors, which target the defective DNA repair pathways to selectively kill cancer cells. The fact that germline mutations have been identified also helps in family counseling since family may be at risk and may be helped by genetic testing and preventive measures.

3. ***Clinical Integration***

Somatic and germline profiling are becoming more used together in modern oncology practice to offer a global genomic evaluation. This combined approach is useful in streamlining therapeutic interventions, establishing preventive interventions, and informing clinical decision-making. As an illustration, a patient can have a tumor with a somatic mutation that can be treated by a particular drug, and have a germline mutation that requires continued monitoring or alerts family members of their risk of cancer. Genetic counseling is vital in interpretation of germline results, risk education and in the process of advising family testing. The combination of the two types of mutations can be used to advance personalized medicine, whereby the treatment choices and preventive measures will be designed to match the molecular specificities of the tumor and the inherited risk factors of the patient.

2.3. TUMOR HETEROGENEITY AND CLONAL EVOLUTION

Tumor heterogeneity is the existence of genetically, epigenetically and phenotypically disparate cancer cell populations in one tumor or different metastases. Such diversity is due to continuous mutations, the influence of the microenvironment and therapeutic interventions, producing subclones that differ in molecular profiles and functional behavior. Multi-region sequencing and single-cell studies have demonstrated that a single tumor that seems homogenous histologically can contain an important amount of intratumoral heterogeneity that can influence the growth rate, metastatic capacity, and response to treatments. This heterogeneity is of great importance to precision oncology since it clarifies the reason why not all patients will respond to identical treatment and why other subclones will survive the treatment to become the cause of relapse.

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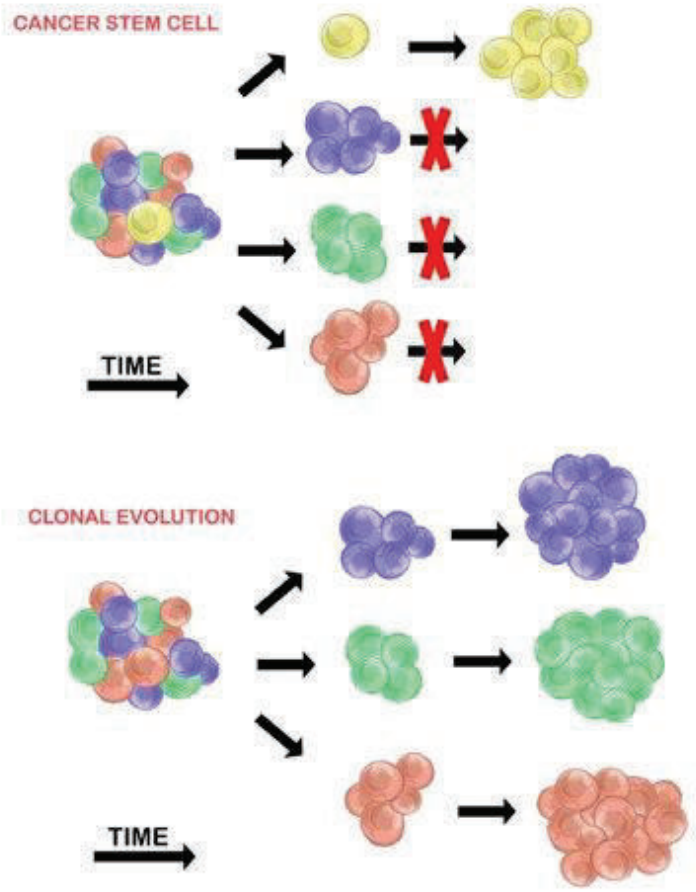

Figure 3: Tumour Heterogeneity

Source: (https://en.wikipedia.org/wiki/Tumour_heterogeneity)

Clonal evolution also makes treatment more complicated as it explains how these subpopulations transform dynamically with time under selective forces including targeted therapies, immunotherapy, or chemotherapy. Liquid biopsies, which measure tumor DNA in circulation and other tumor-derived biomarkers, offer a minimally-invasive way to track clonal evolution, in real time. Clinicians can proactively change treatment plans by monitoring the appearance of resistant subclones or emerging driver mutations, including switching therapies, combining agents or entering patients into clinical trials that are based on emerging tumor profiles. Incorporation of understanding of tumor heterogeneity and clonal evolution into the clinical decision-making process is thus a key to maximizing personalized treatment, resistance overcoming, and better long-term outcomes in cancer care.

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❖ Tumor heterogeneity

Heterogeneity in tumors is a vast diversity at genetic, epigenetic and phenotypic level within an individual tumor as well as among tumors of the same type in different individuals. Such diversity is a result of a set of mutations over time, variable expression of genes, epigenetic changes, and tumor microenvironment effects. There are two general classes of tumor heterogeneity:

- **Intratumoral heterogeneity:** This explains variability that occurs within an individual mass of the tumor. Diverse parts of the tumor can contain different subclones carrying different mutations or different profiles of expression. This heterogeneity leads to therapeutic complications because some subclones may have intrinsic or acquired resistance to certain drugs and total elimination of the tumor will become a challenge. The intratumoral heterogeneity is one of the driving forces behind the failure of therapy and relapse of the disease.
- **Intertumoral heterogeneity:** This is the dissimilarity seen between comparable tumors of the same histological type of different patients. The prognosis, therapeutic response, and susceptibility to metastasis may be affected by genetic and phenotypic differences between the patients. Intertumoral heterogeneity is important because it should be identified to formulate individualized treatment plans and to stratify patients during clinical trials.

❖ Clonal evolution

The dynamic evolution of tumors is associated with accumulating genetic and epigenetic changes in a certain sequence, which results in the differentiation of separate subclones with different biological characteristics. This is what is termed as clonal evolution and is driven by the process of natural selection in which subclones that develop survival benefits, such as chemotherapy resistance or the capacity to avoid immune detection, develop and proliferate, whereas vulnerable clones are killed off. Monitoring of these clonal dynamics with time is important in planning adaptive treatment regimens capable of anticipating resistance and enhance therapeutic results.

❖ Methods to study heterogeneity

A number of more sophisticated methods have been designed to probe tumor heterogeneity on various scales:

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- **Multi-region tumor sequencing:** This is a technique that refers to sampling and sequencing of multiple regions of the same tumor to determine spatial differences in genetic and molecular phenotypes. It assists in showing that there are specific subclones and the distribution of the mutations throughout the tumor mass.
- **Single-cell sequencing:** Single-cell technologies can be used to characterize heterogeneity at the cellular level, un-masking differences between individual tumor cells that can be difficult to see in bulk sequencing. This method gives high-resolution information of mutation spectrums, gene expression and cellular states on tumors.
- **Liquid biopsy:** The subclonal mutations circulating in the blood can be detected by noninvasive techniques like circulating tumor DNA (ctDNA). Real-time monitoring of tumor progression and treatment response with liquid biopsy can be conducted without recurrent invasive tissue biopsy.

❖ **Clinical significance**

Knowledge of tumor heterogeneity and clonal evolution has profound implications to cancer management:

- It guides the rational development of combinations therapy in order to target multiple subclones at a time in order to avoid the occurrence of resistant populations.
- These applications can be used to optimize sequential treatment planning on the basis of the inferred evolutionary dynamics of tumor subclones.
- Heterogeneity information can be used to forecast disease relapse, metastatic possibilities, and the probability of responding to targeted treatment.
- Conclusively, applying knowledge of heterogeneity to clinical practice helps in the evolution of precision oncology approaches that can be specific to the tumor profile of an individual patient.

2.4. INTEGRATING GENOMIC DATA INTO CLINICAL PRACTICE

The process of integrating genomic data into clinical practice is a multi-step process that starts with few steps of collecting high-quality tumor tissue or liquid biopsy samples. Such samples are sequenced by next-generation sequencing (NGS) to reveal actionable mutations, copy number variations, and gene fusions that may inform therapy choices. This results in genomic

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information which is then discussed by a multidisciplinary molecular tumor board, who take into account the clinical situation of the patient, comorbidities and past treatments to come up with individualized care plans. These plans can involve the use of specific therapies, combinatorial treatment plans, or participation of the patient in the associated clinical trials so that specific interventions can be developed based on the specific molecular outline of the cancer in the patient.

Genomic monitoring should also be done continuously, and usually with repeat tissue biopsies or less invasive liquid biopsies to monitor the tumor progression and the development of resistance mutations. Such a dynamic approach enables clinicians to adjust the regimen of treatment on-the-fly to achieve the most efficacy with the least toxicity. Nonetheless, the incorporation of genomic data into clinical practice is fraught with numerous issues, such as differentiating clinically actionable variants versus incidental findings, the high cost and inaccessibility of sequencing technologies, the ethics of genomic germline mutations and privacy of patient data, and a unified integration of genomic outcomes with other omics layers to gain a comprehensive understanding of tumor biology. They are essential to address to achieve the full potential of precision oncology and to provide cancer care at the most individualized level.

Clinical workflow

The application of genomic information in the clinical setting is a multi-step procedure which guarantees proper molecular characterization of tumors and informs individualized treatment plans. This workflow can be classified into sample collection, sequencing and analysis, interpretation, therapeutic decision-making and longitudinal monitoring.

Sample collection and quality control

The initial one is to acquire an appropriate tumor sample. This may be done by invasive (tumor biopsy or tumor resection) or noninvasive (liquid biopsy, the analysis of circulating tumor DNA (ctDNA) in blood or plasma) methods. Nucleic acids (DNA/RNA) purified off of these samples are the most important variables affecting the success of sequencing. Subpar samples may lead to incomplete or distorted genomic data, which may have implications on downstream analysis and clinical judgement. Thus, stringent quality assurances are applied to maintain the integrity of the samples before the act of sequencing.

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Sequencing and bioinformatics analysis

The technologies of next-generation sequencing (NGS) are used to profile the tumor genome in detail. NGS has a broad range of genomic alterations, which include single nucleotide mutations, copy number variations, and gene fusions as well as mutational signatures related to specific cancer phenotype or therapeutic responses. The raw sequencing data are subjected to bioinformatics pipelines, where the matches are made to the reference genomes, variants detected, and prioritized by clinical relevance. Variants are generally classified as actionable (with accessible therapy), likely pathogenic and of undetermined importance to influence further clinical interpretation.

Molecular tumor boards

Genomic data is then reviewed by multidisciplinary molecular tumor boards, which typically include oncologists, pathologists, geneticists, bioinformaticians, and other relevant specialists. These teams collectively interpret the genomic findings, correlate them with clinical and pathological data, and develop personalized treatment recommendations. Decisions may include selecting FDA-approved targeted therapies, determining eligibility for immunotherapy, or considering enrollment in relevant clinical trials for investigational agents. The collaborative approach ensures that complex genomic information is translated into clinically meaningful insights.

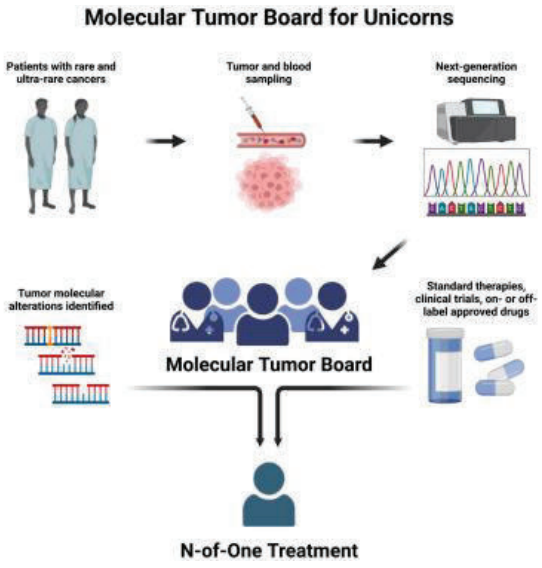

Figure 4: Molecular Tumor Board

Source: (https://www.sciencedirect.com/science/article/pii/S2589004224016900)

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Therapeutic decision-making

Clinicians can pair target therapies or investigational drugs to particular mutations or genomic profiles based on genomic interpretation. Multi-driver mutations or resistant subclones in tumors may only be targeted by a combination of strategies to adequately address all the oncogenic pathways. Individualized treatment regimens are thereby customized to the molecular environment of the tumor of each individual patient, maximizing efficacy and reducing non-therapeutic toxicity.

Monitoring and adaptation

Genomic profiling is not a single event, but serial monitoring with repeat biopsies or liquid biopsies makes it possible to identify resistance mutations or the advent of new subclones. Such dynamic strategy makes it possible to adjust therapy, interfere at an early stage in the case of tumor progress, to increase or decrease the treatment in time, thus enhancing the long-term clinical outcome.

- **Challenges and Considerations**

Although genomic data integration into clinical practice has a promising transformative potential, a number of important issues are associated with the integration, which need to be carefully addressed to integrate genomic data safely, effectively, and equitably. Such challenges include scientific, economic, ethical, and technical.

1. **Clinical validity vs. utility**

The difference between clinical validity and clinical utility is a fundamental issue to apply genomic data to precision oncology. Clinical validity describes the degree to which a genomic variant is linked to a disease or condition and clinical utility describes whether or not the knowledge of a variant can be meaningfully used to inform management or treatment decisions of patients. HTS technologies have vastly increased our capacity to observe genetic changes in tumors and have been able to detect not only familiar mutations but also unfamiliar variants the relevance of which is not yet evident. Not every identified variant is, however, therapeutically actionable. As an example, mutations are directly amenable to existing drugs, producing quantifiable clinical benefits, whereas others are incidental or nonpathogenic and have no current relevance to treatment. The utilization of raw genomic data without critical interpretation can result in wrong interventions, treatments that are unwarranted, or the opportunities to deliver effective treatment.

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In order to overcome this difficulty, genomics has to be integrated in clinical practice with the help of multidisciplinary competencies and strong variant annotation tools. The teams of oncologists, geneticists, bioinformaticians, and molecular pathologists are necessary to distinguish between the clinically actionable mutations and those of uncertain value. The detailed and constantly revised data bases of genetic variants are useful in this process because they list the known therapeutic targets and new evidence of variations. Furthermore, it requires continued studies to build on our knowledge of variant function and its clinical outcome, thus enhancing the translation of genomic discovery to significant patient care. By making sure that the genomic interpretations are scientifically valid and relevant to clinical needs, medical professionals will be able to make informed treatment choices that will have the highest patient outcomes and lowest risks of a misinterpretation.

2. Cost and accessibility

The high prices and unavailability of sophisticated genomic technologies are the major impediments to the embracement of precision oncology. Other methods like next-generation sequencing (NGS) and multi-omics profiling do not only demand costly reagents and equipment but also very specific infrastructure and personnel. These tests are expensive so they can be prohibitive in resource-constrained environments and so many patients do not have the opportunity to access personalized cancer treatment. Even in health care systems that are well funded, the cost of such intensive profiling of the molecule might not allow its use on a routine basis, particularly when many tests are needed to inform the decision to make a treatment choice, and thus create unequal access to precision therapies. In addition to the price, geographic and systemic considerations also limit access to genomic-guided oncology. Full-scale genomic testing is often unevenly distributed across geographical regions, hospitals, and healthcare, with the result that inequities in access to personalized cancer care exist. Rural or underserved patients can also have an extra logistical burden, such as commute to specialized facilities and test results. These economic and structural obstacles are essential to overcome so that precision oncology is not only the privilege of a small group of citizens but it is widely available. Such initiatives as subsidized tests, local genomic centers, scalable and cost-effective technologies are necessary to enable widespread access to genomic-guided therapies and ensure equity between different populations of patients.

3. Ethical considerations

Precision oncology genomic testing frequently reveals incidental germline mutations- variants that are hereditary, can have implications beyond just the patient, and can affect other family

members. Such variants and their discovery present thorny ethical issues, such as the issue of informed consent, disclosure of findings, disclosure, and patient privacy. Before patients are subjected to incidental findings, they should be informed about the occurrence of such findings and its potential implication to the family planning, early health surveillance or prevention measures. These revelations can be emotionally disturbing, anxiety-inducing, or cause hard choices among patients and their family members without a keen handling. Besides this, handling of genomic information requires strict guard against abuse, prejudice and confidentiality invasion. There should be a clear ethical code and legal frameworks that will ensure the right of patients are not violated and also that the clinical utility of genomic data is optimized. This involves specifying access to the information, the possibility of its use in research or clinical practice and handling disclosure of information to family members. With the adoption of strong ethical frameworks, medical workers will be able to utilize genomic knowledge responsibly to support personalized care and prevention and to maintain trust and justice in treating patients.

4. Integration with other omics

Although genomic data offers essential information on tumor biology, this is just a dimension of the multifaceted molecular features of cancer. Combining genomics with other omics proteomics, transcriptomics, metabolomics and epigenomics allow a more detailed view of tumor behavior. Multi-omics strategies can display genetic changes in terms of functional change at protein, metabolite or regulatory tiers, which gives a comprehensive picture of tumor evolution, drug action and resistance. Such holism increases the precision of therapeutic decisions, promotes treatment personalization, and can discover new ways to intervene that would be overlooked by genomics itself. There are however complex technical and analytical issues raised by the integration of the multi-omics data. Computational power, advanced bioinformatics pipelines, and knowledge of data interpretation are needed to handle high-computational-power, large, and heterogeneous datasets due to multiple layers of molecular data. Furthermore, complex molecular signatures require interdisciplinary research between oncologists, molecular biologists, bioinformaticians and computational scientists to translate complex molecular signatures into clinically actionable information. It is necessary to develop standardized data integration, visualization and interpretation to optimize the potential of multi-omics approaches in precision oncology and to guarantee the robustness, reproducibility and clinical relevance of the findings.

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