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Review Article

CRISPR Functional Genomics in Precision Oncology: Integrating Single-Cell Multi-Omics for Cancer Vulnerability Discovery

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ABSTRACT

Precision oncology seeks to identify patient-specific therapeutic vulnerabilities; however, conventional genomic profiling is limited by intratumoral heterogeneity and its inability to distinguish functional driver alterations from passenger mutations, often resulting in incomplete prediction of therapeutic response. Recently, the combination of CRISPR functional genomics with single-cell multi-omics has proven to be a paradigm-shifting strategy for understanding context-specific cancer vulnerabilities by causal functional interrogation. The aim of this review is to critically examine recent progress in the integration of these technologies for discovering vulnerabilities in cancer, and the introduction of a new conceptual model, called the Integrated Functional Precision Oncology (IFPO) Framework, that brings together functional genomic perturbation, single-cell multi-omics, computational systems biology, and clinical translation. Peer-reviewed publications from 2020 to 2026 were considered to reflect recent advances in technology and concepts. Key findings from historic and recent research were analysed to pinpoint methodological innovations, translational studies, limitations, and areas in need of further research. The results reveal that the integrated CRISPR single-cell platforms, such as Perturb-seq, CROP-seq, and ECCITE-seq, can be used to causally interrogate gene function at the single-cell level, allowing for the identification of context-dependent essential genes, synthetic lethal interactions, regulatory networks, and therapeutic resistance mechanisms. All the evidence suggests that therapeutic response is not merely a function of genomic alterations but also the dynamic interplay between genomic alterations, cellular state, epigenetic plasticity, and the tumour microenvironment. the mechanisms by which these functional perturbations, multimodal molecular profiling, and AI-driven integration of data converge to reveal clinically actionable cancer vulnerabilities. This integrated paradigm transforms precision oncology from descriptive molecular profiling to functional systems oncology and offers directions for further progress of precision cancer treatment based on enhanced biomarker discovery, therapeutic target identification, and prospective clinical translation.

Keywords: CRISPR Functional Genomics; Single-cell Multi-Omics; Precision Oncology; Cancer Vulnerability; Therapeutic Resistance

Introduction

Although cancer has shown significant progress in genomics, targeted therapeutics and immunotherapy, there remains a huge gap between molecular characterization of cancer and a sustained clinical benefit, with cancer being a major cause of morbidity and mortality worldwide(1). To address this, precision oncology has emerged as a new area of research, focusing on individual molecular characteristics of tumors instead of their tissue of origin. Its success in clinical practice, however, has proven to be inconsistent, with the current mutation-driven paradigm predicting a therapeutic response with the presence of genomic changes(2). However, this has been challenged by rising evidence that tumors with similar genomic features can have very different phenotypes, drug resistances and evolutionary paths. Such inconsistencies are the result of the dynamic relationship between intratumoral heterogeneity, epigenetic remodeling, cellular plasticity, metabolic adaptation, and the tumor microenvironment, thus indicating that therapeutic vulnerabilities are not only shaped by genetic changes but also by functional cell states(3).

This conceptual constraint has now turned its focus from descriptive genomics to functional cancer genomics, where manipulation of the genome to achieve its causal influence is applied to differentiate biologically relevant driver dependencies non-functional alterations(4). This has now been revolutionized by the development of CRISPR based technologies that allow for the scalable and precise interrogation of gene function through knockout, transcriptional repression and activation and more recently cutting edge editing platforms(5). However, traditional CRISPR screens typically output population-level readouts which fail to sufficiently represent the cell-to-cell variability in responses to therapy, tumour evolution, and recurrence. At the same time, single-cell transcriptomic, epigenomic, proteomic, metabolomic and spatial profiling technologies have uncovered previously unrecognized cellular diversity in and dynamic state transitions across tumours(6). These methods are still largely descriptive and don't link molecules to cause-and-effect relationships.

At the conceptual level, the integration of CRISPR functional genomics with single-cell multi-omics is a major leap forward because it correlates a multidimensional analysis of phenotypes with a causal perturbation analysis(7). Recent technologies, like Perturb-seq, CROP-seq, and ECCITE-seq, have shown that the same genetic perturbation can yield different molecular results in different cell states and in different microenvironments, which has fundamentally called into question the deterministic genotype-to-phenotype model(8). Yet, significant challenges remain, such as the integration of the multimodal data, computational scalability, experimental standardization, and limited clinical validation, that limit translation to routine clinical practice. This review critically summarizes these new technologies and introduces the Integrated Functional Precision Oncology (IFPO) Framework, a systems-level model that combines functional perturbation, single-cell multi-omics, and computational inference to provide insights into context-dependent cancer vulnerabilities as well as to inform the next generation of precision oncology.

Methodology

A thorough literature search was performed to identify relevant publications on functional genomics using the CRISPR tool, single-cell multi-omics, precision oncology, cancer vulnerabilities, therapeutic resistance and emerging applications in cancer research. Literature search was performed by combining keywords and related terms such as “CRISPR functional genomics,” “CRISPR screening,” “CRISPR-induced functional genomics,” “single-cell multi-omics,” “single-cell sequencing,” “precision oncology,” “cancer vulnerability,” “cancer dependency,” “therapeutic resistance,” “drug resistance,” and “multimodal data integration.” Boolean operators (AND/OR) were implemented to help identify related ideas and to expand literature identification.

Peer-reviewed publications from 2020 to 2026 were considered to reflect recent advances in technology and concepts. To provide essential historical background or describe foundational technologies, methodologies, or concepts relevant to the development of CRISPR functional screening, single-cell technologies, and precision oncology, earlier seminal studies were also included.

The literature was chosen due to its relevance, scientific contribution, methodological relevance and according to the objectives of this narrative review. Research advances based on CRISPR-based functional screening, single-cell multi-omics, multimodal data integration, cancer vulnerability mapping, therapeutic resistance, and clinical translation were highlighted. Where appropriate, studies with limited relevance to the topics of the review, duplicate publications with no significant new content, and non-peer-reviewed sources were excluded.

The literature search was not restricted by a formal systematic review protocol or metanalytic framework as the review is narrative in nature. Rather, relevant evidence was critically assessed and thematized. Selected studies were compared to determine methodological advances, areas of consensus and disagreement, strengths and limitations of current approaches, and new research gaps. The integration of functional genomics with single-cell multi-omics has been emphasized as potential to enhance cancer vulnerability elucidation, uncover therapeutic resistance, and guide precision oncology approaches.

Foundations of CRISPR Functional Genomics

The advent of CRISPR technologies has completely transformed the field of functional cancer genomics, transforming the field of precision oncology from identifying genomic alterations to experimentally determining which ones are essential for tumour survival and therapeutic response(9). While initial CRISPR-Cas9 knockout systems proved to make informative causal connections between the loss of a gene and the cancer phenotype, recent studies have revealed that full knockout of a gene is not a complete picture of tumour biology. Even the most comprehensive knockout screens are only able to detect a subset of functional vulnerabilities, as many cancer vulnerabilities are dosage-dependent, reversible and mediated by non-coding regulatory elements(10). The recognition has led to the development of other complementary CRISPR platforms such as CRISPR interference (CRISPRi) and CRISPR activation (CRISPRa) that allow for reversible suppression or activation of endogenous genes and thus discriminate between essential biological functions and complete loss-of-function effects(11). Likewise, both base and prime editing have addressed significant double-DSB issues such as the ability to introduce clinically relevant mutations and minimizing genomic instability and off-target toxicity. These editing platforms, rather than being mutually exclusive, offer complementary functional solutions, increasing the ability to interrogate a variety of tumorigenesis and therapeutic response mechanisms(12).

The development of CRISPR screening approaches is an example of a shift from single dimensional gene discovery to systems-level functional interrogation. Large scale dependency mapping efforts have continued to show that loss of function screens are still essential for discovering new synthetic lethal combinations and essential genes(13). Studies combining CRISPR knockout and CRISPRa, however, have found that the cancer biology associated with acquired drug resistance and cell growth transformation, as well as oncogenic signalling, is often caused by activating, not deleting, the gene. Neither strategy, alone, is enough to faithfully recapitulate functional cancer biology(14). Similarly, genetic interactions and variant-specific effects significantly affect therapeutic vulnerability as revealed by combinatorial perturbation screens and saturation mutagenesis, casting doubt on the idea that each genetic factor acts independently in tumour networks(15).

Conversely, functional genomics is predictive only in the biological context. While high throughput screening is possible with the conventional cell-line models, they do not capture the heterogeneity of tumours and the interactions with stromal cells and evolutionary processes seen in patients(16). As a result, patient-derived organoids, xenografts, genetically engineered mouse models, and in vivo CRISPR screening increasingly have shown to be more clinically relevant by revealing context specific dependencies that are often not seen in 2D cultures. However, the efficiency of editing, the type of editing delivery, experimental standardization, and the interpretation of the results by computer are not without variability, which still restricts reproducibility and clinical translation(17). Together, these hurdles highlight the critical need for the development of multi-omics single-cell methods, as well as the need for the development of more sophisticated computational methods to account for complex functional dependencies between cells, to maximize the impact of CRISPR functional genomics in the future on human cancers.

Single-Cell Multi-Omics: Transforming Functional Cancer Biology

The development of single-cell multi-omics is an indicator of a general paradigm shift in cancer biology from defining molecular heterogeneity to understanding how the interplay of many regulatory levels shape cellular function(18). While single-cell transcriptomics opened the door to a revolution in resolving the cellular diversity that had been undermined by bulk sequencing, many studies have now shown that transcriptomics alone is a partial picture of tumour biology(19). All of these will modify gene expression, which will be continually influenced by genomic changes, epigenetic regulation, protein signalling, metabolic adaptability and microenvironmental interactions, suggesting that none

of these molecular layers alone will explain phenotypic behaviour or therapeutic response(20). However, the shift from single-cell transcriptomics to integrated multi-omics is not only a result of technological advances, but also of the insight that functional cellular states arise when multiple biological systems interact in a coordinated fashion(21).

This change has posed a number of assumptions about the principles of precision oncology. Despite these single-cell genomic analyses that revealed clonal evolution as the basis for tumour progression, subsequent studies repeatedly showed that genetically very similar cells often have very different transcriptional and phenotypic states, especially when subjected to therapeutic or microenvironmental stresses(22). Similarly, increasing evidence indicates that epigenetic changes can precede permanent genetic adaptation, and chromatin plasticity may, in fact, be a major contributor to therapeutic resistance, rather than merely a reflection of genomic evolution. Likewise, the lack of correlation between transcript abundance and protein activity sometimes reported, has underscored the need for more than RNA-based analysis, and spatial transcriptomic studies have confirmed that the behaviour of individual cells has to be considered in the context of tissue architecture and intercellular communication(23). Together, these results show that tumour evolution is regulated by dynamic regulatory networks, not by single molecular changes.

Functional cancer biology has therefore been revolutionized by the development of new integrated sequencing platforms like SHARE-seq, SNARE-seq, Paired-seq, and 10x Multi omics, which allow for complementary layers of molecules to be interrogated simultaneously in each cell. With the growing dimensionality of these data sets, however, the main challenge has switched from data generation to biological interpretation(24). Although artificial intelligence and computational methods based on graphs are now essential for reconstructing regulatory networks, inferring cellular trajectories and extracting clinically relevant cellular states, interpretability, reproducibility, and standardization across platforms remain challenges. The challenges highlight that the value of single-cell technologies will not be limited to the production of new omics datasets, but rather in the development of well-established integrative analytical frameworks for extracting mechanistic and clinically actionable insights from multidimensional molecular information(25). This view offers the rationale for single-cell multi-omics to be combined with CRISPR-based functional genomics, which could jointly yield answers to context-dependent cancer vulnerabilities.

Integrating CRISPR Functional Genomics with Single-Cell Multi-Omics

The convergence of CRISPR functional genomics and single-cell multi-omics is a conceptual and not just a technological leap in precision oncology, since it bridges the gap between the effects of perturbing a gene and the context of cells. In the past, CRISPR screening and single-cell profiling were two independent and complementary techniques(26). Genome-wide CRISPR screens were largely used to establish causal links between gene perturbations and cellular fitness, but they tended to simplify complex biological responses to binary, binary-like, or simpler phenotypic endpoints, such as cell proliferation or cell survival(27). Single-cell multi-omics, on the other hand, could not only capture a high degree of cellular heterogeneity and dynamic molecular states, but also could not go beyond a simple correlation-based analysis and could not resolve the distinction between causal regulators and downstream responses. The convergence of these two approaches serve as a key missing element from both: their connection of functional perturbation directly to multidimensional cellular phenotypes allows genotype–phenotype relationships to be understood in their biological context(28).

The integration has turned the traditional understanding of cancer dependencies on mutation upside down. Integrated perturbation platforms like Perturb-seq, CROP-seq, and ECCITE-seq consistently show that the same perturbation causes different transcriptomic, epigenetic and proteomic responses depending on the microenvironmental context and cellular state(29). From these observations, it may be concluded that therapeutic vulnerability is an emergent property that depends on the dynamic regulatory networks, but not solely on genotype. Thus, functional genomics has evolved from the characterization of key genes to the characterization of the conditions in which key genes become therapeutically useful, and, as such, integrated CRISPR–single-cell methods have become the focus of this field(30).

The most significant effect these technologies have is the ability to reconstruct tumour evolution in a dynamic fashion. Recent research has shown that drug-tolerant cells often result from a reversible, epigenetic and transcriptional shift prior to the establishment of stable genetic resistant cells, as a result of the integration of causal perturbation with single-cell trajectory analysis, hence contradicting classical clonal selection models, which suggest that the origin of

resistance is mainly a pre-existing pool of resistant cells in the population(31). Likewise, combining CRISPR perturbation with transcriptomic, epigenomic, and spatial profiling has shifted the gene regulatory network reconstruction from correlation-based inference to causal modelling as well as uncovered how tumour cells remodel immune and stromal interactions as diseases progress(32). However, with the growing complexity of perturbation data across multiple modes, the main challenge has shifted from experimental capability to computational interpretation. Integrated functional genomics will also require standardization of analytical frameworks and explainable Artificial Intelligence (AI) that can convert multidimensional functional data into precision oncology actions, along with the advancement of techniques for perturbation.

Mapping Cancer Vulnerabilities Through Integrated Functional Genomics

The combination of CRISPR functional genomics and single-cell multi-omics has revolutionized the concept of cancer vulnerability, revealing that tumour dependencies are context-dependent, biological traits that can vary from one tumour to another and not simply deterministic outcomes of recurrent genomic alterations(33). The initial genome-wide CRISPR screening programs, such as the Cancer Dependency Map, identified that the number of genes that are essential for most cancers is relatively small, and that the dependency of most genes depends on tissue lineage, genomic context, and cell state(34). This concept has since been expanded into single-cell perturbation models, which have revealed that cancer vulnerabilities often differ between genetically similar tumour cells, suggesting that these vulnerabilities are more likely to be driven by the tumour genotype, cellular phenotype and tumour ecosystem than by the tumour genome alone.

This system-level approach has changed the fundamental understanding of cancer biology in several aspects. The classical concept of "oncogene addiction" held that dominant oncogenic drivers, like KRAS, MYC and EGFR, caused malignant cells to be constantly addicted. Nevertheless, integrated CRISPR and single-cell analyses reveal that oncogenic dependence is very context-dependent, changing between the developmental states, epigenetic landscapes, and microenvironmental conditions(35). Similarly, while synthetic lethality is one of the most effective ways to uncover selective cancer vulnerabilities, recent reports suggest that synthetic lethal interactions are often context- and state-dependent, meaning that functional dependencies can't be predicted from genomic changes, but must be tested directly(36).

In addition to essential genes and synthetic lethal interactions, integrated functional genomics has broadened the range of vulnerabilities in cancer to encompass lineage-specific, metabolic, epigenetic, and microenvironmental vulnerabilities. Tumors arising from different tissues have specific lineage-specific programmes of growth that give rise to lineage-specific dependencies, and metabolic changes confer selective demands for nutrient utilization and bioenergetic pathways(37). Likewise, many chromatin regulators and epigenetic modifiers play critical roles in defining tumour identity, and tumor-immune and tumor-stromal cell interactions create other context-dependent dependencies that do not occur in primary tumour models. These processes are not independent, but rather they work together to maintain the fitness of the tumour and are a cohesive description of the functional environment of cancer(38).

All this evidence together suggests that cancer susceptibility is best modeled as a property of a complex system of interacting with genetic, epigenetic, metabolic, developmental, and microenvironmental factors. This paradigm change replaces mutation-driven approaches to functional cancer biology with detailed dependency maps, yielding a framework for cancer biology that is rooted in biology and can inform the next generation of therapeutic approaches.

CRISPR Functional Genomics in Therapeutic Resistance

The biggest challenge to lasting precision oncology is therapeutic resistance, as the vulnerabilities of cancer are dynamic and change over time under therapeutic pressure. While CRISPR functional genomics has made major strides to identify actionable cancer dependencies, evidence is growing that effective identification of targets doesn't always lead to sustained clinical success(39). On the contrary, therapeutic intervention begins a process of evolution where tumour cells evolve as a result of dynamic interactions between genetic changes, transcriptional reprogramming, epigenetic plasticity, metabolic remodeling and microenvironmental factors. Thus, resistance is being understood more and more as an adaptive systems-level response, instead of the result of specific molecular events(40).

This changing attitude has led to a reevaluation of the classical viewpoints that saw treatment failure largely as the result of the growth of pre-existing resistant clones with beneficial mutations. But

integrated CRISPR perturbation and single-cell multi-omics studies suggest that for many tumour cells, drug resistance is not a stable genetic state that is acquired after initial resistance, but that it is a reversible drug-tolerant state that occurs before stable resistance. Transcriptional and epigenetic changes occur in these populations, allowing for survival benefits in the short term and then making them susceptible to further selection by drug therapy. Adaptive cellular plasticity and genetic evolution don't seem to be competing processes, but it is rather a combination of both, that is responsible for maintaining tumour persistence and recurrence.

This system-level model of resistance is supported in all four of the major therapeutic modalities (chemotherapy, targeted therapy, immunotherapy, and anti-angiogenic therapy). Even if different therapeutic modalities bring different selective pressures, tumor adaptation is often converging to a few processes such as rewiring of some signaling networks, rewiring of metabolism, immune evasion, and remodeling of the microenvironment(41). These common adaptive mechanisms are responsible for the fact that genetically identical tumors can acquire different resistance mechanisms, and that the same therapeutic targets can lead to different clinical responses in patients. These observations all point to the biological context of treatment being a main determinant of resistance, rather than the primary oncogenic alteration(42).

Clinical Translation and Precision Oncology

Although cancer genomics has made unprecedented progress, the clinical benefits of precision oncology have been relatively limited, as it is often the case that the molecular characterization of cancer trails behind the capacity to rapidly translate the molecular data into therapeutic action(44). While genomic biomarkers have revolutionised the management of certain malignancies, there has been a continued lack of correlation between molecular profiles and functional tumour behaviour, with similar genomic aberrations creating very different clinical outcomes. This constraint has turned the spotlight in precision oncology away from trying to identify genomic alterations and toward trying to identify the therapeutically actionable dependencies in individual patients(45).

Single-cell multi-omics, combined with CRISPR functional genomics, can provide a conceptual solution to this translational gap, as a complementary approach to descriptive biomarkers with experimentally validated functional evidence(46). Unlike mutation-based prediction of therapeutic response, integrated functional profiling allows the direct identification of tumour vulnerabilities and integrates cellular heterogeneity, phenotypic plasticity, and microenvironmental effects, which are often the factors that drive therapeutic response. This shift from mutation-based stratification to function-based precision medicine in which drug selection is influenced by the changing biology of the tumour rather than its permanent genetic makeup is becoming more common(47).

The paradigm also changes drug development. Traditional target discovery has been based on recurrent genomic changes, and these changes are frequently not considered in the context of the biology during clinical evaluation. Functional perturbation coupled with multimodal molecular profiling, on the other hand, identifies targets based on their causal role in tumour survival and enriches patient subsets for specific interventions(48). Furthermore, the increasing application of patient-derived organoids, ex vivo CRISPR screening, and early clinical trials in function highlight a general shift towards functional diagnostics that can assess therapeutic efficacy prior to the start of treatment. Such strategies are especially relevant for rational combination therapies, which require the simultaneous interrogation of multiple pathways, and may predict adaptive resistance and enhance treatment sustainability(49).

But its application is limited clinically because it has not been broadly validated in prospective studies, there are no standardized functional assays, little information on regulatory pathways, and the infrastructure necessary for multimodal data integration is still not available (50). These constraints also have some practical implications regarding scalability: functional profiling on an individual level could take a significant amount of hardware, software, sample processing, specialized staff and sequencing/functional screening platforms to implement, especially in the clinical environment where resources are limited (51). Coordinated expertise in clinical oncology, laboratory science, molecular pathology, bioinformatics and data science is also needed for the interpretation of the high dimensional functional data, which is why workforce training is also critical. In addition, functional assays need to be reliable, reproducible and clinically acceptable in terms of turnaround time and must fit into the current genomic and pathological workflows without being parallel research procedures (52). Therefore, the key translation question is not just if, but whether, functional profiling can provide reliable, fast, cost-

effective clinically relevant vulnerabilities. Incorporating these approaches into precision oncology should thus also be assessed in future studies for feasibility, scalability, cost-effectiveness and clinical utility, as well as for biological validity.

Conceptual Framework: The Integrated Functional Precision Oncology (IFPO) Framework

The Integrated Functional Precision Oncology (IFPO) Framework is proposed as a novel conceptual framework for reconceptualizing cancer vulnerability discovery by combining the key biological, technological and computational advances synthesized across this review into an integrated systems-based framework. The current paradigm of precision oncology has, for the most part, been based on the assumption that genomic changes are the most important drivers of response to treatment (53).

The conceptual novelty of the IFPO Framework lies not in the individual technologies it incorporates, but in their integration into a unified, context-dependent and iterative model of cancer vulnerability discovery. Unlike mutation-centric precision oncology, which primarily links genomic alterations to therapeutic response, or approaches that consider CRISPR functional genomics, single-cell multi-omics and artificial intelligence as relatively independent analytical or experimental modalities, IFPO positions these components as interdependent stages of a causal-to-clinical learning system (54). Specifically, tumour heterogeneity and microenvironmental context define the biological setting; CRISPR perturbation provides causal evidence of functional dependency; single-cell and spatial multi-omics characterize heterogeneous responses; AI and multimodal computational analysis integrate these data into mechanistic models; and clinical outcomes feed back into subsequent functional validation (55). Thus, the principal advance of IFPO is the shift from identifying molecular alterations to identifying context-specific functional dependencies, while linking their discovery directly to biomarker development, therapeutic prioritization, patient stratification and adaptive clinical decision-making.

The IFPO Framework proposes a very different perspective of these branches of research as parallel technologies than is common in existing models, which view these separately as stages of functional knowledge generation, and emphasizes the need to interpret them as successive and interdependent. It starts by acknowledging cancer as a dynamic adaptive system, where heterogeneity of tumours, cellular plasticity and interaction of tumour cells with a microenvironment play a role in shaping disease evolution and therapeutic response. Studies have shown that these factors are each individually linked to the process of tumour evolution, but they have been investigated in isolation, so no comprehensive mechanistic understanding of how tumours evolve has emerged so far (56). The IFPO Framework is designed to solve this conceptual shortcoming, by defining a context for the emergence of functional dependencies biological complexity rather than an experimental source of variation to be controlled.

Within this biological context, CRISPR functional genomics serves as the causal engine of the framework. Genomic profiling is traditional and determines the biological significance by the frequency of mutations, while CRISPR mediated perturbation specifically tests the function of genes by experimental manipulation (57). However, when conducted as individual large-scale CRISPR screens, these assays often boil down to simple two-state (live or dead, grow or not) assays, without accounting for the transcriptional, epigenetic, and phenotypic variation that come with genetic perturbation (58). This does not necessarily mean that causal perturbation is not a limitation of CRISPR technology itself, but rather that the IFPO Framework suggests that multidimensional cellular phenotypes should be considered in the context of causal perturbation. Combining CRISPR knockout, CRISPR interference, and CRISPR activation with single-cell transcriptomics and epigenomics, proteomics, and spatial multi-omics is shifting the paradigm of functional screening from endpoint discovery to interrogation of tumour systems. All three approaches Perturb-seq, CROP-seq and ECCITE-seq show that the same perturbation can often produce different molecular responses in different cells, which aligns with the central tenet of the framework that there is no single molecular dependency that is necessarily defined by a cell's genotype.

The IFPO Framework also suggests that the biggest hurdle in precision oncology is not capturing the molecular data anymore, but rather understanding the biological knowledge in increasingly high dimensions. AI and multimodal computational integration are not then just an analytic tool but can be seen as mechanistic bridges between causal perturbation and biological interpretation. Computational analysis of transcriptomic, epigenomic, proteomic, spatial and functional perturbation data enables reconstruction of gene regulatory networks, deducing state transitions, cell-cell communication networks and uncovering adaptive resistance pathways that are hidden by single-modality analysis(59). Importantly, the framework goes beyond purely

predictive models, suggesting that computational inference should still be constrained by experimentally-validated perturbation data, enhancing the biological interpretability while reducing the need for correlative associations that have been the traditional Achilles' Heel in clinical translation(60).

These integrated processes ultimately result in the discovery of clinically relevant vulnerabilities in cancer, such as context-specific essential genes, synthetic lethal interactions, microenvironment-independent therapeutic targets, and adaptive resistance mechanisms (61). The IFPO Framework calls for clinically relevant biomarkers to be based on experimentally demonstrated functional dependencies in the context of biological processes. Thus, the discovery of new biomarkers, prioritization of drug targets, patient stratification, and rational combination therapy are no longer fixed genomic classifications, but dynamic functions guided by functional evidence. Importantly, the framework also provides a streamlined feedback loop; clinical outcomes, long-term patient tracking, and functional validation inform subsequent perturbative studies and computational models. This "learning cycle" recognizes that a disease like cancer is a dynamic disease and that precision oncology needs to evolve in a way that isn't based solely on a single "snapshot" of a tumour's molecular profile taken at the time of diagnosis (62).

Together, the IFPO Framework facilitates conceptual movement from descriptive precision oncology to functional systems oncology, where complexity is not a barrier to therapeutic prediction, but rather a source of clinically relevant information. The framework brings together the principles of causal experimentation, single-cell systems biology, and computational intelligence, enabling the development of a single theoretical framework that goes beyond mutation-centric approaches to explain the heterogeneity, phenotypic plasticity, and therapeutic resistance of tumour cells. More importantly, it provides a testable conceptual basis for future longitudinal functional studies, standardized multimodal analytical pipelines, and adaptive precision medicine strategies. The IFPO Framework provides an overview of the functional perturbation technologies, spatial multi-omics, and explainable artificial intelligence (XAI) that will be essential for the next generation of personalized cancer therapy, as these tools become increasingly complex and multifaceted. It also provides a roadmap for the translation of increasingly complex biological information into clinically actionable knowledge, as spatial multi-omics, functional perturbation technologies, and explainable artificial intelligence (XAI) will become even more complex and multifaceted in the future.

Figure 1. The Integrated Functional Precision Oncology (IFPO) Framework illustrates the progression from cancer biological complexity through CRISPR-mediated functional perturbation, single-cell multi-omics profiling, and AI-driven computational integration to identify context-specific cancer vulnerabilities. These discoveries inform biomarker development, therapeutic target identification, patient stratification, and personalized therapy, while a continuous feedback loop enables functional validation and iterative refinement of precision oncology strategies.

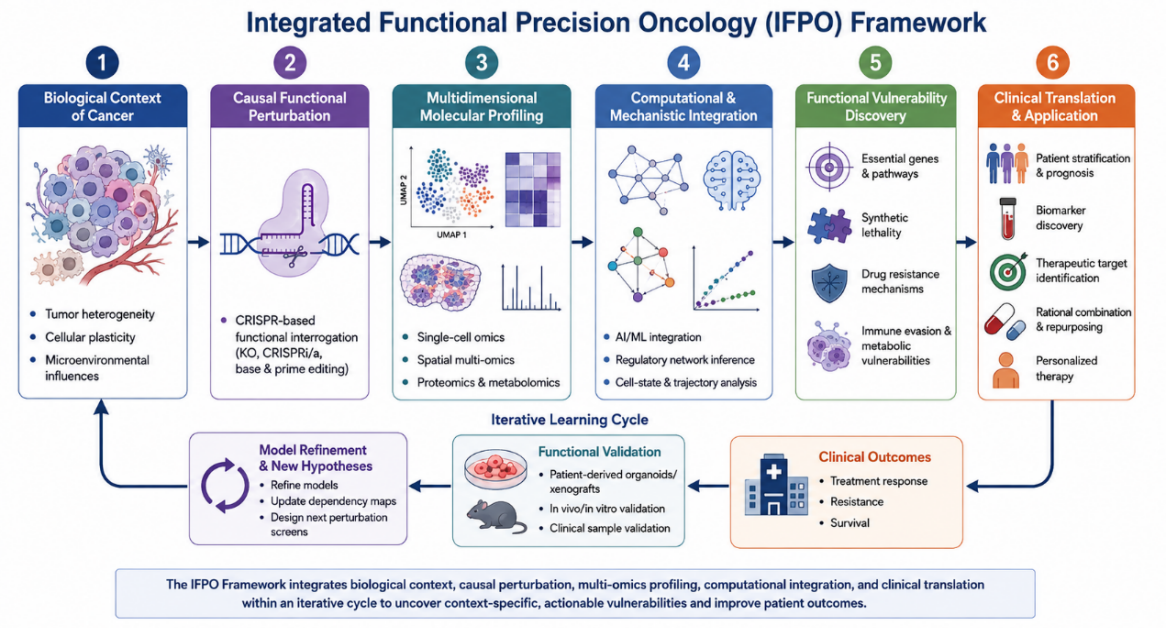

Fig 1. The Integrated Functional Precision Oncology (IFPO) Framework
Source: Author's own illustration

Current Challenges and Future Perspectives

While CRISPR functional genomics and single-cell multi-omics techniques have come a long way, they are still not fully integrated into precision oncology as there exists an important gap between technological capacities and biological and clinical interpretability. The question of results obtained under experimental conditions are reproducible and are biologically relevant across tumour types, cell states and clinical context is, therefore, the larger challenge(63). There is some variation in the efficiency of perturbations, in the performance of the guide-RNAs, and in the delivery systems and experimental protocols used in functional screening that can affect the results, and comparisons between studies may be further complicated by differences in single-cell processing and analysis(64).

Biological complexity is a similar challenge to its interpretation. Longitudinal studies have suggested that therapeutic resistance could be achieved via clonal selection or reversible phenotypic plasticity, but it is unclear which mechanisms are more important or whether their importance differs with the type of tumour, the selective pressure of the treatment and the context of the cells (65). This indicates that there is no single evolutionary model to use in interpreting resistance. Rather, genetic diversification and non-genetic state transitions can occur simultaneously, and thus longitudinal functional studies are needed that can separate out stable dependencies from transitory adaptive states. Therefore, any vulnerability detected in a cellular or experimental context should not necessarily be viewed as a universal therapeutic dependency(66).

Multimodal datasets get more difficult to analyze as they become increasingly dimensional, in that they must distinguish signals, which are biologically informative, from technical variation, and associations that are derived by modeling(67). While AI can enable multimodal integration and prediction, concerns about interpretability, reproducibility and cross-platform generalizability make it difficult to fully trust vulnerabilities computed entirely from data. This strengthens the need for computational models which are both experimentally constrained and independently validated and not just based on predictive performance(68).

The real-world application and use of the field is the final test of clinical implementation. Functional profiling will have to prove to be reproducible and offer a clear benefit compared to existing genomic profiling methods that must also address the requirements for assay standardization, analytical robustness, turnaround time, infrastructure and regulatory oversight and equitable access(69). As functional precision oncology integrates more complex computational and longitudinal data, the importance of ethical governance of genomic and clinical data will further grow. These emerging technologies, such as spatial multi-omics, in situ perturbation, the tracking of tumour cell lineages and more sophisticated computational models, could significantly extend the reach of tumour evolution resolution but only if they provide actionable information as opposed to merely adding to data complexity(70). Clinical adoption in the next five to ten years will have less to do with the complexity of technology and will rely more on the standardization of functional assays, prospective validation of results in a diverse patient population, more reproducible and interpretable computational methods, and scalable and cost effective workflows. Focusing on these areas will inform research priorities for functional vulnerabilities that offer clinical utility in addition to existing genomic approaches and for precision oncology in the clinic. Therefore, a focus on reproducible, causally informative and clinically actionable functional measures will be the key to the future of functional precision oncology, more so than technology accumulation, and validated integration. The "IFPO Framework" here provides a conceptual basis to connect the dots between biological context, causal perturbation, multimodal interpretation and clinical feedback and create an adaptive precision-oncology strategy(71).

Research Gaps

While the fields of CRISPR functional genomics and single-cell multi-omics are rapidly coming together, there are still knowledge gaps that stand in the way of their promise to revolutionize precision oncology(72). A major drawback is the fact that most functional studies are performed within a short time and will therefore not be able to provide information on the molecular response over the entire course of tumour evolution(73). The interplay between genetic changes, epigenetic modification, metabolic adaptation, immune interactions, and microenvironmental remodeling in space is therefore not completely understood. This limits the ability to identify early vulnerable states in the cell and transient therapeutic vulnerabilities, often observed before clinically detectable resistance(74). However, Longitudinal functional studies should be the goal of future research, and should incorporate the use of CRISPR perturbation, lineage tracing, and spatial multi-omics to build predictive models of tumour evolution, not static molecular

snapshots(75).Technological developments have also outstripped methodological standardization. With the advent of more sophisticated genome-editing platforms and multimodal single-cell technologies, there has been a significant increase in the number of ways to interrogate their functions; however, the efficiency of genome-editing, design of perturbations, depth of sequencing, spatial resolution, and experimental workflows remain variable across experiments, making reproducibility between studies challenging(76). Standardized experimental protocols, benchmark datasets, and quality control systems will be crucial to ensure the ability to reliably compare functional genomic data sets across labs and disease contexts.

Computational challenges remain equally significant. Although AI and multimodal machine-learning methods have significantly advanced the integration of data and the predictions of cancer vulnerability, there are still many models that are hard to interpret, poorly generalizable between independent datasets, and sensitive to batch effects and data heterogeneity(77). Future studies should thus focus on explainable AI, standardized computational workflows, interoperable data resources, and prospective validation to preserve the biological relevance and clinical confidence of computational predictions(78).

There are also key translation gaps that remain. A majority of studies that integrate CRISPR and single cell assays are experimental in nature and there are comparatively few studies showing potential clinical applications or use in therapeutic decision making(79). This will take multicenter clinical validation, harmonization of regulatory actions, scalable functional screening platforms, and incorporation of functional genomic data into precision oncology pipeline. Also, unanswered ethical and regulatory issues with genome editing, patient privacy, data ownership, algorithmic bias, equitable access and genome data governance will have an impact on future clinical implementation(80).

Lastly, there is a lack of a common conceptual framework that can help bring together causal perturbation, multidimensional molecular profiling, computational inference and clinical translation into a coherent precision oncology strategy(81). This is a key research need. The opportunities for achieving this integration are unprecedented thanks to emerging technologies: from spatial multi-omics and in situ perturbation screening, to CRISPR lineage tracing, biological foundation models, digital twins and explainable artificial intelligence. Their most important contribution, however, will be to create systems-level frameworks like the proposed Integrated Functional Precision Oncology (IFPO) Framework, which will be able to convert increasingly complex biological information to effective context-specific, clinically actionable therapeutic strategies.

Discussion

The emergence of CRISPR functional genomics and single-cell multi-omics represents a paradigm shift in precision oncology that turns the long-held belief that full molecular characterization is enough for therapeutic action on genomics into upside down(82). Traditional precision oncology has largely been based on descriptive genomics, where genomic, transcriptomic or epigenomic changes have been described and correlated with clinical outcomes(83). This approach has influenced the classification of cancers and helped to develop targeted therapies, but in some cases the genomic alteration has not been predictive of the requirement of the alteration for tumour survival(84). All the evidence collated in this review points to the concept that vulnerability of cancer is not a fixed property of the genome, but a state that is constantly changing, depending on the ongoing interactions between the tumour microenvironment, metabolic adaptation, epigenetic regulation and cellular plasticity, alongside the genetic alterations(85). Precision oncology is therefore moving beyond the era of molecular description towards defining context-dependent biological dependencies in an experimental manner.

This conceptual shift has profoundly changed the way tumour heterogeneity is being understood. Previous models treated intratumor heterogeneity as a biological noise that hindered the discovery of biomarkers, as well as the prediction of treatment. Integrated CRISPR perturbation and single-cell multi-omics, however, have shown that heterogeneity is biologically informative in that it dictates responses of individual cellular populations to the same genetic perturbation(86). Cellular diversity illustrates cell-cell connections and functional interdependencies that determine the evolution, metastasis, immune escape and drug sensitivity of a tumour. Thus, tumour heterogeneity is no longer considered a barrier to precision oncology, but rather the context in which clinically relevant vulnerabilities arise(87).

The combination of causal perturbation with high resolution molecular profiling has also radically changed our understanding of genotype-phenotype relationships. Traditional sequencing methods link mutations with disease phenotypes but do not identify

whether the mutations are causing disease progression or are a by-product(88). Functional genomics using CRISPR can overcome this limitation by experimentally probing gene function, whereas single-cell multi-omics can address the multidimensional nature of the molecular consequences of these perturbations in an individual cell(89). These technologies are combined to move beyond the identification of essential genes to the study of how the dynamics of the tumour ecosystem, transitions in cell states, and regulatory networks all contribute to a therapeutic response. This systems-level view is what makes it that genetically similar tumours often present with vastly different clinical responses and that the genomic changes alone are not good enough to predict therapeutic success(90).

The review also illustrates the profound impact of integrated functional genomics on current views of tumour evolution and treatment resistance. The classical evolutionary arguments have focused on the dominant idea that failure to respond to treatment is due to growth of pre-existing resistant clones that have acquired beneficial mutations(91). In contrast, the evidence from single-cell perturbation with CRISPR always demonstrates that resistance often occurs first through reversible, transcriptional, epigenetic and metabolic changes, prior to stable genetic changes. These findings do not rule out the clonal evolution, but rather suggest that adaptive cellular plasticity and genetic selection work together during the course of a disease(92). This holistic view has helped to give a more complete picture of the mechanism of minimal residual disease, therapeutic relapse, and the different responses seen between patients with similar molecular characteristics.

Furthermore, the functional perturbation, multimodal molecular profiling, and artificial intelligence revolution has changed the focus in precision oncology from providing molecular data to providing mechanistic and clinically actionable knowledge(93). Sequencing technologies are getting better and better, and data generation is now the main constraint. Rather, the use of computational methods has grown in importance to integrate multidimensional data, to reconstruct regulatory networks and to discover functional relationships that are biologically meaningful and clinically repeatable(94). The concept of explainable AI, multimodal integration and systems-based analytic approaches are thus a conceptual advance, not just a computing improvement, over functional genomics(95).

Although these developments have been made, significant hurdles remain to clinical translation. The inefficiency of genome editing, multimodal data integration, data standardization in computation, experimental reproducibility and prospective clinical validation all restrict genome editing as a routine tool(96). The challenges outlined highlight the importance of advancing integration of current technologies into integrated, standardized and clinically viable precision oncology processes rather than the development of new technologies. Addressing ethical governance, regulatory oversight, equitable access, and transparent artificial intelligence will be equally important for ensuring responsible implementation.

Overall, the evidence presented in this review indicates a shift from descriptive precision oncology towards functional systems oncology, in which clinically exploitable vulnerabilities emerge from causal experimentation in the framework of a dynamic tumour ecosystem(97). The concept is manifested in the proposed Integrated Functional Precision Oncology (IFPO) Framework, which unites CRISPR-mediated functional screening, single-cell multi-omics, artificial intelligence-based systems analysis, and clinical translation as a single model for the discovery of cancer vulnerabilities. The framework is not intended as stand-alone technologies, but rather as the parts of a "learning system" which continuously feed into and refine each other through functional interrogation, multidimensional molecular profiling, computational inference, and clinical validation. It offers a mechanistic guide to help discover therapeutic vulnerabilities in context and paves the way for precision oncology moving from molecular characterization to adaptive, predictive, and truly personalized cancer medicine.

Table 1. summarizes the principal challenges limiting the integration of CRISPR functional genomics and single-cell multi-omics in precision oncology, highlighting their associated limitations, key research priorities, and anticipated clinical implications. It provides a concise roadmap for future investigations required to accelerate the translation of functional cancer biology into clinically actionable precision oncology. Source: Author's summary.

Table 1. Current Challenges, Future Research Priorities, and Clinical Implications of Integrating CRISPR Functional Genomics with Single-Cell Multi-Omics in Precision Oncology

Current Challenge	Key Limitation	Future Priority	Potential Clinical Impact
Functional validation of cancer dependencies	Genomic profiling alone cannot distinguish driver dependencies from passenger alterations	Integrate CRISPR perturbation with longitudinal single-cell and spatial multi-omics	More accurate identification of actionable therapeutic targets
Tumour heterogeneity and evolutionary plasticity	Static molecular profiling poorly captures dynamic cellular states	Develop longitudinal and multimodal functional analyses	Improved prediction of tumour evolution and therapeutic response
Computational integration	High-dimensional datasets remain difficult to harmonize and interpret	Standardize multimodal analytical pipelines and develop explainable AI	More reliable biomarker discovery and clinical decision support
Clinical implementation	Functional profiling lacks standardized workflows and prospective validation	Establish scalable functional diagnostics and multicentre clinical trials	Broader adoption of personalized, functionally informed precision oncology

Conclusion

CRISPR functional genomics in combination with the single-cell multi-omics has transformed the field of precision oncology from descriptive molecular profiling to functional characterisation of cancer biology. This review highlights how causal genome perturbation and high-resolution molecular profiling offers unparalleled opportunities to detect context-specific cancer dependencies, beyond those that can be uncovered by conventional genomic approaches. These developments have greatly enhanced knowledge of tumour heterogeneity, functional vulnerability and the dynamic biological processes that drive cancer progression.

Importantly, the evidence reviewed in this review shows that the biggest future advancements in precision oncology will be achieved by combining individual technologies rather than their continued development. In this context, the proposed Integrated Functional Precision Oncology (IFPO) Framework offers a single conceptual model that connects cancer's biological complexity, functional perturbation, single-cell multi-omics, computational intelligence, and clinical translation into a seamless process for the discovery of cancer vulnerabilities and precision oncology.

While there are significant challenges in this area that need to be addressed, ongoing advances in genome editing, multimodal molecular profiling, artificial intelligence and longitudinal functional studies will help to speed the translation of functional discoveries into clinical practice. Overall, the combination of these complementary strategies could revolutionize precision oncology to a truly predictive, adaptive, and functionally informed field, leading to the identification of cancer vulnerabilities that can be acted upon in more precise ways, and the creation of personalized cancer therapies that are more effective.

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