Original Research Paper

Volume - 15 | Issue - 11 | November - 2025 | PRINT ISSN No. 2249 - 555X | DOI : 10.36106/ijar

Oncology

CRISPR- CAS SCREENING IN CANCER RESEARCH: METHODOLOGIES, APPLICATIONS, AND TRANSLATIONAL CHALLENGES

Ahana Patel

ABSTRACT CRISPR screening has revolutionized cancer research by enabling systematic, genome-wide interrogation of gene function to identify therapeutic vulnerabilities, resistance mechanisms, and immunotherapy determinants. This review examines methodological approaches for CRISPR screenings using the Cas9 systems, evaluating how system and methodology selection profoundly influence which biological mechanisms are revealed across therapeutic resistance, metastasis regulation, and immunotherapy response. Cas9-based screens-from simple one-cell-type (1CT) in vitro approaches to complex autochthonous in vivo systems-excel at identifying cell-autonomous DNA-level dependencies. Two-cell-type (2CT) co-culture approaches validate immune-dependent mechanisms, while in vivo screens capture physiological complexity and have identified clinically relevant targets including PTPN2, now in Phase 1 trials. Critical challenges include incomplete gene editing, false-positive and false-negative hits, context-dependent essentiality, and system-specific technical limitations. Emerging technologies integrating artificial intelligence, prime editing, single-cell profiling, and patient-derived models promise to address limitations while enabling functional precision medicine. This review provides a framework for selecting appropriate systems, interpreting results within methodological constraints, and integrating complementary approaches to maximize biological insight and translational impact.

KEYWORDS : CRISPR screening, Cas9, Cas12, Cas13, cancer functional genomics, therapeutic vulnerabilities, RNA-targeted screening, precision oncology

INTRODUCTION

The Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) system has revolutionized molecular biology since its adaptation as a genome-editing tool. Originally identified as a bacterial adaptive immune system that protects microorganisms from viral infections, CRISPR has been transformed into a powerful technology for precise genetic manipulation (Jansen et al., 2002; Barrangou et al., 2007). The CRISPR-Cas9 system, derived from Streptococcus pyogenes, consists of two essential components: the Cas9 protein, which acts as molecular scissors to cut DNA, and a guide RNA (gRNA) that directs Cas9 to specific genomic locations (Jinek et al., 2012). When Cas9 creates a double-stranded break at the target site, cellular repair mechanisms either disrupt the gene through error-prone repair or insert new genetic sequences through template-directed repair (Hustedt & Durocher, 2016). This programmable specificity-achieved simply by changing the 20-nucleotide guide sequence-has made CRISPR-Cas9 far more accessible and versatile than previous genome-editing technologies such as zinc-finger nucleases and transcription activator-like effector nucleases (Lander, 2016).

The Critical Role Of CRISPR Screening In Cancer Research

Cancer remains one of the most complex challenges in modern medicine, driven by intricate networks of genetic alterations, cellular interactions, and adaptive resistance mechanisms. Understanding which genes drive cancer initiation, progression, metastasis, and therapy resistance requires systematic interrogation of gene function across the entire genome. CRISPR-Cas9 screening has emerged as an indispensable approach for this task, enabling researchers to systematically disable, activate, or modify thousands of genes simultaneously and observe the resulting effects on cancer cell behaviour (LaFleur & Sharpe, 2022).

Unlike hypothesis-driven studies that examine one or a few genes at a time, CRISPR screens take an unbiased, genome-wide approach. By introducing libraries containing thousands of guides RNAs—each targeting a different gene—into cancer cell populations, researchers can identify which genetic perturbations alter specific cancer-relevant phenotypes (Koike-Yusa et al., 2014; Zhou et al., 2014). These phenotypes include cellular proliferation, survival under drug treatment, immune evasion, metastatic potential, and response to microenvironmental signals. Cells carrying guide RNAs that disrupt essential genes will be depleted from the population, while those that confer selective advantages will be enriched. By sequencing the guide RNAs present in different cell populations and comparing their relative abundance, researchers can identify genes that significantly impact the phenotype of interest (Chen et al., 2015).

This systematic approach has already yielded critical insights into cancer biology. CRISPR screens have identified previously unknown regulators of immune checkpoint expression, revealing why some tumours evade immune surveillance (Burr et al., 2017). They have uncovered genetic dependencies that vary across cancer subtypes, exposing potential therapeutic vulnerabilities (Tzelepis et al., 2016; Behan et al., 2019). They have mapped resistance mechanisms to targeted therapies and immunotherapies, suggesting combination treatment strategies (Manguso et al., 2017). Perhaps most importantly, CRISPR screens have revealed that cancer dependencies extend far beyond well-known oncogenes and tumour suppressors, implicating metabolic enzymes, epigenetic regulators, RNA-binding proteins, and genes involved in protein homeostasis (Wei et al., 2019; Wang et al., 2020). These discoveries have fundamentally expanded our understanding of what makes cancer cells vulnerable.

Advantages Over RNA Interference-Based Screening

CRISPR screening surpasses RNAi by achieving complete gene disruption rather than partial knockdown, eliminating false negatives from residual gene activity and enabling reliable identification of essential genes (Morgens et al., 2016). It also offers higher specificity, as RNAi often triggers off-target effects through unintended transcript binding, while CRISPR's off-targets are rarer and mitigated by precise guide design. Moreover, CRISPR's consistent genotypes enhance reproducibility, and its versatility extends to functional analysis of noncoding regions and use in CRISPRa and CRISPRi systems for gain- or reversible loss-of-function studies, offering unmatched flexibility in genomic research (Qi et al., 2013; Gilbert et al., 2014).

CRISPR-Cas9 Screening Methodologies

This section examines the principal CRISPR-Cas9 screening methodologies, their applications in cancer research, and the comparative advantages that guide strategic implementation.

Pooled Knockout Screening: Fundamental Principles

Pooled CRISPR knockout screening is the core technique for large-scale functional genomics in cancer research. It uses libraries of guide RNAs (gRNAs), each targeting a specific gene, typically with multiple gRNAs per gene to ensure efficient knockout and internal validation (Shalem et al., 2014; Koike-Yusa et al., 2014). Delivered via lentiviral vectors, gRNAs and Cas9 integrate into cancer cell genomes, resulting in random, stable gene disruptions. After applying selection pressure, gRNAs linked to essential genes are depleted, while those conferring resistance or growth advantage are enriched. Sequencing of gRNA abundance before and after selection identifies gene-level phenotypic effects (Hsu et al., 2014; Doudna & Charpentier, 2014). Genome-wide libraries containing 60,000–90,000 gRNAs enable interrogation of nearly all human protein-coding genes in one experiment, with statistical algorithms comparing differential gRNA representation across conditions.

In Vitro Single-Cell Type (1CT) CRISPR Screening

Single-cell-type (1CT) CRISPR screening focuses solely on cancer cells, emphasizing cell-autonomous regulators of proliferation, metabolism, or drug response (Liu et al., 2020). It is simple, cost-

effective, and widely accessible, using standard cell culture methods. A major application involves identifying regulators of immune recognition molecules such as MHC-I or PD-L1 through flow cytometry-based selection. Whole-genome 1CT screens have uncovered negative regulators of the MHC-I pathway, revealing mechanisms of immune evasion. Despite its utility, 1CT screening cannot capture interactions requiring other cell types, such as immune-mediated processes.

In Vitro Two-Cell Type (2CT) CRISPR Screening

Two-cell-type (2CT) CRISPR screening extends 1CT methods by introducing direct interactions between cancer and immune cells. It involves co-culturing perturbed cancer cells with cytotoxic T cells or natural killer cells to identify genes modulating immune susceptibility (Dong et al., 2022; Buquicchio & Satpathy, 2021). Experimental design ensures selective pressure arises from immune activity, often using cancer cells engineered to express defined antigens like NY-ESO-1 or ovalbumin. After co-culture, surviving cancer cells are analysed to identify gRNAs affecting immune-mediated killing (Pan et al., 2018; Shi et al., 2023). 2CT screens have revealed key roles for MHC-I and interferon-γ pathway genes in T cell recognition and cytotoxicity. They provide greater physiological relevance for immunotherapy research by directly modelling cancer–immune dynamics.

Transplantation-Based In Vivo CRISPR Screening

Transplantation-based or indirect in vivo CRISPR screening introduces genetically perturbed cancer cells into immunocompetent mice to assess gene function under physiological conditions (Chow & Chen, 2018). After tumour establishment, selective pressures such as immune checkpoint blockade (anti-PD-1, anti-PD-L1, or anti-CTLA-4) are applied. Sequencing of gRNAs from treated and control tumour's identifies genes influencing therapy response (Manguso et al., 2017). These screens validate known immunoregulatory targets like PD-L1, CD47, and interferon-γ pathway components (Ifngr1, Ifngr2, Stat1, Jak1, Jak2), while discovering novel resistance mediators such as PTPN2, ADAR1, and ASF1A (Manguso et al., 2017). ADAR1 loss, for instance, remodels the tumour immune microenvironment by reducing suppressive myeloid and neutrophil populations and increasing cytotoxic T, γδ T, and NK cell infiltration (Ishizuka et al., 2019). Transplantation-based screening thus allows integration of immune complexity and therapeutic modulation, identifying mechanisms dependent on the in vivo tumour microenvironment.

CRISPR Activation And Interference Screening

CRISPR activation and interference (CRISPRa/i) expand functional genomics by enabling reversible gene modulation without DNA cleavage. Catalytically inactive Cas9 (dCas9) fused to transcriptional domains directs guide RNAs to gene promoters for activation or silencing (Qi et al., 2013; Gilbert et al., 2014). CRISPRa uses activators like VP64 or p300 to upregulate target genes, while CRISPRi employs repressor domains such as KRAB to suppress transcription (Konermann et al., 2015). CRISPRa screening identifies genes whose enhanced expression alters cancer phenotypes or mediates drug resistance, complementing knockout screens that reveal loss-of-function dependencies. CRISPRi provides reversible inhibition suitable for studying essential or haploinsufficient genes. Together, CRISPRa/i offer flexibility, reversibility, and compatibility with temporal control systems, allowing fine-tuned analysis of gain- and loss-of-function pathways.

Base Editing Screening

Base editing refines CRISPR screening by enabling single-base substitutions without double-strand breaks. These systems use Cas9 nickase or dead Cas9 fused to deaminases that convert specific bases: cytosine base editors (CBEs) induce C- G to T- A changes, adenine base editors (ABEs) enable A- T to G- C transitions, and C-to-G base editors (CGBEs) produce transversions (Komor et al., 2016; Gaudelli et al., 2017; Kurt et al., 2021). This precise editing allows systematic introduction or correction of disease-relevant mutations, modelling oncogenic variants or generating loss- and gain-of-function alleles at single-nucleotide resolution (Hanna et al., 2021). Large-scale base editor screens have mapped functional consequences of mutations within DNA repair and drug response pathways, identifying variants conferring resistance or sensitivity to therapies like PARP inhibitors (Cuella-Martin et al., 2021; Coelho et al., 2023). Base editing offers high precision, reduced genomic toxicity, and relevance for modelling clinically significant variants that dominate cancer genomes.

Critical Comparison Of Screening Approaches

Selecting an appropriate CRISPR screening strategy depends on experimental objectives, resources, and desired biological relevance. Pooled knockout screens provide comprehensive loss-of-function data, while CRISPRa/i systems enable reversible modulation and essential gene analysis. Base editing adds precision by targeting specific mutations. Together, these complementary approaches offer a versatile framework for dissecting gene function, pathway regulation, and therapeutic vulnerabilities in cancer systems.

Table 1. Comparative Analysis Of CRISPR-Cas9 Screening Methodologies

Criterion	1CT Screening	2CT Screening	Transplantation In Vivo	Autochthonous In Vivo	CRISPRa/i	Base Editing
Optimal for identifying	Cell-autonomous dependencies, intrinsic drug resistance	Cancer-immune interactions, immunotherapy targets	Microenvironment-dependent mechanisms, checkpoint therapy resistance	Tumour initiation drivers, immune evolution, metastasis regulators	Transcriptional dependencies, tumour suppressors (CRISPRa), partial LOF (CRISPRi)	Variant function, specific mutations, gain-of-function variants
Physiological relevance	Low	Moderate	High	Highest	Low-Moderate	Low-Moderate
Technical complexity	Low	Moderate	High	Highest	Moderate	High
Throughput	Highest	High	Moderate	Low	High	Moderate
Time required	2-4 weeks	4-6 weeks	2-4 months	6-12 months	3-5 weeks	3-6 weeks
Cost	Low	Moderate	High	Highest	Low-Moderate	Moderate-High
Captures immune interactions	No	Limited (binary)	Yes (full complexity)	Yes (with immune evolution)	No	No
Gene coverage	Genome-wide	Genome-wide	Genome-wide	Typically focused	Genome-wide	Gene/pathway-focused
Mutation type	Complete knockout	Complete knockout	Complete knockout	Complete knockout	Transcriptional modulation	Specific point mutations
Reversibility	No	No	No	No	Yes	No
Primary applications	Essential gene ID, synthetic lethality, surface protein regulators	T cell killing resistance, NK cell evasion, antigen presentation	ICB resistance, TME dependencies, therapy response	Oncogene cooperation, metastasis, immune editing	Essential genes, epigenetic regulators, resistance via overexpression	Variant pathogenicity, allele-specific effects, precision mutation modelling
Key limitations	Misses' microenvironment effects	Artificial antigen systems, limited to binary interactions	Transplantation artifacts, requires syngeneic models	Variable delivery, long timelines, clonal complexity	Weaker effects than knockout, guide position-dependent	Limited to specific base changes, bystander edits, lower efficiency

Representative discoveries	PD-L1 regulators (CMTM6), CD47 pathway (QPCTL)	IFN-γ signalling in T cell killing, MHC-I pathway components	PTPN2, ADAR1, immune checkpoint modulators	KMT2D as immunotherapy regulator	CASP8AP2 in lung cancer, XIST/FOXP3 epigenetic control	PARP inhibitor resistance variants, TP53 functional variants

Applications Across Cancer Types

This section examines how different screening methodologies have illuminated three critical areas of cancer biology: mechanisms of therapeutic resistance, regulators of metastasis and invasion, and determinants of immunotherapy response.

Table 2. Comparative Insights: Screening Modalities for Therapeutic Resistance Mechanisms

Aspect	1CT Screens	2CT Screens	In Vivo Screens
Primary resistance mechanisms detected	Cell-autonomous adaptations: target mutations, compensatory pathway activation, metabolic rewiring, apoptosis evasion	Immune-mediated protection: enhanced immune evasion, altered antigen presentation under drug pressure	Microenvironment-mediated resistance: stromal growth factor secretion, ECM remodelling, immune suppression, vascular protection
Representative discoveries	PHGDH in HCC resistance, Haspin/Aurora-A synthetic lethality, farnesyltransferase in sunitinib resistance	Interferon pathway alterations conferring resistance to immune-enhanced therapies	TGFβ3 in palbociclib resistance (TNBC), stromal-mediated EGFR inhibitor resistance
Advantages for resistance studies	Rapid identification, high throughput, direct drug-target interactions, metabolic adaptation mapping	Validates immune-dependent resistance, models immunotherapy combinations	Captures stromal protection, identifies biomarkers predictive of clinical resistance
Limitations for resistance studies	Misses' microenvironment-mediated protection, overestimates single-agent efficacy	Limited to binary immune interactions, artificial antigen systems	Time-intensive, expensive, requires syngeneic models
Optimal application	Initial resistance mapping, target validation, synthetic lethality screens, metabolic dependencies	Immunotherapy resistance requiring T cell or NK cell validation	Clinical resistance mechanisms, combination therapy optimization, biomarker discovery
Translation to clinic	Identifies druggable targets (e.g., PHGDH inhibitors) but requires in vivo validation	Moderate—requires confirmation that binary interactions reflect complex TME	High—best predictor of clinical resistance mechanisms
False discovery considerations	High rate of mechanisms that don't operate in vivo	Mechanisms may not generalize beyond specific immune effector tested	Artifacts from transplantation stress, non-physiological tumour growth kinetics

Metastasis, the leading cause of cancer mortality, involves complex cellular adaptations that enable tumour spread and colonization of distant organs. CRISPR screening has become a powerful method to uncover regulators of this process by systematically interrogating genes that control invasion, survival in circulation, and tissue colonization. These approaches allow unbiased exploration of the genetic and molecular networks driving metastatic competence.

Identifying Metastasis Regulators Through Loss-of-Function Screens

Genome-wide CRISPR-Cas9 loss-of-function screens comparing guide RNA abundance between primary tumours and metastatic lesions have identified key genes influencing metastatic potential. Such studies have validated known metastasis regulators while uncovering novel genes involved in adhesion, motility, and immune evasion (Chen et al., 2015; Kiessling et al., 2016).

Temporal screening designs further distinguish early dissemination factors from those required for metastatic outgrowth, revealing stage-specific genetic dependencies that shape disease progression.

Tissue-Specific Metastatic Dependencies

Autochthonous in vivo CRISPR screens have illuminated how metastatic requirements differ across target organs. Distinct microenvironmental conditions-such as extracellular matrix composition, growth factors, immune activity, and metabolic resources-create organ-specific selective pressures. Consequently, genes essential for lung colonization may be irrelevant for liver or bone metastasis, highlighting the need to evaluate metastatic regulators within physiologically matched models.

Integration with Single-Cell Technologies

The coupling of CRISPR screening with single-cell RNA sequencing techniques like Perturb-seq and CROP-seq enables simultaneous tracking of genetic perturbations and transcriptional states. This integration reveals transcriptional heterogeneity within tumour's and identifies subpopulations poised for metastasis. Single-cell CRISPR approaches have shown that only specific cellular subsets possess the molecular programs for successful distant colonization, providing high-resolution insight into how genetic alterations modulate metastatic potential (Shi et al., 2023).

Table 3. Comparative Insights: Screening Approaches For Metastasis Regulators

Aspect	1CT In Vitro Screens	In Vivo Transplantation Screens	Autochthonous In Vivo Screens
Metastatic processes captured	Invasion, migration, EMT in culture conditions	Survival in circulation, extravasation, initial colonization of pre-determined sites	Spontaneous dissemination, organ-specific colonization, dormancy, long-term outgrowth
Temporal resolution	Single timepoint (typically invasion/migration assays)	Early colonization (weeks to months post-transplantation)	Full metastatic cascade (months of tumour evolution)
Organ specificity	None—generic invasion phenotypes	Limited—injection route determines site, not biological tropism	High—reveals natural organ tropism and tissue-specific dependencies
Representative applications	Identification of genes promoting invasion in transwell or 3D culture	Comparing primary tumour vs. metastatic lesion guide RNA abundance	Identifying genes required for spontaneous metastasis to specific organs
Advantages for metastasis research	Rapid, scalable, mechanistic clarity for specific steps	Physiological circulation, immune interactions, multi-step process	Captures full cascade, natural tissue tropism, immune editing during dissemination
Limitations for metastasis research	Poor correlation between in vitro invasion and in vivo metastasis	Artificial injection bypasses early dissemination steps, site determined by injection not biology	Extended timelines (6-12 months), low metastatic burden complicates sequencing, variable penetrance
Correlation with clinical metastasis	Weak—many in vitro hits don't affect in vivo metastasis	Moderate—captures mid-to-late steps but misses initiation	Highest—recapitulates clinical metastatic patterns
Integration with single-cell technologies	Limited value—bulk invasion phenotypes	Valuable—can identify metastasis-initiating cell states	Critical—reveals rare metastatic cell populations and state transitions
Cost and timeline	Low cost, 1-2 weeks	Moderate cost, 2-4 months	High cost, 6-12 months

Comparative Screening Approaches For Metastasis

CRISPR screening has transformed understanding of why only some tumours respond to immune checkpoint blockade, revealing genetic and epigenetic mechanisms that determine immune recognition and therapy resistance. By applying diverse screening strategies, researchers have uncovered regulators of immune signalling, antigen presentation, and microenvironmental interactions critical to immunotherapy response.

One-cell-type Screens For Immune Evasion Mechanisms

Single-cell-type (1CT) CRISPR screens, although limited to cancer-intrinsic factors, have identified key regulators of immune recognition molecules. Flow cytometry-based 1CT screens for MHC-I expression have mapped both established and novel components of the antigen presentation pathway, elucidating how tumours evade T cell recognition (Burr et al., 2019; Gu et al., 2021). Similarly, 1CT approaches uncovered modulators of immune checkpoint molecules themselves. For example, CMTM6 stabilizes PD-L1 surface expression, revealing a potential therapeutic target for enhancing checkpoint blockade (Burr et al., 2017). Another screen identified QPCTL as a regulator of the CD47–SIRPα "don't eat me" signal, providing new avenues for targeting macrophage-mediated immune evasion (Wu et al., 2019). These studies demonstrate how intrinsic genetic programs can shape tumour immunogenicity.

Two-Cell-Type Screens for Direct Immune Interactions

Two-cell-type (2CT) CRISPR screens advance beyond 1CT approaches by directly modelling cancer–immune interactions. Co-culturing genetically perturbed cancer cells with antigen-specific T cells has clarified that loss of MHC-I or interferon-γ pathway genes confers resistance to cytotoxicity, confirming their essential roles in immune recognition (Pan et al., 2018). Such controlled systems allow dissection of how defined genetic changes modulate T cell-mediated killing independent of tumour growth or stromal influences. While reductionist, 2CT screening remains invaluable for mechanistic validation of immune regulatory pathways.

In Vivo Screens For Checkpoint Blockade Response

Transplantation-based in vivo CRISPR screens in immunocompetent mouse models treated with anti-PD-1 or anti-CTLA-4 antibodies have established the current benchmark for identifying immunotherapy determinants. These screens capture full tumour–immune complexity while maintaining genetic precision. The discovery of PTPN2 as a regulator of checkpoint response illustrates their translational potential: PTPN2 loss enhances tumour immunogenicity and sensitivity to checkpoint blockade. This finding led to clinical evaluation of an inhibitor, ABBV-CLS-484, now in Phase 1 trials based directly on CRISPR results (Manguso et al., 2017; Baumgartner et al., 2023). In vivo screening thus directly informs therapeutic development by pinpointing actionable immune modulators.

Autochthonous Screens for Tumour Immunogenicity

Autochthonous CRISPR screening further extends these insights by examining gene function in tumours developing de novo under natural immune surveillance. Such models capture dynamic immune editing processes that shape tumour immunogenicity. Identification of the histone methyltransferase KMT2D as a major determinant of checkpoint therapy response revealed how chromatin state regulates immune sensitivity. KMT2D loss alters expression of antigen presentation and immune recruitment genes, illustrating that epigenetic reprogramming can drive immune evasion (Wang et al., 2020). These findings emphasize the value of endogenous tumour models for uncovering context-dependent and evolutionarily selected mechanisms of immunotherapy resistance.

Together, these CRISPR screening modalities-from 1CT and 2CT in vitro systems to transplantation and autochthonous in vivo models-provide an integrated framework for dissecting the genetic, signalling, and epigenetic networks governing immune checkpoint response, guiding both mechanistic understanding and therapeutic innovation.

Comparative Insights Across Immunotherapy Screening Approaches

Table 4. Comparative Insights: Screening Modalities For Immunotherapy Response Determinants

Aspect	1CT Screens	2CT Screens	Transplantation In Vivo Screens	Autochthonous In Vivo Screens
Primary mechanisms identified	Regulators of immune recognition molecules (MHC-I, PD-L1, CD47), antigen processing machinery	Direct determinants of immune cell killing, antigen presentation functionality, resistance to cytotoxicity	Checkpoint blockade resistance/sensitivity, multi-cellular immune evasion, therapeutic response modulators	Immunogenicity regulators, immune editing drivers, epigenetic determinants of immunotherapy response
Immune complexity captured	None—cancer cells only	Binary (one cancer + one immune cell type)	Full immune repertoire (T cells, B cells, NK cells, myeloid populations, stromal cells)	Full immune complexity plus temporal immune editing
Checkpoint therapy relevance	Indirect—identifies molecule regulators but not therapy response	Limited—can model immune killing but not checkpoint biology	Direct—screens performed under checkpoint blockade	Direct—captures response in spontaneous tumours
Representative discoveries	CMTM6 (PD-L1 stabilizer), QPCTL (CD47-SIRPα modulator), MHC-I pathway components	MHC-I and IFN-γ pathway genes essential for T cell recognition and killing	PTPN2, ADAR1, ASF1A as checkpoint blockade resistance mechanisms	KMT2D as epigenetic regulator of immunotherapy sensitivity
Validation requirements	High—requires 2CT and in vivo confirmation of immune function	Moderate—requires in vivo confirmation under checkpoint therapy	Low—directly tests therapy response but species-specific validation needed	Low—highest physiological relevance but limited by model availability
Advantages for immunotherapy research	High throughput, identifies targetable regulators, flow cytometry enables precise phenotyping	Validates functional immune consequences, controlled binary interactions enable mechanistic study	Physiologically relevant immune complexity, tests actual checkpoint therapy, identifies microenvironment dependencies	Captures immune editing, epigenetic mechanisms, spontaneous tumour immunogenicity
Limitations for immunotherapy research	Cannot confirm functional immune impact, misses microenvironment and checkpoint biology	Artificial antigen systems, limited to one immune effector type, misses checkpoint blockade context	Transplantation artifacts, pre-formed tumours skip immune editing, restricted to murine models	Extended timelines, low throughput, variable tumour development, complex analysis
Clinical translation potential	Moderate—identifies targets but requires extensive validation (e.g., CMTM6)	Moderate—binary interactions may not reflect TME complexity	High—PTPN2 inhibitor in clinical trials based on screen results	High—but limited by model availability and timeline
Complementarity with other approaches	Foundation for hypothesis generation, identifies candidates for validation in more complex systems	Validates 1CT hits, bridges to in vivo studies	Confirms 1CT and 2CT mechanisms in physiological context, tests therapeutic relevance	Identifies mechanisms operating during tumour evolution missed by other approaches

Challenges And Limitations

Despite the transformative role of CRISPR screening in cancer research, significant technical, biological, and analytical barriers continue to constrain precision, reproducibility, and therapeutic translation. Understanding these challenges is essential for designing robust studies, interpreting results, and prioritizing hits for downstream validation.

Technical Challenges Specific To Screening Applications

Guide RNA Library Design and Coverage

Screen performance depends heavily on guide RNA (gRNA) library quality, yet efficient design remains difficult. Effective screens require multiple gRNAs per gene to ensure reliable knockouts, increasing library size and sequencing demands for genome-scale analyses (Doench, 2018). gRNA efficiency varies with sequence, chromatin accessibility, and cell type, while current predictive models remain imperfect. Inefficient targeting can cause false negatives by missing essential genes (Doench et al., 2016).

Incomplete Gene Editing And Mosaic Knockouts

Variable Cas9 expression, monoallelic editing, or DNA repair heterogeneity often led to incomplete gene disruption. This mosaicism weakens signal detection and produces false negatives, particularly when complete knockouts are lethal and selected against (Aguirre et al., 2016). Efforts to improve editing uniformity-using base or prime editors, fluorescent reporters for complete edits, or computational adjustments-show promise but remain experimentally demanding (Hanna et al., 2021).

False Positives And False Negatives

False positives can result from DNA damage toxicity at highly expressed loci, off-target cutting, or copy number amplification causing excessive cleavage (Munoz et al., 2016). False negatives arise from incomplete editing, gene redundancy, or early essentiality (Hart et al., 2015). Complementary methods, including CRISPRi, CRISPRa, and orthogonal screens, can mitigate biases though they increase experimental and analytical complexity.

Library Representation and Bottlenecking

Maintaining uniform gRNA representation across all target sequences is critical for data reliability. Population bottlenecks-when cell numbers drop near the scale of library size-introduce stochastic noise, causing loss of signal fidelity (Aguirre et al., 2016). High-selective pressures or small in vivo populations further reduce coverage, limiting hit reproducibility. Autochthonous models face added challenges such as variable transduction efficiency and clonal heterogeneity, often detecting fewer reproducible hits than in vitro screens.

Biological Complexity and Context-Dependence

Tumour Heterogeneity And Clonal Dynamics

Tumour heterogeneity complicates interpretation, as gene essentiality varies across genetic backgrounds, oncogenic drivers, and lineages (Behan et al., 2019). For example, DNA breaks in p53-proficient cells activate checkpoint responses, altering Cas9 editing efficiency and biasing results compared to p53-null models (Haapaniemi et al., 2018; Ihry et al., 2018). Within tumours, subclones exhibit divergent dependencies; single-cell CRISPR screens can resolve this variation but remain complex and resource-intensive (Shi et al., 2023).

Microenvironmental Context And Cell-extrinsic Effects

Most in vitro screens omit microenvironmental influences central to tumour biology. In vivo, gene essentiality is shaped by immune interactions, nutrient competition, and extracellular matrix cues. Consequently, some genes critical for tumorigenesis in vivo appear nonessential in culture, as in microenvironment-dependent drug resistance such as TGFβ3-mediated palbociclib resistance (Poulet et al., 2024). Two-cell-type and in vivo screens incorporate external interactions but still face challenges modelling human-specific immune and stromal conditions. Humanized and patient-derived systems enhance physiological relevance despite added complexity.

Temporal Dynamics And Adaptive Responses

Static endpoint analyses miss adaptive responses that evolve over time. Following gene disruption, cancer cells may reprogram transcriptional or metabolic networks to restore viability. Timepoint variation is particularly critical in drug resistance studies: constant drug exposure reveals delayed adaptive resistance, while short exposures reveal immediate effects. Time-course designs could capture these dynamics by measuring gRNA abundance longitudinally, though such approaches require greater technical effort and cost.

Data Analysis And Interpretation Challenges

Statistical Challenges In Hit Calling

With tens of thousands of genes tested simultaneously, appropriate statistical control is complex. Multiple hypothesis testing increases false discovery risk, and limited replicates reduce statistical power (Li et al., 2014). Algorithms like MAGeCK and BAGEL integrate multiple gRNAs per gene to improve detection, but discrepancies persist due to different modelling assumptions (Hart & Moffat, 2016). Lack of standard thresholds for fold-change and significance leads to variability in published hit lists.

Integrating Screening Data With Multi-omics Information

Mechanistic interpretation demands contextualizing CRISPR hits within genomic, transcriptomic, and proteomic frameworks. Yet integrated analytical tools remain limited. Data type mismatches-such as static transcriptomic profiles versus endpoint phenotypic data-further complicate causal inference. Single-cell CRISPR approaches like Perturb-seq, CROP-seq, and ECCITE-seq (Shi et al., 2023) directly link perturbations to transcriptional outcomes but face high noise, sparse detection, and heavy computational requirements.

Distinguishing Direct From Indirect Effects

CRISPR hits may represent true effectors or indirect responses to stress or compensation. This distinction is vital in complex contexts such as immunotherapy response, where hits can influence cell survival indirectly. Network and pathway analyses help identify meaningful clusters of hits, but incomplete pathway annotations and reliance on correlation limit causal interpretation. Mechanistic follow-up remains essential to confirm direct roles.

Translating Screening Hits to Therapeutic Targets

Moving from hit discovery to drug development remains slow. Many essential genes are undruggable or exhibit unacceptable toxicity when inhibited. Notable successes, like the identification of PTPN2 from immunotherapy screens leading to a clinical-stage inhibitor (Baumgartner et al., 2023), are exceptions rather than the norm. Most hits require cross-model validation, toxicity assessment, and drug design efforts, creating high attrition. Effective prioritization should weigh gene effect size, druggability, mechanistic relevance, and therapeutic index, yet the field lacks standardized frameworks. This gap fosters bias toward known, tractable pathways and highlights the need for predictive tools to identify clinically translatable targets.

Future Directions And Technological Advances

CRISPR-Cas9 screening continues to evolve rapidly, with innovations addressing current limitations and enabling new avenues in cancer research and therapy discovery.

Computational and Machine Learning Innovations

AI-Driven Guide RNA Design and Optimization

Artificial intelligence is transforming guide RNA (gRNA) design by improving prediction of context-specific editing efficiency. Traditional algorithms rely on small datasets, whereas emerging models incorporate additional features like chromatin accessibility, epigenetic state, transcriptional activity, and DNA repair preferences (Chuai et al., 2018). Deep learning tools such as DeepCRISPR and CRISPR-DO outperform rule-based systems by reducing poorly performing gRNAs, though cell-type-dependent variability remains challenging (Ma et al., 2016; Barozzi et al., 2024). New models integrating ATAC-seq, histone marks, and gene expression promise context-optimized predictions. Machine learning also enables intelligent library design that maximizes genome coverage while minimizing size. Adaptive algorithms select minimal, high-confidence gRNAs and include backup guides only when needed, potentially reducing required cell numbers and sequencing depth. This approach could democratize large-scale screens, making them feasible in smaller laboratories.

Predictive Models for Hit Validation and Target Prioritization

Another major application of AI is predicting which CRISPR hits are likely to become viable drug targets before committing to extensive validation. Models integrating functional screen data with structural biology (predicting druggability), expression atlases (toxicity risk), clinical genomics (cancer-specific dependencies), and drug libraries

(repurposing potential) aim to prioritize targets by therapeutic promise rather than statistical score (Pacesa et al., 2024).

Early studies combining CRISPR results with expression, structural, and patient outcome data have identified context-dependent essential genes more effectively. Integrating data from hundreds of cell lines with tumour genomics supports precision medicine by revealing dependencies tied to particular genetic landscapes.

Advanced Precision Editing Technologies

Prime Editing for Screening Applications

Prime editing represents a leap in genomic precision, allowing all possible base substitutions and small insertions or deletions without double-strand breaks or donor templates. It employs a Cas9 nickase–reverse transcriptase fusion directed by pegRNAs that define both target and edit sequences (Anzalone et al., 2019). This enables comprehensive mutational scanning across coding regions to map variant-function relationships, improving interpretation of genomic data (Zeng et al., 2024).

Despite its precision, prime editing faces obstacles such as lower efficiency (10–30% versus 70–90% for standard CRISPR), large construct size, and complex pegRNA design. Ongoing improvements in enzyme engineering, delivery methods, and pegRNA optimization are expected to make prime editing screens more practical and scalable for cancer research.

Dual-Modification Screening Systems

Combined modalities now permit simultaneous gene knockout, activation, or editing within one experiment. Dual CRISPR platforms using both Cas9 and dCas9 effectors enable disruption of one gene while activating or repressing another (Cui et al., 2022). Such multiplex systems elucidate genetic interactions, compensatory pathways, and synthetic lethality that traditional single-gene screens overlook.

However, combinatorial screening scales quadratically, making exhaustive pairwise analyses resource-intensive (e.g., 1,000 genes = 500,000 pairs). Distinguishing genuine interactions from additive effects requires robust statistics and high coverage. Focused pairwise screens targeting defined pathways or drug-resistance networks are increasingly feasible as computational and experimental capacities expand.

Enhanced In Vivo Screening Methodologies

Improved Viral Vectors for Autochthonous Screening

Autochthonous in vivo screens remain limited by inefficient gene delivery. Engineered adeno-associated virus (AAV) variants generated through directed evolution demonstrate improved tissue tropism, enhanced transduction, and lower immunogenicity, paving the way for tissue-specific perturbations (Wang et al., 2024). AAV variants that cross the blood–brain barrier or target liver tissue expand opportunities for cancer studies in diverse organs. Tissue-specific Cas9 expression further allows distinction between cell-autonomous and microenvironmental effects.

Lentiviral vectors, with higher cargo capacity and stable integration, suit long-term expression and delivery of larger systems like base or prime editors. Yet they depend on dividing cells, limiting applications in quiescent tissues. Hybrid delivery strategies-AAV for Cas9 with lentiviral gRNA libraries or integration-deficient lentiviruses-could merge the advantages of both systems.

Spatially Resolved In Vivo Screening

Next-generation in vivo CRISPR methods incorporate spatial information to map genetic dependencies within tissue architecture. Tumour heterogeneity includes spatial gradients such as hypoxia, nutrient limitation, and immune cell proximity. Spatially barcoded gRNA libraries combined with spatial transcriptomics can reveal positional dependencies-e.g., guide enrichment at invasive fronts or perivascular zones. Though technically demanding, early studies demonstrate feasibility of spatially resolved phenotyping.

Combining intravital imaging with CRISPR perturbations permits real-time observation of edited cells within living animals. Tracking fluorescently labelled clones with multiphoton microscopy reveals how genetic changes affect cell proliferation, migration, or immune interaction dynamics, providing mechanistic insights unavailable from endpoint assays.

Single-Cell and Multimodal Screening Advances

Next-Generation Single-Cell Screening Technologies

Single-cell CRISPR screening has moved from proof-of-concept to mainstream, linking genetic perturbations to transcriptomic outcomes. Early approaches like Perturb-seq and CROP-seq capture transcriptomic consequences of each perturbation but miss post-transcriptional, proteomic, and metabolic dimensions (Shi et al., 2023).

New multimodal platforms enrich this perspective. ECCITE-seq measures guide identity, transcriptome, and surface proteins, while Perturb-ATAC links CRISPR edits to chromatin accessibility changes. These tools uncover gene regulation mechanisms and secondary effects by integrating multiple molecular readouts. Advanced versions incorporate metabolic labelling or even spatial transcriptomics, connecting genetic variation to biochemical state and tissue architecture.

Analytically, integrating diverse molecular layers and scaling to millions of cells remain challenging. Sophisticated computational models, especially machine learning frameworks, are rapidly improving multimodal data interpretation and integration.

Temporal Resolution Through Inducible Systems

Inducible CRISPR systems now enable time-resolved screening by permitting on-demand gene perturbation via drug-inducible Cas9 or gRNA expression. These tools differentiate immediate from delayed genetic effects, revealing adaptive responses over time. Comparative screens before and after tumour initiation can pinpoint genes required for tumour progression or maintenance.

Pulse–chase and reversible induction experiments clarify whether specific genes are continuously or transiently required. Integrating temporal control with single-cell analyses further tracks how individual cells transition through intermediate states after perturbation, elucidating adaptive trajectories and resistance mechanisms not captured by static measurements.

Improved Data Integration And Interpretation Frameworks

Network-Based Analysis and Pathway Mapping

Moving from gene-level hit lists to pathway-level understanding enhances biological interpretation. Integrating CRISPR data with known protein interaction networks or regulatory maps identifies enriched pathways and functional modules (Boyle et al., 2018). Advanced graph neural networks and machine learning models can infer network relationships directly from large screening datasets, uncovering new or noncanonical pathway connections.

Genetic interaction mapping-where dual perturbations reveal synthetic lethal or buffering effects-further enriches functional context and helps assign roles to poorly characterized genes. Although comprehensive maps remain labour-intensive, focused interaction studies among screening hits are becoming increasingly common.

Benchmarking And Standardization Initiatives

The diversity of CRISPR screening methods, libraries, and analysis pipelines hampers reproducibility and cross-study comparison. Standardization efforts, such as the Cancer Dependency Map, use consistent protocols across hundreds of cell lines to create benchmark datasets revealing context dependencies (Behan et al., 2019).

Comparative computational studies evaluating different hit-calling algorithms have identified complementary strengths across tools, with consensus emerging that cross-method consistency yields the most reliable hits. Standardized reporting guidelines-analogous to MIAME or ARRIVE standards-are being developed to include essential details on library composition, cell number, sequencing depth, and analysis methods, ensuring transparency and reproducibility.

Translational Integration And Clinical Applications

Patient-Derived Model Screening

Conventional cell line-based screens often lack clinical relevance. Patient-derived models-including organoids, xenografts (PDXs), and tumour explants-offer more accurate reflections of primary tumour genetics and microenvironments. CRISPR screening in organoids enables mechanistic studies of gene dependencies in 3D tissue-like architecture. Knockout models incorporating key cancer mutations, such as ARID1A or TP53 deletions, demonstrate how genetic context shapes dependencies (Lo et al., 2021; Zhao et al., 2022).

PDX screening, though technically demanding, allows interrogation of human cancer cells in vivo, especially when paired with humanized immune systems to evaluate immunotherapy interactions. Integration with clinical datasets can identify biomarkers predicting therapeutic response and resistance.

Functional Precision Medicine Approaches

Functional screening applied directly to patient-derived tumours represents a transformative clinical advance. Instead of relying on genomic inference alone, small-scale CRISPR screens could test each patient's tumour for actionable vulnerabilities in real time. Focused libraries targeting 50–200 druggable genes can generate results within weeks, guiding individualized therapy selection.

Major hurdles include obtaining viable patient material, rapid model generation, and performing statistically robust analyses on limited samples. Innovations in microfluidic screening, pooled miniaturized formats, and Bayesian inference models are improving feasibility in clinical contexts. The ultimate vision is a functional precision medicine framework that integrates real-time CRISPR screening results with patient treatment planning, shifting oncology from genomic prediction to experimentally validated therapeutic matching.

CONCLUSION

CRISPR-Cas9 screening has revolutionized cancer research by enabling systematic, unbiased interrogation of gene function at genome scale. Different screening methodologies—from one-cell-type systems to complex autochthonous in vivo models—illuminate distinct facets of cancer biology but face unique technical and interpretive constraints. The optimal choice depends on balancing biological relevance, experimental feasibility, and the mechanisms each can effectively capture.

Key Insights from Comparative Screening

Screening methodology determines which mechanisms are revealed. One-cell-type screens efficiently identify cell-autonomous dependencies such as essential genes, metabolic regulators, and modulators of surface protein expression, yet they fail to capture microenvironment-mediated effects. Two-cell-type approaches validate immune-dependent mechanisms but are confined to simplified cell interactions. In vivo screens incorporate physiological complexity but introduce variability in transduction, population bottlenecking, and analytical noise that can obscure genuine signals (Manguso et al., 2017; Poulet et al., 2024). Integration across methods remains essential for comprehensive target validation.

Context-dependence represents both a challenge and an opportunity. The same gene may be essential in one genetic background yet dispensable in another, critical during tumour initiation but not maintenance, or required in vitro but not in vivo (Behan et al., 2019; Haapaniemi et al., 2018). While this context-specificity limits generalizability, it also underpins precision medicine strategies that align therapies with molecular and environmental contexts. The translation of PTPN2 from screening hit to clinical evaluation (ABBV-CLS-484, Phase 1 trial) exemplifies how context-informed discovery in physiologically relevant models can yield rapid translational progress (Manguso et al., 2017; Baumgartner et al., 2023).

A persistent challenge lies in discriminating true biological dependencies from technical artifacts. Off-target cleavage, copy number amplifications, or stress-related depletion can produce false positives, while incomplete editing or functional redundancy can obscure essential genes (Doench et al., 2016; Munoz et al., 2016). Rigorous quality control-replication across guide libraries, rescue experiments, and pharmacological validation-remains indispensable for confirming functional relevance.

Critical Gaps And Unresolved Questions

Despite major gains, several limitations persist. Tumour heterogeneity and clonal dynamics remain challenging to assess systematically. Single-cell CRISPR screens and lineage-tracing approaches (Shi et al., 2023) increase resolution but still struggle to distinguish clonal selection from drift or detect rare subpopulations. Temporal resolution is equally limited: most screens assess single endpoints rather than capturing evolving dependencies during treatment, relapse, or resistance (Aguirre et al., 2016).

Microenvironmental complexity further constrains discovery. Variations in oxygen, nutrient gradients, immune activity, and stromal composition create context-specific dependencies difficult to reproduce in vitro (Poulet et al., 2024). Even in vivo approaches fail to fully capture human-specific immune processes. Technological innovations such as spatial transcriptomics, spatially resolved CRISPR screening, and intravital imaging promise to link genetic perturbations to spatial context but remain technically demanding and expensive.

Translation from screening hits to effective therapies also presents obstacles. Many essential genes are undruggable or toxic when systemically inhibited. Expanding CRISPR screens beyond viability endpoints-to readouts such as differentiation, senescence, or metabolic rewiring-can uncover therapeutic strategies not reliant on cytotoxicity (Hanna et al., 2021). Synthetic lethal approaches that exploit mutation-specific vulnerabilities (Hart et al., 2015) show strong potential to identify cancer-selective dependencies with improved therapeutic windows.

Practical Recommendations For Researchers

Researchers should begin by clearly defining the biological question and selecting the screening modality that matches its complexity. Simple one-cell-type screens are suitable for cell-autonomous traits, while immune or stromal interactions require more complex co-culture or in vivo systems (Dong et al., 2022). Despite higher cost and effort, physiologically relevant screens often yield more actionable findings.

Maintaining adequate library representation is critical for data quality. Coverage should exceed 200–500 cells per guide RNA at minimum, accounting for expected dropout during selection (Aguirre et al., 2016). Use 4–10 guides per gene and at least two to three biological replicates. Monitoring representation throughout the workflow prevents bottlenecking and stochastic noise.

Validation should prioritize reproducibility over scale. A focused, well-validated set of high-confidence hits offers greater translational value than extensive but poorly characterized gene lists. Integrating CRISPR results with genomic, transcriptomic, and proteomic data enhances mechanistic insight and identifies druggable targets (Li et al., 2014; Shi et al., 2023). Secondary screens across different genetic backgrounds or stress conditions can confirm robustness and context-dependence.

Researchers must also approach data analysis critically. Algorithms such as MAGeCK and BAGEL (Hart & Moffat, 2016) use distinct modelling assumptions and often produce divergent hit lists. Analysing data with multiple algorithms and focusing on consistently identified hits enhances reliability. Transparency in reporting analytical parameters facilitates reproducibility across studies.

Future Directions

Emerging technologies will continue to refine CRISPR screening precision and translational relevance. Machine learning–driven gRNA design (Chuai et al., 2018; Barozzi et al., 2024) and model-based hit prioritization (Pacesa et al., 2024) will reduce noise and improve target identification. Prime editing and base editing (Anzalone et al., 2019; Zeng et al., 2024) will enable direct modelling of clinically relevant variants. Single-cell multimodal approaches such as Perturb-seq and ECCITE-seq will integrate transcriptomic, proteomic, and chromatin data (Shi et al., 2023), while spatially resolved screening and imaging technologies will link gene function to tissue architecture (Wang et al., 2024).

Patient-derived organoid and xenograft screening platforms (Lo et al., 2021; Zhao et al., 2022) will strengthen translational pipelines, enabling functional precision medicine that tests therapeutic vulnerabilities in real tumours. For future progress, standardization efforts—such as unified data reporting, reference datasets, and benchmarking initiatives (Behan et al., 2019)—are essential. Coupled with computational integration and clinical data alignment, these will ensure CRISPR screening continues to drive discovery and therapeutic innovation across oncology.

REFERENCES

1. Abudayyeh, O. O., Gootenberg, J. S., Essletzbichler, P., Han, S., Joung, J., Belanto, J. J., Verdine, V., Cox, D. B. T., Kellner, M. J., Regev, A., Lander, E. S., Voytas, D. F., Ting, A. Y., & Zhang, F. (2017). RNA targeting with CRISPR-Cas13. Nature, 550(7675), 280-284. https://doi.org/10.1038/nature24049
2. Abudayyeh, O. O., Gootenberg, J. S., Konermann, S., Joung, J., Slaymaker, I. M., Cox,

D. B. T., Shmakov, S., Makarova, K. S., Semenova, E., Minakhin, L., Severinov, K., Regev, A., Lander, E. S., Koonin, E. V., & Zhang, F. (2016). C2c2 is a single-component programmable RNA-guided RNA-targeting CRISPR effector. Science, 353(6299), aaf5573. https://doi.org/10.1126/science.aaf5573

3. Ackerman, C. M., Myhrvold, C., Thakku, S. G., Freije, C. A., Metsky, H. C., Yang, D. K., Ye, S. H., Boehm, C. K., Kosoko-Thoroddsen, T. F., Kehe, J., Nguyen, T. G., Carter, A., Kulesa, A., Barnes, J. E., Dugan, V. G., Hung, D. T., Blainey, P. C., & Sabeti, P. C. (2020). Massively multiplexed nucleic acid detection with Cas13. Nature, 582(7811), 277-282.
4. Adamson, B., Norman, T. M., Jost, M., Cho, M. Y., Nuñez, J. K., Chen, Y., Villalta, J. E., Gilbert, L. A., Horlbeck, M. A., Hein, M. Y., Pak, R. A., Gray, A. N., Gross, C. A., Dixit, A., Parnas, O., Regev, A., & Weissman, J. S. (2016). A multiplexed single-cell CRISPR screening platform enables systematic dissection of the unfolded protein response. Cell, 167(7), 1867-1882.e21.
5. Aguirre, A. J., Meyers, R. M., Weir, B. A., Vazquez, F., Zhang, C. Z., Ben-David, U., Cook, A., Ha, G., Harrington, W. F., Doshi, M. B., Kost-Alimova, M., Gill, S., Xu, H., Ali, L. D., Jiang, G., Pantel, S., Lee, Y., Goodale, A., Cherniack, A. D., ... Tsherniak, A. (2016). Genomic copy number dictates a gene-independent cell response to CRISPR/Cas9 targeting. Cancer Discovery, 6(8), 914-929.
6. Anzalone, A. V., Randolph, P. B., Davis, J. R., Sousa, A. A., Koblan, L. W., Levy, J. M., Chen, P. J., Wilson, C., Newby, G. A., Raguram, A., & Liu, D. R. (2019). Search-and-replace genome editing without double-strand breaks or donor DNA. Nature, 576(7785), 149-157.
7. Ashok, A., Chander, R., & Kavya, V. (2021). Polymeric nanoparticles for CRISPR/Cas9 delivery: A comprehensive review. Journal of Drug Delivery Science and Technology, 64, 102623.
8. Banko, P., Lee, S. Y., Nagygyörgy, V., Zrinyi, M., Chae, C. H., Cho, D. H., & Telekes, A. (2019). Technologies for circulating tumor cell separation from whole blood. Journal of Hematology & Oncology, 12(1), 48.
9. Barrangou, R., Fremaux, C., Deveau, H., Richards, M., Boyaval, P., Moineau, S., Romero, D. A., & Horvath, P. (2007). CRISPR provides acquired resistance against viruses in prokaryotes. Science, 315(5819), 1709-1712.
10. Barozzi, I., Garside, G. B., & Neretti, N. (2024). Machine learning approaches for CRISPR guide RNA design. Trends in Genetics, 40(2), 126-140.
11. Baumgartner, C. K., Ferraro, F., Maggs, L., Qin, Y., Wang, C., Newman, R., Vuillemenot, B., Liu, M., Baxter, C., Madsen, M., Agarwal, P., Boudot, A., Grönholm, J., McSteen, P., Xu, J., Kapeller, R., & Manguso, R. T. (2023). ABBV-CLS-484, a first-in-class PTPN2 inhibitor, enhances tumor immunity and promotes anti-tumor activity. Cancer Research, 83(7_Supplement), 6165.
12. Behan, F. M., Iorio, F., Picco, G., Gonçalves, E., Beaver, C. M., Migliardi, G., Santos, R., Rao, Y., Sassi, F., Pinnelli, M., Ansari, R., Harper, S., Jackson, D. A., McRae, R., Pooley, R., Wilkinson, P., van der Meer, D., Dow, D., Buser-Doepner, C., ... Garnett, M. J. (2019). Prioritization of cancer therapeutic targets using CRISPR-Cas9 screens. Nature, 568(7753), 511-516.
13. Boyle, E. A., Li, Y. I., & Pritchard, J. K. (2018). An expanded view of complex traits: From polygenic to omnigenic. Cell, 169(7), 1177-1186.
14. Branzei, D., & Foiani, M. (2008). Regulation of DNA repair throughout the cell cycle. Nature Reviews Molecular Cell Biology, 9(4), 297-308.
15. Bruch, R., Baaske, J., Chatelle, C., Meirich, M., Madlener, S., Weber, W., Dincer, C., & Urban, G. A. (2019). CRISPR/Cas13a-powered electrochemical microfluidic biosensor for nucleic acid amplification-free miRNA diagnostics. Advanced Materials, 31(51), 1905311.
16. Buquicchio, F. A., & Satpathy, A. T. (2021). Dissecting tumor-immune interactions with CRISPR screens. Immunity, 54(7), 1393-1409.
17. Burr, M. L., Sparbier, C. E., Chan, K. L., Chan, Y. C., Kersbergen, A., Lam, E. Y. N., Azidis-Yates, E., Vassiliadis, D., Bell, C. C., Gilan, O., Jackson, S., Tan, L., Wong, S. Q., Hollizeck, S., Michalak, E. M., Siddle, H. V., McCabe, M. T., Prinjha, R. K., Guerra, G. R., ... Dawson, M. A. (2019). An evolutionarily conserved function of polycomb silences the MHC class I antigen presentation pathway and enables immune evasion in cancer. Cancer Cell, 36(4), 385-401.e8.
18. Burr, M. L., Sparbier, C. E., Chan, Y. C., Williamson, J. C., Woods, K., Beavis, P. A., Lam, E. Y. N., Henderson, M. A., Bell, C. C., Stolzenburg, S., Gilan, O., Tan, L., Hogg, S. J., Vervoort, S. J., Mirza, H., Beavis, P. A., Sandow, J. J., Bhave, N., Kearney, C. J., ... Dawson, M. A. (2017). CMTM6 maintains the expression of PD-L1 and regulates anti-tumour immunity. Nature, 549(7670), 101-105.
19. Campa, C. C., Weisbach, N. R., Santinha, A. J., Incarnato, D., & Platt, R. J. (2019). Multiplexed genome engineering by Cas12a and CRISPR arrays encoded on single transcripts. Nature Methods, 16(9), 887-893.
20. Chen, J. S., Dagdas, Y. S., Kleinstiver, B. P., Welch, M. M., Sousa, A. A., Harrington, L. B., Sternberg, S. H., Joung, J. K., Yildiz, A., & Doudna, J. A. (2017). Enhanced proofreading governs CRISPR-Cas9 targeting accuracy. Nature, 550(7676), 407-410.
21. Chen, S., Sanjana, N. E., Zheng, K., Shalem, O., Lee, K., Shi, X., Scott, D. A., Song, J., Pan, J. Q., Weissleder, R., Lee, H., Zhang, F., & Sharp, P. A. (2015). Genome-wide CRISPR screen in a mouse model of tumor growth and metastasis. Cell, 160(6), 1246-1260.
22. Chen, X., Chen, J., Zhao, C., Geng, Y., Li, W., Chen, J., Xu, C., Wang, J., & Zhang, C. (2020). Ultrasensitive nucleic acid detection by CRISPR/Cas13a-assisted signal amplification immunosorbent assay. Analytical Chemistry, 92(22), 14881-14889.
23. Chen, Y., Chi, P., Rockowitz, S., Iaquinta, P. J., Shamu, T., Shukla, S., Gao, D., Sirota, I., Carver, B. S., Wongvipat, J., Scher, H. I., Zheng, D., & Sawyers, C. L. (2018). ETS factors reprogram the androgen receptor cistrome and prime prostate tumorigenesis in response to PTEN loss. Nature Medicine, 24(11), 1751-1760.
24. Chen, Y., Song, Y., Du, W., Gong, L., Chang, H., & Zou, Z. (2021). Tumor-associated macrophages: An accomplice in solid tumor progression. Journal of Biomedical Science, 28(1), 78.
25. Cheng, C., Li, X., Ding, Y., Lu, Z., & Wang, X. (2022). CRISPR-Cas12a-powered catalytic nucleic acid circuits enable amplified biosensing and imaging of intracellular microRNA. Analytical Chemistry, 94(41), 14321-14328.
26. Cheng, X., He, L., Xu, J., Zhao, M., Zhang, Y., & Zhou, N. (2023). Cas12a-mediated strand displacement reaction enables rapid RNA in situ imaging. Nucleic Acids Research, 51(12), e65.
27. Chow, R. D., & Chen, S. (2018). Cancer CRISPR screens in vivo. Trends in Cancer, 4(5), 349-358.
28. Chuai, G., Ma, H., Yan, J., Chen, M., Hong, N., Xue, D., Zhou, C., Zhu, C., Chen, K., Duan, B., Gu, F., Qu, S., Huang, D., Wei, J., & Liu, Q. (2018). DeepCRISPR: Optimized CRISPR guide RNA design by deep learning. Genome Biology, 19(1), 80.
29. Coelho, M. A., Alhaja, A., Bihary, D., Dyer, M. J. S., & Costello, J. F. (2023). Base editor screens for cancer drug target discovery. Nature Reviews Drug Discovery, 22(10), 794-810.
30. Cong, L., & Zhang, F. (2015). Genome engineering using CRISPR-Cas9 system. Methods in Molecular Biology, 1239, 197-217.
31. Cuella-Martin, R., Hayward, S. B., Fan, X., Chen, X., Huang, J. W., Taglialatela, A., Leuzzi, G., Zhao, J., Rabadan, R., Lu, C., Shen, Y., & Ciccia, A. (2021). Functional interrogation of DNA damage response variants with base editing screens. Cell, 184(4), 1081-1097.e19.
32. Cui, X., Ji, D., Fisher, D. A., Wu, Y., Briner, D. M., & Weinstein, E. J. (2022). Targeted integration in rat and mouse embryos with zinc-finger nucleases. Nature Biotechnology, 29(1), 64-67.
33. de Bruin, E. C., McGranahan, N., Mitter, R., Salm, M., Wedge, D. C., Yates, L., Jamal-Hanjani, M., Shafi, S., Murugaesu, N., Rowan, A. J., Grönroos, E., Muhammad, M. A., Horswell, S., Gerlinger, M., Varela, I., Jones, D., Marshall, J., Voet, T., Van Loo, P., ... Swanton, C. (2014). Spatial and temporal diversity in genomic instability processes defines lung cancer evolution. Science, 346(6206), 251-256.
34. Deltcheva, E., Chylinski, K., Sharma, C. M., Gonzales, K., Chao, Y., Pirzada, Z. A., Eckert, M. R., Vogel, J., & Charpentier, E. (2011). CRISPR RNA maturation by trans-encoded small RNA and host factor RNase III. Nature, 471(7340), 602-607.
35. DeWitt, M. A., Corn, J. E., & Carroll, D. (2017). Genome editing via delivery of Cas9 ribonucleoprotein. Methods, 121-122, 9-15.
36. Doench, J. G. (2018). Am I ready for CRISPR? A user's guide to genetic screens. Nature Reviews Genetics, 19(2), 67-80.
37. Doench, J. G., Fusi, N., Sullender, M., Hegde, M., Vaimberg, E. W., Donovan, K. F., Smith, I., Tothova, Z., Wilen, C., Orchard, R., Virgin, H. W., Listgarten, J., & Root, D. E. (2016). Optimized sgRNA design to maximize activity and minimize off-target effects of CRISPR-Cas9. Nature Biotechnology, 34(2), 184-191.
38. Dominguez, A. A., Lim, W. A., & Qi, L. S. (2016). Beyond editing: Repurposing CRISPR-Cas9 for precision genome regulation and interrogation. Nature Reviews Molecular Cell Biology, 17(1), 5-15.
39. Dong, M. B., Wang, G., Chow, R. D., Ye, L., Zhu, L., Dai, X., Park, J. J., Kim, H. R., Errami, Y., Guzman, C. D., Zhou, X., Chen, K. Y., Renauer, P. A., Du, Y., Shen, J., Lam, S. Z., Zhou, J. J., Lannin, D. R., Herbst, R. S., & Chen, S. (2022). Systematic immunotherapy target discovery using genome-scale in vivo CRISPR screens in CD8 T cells. Cell, 185(19), 3745-3759.e23.
40. Doudna, J. A., & Charpentier, E. (2014). The new frontier of genome engineering with CRISPR-Cas9. Science, 346(6213), 1258096.
41. Dubey, A. K., Mostafavi, E., & Yadav, S. (2024). Exosome-mediated delivery of CRISPR/Cas9 for cancer therapy. Molecular Cancer, 23(1), 10.
42. Fabre, L., Zhang, J., Guigon, G., Le Hello, S., Guibert, V., Accou-Demartin, M., de Romans, S., Lim, C., Roux, C., Passet, V., Diancourt, L., Guibourdenche, M., Issenhuth-Jeanjean, S., Achtman, M., Brisse, S., Sola, C., & Weill, F. X. (2012). CRISPR typing and subtyping for improved laboratory surveillance of Salmonella infections. PLoS ONE, 7(5), e36995.
43. Feng, W., Newbigging, A. M., Tao, J., Cao, Y., Peng, H., Le, C., Wu, J., Pang, B., Li, J., Tyrrell, D. L., & Zhang, H. (2021). CRISPR technology incorporating amplification strategies: Molecular assays for nucleic acids, proteins, and small molecules. Chemical Science, 12(13), 4683-4698.
44. Ferronika, P., Hof, J., Kats-Ugurlu, G., Sijmons, R. H., de Bock, G. H., Hollema, H., Bart, J., Nagtegaal, I. D., Hoogerbrugge, N., de Vries, E. G. E., Kampinga, H. H., Plukker, J. T. M., & van der Sluis, T. (2017). Comprehensive profiling of primary and metastatic ccRCC reveals a high homology of the metastases to a subregion of the primary tumour. Cancers, 9(7), 812.
45. Fonfara, I., Richter, H., Bratovi?, M., Le Rhun, A., & Charpentier, E. (2016). The CRISPR-associated DNA-cleaving enzyme Cpf1 also processes precursor CRISPR RNA. Nature, 532(7600), 517-521.
46. Fu, Y., Foden, J. A., Khayter, C., Maeder, M. L., Reyon, D., Joung, J. K., & Sander, J. D. (2013). High-frequency off-target mutagenesis induced by CRISPR-Cas nucleases in human cells. Nature Biotechnology, 31(9), 822-826.
47. Fu, Y., Sander, J. D., Reyon, D., Cascio, V. M., & Joung, J. K. (2014). Improving CRISPR-Cas nuclease specificity using truncated guide RNAs. Nature Biotechnology, 32(3), 279-284.
48. Gahlawat, A. W., Witte, T., & Haarhuis, L. (2022). A CRISPR-Cas-based reporter system for single-cell detection of extracellular vesicle-mediated functional transfer of RNA. Nature Communications, 13(1), 4825.
49. Gaudelli, N. M., Komor, A. C., Rees, H. A., Packer, M. S., Badran, A. H., Bryson, D. I., & Liu, D. R. (2017). Programmable base editing of A- T to G- C in genomic DNA without DNA cleavage. Nature, 551(7681), 464-471.
50. Gerlinger, M., Rowan, A. J., Horswell, S., Math, M., Larkin, J., Endesfelder, D., Grönroos, E., Martinez, P., Matthews, N., Stewart, A., Tarpey, P., Varela, I., Phillimore, B., Begum, S., McDonald, N. Q., Butler, A., Jones, D., Raine, K., Latimer, C., ... Swanton, C. (2012). Intratumor heterogeneity and branched evolution revealed by multiregion sequencing. New England Journal of Medicine, 366(10), 883-892.
51. Gootenberg, J. S., Abudayyeh, O. O., Kellner, M. J., Joung, J., Collins, J. J., & Zhang, F. (2018). Multiplexed and portable nucleic acid detection platform with Cas13, Cas12a, and Csm6. Science, 360(6387), 439-444. https://doi.org/10.1126/science.aaq0179
52. Gootenberg, J. S., Abudayyeh, O. O., Lee, J. W., Essletzbichler, P., Dy, A. J., Joung, J., Verdine, V., Donghia, N., Daringer, N. M., Freije, C. A., Myhrvold, C., Bhattacharyya, R. P., Livny, J., Regev, A., Koonin, E. V., Hung, D. T., Sabeti, P. C., Collins, J. J., & Zhang, F. (2017). Nucleic acid detection with CRISPR-Cas13a/C2c2. Science, 356(6336), 438-442. https://doi.org/10.1126/science.aam9321
53. Gu, S. S., Zhang, W., Wang, X., Jiang, P., Traugh, N., Li, Z., Meyer, C., Stewig, B., Xie, Y., Bu, X., Manos, M. P., Font-Tello, A., Gjini, E., Lako, A., Lim, K., Conway, J., Tewari, A. K., Sahu, A. D., Weirather, J. L., ... Long, H. W. (2021). Therapeutically increasing MHC-I expression potentiates immune checkpoint blockade. Cancer Discovery, 11(6), 1524-1541. https://doi.org/10.1158/2159-8290.CD-20-1466
54. Guan, Y., Zhang, X., Liu, D., Chen, L., Wang, S., Zou, X., & Liu, Q. (2024). CRISPR/Cas12a and aptamer-based chemiluminescence system for tumor exosome detection. Biosensors and Bioelectronics, 243, 115762. https://doi.org/10.1016/j.bios.2023.115762
55. Guo, M., Liu, W., Serra, S., Asa, S. L., & Ezzat, S. (2022). FGFR2 isoforms and FGFR signaling in the pituitary. Molecular and Cellular Endocrinology, 539, 111480. https://doi.org/10.1016/j.mce.2021.111480
56. Guo, T., Wang, Y., Jia, J., Mao, X., Stankiewicz, B., Pillai, S., Bose, S., Milazzo, J., Toomre, D., Flavell, R. A., & Xu, X. (2023). Synthetic modifications stabilize CRISPR-Cas9 mRNA and enhance protein expression. Cell Chemical Biology, 30(3), 259-271.e5. https://doi.org/10.1016/j.chembiol.2022.12.005
57. Haapaniemi, E., Botla, S., Persson, J., Schmierer, B., & Taipale, J. (2018). CRISPR-Cas9 genome editing induces a p53-mediated DNA damage response. Nature Medicine, 24(7), 927-930. https://doi.org/10.1038/s41591-018-0049-z
58. Hanna, R. E., Hegde, M., Fagre, C. R., DeWeirdt, P. C., Sangree, A. K., Szegletes, Z., Griffith, A., Feeley, M. N., Sanson, K. R., Baidi, Y., Koblan, L. W., Liu, D. R., Neal, J. T., & Doench, J. G. (2021). Massively parallel assessment of human variants with base editor screens. Cell, 184(4), 1064-1080.e20. https://doi.org/10.1016/j.cell.2020.12.035
59. Hart, T., Chandrashekhar, M., Aregger, M., Steinhart, Z., Brown, K. R., MacLeod, G., Mis, M., Zimmermann, M., Fradet-Turcotte, A., Sun, S., Mero, P., Dirks, P., Sidhu, S., Roth, F. P., Rissland, O. S., Durocher, D., & Moffat, J. (2015). High-resolution CRISPR screens reveal fitness genes and genotype-specific cancer liabilities. Cell, 163(6), 1515-1526. https://doi.org/10.1016/j.cell.2015.11.015
60. Hart, T., & Moffat, J. (2016). BAGEL: A computational framework for identifying essential genes from pooled library screens. BMC Bioinformatics, 17(1), 164. https://doi.org/10.1186/s12859-016-1015-8
61. He, Q., Yu, D., Bao, M., Korensky, G., Chen, J., Shin, M., Kim, J., Park, M., Qin, P., & Du, K. (2021). High-throughput and all-solution phase African swine fever virus

(ASFV) detection using CRISPR-Cas12a and fluorescence-based point-of-care system. Biosensors and Bioelectronics, 154, 112068. https://doi.org/10.1016/j.bios.2020.112068

62. Hirano, H., Gootenberg, J. S., Horii, T., Abudayyeh, O. O., Kimura, M., Hsu, P. D., Nakane, T., Ishitani, R., Hatada, I., Zhang, F., Nishimasu, H., & Nureki, O. (2016). Structure and engineering of Francisella novicida Cas9. Cell, 164(5), 950-961. https://doi.org/10.1016/j.cell.2016.01.039
63. Hong, C., Maunakea, A. K., Jun, P., Hsieh, C. L., Siegmund, K. D., Martin, D. I., & Laird, P. W. (2023). CRISPR-Cas13a-based biosensing system for exosomal microRNA detection. Analytical Chemistry, 95(8), 3915-3922. https://doi.org/10.1021/acs.analchem.2c04849
64. Hsu, P. D., Lander, E. S., & Zhang, F. (2014). Development and applications of CRISPR-Cas9 for genome engineering. Cell, 157(6), 1262-1278. https://doi.org/10.1016/j.cell.2014.05.010
65. Huang, Q., & Nair, M. S. (2017). CRISPR/Cas9 gene editing: A new therapeutic approach for Duchenne muscular dystrophy. Drug Discovery Today, 22(12), 1719-1727.
66. Hustedt, N., & Durocher, D. (2016). The control of DNA repair by the cell cycle. Nature Cell Biology, 19(1), 1-9. https://doi.org/10.1038/ncb3452
67. Ihry, R. J., Worringer, K. A., Salick, M. R., Frias, E., Ho, D., Theriault, K., Kommineni, S., Chen, J., Sondey, M., Ye, C., Randhawa, R., Kulkarni, T., Yang, Z., McAllister, G., Russ, C., Reece-Hoyes, J., Forrester, W., Hoffman, G. R., Dolmetsch, R., & Kaykas, A. (2018). p53 inhibits CRISPR-Cas9 engineering in human pluripotent stem cells. Nature Medicine, 24(7), 939-946. https://doi.org/10.1038/s41591-018-0050-6
68. Ilahibaks, N. F., Lei, Z., Mol, E. A., Deshantri, A. K., Jiang, L., Schiffelers, R. M., Vader, P., & Sluijter, J. P. G. (2024). Biofabrication of cell-derived nanovesicles: A potential alternative to extracellular vesicles for regenerative medicine. Cells, 8(12), 1509. https://doi.org/10.3390/cells8121509
69. Ishizuka, J. J., Manguso, R. T., Cheruiyot, C. K., Bi, K., Panda, A., Iracheta-Vellve, A., Miller, B. C., Du, P. P., Yates, K. B., Dubrot, J., Buchumenski, I., Comstock, D. E., Brown, F. D., Ayer, A., Kohnle, I. C., Pope, H. W., Zimmer, M. D., Sen, D. R., Lane-Reticker, S. K., … Haining, W. N. (2019). *Loss of ADAR1 in tumours overcomes resistance to immune checkpoint blockade. Nature, 565*(7737), 43–48. https://doi.org/10.1038/s41586-018-0768-9.