Narrative Review Article

CRISPR-Mediated Therapeutic Engineering and Genetic Screening in Leukemia: A Narrative Review

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ABSTRACT

Leukemia remains a leading cause of hematologic cancer deaths despite advances in chemotherapy and immunotherapies such as Chimeric Antigen Receptor (CAR) T-cells, largely due to drug resistance, immune evasion, and relapse. This narrative review examines how CRISPR-based technologies are being applied to leukemia therapeutics through precise genome editing and functional genetic screens. It first synthesizes current literature on how CRISPR-Cas9 and next-generation editors (including Cas12a, Cas13, base editors, and prime editors) enable the engineering of universal allogeneic CAR-T cells and hematopoietic stem cells through disrupting immune checkpoints and Human Leukocyte Antigen (HLA) genes, correcting pathogenic mutations, and reducing graft-versus-host disease. The review then discusses pooled and arrayed CRISPR knockout, interference, and activation screens in which leukemia cells are subjected to selective forces, including chemotherapeutic agents and immune-mediated selective pressures, to enable the identification of genetic regulators of cell growth, survival, and antigen escape. Genome-wide dropout screens in acute myeloid leukemia have revealed leukemia-specific "fitness genes" in chromatin regulation, DNA repair, and metabolism whose pharmacologic inhibition recapitulates CRISPR-induced cytotoxicity. In addition, focused screens have identified subtype-specific dependencies, such as the vesicle-tethering factor Unconventional Secretion Organelle 1 (USO1) in B-cell acute lymphoblastic leukemia, as well as synthetic lethal gene pairs that provide a framework for rational combination therapies. Comparative analyses with RNA interference and computational essentiality frameworks further sharpen the distinction between core-essential and leukemia-selective targets. Collectively, the studies reviewed demonstrate how CRISPR-based screening and therapeutic strategies are reshaping target discovery pipelines and accelerating the development of more precise, effective, and less toxic treatment approaches across leukemia subtypes.

Keywords: Targeted Therapies; Leukemia; CRISPR Screens; Genetic Dependencies; CAR-T Cell Therapy; CRISPR-Cas9

INTRODUCTION

Leukemia is a collection of blood and bone marrow cancers characterized by the malignant transformation of myeloid or lymphoid lineages, with over 60,000 new cases reported annually in the United States, making it one of the most fatal hematologic malignancies (1, 2). While current treatments such as chemotherapy and

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Chimeric Antigen Receptor (CAR) T-cell therapy have improved outcomes, they are frequently limited by systemic toxicity, drug resistance, and "on-target, off-tumor" effects like B-cell aplasia (3, 4). Despite these advances, there remains a fragmented understanding of how diverse CRISPR modalities can be systematically applied to overcome these therapeutic hurdles. This narrative review synthesizes current evidence on CRISPR-based genetic screens and genome editing strategies that inform leukemia vulnerability mapping and CAR-T cell optimization.

LITERATURE REVIEW

Leukemia: Disease Initiation, Progression, and Current CAR-T Therapies

Leukemia cells, like other cancer cells, exhibit the hallmarks of cancer, including uncontrolled cell proliferation, resistance to apoptosis, and immune evasion (5). However, unlike solid tumors, leukemia cells do not form a localized mass; instead, they circulate throughout the blood and infiltrate the bone marrow (6). This widespread distribution directly impacts normal hematopoiesis, leading to a decrease in the production of functional red blood cells, platelets, and immune cells, which results in anemia, bleeding tendencies, and increased susceptibility to infections (5).

The behavior of leukemia is dictated by specific molecular drivers that provide a roadmap for genetic intervention. In myeloid lineages, Acute Myeloid Leukemia (AML) is often characterized by rapid myeloblasts expansion driven most commonly by NPM1 and FLT3, accounting for ~30% each, while Chronic Myeloid Leukemia (CML) is often associated with the Philadelphia chromosome translocation (t(9;22)), resulting in the BCR-ABL fusion gene, which drives the uncontrolled proliferation of myeloid cells (7-9).

In contrast, lymphoid malignancies occur due to the rapid proliferation of lymphoid cells (10). Acute lymphoblastic leukemia (ALL) is an aggressive hematologic malignancy characterized by the rapid expansion of immature lymphoid progenitors, most commonly of B-cell lineage, driven by recurrent genetic alterations in key regulators of cell signaling, cell-cycle control, and lymphoid differentiation, including KRAS, CDKN2A/B, and PAX (10, 11). Chronic lymphocytic leukemia (CLL) progresses slowly and involves the accumulation of defective B-lymphocytes over time (12). The genetic drivers of this disease include SF3B1, NOTCH, and TP53 (13). These diverse genetic signatures necessitate the use of high-throughput tools to identify lineage-specific vulnerabilities that can be targeted without compromising healthy hematopoiesis.

Challenges of Chemotherapy and Current CAR-T Therapy

Chemotherapy remains the frontline treatment for aggressive leukemias like ALL and AML, acting by inducing apoptosis in rapidly dividing cells (14-17). Its lack of tumor specificity causes off-target toxicity, and relapsed leukemia often develops resistance through mutations in NT5C2, which inactivates thiopurine drugs, or through alterations in apoptotic pathways, such as TP53 loss or BCL-2 overexpression (18, 19). These molecular escape routes highlight the need for precision approaches, such as CRISPR screens, that can systematically map such vulnerabilities and identify novel therapeutic targets.

Chimeric Antigen Receptor (CAR) T-cell therapy is an immunotherapeutic approach in which the patient's or donor's T lymphocytes are engineered to recognize tumor-associated surface antigens, enabling direct and antigen-specific elimination of malignant cells (20). Unlike conventional T-cell activation, CAR constructs bypass Major Histocompatibility Complex (MHC) restriction, allowing engineered T cells to respond effectively to predefined leukemia antigens (21). Since different cancer cells display distinct antigenic profiles, T-cells can be tailored to target tumor-specific markers (21).

Despite these advances, CAR-T therapy is constrained by substantial safety and durability challenges. Excessive immune activation can trigger cytokine release syndrome (CRS), a systemic inflammatory response caused by rapid immune activation and production of large amounts of cytokines, which can lead to high fever, hypotension, and multi-organ dysfunction (22). In addition, CAR-T cells frequently exhibit "on-target, off-target" toxicity when target antigens are shared between malignant and healthy cells (23). For example, most CAR-T therapies for B-cell leukemias target CD19, a surface protein expressed on malignant B cells but also present on normal B lymphocytes (3, 24). As a result, patients frequently develop B-cell aplasia, the near-complete depletion of healthy B cells (25). In addition, on-target, off-tumor toxicity limits the range of safe antigens for CAR-T development, as many potential tumor-associated targets are also expressed at low levels in vital normal tissues (26). This trade-off between efficacy and safety remains a central obstacle in expanding CAR-T therapy

in leukemia.

Taken together, these challenges underscore that CAR-T cell performance is fundamentally determined by genetically programmed signaling circuits, antigen sensitivity parameters, and immune regulatory mechanisms, positioning CAR-T therapy as a powerful system for CRISPR-mediated genetic optimization.

CRISPR

To address the limitations of traditional therapies, researchers have turned to genome editing technologies – foremost among them, CRISPR-Cas systems. Originally identified as an adaptive immune mechanism in bacteria, CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) systems use RNA-guided nucleases to recognize and cleave foreign nucleic acids, a property that has been repurposed to enable programmable genetic perturbation in mammalian cells (27-31).

In its canonical form, CRISPR-Cas9 introduces targeted double-strand DNA breaks that are repaired through endogenous pathways, including error-prone non-homologous end joining (NHEJ), leading to gene disruption, or homology-directed repair (HDR) when a donor template is provided, allowing for precise correction or insertion (32). In leukemia research, these capabilities are particularly valuable for interrogating oncogenic drivers, resistance mechanisms, and lineage-specific dependencies that are difficult to resolve using therapeutic approaches alone (33). By harnessing these repair pathways, CRISPR-Cas9 has become a transformative tool to knock out genes, correct mutations, and introduce new sequences, with applications ranging from functional genomics to potential therapies for genetic diseases such as sickle cell anemia, β-thalassemia, and leukemias (32, 34).

Beyond CRISPR-Cas9, Cas variant technologies such as Cas12a, Cas13a, base editing, and prime editing have revolutionized gene editing. Cas12a, also known as Cpf1, has the ability to perform two distinct nuclease activities using the RuvC domain: site-specific cleavage of target dsDNA (cis-activity) and indiscriminate degradation of ssDNA (trans-activity) (35, 36). Cas12a is mainly used for nucleic acid detection and viral diagnosis, like the DETECTR method (DNA Endonuclease Targeted CRISPR Trans Reporter) (35, 36). Cas13 systems extend CRISPR functionality to the transcriptome, enabling programmable RNA knockdown without permanent genomic alteration, an attractive feature for studying dosage-sensitive genes or preserving genomic integrity in normal hematopoietic cells (37).

Unlike the prior CRISPR technologies mentioned before, base-editing and prime-editing enable the irreversible conversion of one target DNA base into another without requiring dsDNA cleavage or a donor template (38). In base-editing, a catalytically dead Cas9 (dCas9) enzyme is used, which can bind DNA in a guide RNA-programmed manner but does not cleave the DNA backbone (38). However, base editors allow specific transition mutations, while prime editors enable all possible base substitutions as well as small insertions and deletions through a reverse transcriptase–mediated mechanism (38, 39).

Prime-editing uses a modified Cas9 nickase fused to a reverse transcriptase and guided by a prime editing guide RNA (pegRNA) to introduce precise DNA changes (39). These approaches reduce reliance on endogenous DNA repair pathways, thereby minimizing unintended insertions or deletions and improving safety profiles, an important consideration for therapeutic applications in treatment for leukemia (40).

Ultimately, the best Cas variant for leukemia screening depends on the biological question and safety constraints of the experimental context. While Cas9 remains the standard in genome-wide loss-of-function studies, Cas12a is uniquely suited for investigating complex genetic interactions due to its inherent ability to process multiplexed crRNA arrays (41-43). For experiments where complete gene ablation is cytotoxic or lethal to healthy hematopoietic cells, dCas9-based CRISPRi and CRISPRa provide a safer, reversible alternative through transcriptional modulation (44). Finally, Cas13 is particularly well suited for contexts where transcript-level control is desired or where preserving genomic stability is crucial (45). Taken together, the expanding CRISPR toolkit enables leukemia screens to be precisely matched to biological complexity, genetic context, and clinical relevance, allowing researchers to balance scalability, mechanistic resolution, and safety while interrogating vulnerabilities that range from essential genes to subtle, context-dependent regulatory dependencies.

CRISPR-BASED THERAPEUTICS

CAR-T Cell Engineering using CRISPR

CRISPR-Cas9 can be used to delete immune checkpoint genes like PD-1 and CTLA-4 from CAR-T cells, which normally impede T-cell activity and allow cancer cells to evade immune attacks (46) (Figure 1).

While autologous CAR-T cells avoid rejection, they

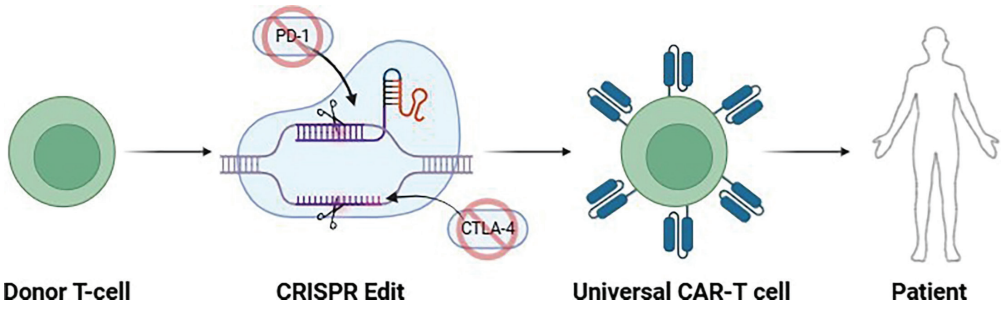

Figure 1. Generation of universal CRISPR-edited CAR-T cells for cancer immunotherapy. *T-cells are harvested from a donor and modified using CRISPR-Cas9 technology to disrupt immune checkpoint genes, such as PD-1 and CTLA-4, which enhances their anti-tumor activity by preventing immune evasion. Genetic editing is also applied to T-cell receptors and HLA genes to create allogeneic, universal CAR-T cells that minimize the risk of GvHD. These engineered cells are equipped with CARs and administered to the patient to induce remission, particularly in cases of relapsed leukemia, while minimizing systemic side effects like cytokine release syndrome.*

are costly and time-consuming to produce, limiting accessibility (46). CRISPR-Cas9 enables the generation of allogeneic or universal CAR-T cells by editing T-cell receptors and human leucocyte antigen (HLA) genes to prevent graft-versus-host disease (GvHD) (46). Clinical products such as UCART19 have been reported to induce remission in relapsed ALL patients, showing the utility of universal CAR-T cell approaches (47). Furthermore, CRISPR can be applied to minimize CRS by designing multiantigen CARs that restrict T-cell activation to cells expressing multiple tumor antigens, thereby reducing systemic cytokine production, while preventing escape of antigens like CD19 (22).

Stem Cell Therapy using CRISPR

Another promising therapy for leukemia using CRISPR technology is stem cell therapy, which involves editing hematopoietic stem cells (HSCs) to enhance their therapeutic potential. In conventional allogeneic bone marrow transplantation, donor HSCs can restore normal hematopoiesis but risk GvHD, relapse, or rejection (48). CRISPR-Cas9 offers solutions by precisely deleting or modifying genes that drive immune incompatibility, such as human leukocyte antigen (HLA) molecules, thereby enabling the generation of universal donor stem cells that reduce GvHD while maintaining immune functionality (46).

Beyond immune engineering, CRISPR can directly replace infected or leukemic HSCs with edited, healthy counterparts. By isolating a patient's own HSCs, correcting genetic abnormalities, and reinfusing the edited cells, researchers can restore normal blood cell formation and potentially eliminate leukemia-causing genes at their source (34, 49).

CRISPR SCREENS

Beyond direct therapeutic applications, CRISPR technologies have transformed cancer research by enabling systematic, genome-scale interrogation of gene function. A CRISPR screen is a large-scale genetic experiment that uses the CRISPR-Cas system to systematically perturb genes across the genome in order to study their functions (50). By linking specific gene disruptions to measurable cellular outcomes, CRISPR screening enables the unbiased discovery of essential genes and therapeutic targets across diverse biological contexts (50, 51).

Prior to the development of CRISPR screening, RNA interference (RNAi) was the primary method for large-scale loss-of-function studies (52). RNAi works by introducing short interfering RNAs (siRNAs) or short hairpin RNAs (shRNAs) that bind to messenger RNA (mRNA) transcripts and trigger their degradation (53). However, RNAi-based studies often suffer from incomplete protein knockdown and significant off-target effects, leading to inaccurate data collection (53). Furthermore, the development of CRISPR interference (CRISPRi) and CRISPR activation (CRISPRa) allows researchers to precisely silence and overexpress genes, providing more accurate and reproducible results (42, 54).

Methodology of CRISPR Screening

CRISPR screening consists of two main phases: the perturbation phase and the screening phase (Figure 2). In the perturbation phase, a large library of single-guide RNAs (sgRNAs) targeting thousands of genes is designed and administered to a population of cells along with a Cas protein (50). Cells can either be engineered

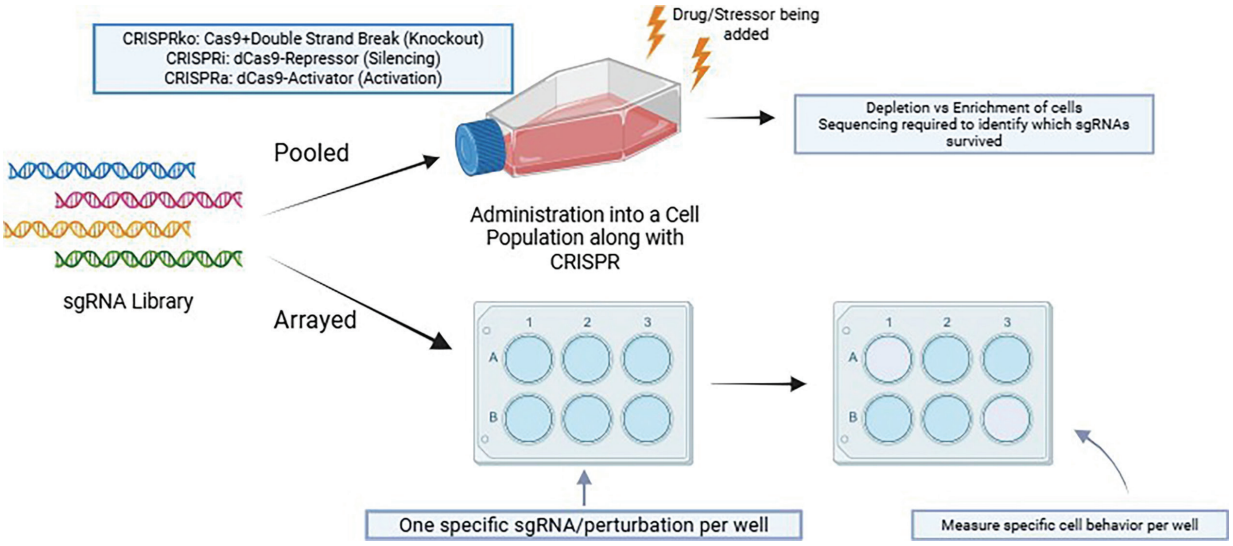

Figure 2. ***Genetic screening using CRISPR-based perturbations in pooled and arrayed formats for functional genomics.*** *A library of single-guide RNAs (sgRNAs) is designed to target specific genes using CRISPR knockout (CRISPRko), interference (CRISPRi), or activation (CRISPRa) systems. In a pooled screen, the entire library is administered to a single population of cells, which are then subjected to selective pressure, such as a drug or stressor; next-generation sequencing is then required to identify which sgRNAs were enriched or depleted based on cell survival. In an arrayed screen, each specific sgRNA or perturbation is isolated in a separate well of a multi-well plate, allowing for the direct measurement of specific cell behaviors or phenotypes on a per-well basis without the need for sequencing.*

to stably express Cas9, or Cas9 can be co-delivered with the sgRNA library using lentiviral or plasmid vectors (50, 51). The specific Cas system employed depends on the experimental goal: CRISPR knockout (CRISPR-ko) uses Cas9 to introduce double-strand breaks for loss-of-function studies, while CRISPR interference (CRISPRi) employs a catalytically inactive dCas9 fused to a repressor domain to reversibly silence transcription without cleaving the DNA, and CRISPR activation (CRISPRa) uses dCas9 fused to transcriptional activators to drive gene expression, enabling gain-of-function studies (42).

At this stage, CRISPR screens diverge into two main formats: pooled and arrayed. In pooled screens, a large sgRNA library is introduced into a mixed population of cells at a low multiplicity of infection (MOI), ensuring each cell receives a single perturbation (51). This creates a diverse pool of mutant cells, each carrying a different gene knockout or modulation (50). In contrast, arrayed screens deliver individual sgRNAs into separate wells of a multiwell plate, keeping each perturbation physically isolated (55).

While pooled screens require sequencing-based readouts to deconvolute which perturbations are enriched or depleted, arrayed screens allow direct phenotype measurement in each well, including imaging-based or functional assays that cannot be reduced to survival alone (55, 56).

In the screening phase, selective pressures such as chemotherapy drugs, nutrient limitations, or immune effector cells are applied to the engineered populations. In pooled screens, sequencing of the integrated sgRNAs reveals which gene knockouts are enriched or depleted, identifying genes required for survival or sensitivity to treatment (50, 51, 56). In arrayed screens, phenotypic changes, such as altered morphology, protein localization, or signaling activity, can be directly quantified at the single-well level, enabling the study of complex cellular outcomes (55).

Together, pooled and arrayed CRISPR screens provide complementary strategies: pooled screens enable cost-effective, high-throughput discovery at the genome scale, while arrayed screens allow high-resolution functional analysis of specific phenotypes (50, 57).

CRISPR Screening in Leukemia Research

CRISPR-based genetic screening has proven incredibly useful for identifying genetic dependencies,

resistance mechanisms, and therapeutic vulnerabilities in leukemia by enabling genome-wide perturbation of gene function (50, 51). By linking specific gene disruptions to survival, proliferation, or immune sensitivity phenotypes, CRISPR screens allow researchers to move beyond candidate-based approaches and map functional gene networks that sustain leukemic growth (58, 59). This systematic approach has been particularly instrumental in three areas: defining the "essentialome" of leukemia, uncovering drug resistance mechanisms, and identifying subtype-specific vulnerabilities (60).

A primary goal in functional genomics is distinguishing genes required for general cell survival from those specifically required by leukemic cells. In AML, genome-wide dropout screens have revealed specific "fitness genes" that sustain leukemic proliferation but are not required for normal hematopoietic stem cell function (61). Building on this, Tzelepis *et al.* (2016) utilized CRISPR-Cas9 to identify essential regulators of chromatin modification, DNA repair, and metabolism whose loss selectively impaired AML cell survival (42). These genes, many of which encode transcriptional cofactors or epigenetic enzymes, represent potential drug targets for future precision therapies (42). Follow-up studies demonstrated that inhibiting these pathways recapitulated the cytotoxic effects of genetic knockout, validating screen-derived hits as translationally relevant targets (42). Computational frameworks such as BAGEL (Bayesian Analysis of Gene EssentiaLity) have further refined this process by distinguishing core-essential genes from leukemia-specific vulnerabilities, improving selectivity and reducing toxicity risks (56).

Research regarding drug resistance reveals a recurring theme: the disruption of DNA repair and apoptotic pathways. Early genome-wide CRISPR knockout screens, such as Wang *et al*., provided the initial proof-of-concept by demonstrating that the loss of DNA mismatch repair (MMR) genes, specifically MSH2, MSH6, MLH1, and PMS2, confers resistance to thiopurine chemotherapy (6-TG) in leukemia models (51). This discovery laid the groundwork for subsequent studies that explored resistance to modern targeted therapies. For example, recent screens have identified that resistance to Venetoclax (a BCL-2 inhibitor used in AML) is often driven by the upregulation of alternative anti-apoptotic proteins like *MCL-1* or the loss of mitochondrial chaperones, thereby bypassing chemotherapy-induced cell death pathways (62).

Recent research has shifted toward identifying vulnerabilities unique to specific genetic subtypes of leukemia, moving away from "one-size-fits-all" targets in favor of precision medicine (63). This evolution is exemplified by the work of Jaiswal *et al.* (2021), who identified USO1, a vesicle tethering factor, as a critical dependency specifically in B-ALL (41). Their findings demonstrated that USO1 loss induces apoptosis in leukemic cells while sparing healthy lymphocytes, suggesting a highly selective therapeutic window (41). This shift toward identifying lineage-specific vulnerabilities highlights how functional genomics is successfully narrowing the gap between basic laboratory discovery and the clinical implementation of targeted oncology.

CONCLUSION

While conventional chemotherapy remains essential for acute leukemias, its efficacy is continuously undermined by drug resistance driven by mechanisms like gene mutations as well as systemic toxicity. The advent of CAR-T cell therapy, as seen by success against B-ALL, represents a monumental shift in personalized medicine, yet it introduces its own set of challenges, such as severe cytokine release syndrome and the critical safety concern of on-target, off-tumor toxicity, leading to B-cell aplasia and GvHD. To overcome these obstacles, the field is moving towards precision gene editing with the CRISPR-Cas system. CRISPR's accuracy is being leveraged in two ways: therapeutic intervention and target discovery. Therapeutically, CRISPR-Cas9 enables the generation of universal allogeneic CAR-T cells (e.g., UCART19) by editing T-cell receptors and HLA genes to mitigate GvHD and accelerate production. Furthermore, it holds promise for engineering HSCs to correct genetic defects and restore normal hematopoiesis.

Crucially, CRISPR screening has become the engine of discovery, moving beyond the guesswork of previous genetic studies towards unbiased, genome-wide identification of therapeutic vulnerabilities and resistance mechanisms. Genome-scale dropout screens, validated in models like KBM7, are uncovering essential genes and leukemia-specific vulnerabilities (such as USO1 in B-ALL). By coupling CRISPR perturbations with relevant selective pressures, researchers are identifying the precise genetic networks responsible for resistance and relapse. This convergence of genetic mapping and precise gene editing is not just refining existing therapies; it is defining a new era of leukemia management, aiming to generate highly selective, durable, and less toxic therapeutic strategies for all leukemia subtypes.

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