From Diagnostic Limitations to Precision Medicine: AI-Enhanced CRISPR for Gallbladder Cancer Detection

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Abstract

Gallbladder cancer (GBC) is a highly lethal disease, often diagnosed at advanced stages due to vague symptoms and lack of reliable screening methods. This paper explores how AI-enhanced CRISPR can contribute to an earlier and more precise detection of GBC. It first details the diagnostic challenges of GBC. Next, it introduces CRISPR, a genome editing technology including its ability to identify genetic alterations associated with cancer. Then, it explores how CRISPR has revolutionised medical diagnostics and treatment, particularly when combined with AI. AI can improve accuracy by reducing off-target effects and guiding the RNA, helping improve accuracy. It will also examine existing AI-integrated CRISPR tools. We hypothesize that this AI-enhanced CRISPR-based approach will enable the development of more precise and sensitive diagnostic systems, offering a critical opportunity to improve early detection of GBC and patient outcomes.
Keywords: Gallbladder cancer, CRISPR, artificial intelligence, diagnostics

1. Introduction

Gallbladder cancer (GBC), while rare, remains a highly lethal disease, with approximately 115,000 new cases and 10,000 deaths globally each year (Bray et al., 2018; Hundal & Shaffer, 2014). GBC develops when aberrant and malignant cells form in the epithelial lining of the gallbladder, an organ located beneath the liver and on the upper right part of the abdomen (Hundal & Shaffer, 2014). The most common symptoms include: abdominal pain or discomfort, jaundice, and rapid weight loss. Other symptoms include abdominal lumps, nausea, vomiting, bloating, and fever (Duffy et al., 2019). GBC most commonly affects females, particularly those with obesity, gallstones, inflammatory bowel disease, and individuals of Hispanic, Native American, South, or East Asian ethnicity (Hundal & Shaffer, 2014; Randi et al., 2006). Additionally, patients with colitis or Crohn's disease also have an increased risk of gallbladder cancer (Bernstein et al., 2001).

During its early stages, GBC often presents with symptoms that overlap with other hepatobiliary diseases, such as liver or bile duct cancer, leading to late or incorrect diagnoses (Duffy et al., 2019). Some ways to detect GBC are imaging techniques, such as ultrasound, CT scans, MRI, and PET scans, then histopathological examination of biopsy samples and lastly, blood tests. Indeed, gallbladder cancer is often misdiagnosed as gallstones or a bile duct

blockage, as they share similar symptoms (Duffy et al., 2019). This frequently leads to late detection, at which point, the cancer often metastasizes to the liver, lymph nodes, and peritoneum (Hundal & Shaffer, 2014). Better early detection methods are therefore crucial for GBC, as it is typically diagnosed in advanced stages when treatment options are limited (Valle et al., 2016). While early-stage treatment involves surgery to remove the gallbladder, lymph nodes, and parts of the liver (Aloia et al., 2015), metastatic diseases necessitate additional approaches like chemotherapy, immunotherapy, and targeted therapy (Aloia et al., 2015; Valle et al., 2016). Therefore, identifying GBC earlier provides more opportunities for effective intervention, such as curative surgery (Hundal & Shaffer, 2014).

The application of CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) technology, particularly in cancer diagnostics, offers an exciting opportunity to improve GBC detection (Li et al., 2023; Hsu et al., 2014). Beyond its broad applications in creating disease models and new therapies for conditions like cancer, HIV, and sickle cell anemia, CRISPR holds immense promise as a diagnostic tool (Barrangou et al., 2020; Li et al., 2023). This is facilitated through CRISPR-enabled creation of specific biosensors to detect disease biomarkers in DNA (Li et al., 2023). Compared to conventional means of detecting cancer, such as blood tests for tumor-associated proteins, CRISPR technology offers benefits, including low cost, high efficiency, and adaptability across multiple organisms, making it particularly attractive for aiding early detection in disease (Barrangou et al., 2020)

Diagnostic platforms based on CRISPR exhibit high sensitivity and rapid detection capabilities (Li et al., 2022). However, the high complexity of biological data required for disease diagnostics reduces this potential. The incorporation of artificial intelligence and machine learning (AI/ML) provides an avenue to overcome these barriers by providing mechanisms to analyze large genomic databases in an efficient manner, optimize guide RNA design, and increase the specificity of targets for CRISPR technology. AI/ML permits prediction of off-target effects and improves the interpretation of signals, thus enhancing the accuracy and reliability of CRISPR diagnostics. In relation to the detection of GBC, this collaborative system enables the rapid, non-invasive identification of nucleic acid biomarkers associated with GBC, miR-552-3p, miR-581, miR-146a, HOTAIR, and MEG3, in the early stages of the disease, thus facilitating the development of innovative diagnostic technologies, consistent with the intent of this study.

I hypothesize that integrating AI/ML with CRISPR-based biosensing platforms will enable the development of a highly precise and sensitive diagnostic system, capable of non-invasively detecting GBC–specific nucleic acid biomarkers at the earliest stages of disease. This invites opportunity in gallbladder cancer to help improve early and precise detection (Li et al., 2023; Barrangou et al., 2020).

2. Challenges in Gallbladder Cancer Diagnostics

Gallbladder cancer remains a fatal disease that is often detected very late (stage III-IV), primarily due to its symptoms overlapping with other hepatobiliary conditions like liver or bile

duct cancer, leading to late or incorrect diagnoses (Rawla et al., 2019). Furthermore, the gallbladder's deep anatomical location beneath the liver makes early tumors challenging to detect through physical examinations or traditional imaging techniques. Consequently, most GBC cases are only detected once the cancer has metastasized, severely impacting treatment options and prognosis (American Cancer Society, 2024).

The most common detection methods for GBC include imaging techniques like ultrasound, computed tomography (CT) scans, and magnetic resonance imaging (MRI). Ultrasound machines use high-frequency ultrasound waves to generate images of organs and tissues present inside the human body (Sharma et al., 2023). While abdominal ultrasound is used to check for thickening of the gallbladder wall, it often struggles to reliably differentiate between non-cancerous (benign) and cancerous (malignant) tissue in the gallbladder. This frequently leads to misdiagnosis like cholecystitis, gallstones, or polyps (Ueno et al., 2021). Additionally, ultrasound image quality is operator-dependent and can be poor in obese patients, causing small cancers that resemble gallstones to go unnoticed during routine scans (Sharma et al., 2023). Endoscopic ultrasound (EUS) combines endoscopy and ultrasound, using a thin flexible tube inserted into the digestive tract with an ultrasound device as its tip to produce images of the digestive tract and surrounding organ tissues (Penn Medicine, 2024). Similar to conventional ultrasound, EUS faces difficulty in distinguishing between benign and malignant gallbladder diseases (Penn Medicine, 2024).

CT scans, which use a series of x-ray images from different angles to create detailed cross-sectional layouts of the body, similarly struggle to delineate the boundaries between cancer cells and inflamed tissue in a precise manner (Medscape, 2024). This limitation means tiny cancer cells in the abdominal lining or small lymph nodes with cancer spread may not appear on CT scans. The visual similarity between inflammation and cancer often leads to misdiagnosis (Medscape, 2024).

Another diagnostic tool, MRI, uses magnetic fields and radio waves to create detailed pictures of organs and tissues present in our body. However, MRI is typically ineffective at detecting GBC in its early stages because the gallbladder is small and tucked under the liver, making subtle changes difficult to visualize. Early-stage cancer often manifests as mild inflammation or abdominal wall thickening, which can be mistaken for benign conditions like gallstones or cholecystitis. Furthermore, MRI often lacks the resolution required to accurately detect subtle or extremely small tumors, meaning it is more useful for staging and determining the extent of the cancer than for early detection (Mayo Clinic, 2024).

Cholangiography, which involves imaging of the bile duct via x-rays with a contrast medium, is primarily designed to visualize the bile ducts rather than the gallbladder itself. Consequently, it is largely ineffective for early GBC detection. In its initial stages, cancer may only cause minor wall alterations or tiny lesions that do not obstruct or distort the ducts, leading to healthy-appearing images. Abnormalities such as irregular narrowing of the duct only become apparent once the tumor has spread to or compressed the bile ducts, making cholangiography

more helpful for determining the degree of obstruction than for making an early diagnosis (Mayo Clinic, 2024).

GBC can also be detected through needle and laparoscopic biopsies, where tissue from the organ and surrounding areas is removed and examined by a pathologist. While needle biopsies use ultrasound guidance to collect tissue samples and laparoscopic biopsies involve keyhole surgery for broader tissue collection, both methods present significant challenges. Firstly, there is a risk of tumor seeding, where cancer cells can spread if the needle is inserted into the gallbladder. Even when this risk is avoided, biopsies can suffer from sampling error, where only a small section of the tumor is evaluated, potentially missing the cancerous area (false negative diagnosis). The gallbladder's hidden location beneath the liver also makes biopsies technically difficult and risky. These factors can lead to biopsies being avoided before surgery, delaying diagnosis, and sometimes, GBC is only discovered incidentally after surgery (Sharma et al., 2023).

Traditional serum tumor markers, such as CEA (Carcinoembryonic Antigen), while overexpressed in tumors, are also expressed by normal, healthy cells. This non-specificity makes it challenging to distinguish between malignant and benign (non-malignant) conditions, particularly in early stages of cancer development (Medscape, 2024). In contrast, new molecular biomarkers like circulating miRNAs (miR-552-3p, miR-581, miR-146a) and long non-coding RNAs (HOTAIR, MEG3) offer greater accuracy (Wang et al., 2022). By shedding light on genetic changes and the molecular processes underlying tumor growth, these biomarkers may be more precise than conventional serum markers. This could aid in prognostic assessment and treatment response.

Given the limitations and challenges of traditional detection methods, there is an opportunity to improve early detection for GBC. CRISPR technology is a powerful tool that can be used to detect specific genetic mutations or biomarkers associated with tumour cells. CRISPR Cas-9 can be programmed to recognise cancer-related DNA and RNA sequences. This can help doctors and researchers identify these mutations with sensitivity and precision and can allow for early cancer detection and more accurate diagnostics with the potential for personalized treatment options (Li et al., 2023).

3. Principles of CRISPR and Its Diagnostic Potential in Gallbladder Cancer

3.1 Principles of CRISPR

CRISPR is a revolutionary gene-editing tool, originally discovered as a bacterial defense mechanism against viruses, that can be harnessed to address lifelong diseases such as cancer (Hsu et al., 2014). It is currently utilized for genome editing, where a Cas9 nuclease enzyme is guided by a single guide RNA (sgRNA) to make a specific cut in the DNA (Hsu et al., 2014; Doudna & Charpentier, 2014). When the Cas enzyme creates a double-strand break, DNA repair pathways take over: non-homologous end joining may disrupt genes through insertions or

deletions, whereas homology-directed repair can insert or correct specific sequences using a template (Figure 1) (Doudna & Charpentier, 2014). In bacteria, CRISPR arrays store viral DNA, enabling the bacteria's immune system to detect and destroy viral DNA upon subsequent attacks Makarova et al., 2020; Jinek et al., 2012)

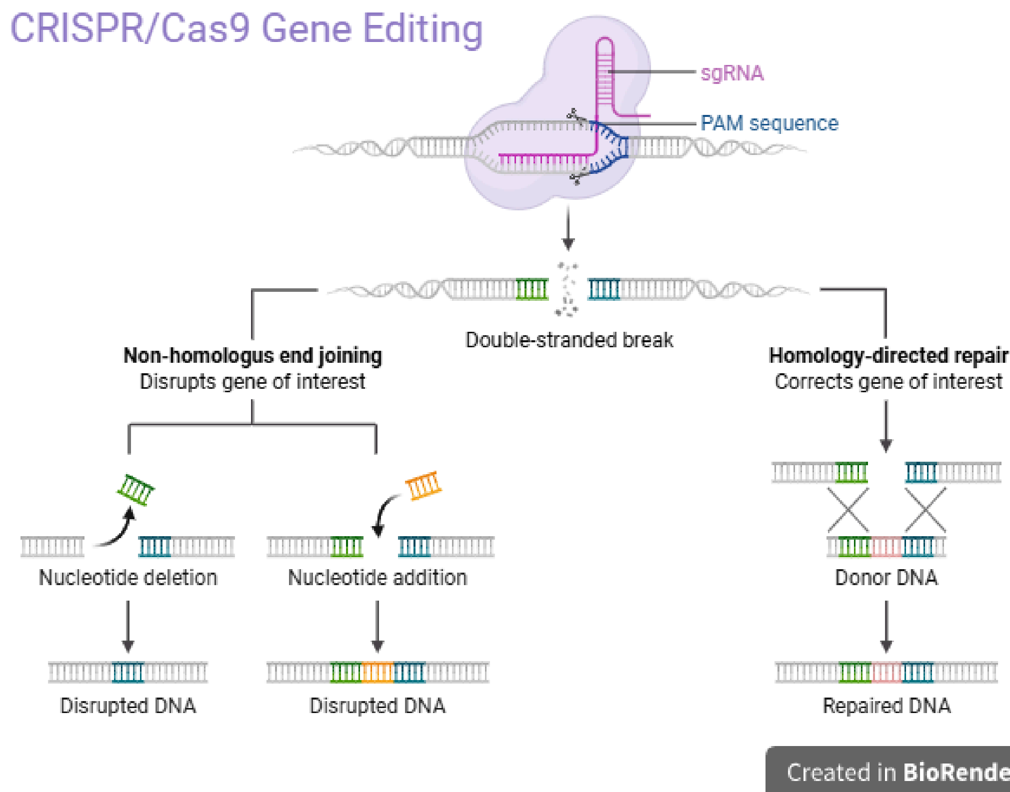

Figure 1. Cas9 Gene editing (Source: BioRender, Dragt, E. (2026)).

There are two main parts of the CRISPR system: a Cas nuclease enzyme (such as Cas9), which cuts the DNA, and a guide RNA, which tells the enzyme where to cut along the DNA. The Cas and guide RNA form a complex and move through the cell to attach to the DNA in the patient. Crucially, the Cas enzyme will only cleave the DNA if a specific protospacer adjacent motif (PAM) sequence is present immediately adjacent to the target site (Royal Society Publishing, 2016). PAM is crucial as it allows the Cas enzyme to identify and cut only foreign DNA and protect the organism's own genome from being targeted by mistake.

Cas9 then generates a double-stranded break in the DNA, as seen in figure 1, at the exact location that matches the gRNA sequence. The cell repairs the double-stranded break through DNA repair mechanisms such as non-homologous end joining (NHEJ) or homology-directed repair (HDR). NHEJ disrupts genes by introducing deletions or insertions, creating a small change to inactivate a gene. The cell simply rejoins the ends that have been

cut. HDR, conversely, is useful for inserting or correcting specific sequences using a template (Jinek et al. 2012; Adli 2018).

All together, these steps allow CRISPR-Cas9 to act in a programmable and a highly specific manner genome editing tool. Once cut, scientists can delete, insert, or replace genetic information, making CRISPR efficient, precise, and adaptable across organisms (CRISPR Therapeutics, 2024). For example, CRISPR-Cas9 has been tested in treating sickle cell disease. In a clinical trial, CRISPR-Cas9 was used to edit hematopoietic stem cells to increase fetal hemoglobin production. This significantly improved symptoms in patients. Furthermore, the CRISPR gene-editing treatment has received regulatory approval from the United States (FDA 2023) This example shows how CRISPR-cas9 has been utilized and applied in humans and it can be used for genome editing.

3.2 CRISPR-based Diagnostics for Gallbladder Cancer

The diagnosis of GBC increasingly relies on detection of ultra-low abundance biomarkers from liquid biopsies, such as mutated DNA or RNA fragments derived from circulating tumor cells in a body. This represents a non-invasive technique with significant potential for early diagnosis, enabling real-time tracking of cancer development. There are two main conventional techniques to do this: 1) quantitative polymerase chain reaction (qPCR), which amplifies specific DNA sequences for detection, and 2) next-generation sequencing (NGS), which enables high-throughput analysis of genetic material. These two are commonly used for identifying cancer-related biomarkers. While these traditional technologies offer high sensitivity, they are often limited by complex primer design or relatively high-cost.

CRISPR-based diagnostics offer a transformative alternative by leveraging the programmable nature of Cas enzymes to achieve rapid, single-nucleotide specificity without the need for intensive thermal cycling (Kaminski et al., 2021). The goal of the CRISPR diagnostics is to develop a more precise, rapid and non-invasive way of detecting these molecular alterations. In doing so, diagnostic tests can detect gallbladder cancer very early. The molecular biomarkers of gallbladder cancer, such as TP53 and KRAS mutations and altered microRNA expression are often lowly abundant and difficult to detect using traditional diagnostic methods, such as qPCR (Hundal and Shaffer, 2014; Lazcano-Ponce et al., 2001). Therefore, the use of CRISPR-based diagnostics may help improve the sensitivity of detecting these molecular biomarkers in liquid biopsy specimens.

The ultra-sensitivity of CRISPR diagnostic technologies is mainly attributable to collateral cleavage of reporter molecules by Cas12/13 proteins when they recognize their target(s). This enables the use of CRISPR diagnostic technologies to provide enhanced signals that are detectable. Cas12 and Cas13-based systems can detect specific DNA mutations or RNA expression profiles that are particular to a cancer cell by leveraging collateral cleavage to generate a signal associated with the presence of these biomarkers (Figure 2). Collateral

cleavage is a key mechanism for signal amplification that allows for ultra-sensitive diagnostics. Once a target sequence, such as a biomarker of cancer, is detected, collateral cleavage is triggered, and it cleaves reporter molecules, a reporter is a labelled DNA or RNA molecule that produces a detectable signal when cleaved by activated Cas proteins generating a signal that is detectable (Chen et al., 2018). This signal amplification helps improve the sensitivity and response time of diagnostic platforms (Collateral Cleavage Mechanisms, 2023).This kind of non-invasive, blood test-based molecular diagnosis can modernize cancer detection and thereby stabilize proliferative cellular illnesses with greater specificity and speed over traditional methodologies (Kellner et al., 2019; Broughton et al., 2020).

The process of collateral cleavage results in the amplification of signals and contributes significantly to improved sensitivity for diagnostic testing to detect GBC. For example, the identification of a specific mutant found in the tumor's DNA or RNA, such as the R273H mutation on TP53 (not only applied to GBC but other diseases as well) or the G12D mutation on KRAS, can occur through detecting circulating tumor cell (CTC) and/or cell-free (cf) DNA from either liquid biopsies (blood plasma and/or bile) or a combination of both, and thus allow for ultra-sensitive detection of GBC at levels as low as attomolar (10^–18). Collateral cleavage will also generate a detectable fluorescent and/or chromogenic signal when an oligonucleotide-based reporter molecule that is labeled with a fluorophore-quencher is cleaved by collateral cleavage. This is performed within an hour or less and is capable of being performed without the need for thermal cycling, which makes it portable (Smith et al., 2025; Gootenberg et al., 2018) compared to qPCR. Furthermore, the collateral cleavage assay will have the same SNP specificity as that of other SNP assays. This would allow for accurate identification of minimal residual disease following curative treatments for early-stage GBC or chemotherapy.

SHERLOCK and DETECTR are two platforms that utilize collateral cleavage, not only for GBC but for other diseases as well (Figure 2). SHERLOCK (Specific High-sensitivity Enzymatic Reporter unLOCKing) utilizes Cas13 enzymes, which specifically target RNA sequences and have collateral cleavage of single-stranded RNA reporters. The SHERLOCK technology provides an important method to detect RNA biomarkers (e.g., aberrant expression of microRNA) that have been linked to gallbladder cancer. By utilizing the Cas13 enzyme, which will become activated upon binding to its target RNA sequence, SHERLOCK can cleave adjacent reporter molecules producing a detectable signal (Figure 2). Thus, SHERLOCK technology can provide both high sensitivity and specificity for low-abundance nucleic acid targets from liquid biopsy samples (Gootenberg et al. 2017; Kellner et al. 2019).

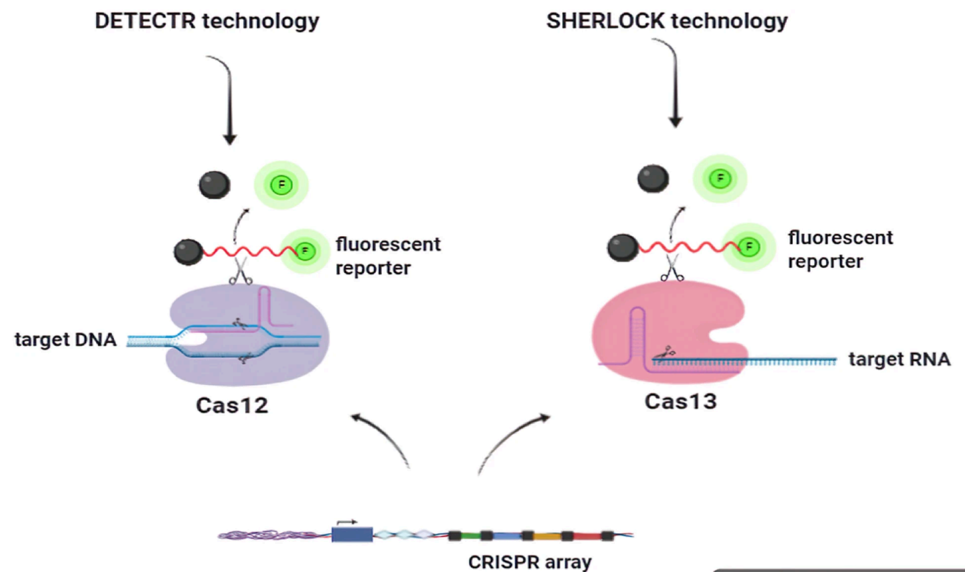

Figure 2. Collateral cleavage activity using SHERLOCK and DETECTR technologies. In SHERLOCK (right), Cas13 is guided by the single gRNA to cleave ssRNA or mRNA,leading to cleavage of the reporter molecule nonspecifically after which the fluorescent signal is detectable. In DETECTR (left), after binding the Cas12-gRNA complex to its target (dsDNA) the collateral nuclease activity of the Cas12 leads to cleavage of the reporter molecule nonspecifically after which the fluorescent signal is detectable (Source: Jolany Vangah, S., Katalani, C., Boone, H.A. et al., 2020).

The SHERLOCK detection method offers advantages over conventional nucleic acid detection methods. To begin with, it is highly sensitive, being able to detect single molecules of DNA and RNA.The SHERLOCK technology offers speedier results than many lab-based assay techniques because it does not require thermal cycling. In fact, it allows for rapid, point-of-care testing in clinics, in remote locations, and even at the patient's home because a healthcare worker can simply collect a specimen from a patient and test it on-site using SHERLOCK. Results can be obtained quickly (within 10–15 minutes); therefore, healthcare providers can make timely diagnostic and treatment decisions. In addition, the ability to freeze-dry (lyophilize) the reaction, store it, and use it to perform tests over extended periods of time, with near 99.9% accuracy and ease of use, enables the possibility of using these tests as a platform for a large number of screening programs across many locations worldwide (cite).

There are some limitations associated with SHERLOCK's use. First, as SHERLOCK's assay requires expert-level skill to prepare many of the reaction components, this has made access to SHERLOCK very limited as a platform used routinely for detection. Second, the lack of widely available predesigned commercial kits further hinders standardization and the ability for ease of adoption. SHERLOCK has performed well for producing relative quantitative results, pathogens like SARS-CoV-2, Zika, and Ebola; however, it cannot obtain absolute digital

quantification similar to digital droplet PCR and may not be able to detect changes in target concentration that are less than two-fold. Thus, SHERLOCK is probably not as suitable for applications requiring highly precise quantitative results (Mustafa & Makhawi, 2021).

Another similar and relatively new CRISPR-based diagnostic tool, DETECTR (DNA Endonuclease Targeted CRISPR Trans Reporter), allows for the detection of specific RNA biomarker(s) using amplification techniques at constant temperatures. DETECTR is specific and sensitive to the biomarker(s) of interest (e.g., TP53/KRAS mutations). In particular, DETECTR uses different Cas12 proteins for the detection of specific DNA sequences (e.g., TP53/KRAS mutations in gallbladder tumors via PAM sites) (Figure 2). The Cas12 proteins used in DETECTR also cleave ssDNA reporters that are adjacent to the target, which allows the results to be detected rapidly by either a lateral flow assay or plate reading within 30 minutes (Smith et al., 2025; Gootenberg et al., 2018) (Figure 3). DETECTR is one of the fastest CRISPR-based diagnostic platforms. Having numerous advantages, such as high reliability and high portability, makes it a tool for early cancer detection.

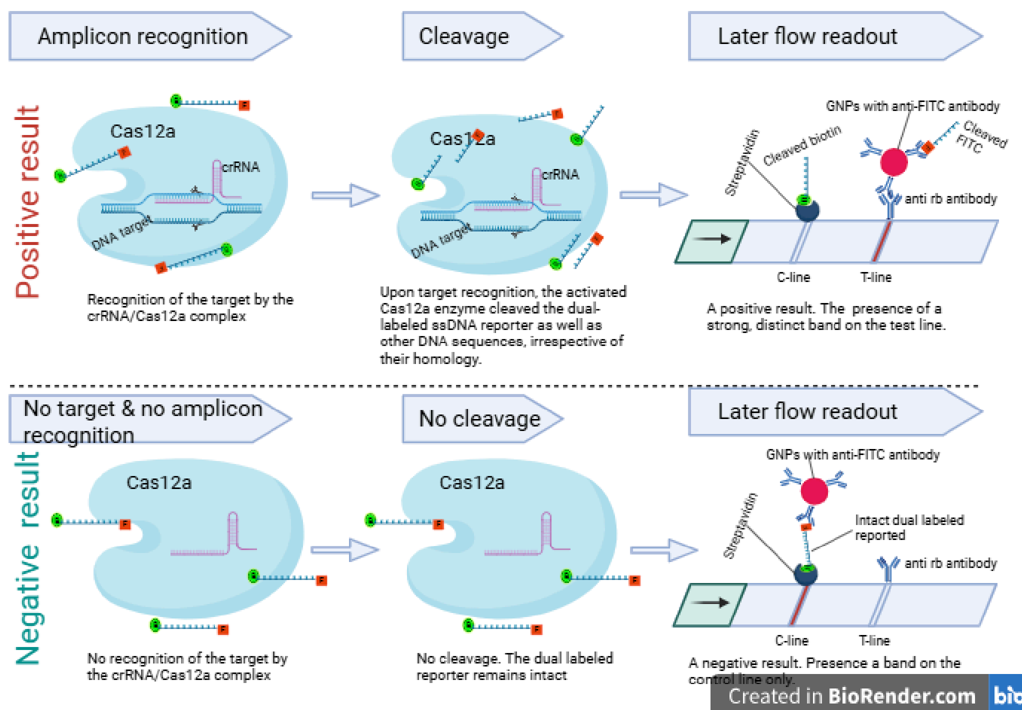

Figure 3. CRISPR-Cas12a lateral flow detection (Source: BioRender, Regassa, S. L. (2026)).

Although DETECTR has many advantages, it also has some disadvantages. For example, it has a lower sensitivity than SHERLOCK because the detection of DETECTR usually occurs at higher target concentrations than those used by SHERLOCK. Furthermore, the primary application of DETECTR is to detect the DNA target; thus, the use of this detection tool

for the direct determination of RNA requires additional processing steps before applying the detection methodology. For DETECTR to produce effective results, researchers must use the design of the guide RNAs and the optimization of the reaction conditions to improve the reproducibility and consistency across different applications (Mustafa & Makhawi, 2021).

In summary, compared to traditional technologies such as qPCR and NGS, CRISPR offers several advantages in speed and accuracy. However, there remain disadvantages to using CRISPR for clinical diagnostics versus these more commonly-used platforms. CRISPR diagnostic platforms are typically much less sensitive (without pre-amplification) than qPCR platforms due to the lower catalytic efficiency of the various enzymes used. The dilution factor of a large reaction volume means that there is much less sensitivity compared to the increase in response time of the reaction compared to qPCR. Additionally, there will be much greater differences in response times for reactions compared to both aggregates of responses with and without amplification factors, and therefore there will be a larger difference in sensitivity versus speed compared to qPCR (even considering qPCR digital counts). Potential issues with using CRISPR for diagnostics include inaccurate cutting (or off-target cuts) by the target site enzyme because of impending reactions, difficulties with delivering the CRISPR components to their target cells, the potential of the Cas proteins to trigger an immune response.

4. AI/ML in CRISPR Diagnostics: Implications for Gallbladder Cancer

The combination of artificial intelligence and machine learning in medicine marks a transformative change in healthcare (Esteva et al., 2017). Recent studies involving ML to design CRISPR diagnostic guide RNAs, such as TIGER deep learning model, have shown improved targeting specificity and reduced off target activity by accurately predicting guide RNA efficiency (Wessels et al. 2023). AI-driven algorithms can detect precise patterns of disease such as cancer, redefining how cancers can be detected, monitored, and treated (Litjens et al., 2017; Topol, 2019) for instance, AI is used to design CRISPR-based sensors that identify circulating tumor DNA (ctDNA) in blood samples, allowing for the early detection of lung or breast cancer mutations.

AI/ML can help overcome limitations associated with CRISPR technology (Hsu et al., 2014). AI/ML can improve the precision, efficiency, and safety of genome editing systems (Wang et al., 2019). In particular, these algorithms help reduce the off-target effects caused by the Cas enzyme's tolerance for DNA mismatches, allowing delivery of CRISPR components into target cells, and lastly, predicting and reducing immune responses to Cas proteins (Chuai et al., 2018). This section will explore how AI/ML contribute to the CRISPR limitations discussed above.

Off-target effects are unintended actions by a gene editing tool, drug, or other molecule causing effects in regions other than the targeted locations in the body(Fu et al., 2013). Multiple procedures can be done to reduce off-target effects by CRISPR (Hsu et al., 2014). AI and deep learning models, such as DeepCRISPR, use large datasets of CRISPR experiments to learn patterns of where the off-target editing occurs (Chuai et al., 2018). Then these models integrate

the sequence and epigenetic features to predict where the unintended cuts will occur (Chuai et al., 2018). This helps researchers select guide RNA with fewer off-target effects than before (Doench et al., 2016). Secondly, AI algorithms can use guide RNA sequences to predict how likely a candidate is to bind with the intended target region rather than similar non-target sequences (Wang et al., 2019). This reduces the risk of off-target detection signals so it improves the specificity and reliability of CRISPR-based diagnostic model (Fu et al., 2013). Lastly, advanced machine learning, pretrained language models for RNA and DNA, or deep neural networks, can detect complex patterns, allowing them to predict off-target effects in genomic contexts, which traditional tools often struggle with (Zhang et al., 2021). AI models, such as deep learning platforms like TIGER, can classify cancer subtypes using transcriptomic and gene expression data, enabling more precise guide RNA selection and improving the accuracy of CRISPR-based diagnostics (Wessels et al., 2023). For example, researchers at the Broad Institute have used AI to identify unique cancer subtypes based on gene expression profiles. This supports a more targeted CRISPR diagnostic approach for cancers such as gallbladder cancer. This improves the reliability of predictions across cell types (Chuai et al., 2018).

Efficient delivery of CRISPR components to target cells is a limitation in CRISPR technology (Lino et al., 2018). This can be improved by implementing AI/ML (Wang et al., 2019). AI and ML can analyze a large number of experimental datasets, creating predictive models that may give insight into how well the method of delivery (viral, lipid nanoparticle, or exosome-based) may work with a certain cell type or tissue (Khalil et al., 2020). It is possible to predict the level of efficiency of the delivery means and their cellular uptake, thereby eliminating a major barrier to creating an effective experimental strategy (Zhang et al., 2020). In addition, AI is assisting with the development of nanoparticles for CRISPR delivery with optimal size, charge, and composition to improve cellular entry, intracellular release, and reduce potential for cytotoxicity (Yang et al., 2022). By supporting both targeted delivery methods at the cell-type level and improved delivery efficiency, AI/ML increases the feasibility and safety of CRISPR-based therapies (Wang et al., 2019).

Immune responses to Cas proteins are a significant limitation in CRISPR technology (Charlesworth et al., 2019). AI/ ML help in predicting the immunogenicity of different Cas proteins using protein sequence and structural data (Sarkisyan et al., 2016). ML and AI-powered models allow researchers to identify and develop modified Cas proteins that have a decreased level of immunogenicity while still retaining gene-editing capabilities for use in CRISPR technology (Crudele & Chamberlain, 2018). Furthermore, ML tools can support analysis of immunological data at the population level, helping to identify potential immunological barriers and aiding in the selection of safer Cas enzymes (Charlesworth et al., 2019). Moreover, AI accelerates the identification of extinct and currently new Cas proteins (Burstein et al., 2017). Using AI to continuously integrate knowledge of immunology with the design and delivery of proteins will continue to improve the viability of CRISPR as a therapeutic tool (Topol, 2019).

Taken together, the integration of AI/ML with CRISPR technology helps improve several barriers for its clinical application (Wang et al., 2019). Improving guide RNA specificity helps reduce off-target effects, allowing for efficient cellular uptake and mitigating immune response to Cas proteins. AI/ML improve both accuracy and safety when editing genomes (Chuai et al., 2018). Advances in the application of AI have shown great potential for translating CRISPR technology from research to therapeutic uses (Topol, 2019).

5. Discussion

Integrating artificial intelligence with CRISPR technology offers a powerful approach for early and precise detection of gallbladder cancer (Topol 2019; Chen and Asch 2017). CRISPR enables the identification of highly specific cancer biomarkers while AI enhances data analysis by reducing errors and improving signal interpretation (Gootenberg et al. 2017; Chen et al. 2018). By combining these two different methodologies, early and better quality detection of cancers is expected, as well as increased monitoring for the presence of minimal residual disease, and the development of personalized treatment approaches (Esteva et al. 2019; Topol 2019). By increasing the accuracy of diagnostics and lowering false positives, this partnership may result in improved patient outcomes, less reliance on invasive procedures, and reduced overall costs due to quicker turnaround times in diagnosis (Jiang et al. 2020; Chen and Asch, 2017).

The ability to utilize AI-based models such as DeepCRISPR will improve the accuracy of CRISPR-based diagnostics (Wang et al., 2019). AI will provide improvements to CRISPR by designing guides for RNA, predicting on/off targets, and increasing the specificity of the target sites using the latest advances in pattern recognition technology (Wang et al., 2019; Chuai et al., 2018). Machine learning methods applied to protein sequence data can minimize immune responses to Cas protein, maximizing the effectiveness of CRISPR systems and increasing the overall security and reliability of these systems (Adli, 2018; Topol, 2019). In the case of gallbladder cancer, where current diagnostic methods (biopsies) are invasive and pose significant risk, and because serum biomarkers lack the necessary specificity to identify early stages of disease, AI and CRISPR are able to provide significant advantages (Hundal and Shaffer 2014). In comparison to current methodologies that utilize imaging and biopsy, CRISPR-based molecular diagnostics powered by AI allow for quicker, non-invasive, and much more precise detection, especially in the early stages of diseases (Kellner et al. 2019; Broughton et al, 2020). With these different technological aspects combined together, they help provide solutions to many of the current limitations faced in diagnosing gallbladder cancer. The result of these improvements on patient outcomes is increasing by creating new opportunities for early detection and transforming how diseases are identified.

Despite its innovative approach, several limitations in implementing AI-enhanced CRISPR diagnostics remain (Topol, 2019). Firstly, there is a lack of availability of large, high-quality datasets required to train AI and machine learning models to detect patterns for diagnostics (Esteva et al., 2019). This poses a problem as biased or inefficient data can reduce

accuracy for cancer diagnostics (Chen and Asch, 2017). Future research should focus on the development of diverse and large high quality datasets with international researchers (Topol, 2019). Secondly, CRISPR diagnostics may still be affected by off-target effects, leading to false signals if not carefully controlled (Adli 2018). To reduce off-target effects, future research could combine CRISPR enzymes with AI guided RNA to reduce offtarget effects and false signals (Wang et al, 2019). Thirdly, extensive validation from a diverse population is required to ensure reliability (Esteva et al., 2019). Moreover, there is a lack of large-scale clinical validation. Furthermore, regulatory approval is complex due to data privacy and the ethical use of AI and CRISPR in medicine which may slow down its implementation (Topol, 2019). It is essential to address these limitations before AI-enhanced CRISPR diagnostics can be widely used.

The goal of this review was to evaluate how CRISPR technology can be leveraged for the early and specific detection of gallbladder cancer biomarkers, and the specific role of AI in optimizing this process. Looking ahead, with the advancements of AI algorithms, data availability, and clinical validation, AI-enhanced CRISPR diagnostics can be clinically used. Finally, the development of AI-enhanced CRISPR diagnostics is significant for earlier detection of gallbladder cancer, improving patient outcomes and diagnostics in general.

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