Exploring the Potential of Star Polymer Tagged with siRNA for Lung Cancer Treatment

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ABSTRACT

Lung cancer remains one of the most common and harmful forms of cancer worldwide, making it critical to explore new treatment strategies that can improve patient outcomes. One of the most promising approaches in this field is the development of nanoparticle-based therapies, particularly those utilising star polymer-siRNA nanoparticles. These nanoparticles represent a revolutionary platform for targeted drug delivery in lung cancer treatment.

Star polymer-siRNA nanoparticles are particularly advantageous due to their enhanced stability, controlled release, and biocompatibility, which make them ideal candidates for delivering small interfering RNA (siRNA) molecules that aim to silence specific genes involved in cancer progression.

The creation of star polymer-siRNA nanoparticles begins with an extensive bioinformatics analysis of the genomic and molecular data. This analysis is crucial for identifying unique molecular targets within the cancer cells, enabling researchers to understand the specific genetic mutations and molecular pathways driving the patient's cancer. By pinpointing these specific targets, custom siRNA sequences can be designed to silence oncogenes or other genes critical for cancer cell survival and growth.

Once the specific molecular targets are identified, custom siRNA sequences are developed and encapsulated within star polymer nanoparticles. The star-shaped structure of these polymers not only provides stability to the siRNA, protecting it from degradation in the bloodstream, but also ensures controlled release, so the siRNA remains intact until it reaches the target cells. This structural integrity is vital for the effectiveness of the therapy, as it ensures that the siRNA molecules are delivered precisely where they are needed most.

To further enhance the precision of the delivery, these nanoparticles are functionalized with targeting ligands. These substances are known as ligands, and they have the ability to selectively attach to receptors that are overexpressed on the exterior of cancer cells. With the help of this targeting mechanism, the nanoparticles are

guaranteed to specifically target cancer cells, minimising any negative effects on healthy tissues and lowering the possibility of adverse reactions. One important benefit of this strategy is that it targets cancer cells precisely, which increases the therapeutic agent's potency and delivers it straight to the tumour site for maximum treatment effectiveness.

Upon reaching the cancer cells, the star polymer-siRNA nanoparticles are internalised through a process known as endocytosis. After entering the cell, the siRNA enters into the cytoplasm, where it engages in interactions with the RNA-induced silencing complex (RISC). Guided by the siRNA, the RISC binds to mRNA of the target gene, leading to its degradation and effectively silencing the gene. This process inhibits the production of proteins essential for tumor growth and survival, allowing these nanoparticles to significantly suppress tumor growth and potentially overcome mechanisms of drug resistance.

To maximize the therapeutic benefits of this approach, it is essential to monitor the treatment response in real time. Using patient-specific biomarkers, clinicians can track how the cancer responds to the therapy and make necessary adjustments. This adaptive approach allows for modifications in the siRNA sequences or dosing regimens based on the patient's response, optimizing the treatment and enhancing its efficacy. Real-time monitoring also enables the identification of any emerging resistance mechanisms, allowing for prompt intervention and adjustment of the therapeutic strategy.

The development of star polymer siRNA nanoparticles represents a significant advancement in lung cancer therapy. By integrating bioinformatics for precise target identification, designing custom siRNA sequences, and utilising star polymer nanoparticles for targeted delivery, this approach offers a highly specific and effective treatment modality. The ability to selectively inhibit oncogenic pathways, suppress tumor growth, and overcome drug resistance mechanisms positions these personalized nanoparticles as a promising strategy in the fight against lung cancer. Incorporating real-time monitoring and adaptive therapy further enhances their potential, promising improved patient outcomes and paving the way for more tailored and effective cancer treatments.

The application of star polymer-siRNA nanoparticles holds great promise for revolutionising lung cancer therapy and enhancing the quality of life for patients dealing with this difficult disease as research and development in this field continue. The combination of state-of-the-art materials science, molecular biology, and bioinformatics approaches is about to transform the treatment of lung cancer and provide hope for more potent, individualised, and less toxic treatments. Through the utilisation of siRNA molecules' specificity and the distinct qualities of star polymers, tailored nanoparticles can effectively suppress tumour growth, inhibit cancerous pathways, and circumvent drug resistance mechanisms. This multi-faceted approach, combining targeted delivery, real-time monitoring, and adaptive therapy, represents a significant step forward in the ongoing battle against lung cancer, offering the potential for greatly improved patient outcomes and a new era of personalized cancer treatment.

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CHAPTER 1: INTRODUCTION

Lung cancer is a cancerous tumour in the lung. It is the most common of all cancers worldwide and is typically caused by smoking. The two types of primary lung cancer are small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC) and have different prognosis and treatment plan. [1]

The 5-year survival rate is around 61% higher for lung cancer that is still localised, or restricted to the lung, but if the rate drops to 35% in cases where the cancer spreads to the surrounding tissues or lymph nodes. Distant metastatic lung cancer, which is the cancer that spreads to the distant organs or tissues, gives a meagre 6% survival chance for patients with this level of diagnosis. [1] [2]

These numbers are based on people diagnosed with NSCLC between 2012 and 2018.

SEER stage	5-year relative survival rate
Localized	65%
Regional	37%
Distant	9%
All SEER stages combined	28%

Figure 1: 5-year relative survival rates for NSLC [1]

These numbers are based on people diagnosed with SCLC between 2012 and 2018.

SEER stage	5-year relative survival rate
Localized	30%
Regional	18%
Distant	3%
All SEER stages combined	7%

Figure 2: 5-year relative survival rates for SCLC [1]

Certain patients have experienced better results as a result of advancements in treatment options. These include immunotherapy targeted therapy and chemotherapy. Radiation therapy and surgery are also notable advancements. Early detection through screening programs for high-risk individuals is crucial. They can lead to earlier treatment and better survival rates. Nonetheless, lung cancer remains a significant health challenge globally. This highlights the importance of prevention. It underscores early detection. It emphasises ongoing research into more effective treatments..

SiRNA targeted treatment of lung cancer represents a groundbreaking strategy aimed at enhancing patient outcomes by tailoring therapies to unique genetic and molecular profiles of individuals This project describes the potential development of star polymer siRNA nanoparticles for the treatment of lung cancer. Mainly star polymer-siRNA nanoparticles emerge as a versatile platform for targeted drug delivery. They boast advantages such as enhanced stability. Controlled release and biocompatibility are also key benefits. The procedure comprises evaluating genomic and molecular data using bioinformatics. This helps to identify precise molecular targets. Distinct siRNA sequences are then generated. Subsequently, these sequences are enclosed in star polymer nanoparticles. The nanoparticles undergo additional functionalization through the addition of targeting ligands to guarantee accurate delivery to cancer cells.

Drug resistance mechanisms can be defeated. Tumour growth can be suppressed and oncogenic pathways selectively inhibited by utilising special qualities of star polymers in combination with specificity of siRNA molecules. Furthermore the ability to monitor treatment response in real time and adjust therapy accordingly based on biomarkers unique to each patient has the potential to greatly increase therapeutic efficacy. This also reduces side effects. The development of patient-specific star polymer-siRNA nanoparticles is thus poised to substantially advance lung cancer therapy. This heralds a new era of more effective and tailored treatment approaches.

The targeted genes βIII-tubulin and Polo-like kinase (PLK1), have been found to be essential facilitators of lung cancer progression. As well as chemoresistance. This therapeutic model uses the principles of polymer chemistry. PDMAEMA and POEGMA segments conjugated into star polymer nanoparticles .

Bioinformatics is used in the design of siRNA targeting βIII-tubulin and PLK1. Through the genomic and transcriptomic information of lung cancer patients, the expression of genes which are abnormally expressed and the pathways which are not regulated in the patients. They have identified βIII-tubulin and PLK1 as potential targets for a therapeutic approach. Advanced bioinformatics algorithms can predict siRNA with minimal off-targets and optimal efficacy. These siRNA have been validated experimentally. They have been shown to efficiently reduce βIII-tubulin and PLK1 expression in cancer cells. This result decreases tumour cell proliferation and migration. These results accentuate the power of bioinformatics-guided siRNA design in the development of lung cancer treatment models and open the door for the use of personalized treatment protocols based on unique patient profiles.

Targeting therapy uses star polymer nanoparticles, which have been aerosolized. They target overexpressed genes in a patient's lung cancer cells. This provides a highly targeted approach and, hence, reduces the toxicity levels, which are normally seen with other treatment approaches. They are used in a minimally invasive administration device. The minimally invasive administration device generates less toxicity and side effects than other traditional forms of administration. Nanopumps are used which atomize the drug to micron size particles. this enables administration of the drugs to deeper sites in the lung. The design of star polymer nanoparticles makes them more stable to deliver as an aerosol. POEGMA segments stabilize the nanoparticles. They protect them from anionic mucins. This allows them to remain in the body for longer

Precise targeting capability of star polymer nanoparticles ensures delivery of siRNA to cancer cells, thus sparing healthy tissues and minimizing off-target effects. The enhanced stability and bioavailability of the siRNA molecules are protected from degradation, leading to prolonged circulation time. They further improve the bioavailability of the drug in the tumour site. Efficient cellular uptake of siRNA into the cancer cells will enable potent gene silencing effects. This ensures that therapeutic effects are realized. The targeting delivery approach reduces off-target or systemic toxicity and side effects. It enhances patient safety and tolerability. Furthermore, star polymer nanoparticles can encapsulate multiple therapeutic agents, enabling combination therapy approaches targeting multiple pathways that drive lung cancer progression. This, therefore, will enable overcoming mechanisms of treatment

resistance. It will tackle the challenges of tumour heterogeneity and drug resistance in lung cancer treatment.

In summary, the generation of star polymer siRNA nanoparticles is a groundbreaking strategy in lung cancer treatment. Nanoparticles are designed by merging the cutting-edge state of nanotechnology and bioinformatics. Every particle is tailored to target a unique molecular signature. They target the genetic abnormalities of each patient's tumour. This new therapeutic method holds tremendous promise. It might change lung cancer treatment. Providing a personalized, targeted, and effective remedy could tackle this lethal disease.

This enables combination therapy approaches that target multiple pathways involved in lung cancer progression. This synergistic approach has potential to overcome treatment resistance mechanisms. It addresses the challenges of tumour heterogeneity and drug resistance in lung cancer treatment.

In summary, one of the groundbreaking strategies to treat lung cancer is the generation of star polymer siRNA nanoparticles. These are synthesized with the integration of advanced nanotechnology with bioinformatics. Each nanoparticle is designed to target distinct molecular signatures to home in on the genetic abnormalities of each patient's tumour. This unprecedented therapeutic approach has boundless potential and could very well revolutionize lung cancer treatment. Offering a personalized, targeted and effective solution could combat this devastating disease.

1.1 OBJECTIVES

1. To study the development and generation star polymer nanoparticles
2. to identify and select important target proteins that are overexpressed in lung cancer cells
3. to assess and develop siRNA sequences targeting markers of interest
4. to conduct step-by-Step procedure for siRNA Prediction and Analysis using various bioinformatics software.

5. To analyse and check the interaction between target protein and designed siRNA

CHAPTER 2: REVIEW OF LITERATURE

2.1 LUNG CANCER

Uncontrollably growing aberrant cells in lung tissue is the hallmark of lung cancer. It is a complicated illness. Smoking serves as the main risk factor. It is the primary cause of cancer-related deaths globally. Lung cancer can also strike non-smokers for a variety of reasons. These include genetic predispositions, asbestos exposure, radon gas exposure, air pollution and second hand smoke exposure.

2.1.1 TYPES OF LUNG CANCER [2] [3] [4]

Small cell lung cancer and non-small cell lung cancer are the two important forms of lung cancer. About 85% of all cases of lung cancer are classified as non-small cell lung cancer (NSCLC), which includes subtypes like adenocarcinoma, squamous cell carcinoma, and large cell carcinoma. Though it is the least common, SCLC tends to be faster-growing and spreads more quickly. [2] [3] [4]

- Non-Small Cell Lung Cancer (NSCLC)

Currently, NSCLC accounts for 80–85% of all cases of lung cancer, making it the most common type of lung cancer [2] [3]. The growth of abnormal cells in the lining of the lung, which can metastasize to other parts of the body is its characteristic feature. The non-small cell lung cancer has multiple subtypes, which include:

  - Adenocarcinoma: This is the most common type of NSCLC, which nearly accounts for about 30 % of all lung cancer cases. It occurs at the periphery of the lungs and is often attributed to the cause of smoking.
  - Large cell carcinoma: This is the type of NSCLC marked by the growth of large abnormal cells that usually grows rapidly and metastasizes to other parts of the body.

- Small Cell Lung Cancer (SCLC)
- The most common type of SCLC is small cell carcinoma. It is characterised by the growth of abnormal small cells in the lungs. [2] [3].
- There are mainly two types of SCLC which include:

- Small cell carcinoma: The most common type of SCLC, is called small cell carcinoma, is characterised by the growth of small abnormal cells in the lungs.
- Combined small cell carcinoma: Small cell carcinoma and another type of lung cancer combine to form this type of SCLC [2] [3].

2.1.2 STAGES OF LUNG CANCER

Lung cancer is staged depending on the amount of the disease which is determined by size of the tumour. The stages of lung cancer are:

- Stage 1 The tumour is small. It has not yet disbursed to other parts of the body.
- Stage 2: The tumour is larger and has shifted to lymph nodes
- Stage 3 The tumour has travelled to lymph nodes. It has also spread to parts of the body, such as diaphragm.
- Stage 4: The tumour has conjugated to other parts of the body such as brain, liver, etc.[3] [4]

2.1.3 TREATMENT OPTIONS FOR LUNG CANCER

Treatment possibilities for lung cancer depend on the stage and type of the disease. current treatments involve:

- Surgery: Surgery is often the first option to remove the tumour and any affected lymph nodes.
- Chemotherapy: It destroys cancer cells and can be done pre-surgery or post-surgery.
- Radiation therapy: Through the use of radiation, it kills the cancer cells.
- Targeted therapy: Targeted therapy zeroes in on specific genes or antigens or factors that play a part in the growth and spread of lung cancer.
- Immunotherapy: It boosts the body's immune system to fight lung cancer [2] [3] [4] [5]

Symptoms for lung cancer may vary but most often include chest pain, shortness of breath, fatigue, weight loss, and recurrence of respiratory infections. Unfortunately, early detection is difficult because these symptoms frequently do not show up until the disease has progressed to later stages.

2.2 siRNA

A particular class of small RNA molecule known as siRNA (short interfering RNA) is essential to the RNA interference (RNAi) pathway. Through the silencing of genes RNA interference (RNAi) is a naturally occurring biological mechanism that aids cells in controlling gene expression. This process decreases the expression of the targeted gene. SiRNA is engineered to selectively target and degrade messenger RNA sequences that are complementary to the siRNA sequence.

Typically ranging from 21 to 23 nucleotides in length, siRNA has a specific sequence meant to bind to the target mRNA. The RNA-induced silencing complex (RISC) is formed when siRNA is introduced into cells. This occurs either through chemical synthesis or in vitro transcription. After processing by the RNAi machinery RISC recognizes and degrades the target mRNA. The targeted gene is then silenced as a result.

siRNA design is a critical step in the gene silencing process. It determines the specificity and efficacy of the silencing. The design of siRNA involves selection of the target gene. The siRNA sequence is designed to ensure specificity to the target gene. This prevents binding to other non-targeted regions.

siRNA has been used extensively in research. It has shown promise in various therapeutic applications. These include the treatment of diseases such as cancer viral infections and genetic disorders. Development of siRNA therapeutics is an active area of research. Ongoing efforts aim to improve the design. Researchers also focus on delivery and efficacy of siRNA-based treatments.

2.2.1 MECHANISM OF SILENCING VIA siRNA

- **siRNA Design**:
    - siRNA is made for complementary sequence on the target mRNA sequence to ensure specific binding and silencing of the target gene.
    - The siRNA is usually 21-23 nt long, with a 2-nt overhang at the 3' end.
    - The sequence of the siRNA is properly selected to avoid off-target effects. [7] [8] [9] [10].
- **siRNA Delivery**:
    - The synthesised siRNA should be delivered to the target cells to induce gene silencing.
    - Various delivery methods are used, such as lipofection (using cationic lipids), electroporation (using electrical pulses), or viral vectors (using modified viruses).
    - The choice of delivery method depends on the cell type, tissue, and specific application [8] [9] [10].
- **siRNA Processing**:
    - Once inside the cell, the siRNA is recognized by the RISC.
    - RISC unwinds the siRNA duplex, retaining only the guide strand (antisense strand) that is complementary to the target mRNA.
    - The passenger strand (sense strand) is degraded or released from the complex [8] [9] [10].
- **Target Recognition**:
    - The guide strand of the siRNA within the RISC complex binds to the complementary sequence on the target mRNA.

  - This binding is facilitated by Watson-Crick base pairing between the siRNA and the target mRNA.
  - The specificity of the siRNA-mRNA interaction is crucial for ensuring that only the intended target is silenced [10] [11] [12] [13].
- **Target Cleavage**:
  - Once the siRNA-RISC complex is attached to the target mRNA, the endonuclease activity of RISC cleaves the mRNA.
  - The cleavage occurs approximately in the middle of the siRNA-mRNA duplex, resulting in the generation of two mRNA fragments.
  - The cleaved mRNA fragments are then rapidly degraded by cellular exonucleases, preventing the translation of the target gene [12] [13] [14].
- **Gene Silencing**:

The degradation of the target mRNA leads to significant reduction in expression of the corresponding protein. This silencing of the target gene can result in various cellular effects. These effects include inhibition of cell proliferation. They also include migration invasion, or induction of apoptosis depending on the function of the target gene. The degree of gene silencing depends on factors such as efficiency of siRNA delivery. It also depends on the stability of the siRNA and the turnover rate of the target mRNA and protein. [13] [14] [15] [16].

- **Off-Target Effects:**

It is potential that siRNA could bind to and silence other genes which share sequence similarity to the intended target. These are called off-target effects, and they can be minimized. Minimization is done by careful design of the siRNA sequence for it not to have significant complementarity to other transcripts. During the siRNA design process, bioinformatics tools and algorithms are used to predict and minimize potential off-target interactions. [13] [14] [15] [16]

- **Delivery and Uptake:**

1. An effective delivery and uptake of the siRNA into the target cells are crucial for efficient gene silencing.
2. Various delivery methods are in use to facilitate the entry of siRNA into cells, they include lipofection, electroporation, or viral vectors.
3. The choice of delivery method includes factors like cell type, tissue and the specific application [12] [14] [15] [16] [17]

- **Monitoring and Evaluation:**

1. The efficiency of the gene silencing mediated by siRNA can be monitored and evaluated by means of these technologies at different levels, such as Western blot, qRT-PCR and Immunohistochemistry.
2. Western Blot analyses find the levels of target proteins in deciding the reduction produced by siRNA treatment.
3. The targeted reduction in the expression level of the mRNA is quantified using qRT-PCR, and it thus gives an early indication of silencing.
4. Through immunohistochemistry, one can observe the spatial distribution and the localization of the target protein in the tissue sections.
5. These methods provide an indication of the level of gene silencing and the effectiveness of the siRNA under different experimental conditions. [12] [13] [14] [15] [16]

2.2.2 SiRNA DESIGN

Design of the siRNA (short interfering RNA) is the most crucial step in developing RNAi therapeutics. Following are the points to consider while designing siRNA:

- Selection of Target Gene: To decide the target gene to be silenced, one must first identify the gene/genes responsible for the cause of a disease or a condition.
- Design the Sequence of siRNA: The next step after selecting the target gene is to design the sequence of siRNA. The sequence should be designed

complementarily with the target gene; the antisense strand is the guide strand, while the sense strand is the passenger strand.

- Seed Region: This is a 2-nt overhang at the 3' end of the siRNA. This region is very important for the recognition of the target gene by RISC.
- Nucleotide composition: The sequence of nucleotides in the siRNA is also quite important. A general recommendation is to refrain from sequences that have high GC content (above 55%) since these are less likely to be synthesized efficiently and their efficacy could be lowered.
- Delivery vehicle: Safe delivery of siRNA to the target cells requires an appropriate delivery vehicle. There are several methods involved in this process, among which are lipofection, electroporation, and viral vectors.
- Scoring systems: The scoring systems are used to evaluate the efficiency of siRNA sequences. Based on the seed region, nucleotide composition, and off-target activity, the scoring systems are formulated.
- Validation: Validation of the siRNA sequence is critical to ensure that it is effective and specific. This will involve testing the siRNA in cell culture or animal models, and hence, confirming it to be effective and specific. [10] [11] [12] [13] [14] [15] [16]

2.3 STAR POLYMER NANOPARTICLES

Star polymer nanoparticles are new designs of numerous linear branches with numerous functional attachments covalently linked to a central core. This class of nanoparticle has been receiving tremendous interest within the context of nanomedicine due to their special characteristics and numerous potential uses in biocatalysis and drug delivery.

Recent studies have demonstrated that star polymer nanoparticles are highly efficient and versatile in function under various biological environments. For example, to demonstrate the potential of star polymer nanoparticles in precision medicine for cancer therapy, they have been used in the delivery of short-interfering RNA to pancreatic tumours in mouse models of pancreatic cancer. For instance, such novel functionalised star polymer nanoparticles synthesized through RAFT polymerization have been effective in constructing multi-enzyme systems with enhanced thermal

stability and enzyme activity for biocatalysis reactions and biosensors.18] [19] [20] [21]

2.3.1 SYNTHESIS OF STAR POLYMER NANOPARTICLES

Star polymer nanoparticles belong to the class of macromolecules that have a central core from which multiple polymer arms radiate. As a result of this unique architecture, they tend to have very distinctive properties that make them highly valued in a wide range of applications, including drug delivery, nanomedicine, and advanced materials.

Synthesis Methods:

- Core-First Method : The core-first approach starts with the synthesis of a multifunctional core, which could be a small molecule or a macromolecule with multiple functional groups. Controlled polymerization methods, such as ring-opening polymerization (ROP), reversible addition-fragmentation chain transfer polymerization, or atom transfer radical polymerization, can then be used to grow polymer chains from the core. For example, using ROP, a core molecule containing several hydroxyl groups can support growth to obtain PEG chains.

- Arm-First Method: In the arm-first method, linear polymer chains with reactive end groups are synthesised first. These chains are subsequently attached to a multifunctional core through coupling or crosslinking reactions. An example involves PEG chains with terminal carboxyl groups reacting with an amine-functionalized core to create star-shaped PEG .

- “In-Out” Method : The "in-out" method involves using a core molecule to initiate the polymerization of monomers, forming polymer arms. After the arms are created, a different monomer can be added to grow another layer of polymer extending outward. For instance, a dendritic core can initiate the polymerization of styrene, followed by the addition of another monomer like methyl methacrylate. [18] [19] [20] [21]

2.3.2 CHARACTERIZATION TECHNIQUES

Proper formation and functionality of star polymer nanoparticles are verified using several characterization techniques:

- Nuclear Magnetic Resonance (NMR) Spectroscopy: Identifies the chemical structure and composition.
- Gel Permeation Chromatography (GPC): Determines molecular weight and polydispersity index.
- Dynamic Light Scattering (DLS): Measures particle size and size distribution.
- Transmission Electron Microscopy (TEM) or Scanning Electron Microscopy (SEM): Provides detailed images of nanoparticle morphology.
- Differential Scanning Calorimetry (DSC) and Thermogravimetric Analysis (TGA): Evaluates thermal properties and stability. [18] [19] [20] [21]

For this project, star-POEGMA polymers is seen as the appropriate choice.

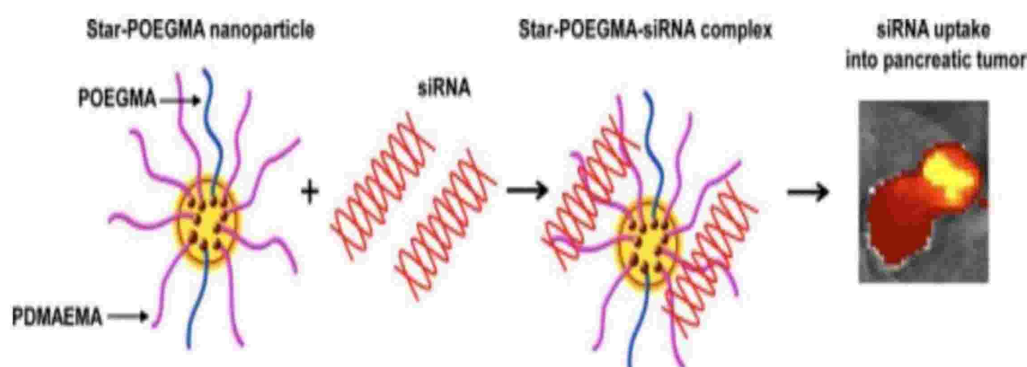


Figure 3: Diagrammatic representation of star-POEGMA nanoparticle tagged with siRNA in pancreatic cancer treatment [43]

The development of star polymer nanoparticle involved a multi-step process utilising RAFT polymerization. Here is the procedure:

- Design of Polymers: The first step is the rational design of star-shaped polymers. These polymers consist of a core that radiates multiple arms of polymers. The polymers used in this study were engineered to include varying concentrations

of poly (oligo (ethylene glycol) methyl ether methacrylate) (POEGMA) and cationic poly(dimethyl aminoethyl methacrylate) (PDMAEMA) side-arms. Although POEGMA improves the polymer's solubility and biocompatibility, PDMAEMA supplies the cationic charge required for binding to the negatively charged siRNA.

- Synthesis Using RAFT Polymerization: RAFT polymerization is employed in this synthesis to create the star polymers. This process enables the control of molecular weight and polymer composition. The RAFT agent acts like a central core, and from there, the polymer arms grow to give rise to the star architecture.
- Self-Assembling with siRNA: Subsequently, synthesised star-POEGMA is admixed with siRNA. The cationic PDMAEMA arms of the polymers interact electrostatically with the anionic siRNA, causing self-assembly of the polymers and siRNA into nanoparticles. It is driven by neutralisation of the charges, forming compact and stable nanoparticles.
- Physicochemical Characterization: This involves the characterization of nanoparticles, such as size, surface charge, stability, and morphology. These are usually performed with techniques like DLS and TEM. The two types of characterizations ensure the nanoparticles are within the desired size, usually in the range of nanometers, and with the right surface charge to ensure effective cellular uptake.
- Testing: In vitro testing of the nanoparticles is conducted using pancreatic cancer cell lines to check whether the siRNA is carried and thus silences the target gene, TUBB3/βIII-tubulin, effectively. Cytotoxicity assays are carried out, ensuring the least toxicity for normal cells. Positive in vitro findings make the application move forward to in vivo studies in animal models that are mostly mice having orthotopic pancreatic tumours. The nanoparticles are administered systematically, and their distribution, accumulation in the tumours, and gene silencing capacity are analysed. The reduction in TUBB3/βIII-tubulin expression is determined at both the protein and gene levels.
- Data Analysis and Optimization: This is where the data obtained in in vivo studies are analyzed toward the scrutiny of the therapeutic potential of the

nanoparticles. These outcomes usually lead to further optimization of the polymer structure and formulation to enhance the efficacy and safety of the nanoparticles. [21] [22] [23] [24] [25] [26] [27] [28] [29] [30] [31] [32]

2.3.3 POTENTIAL OF STAR POLYMER NANOPARTICLES TO ENHANCE THE DELIVERY OF siRNA IN CANCER TREATMENT

- Targeted Delivery: The star polymer nanoparticles can be designed in such a way that they reach particular cells or tissue, for example, cancer cells, with the help of targeting ligands or antibodies on their surface. Through this targeted delivery, siRNA will be efficiently delivered to the cancer cells, reducing off-target effects, and enhancing the efficacy of the treatment.
- Increased Cellular Uptake: Star polymer nanoparticles have been shown to increase the cellular uptake of siRNA through inherent cellular internalization pathways. For instance, they can be internalized in cells through the clathrin-mediated endocytosis pathway, which is relatively efficient and targeted compared to some other methods.
- Endosomal Escape: Star polymer nanoparticles have been designed with the ability to escape the endo-lysosomal pathway, which is often a major barrier to siRNA delivery. It can lead to increase or enhance gene silencing by providing a more efficient and targeted means of siRNA delivery to the cytoplasm. This can be done through the use of more specific methods, leading to increased efficacy and reduced side effects compared to other methods.
- Improved Stability: Star polymer nanoparticles can provide increased stability to siRNA against degradation and improve its stability during transport. This may increase efficacy and decrease side effects compared to other means of siRNA delivery.
- Reduced Off-Target Effects: Star polymer nanoparticles could lower off-target effects by targeting particular cells or tissues that cause the disease state, hence reducing the delivery of the siRNA to nontargeted cells. This could increase efficacy and reduce side effects compared to other delivery methods.
- Increased Bioavailability: Star polymer nanoparticles can enhance the bioavailability of siRNA, making it an efficient and targeted means of delivering

the same to the cytoplasm. This could potentially help increase the efficacy and, on the flip side, lower side effects compared to other delivery methods. These siRNA therapies could better the therapeutic index of currently known strategies, which deliver their active ingredient in an untargeted manner to all rapidly proliferating cells. In turn, such targeted therapies could increase efficacy with fewer side effects than other delivery methods. [18–26]

2.3.4 STAR POLYMER WITH siRNA NANOPARTICLES: POTENTIAL BREAKTHROUGH IN LUNG CANCER TREATMENT

Deadly lung cancer is a leading cause of mortality worldwide, urging for new innovative therapeutic modalities. Recent advances in the field of nanotechnology have led to the synthesis of star polymer-siRNA nanoparticles that possess enormous potential in treating lung cancer. These nanoparticles can effectively deliver small interference RNA to the lung tumour, thus inhibiting the expression of important genes in the promotion of tumour growth and progression.

But the major issue in the siRNA-based therapy of lung cancer is to deliver these molecules in proper quantity at the tumour site.

The star-polymer siRNA nanoparticles bypass this challenge by using a novel architecture that offers better stability, biocompatibility, and targeting. It consists of a star-shaped polymer backbone, which provides a high surface area for loading siRNA and a compact structure to facilitate the endocytosis of its contents into cells.

Specific in vitro studies have shown that siRNA nanoparticles of star polymer have successfully internalized in cancerous cells, escaped the endo-lysosomal pathway, and undergone gene silencing in relation to the target gene,

overexpressed in cases of lung cancer. A recent study has even proved that the star polymer-siRNA nanoparticles complexed with siRNA for βIII-tubulin

and PLK1 could silence these genes in the lung cancer cells and cause significant inhibition of the tumour growth and proliferation. [31] [32] [33] [34] [35]

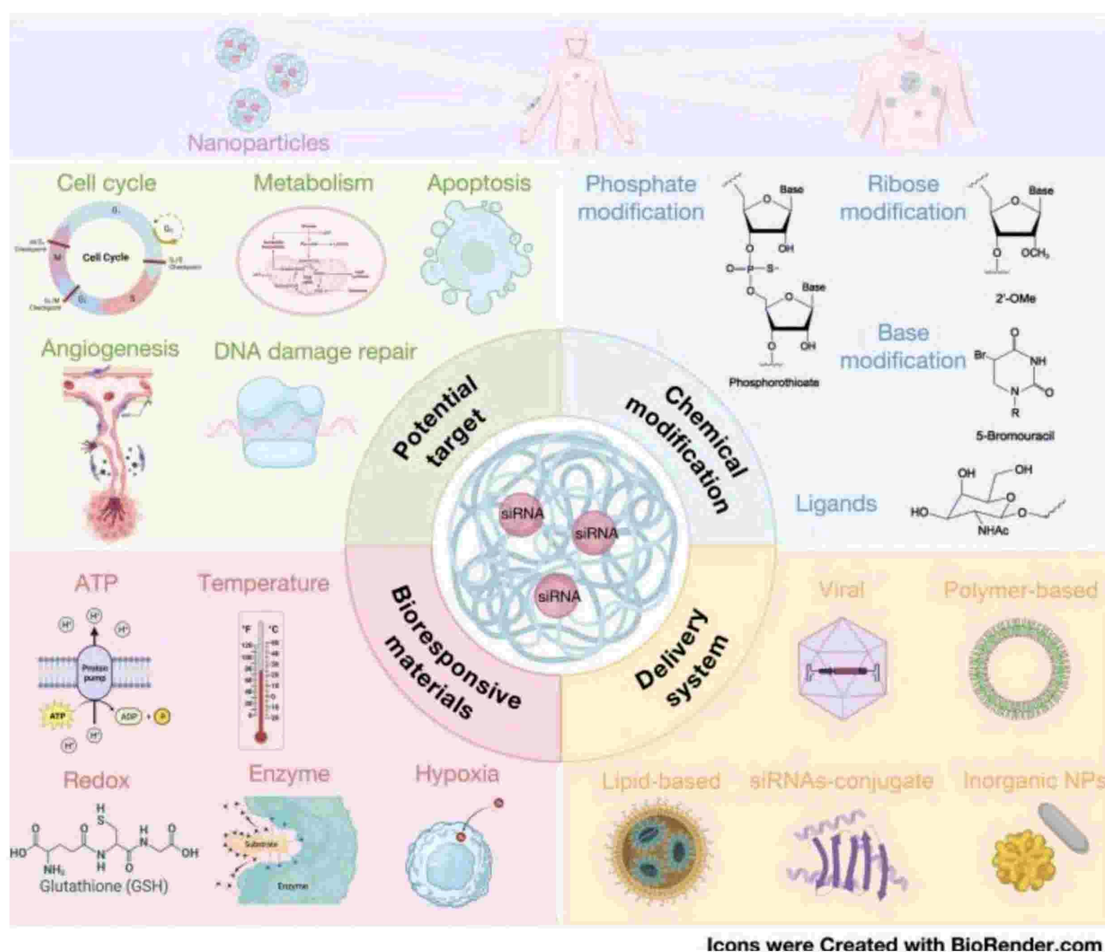


Figure 4: Star Polymer with siRNA Nanoparticles [35]

Additionally, it has been probed that combinations of the use of star polymer-siRNA nanoparticles with other therapeutic methods are possible. For example, just recently, it was demonstrated that the application of nebulized nanoparticles containing Let-7b miRNA, when combined with chemotherapy, resulted in a considerable suppression of lung tumour growth in mice. These findings can be seen as showing the development of delivery of star polymer-siRNA nanoparticles as a novel and promising therapeutic approach for lung cancer.

2.4 HOW DO STAR POLYMER-siRNA NANOPARTICLES EVADE THE IMMUNE SYSTEM

Star polymer-siRNA nanoparticles will be designed to harness this structure for immune escape, better stability, biocompatibility, and improved targeting traits. The

nanoparticles will be based on the star polymer backbone, having a higher surface area for the loading of siRNA and a compact structure enabling cellular uptake.
Star polymer architecture allows the nanoparticles to internalise to lung cancer cells and escape the endolysosomal pathway, thus avoiding rapid clearance by the reticuloendothelial system and limited siRNA release from endosomes once inside the cell.
Furthermore, it allows for the targeted delivery of the nanoparticles to the lung tumours, limiting systemic toxicity and enhancing therapeutic efficacy. The targeting delivery way avoids that nanoparticles possibly contact the immune system and are recognized as foreign, so it results in reducing immune system evasion.

2.5 RECENT STUDIES

The key studies for star polymer-tagged siRNA for lung cancer treatment are:

2.5.1 AEROSOL DELIVERY OF STAR POLYMER-SIRNA NANOPARTICLES FOR LUNG CANCER TREATMENT:

This study explored the potential of nebulized star-siRNA nanoparticles to home into orthotopic mouse lung tumours and to target the downregulation of genes, such as βIII-tubulin and Polo-Like Kinase 1 (PLK1), that are overexpressed in lung cancer cells. The results demonstrated that star-siRNA nanoparticle aerosol delivery is an effective new therapeutic approach to stopping the growth of lung tumours. [38] [39] [40]

2.5.2 INHALABLE SIRNA NANOPARTICLES FOR ENHANCED TUMOUR-TARGETING TREATMENT OF KRAS-MUTANT NON-SMALL-CELL LUNG CANCER [41]:

In this study, inhalable siRNA nanoparticles have been developed against KRAS-mutant non-small-cell lung cancer. The study demonstrated the striking tumour-targeting capability and enhanced antitumor activity, showing the potential of this approach for precision gene therapy in lung cancers [41].

These data provide a potential application of the star polymer-tagged siRNA nanoparticles in lung cancer treatment with special attention to target delivery, gene silencing activity, and therapeutic efficacy.

2.6 HOW DO STAR POLYMER-SIRNA NANOPARTICLES AFFECT THE TUMOUR MICROENVIRONMENT

The tumour microenvironment is one of the most complex and dynamic environments which are seriously essential during cancer development. Star polymer-siRNA nanoparticles are developed in a way that allows them to internalise into the lung cancer cells and be able to interact

With this microenvironment, the expression of genes which promote the growth of tumours is retarded. As this interaction is due to their targeting potential along with highly suppressive activity against the genes helping in tumour growth and progression with extremely high selectivity, the effectiveness of the therapy of these nanoparticles relies on the latter. [44] [45] [46] [47] [48]

2.7 LATEST ADVANCEMENTS

The latest advance in the technology of star polymer-siRNA nanoparticles brings great promise for the treatment of lung cancer, because such nanoparticles have been demonstrated to effectively deliver siRNA to lung tumors and suppress the expression of important genes that drive tumor growth.

In recent studies, it was demonstrated that star polymer nanoparticles of PDMAEMA-POEGMA, complexed with siRNA, entered lung cancer cells, escaped the endo-lysosomal pathway, and silenced the expression of targeted genes, such as βIII-tubulin and PLK1, upregulated in lung cancer. Nebulized star-siRNA nanoparticles localized in mouse lung tumors and suppressed these targeted genes, significantly delaying aggressive tumor growth with no gross evidence of side effects.

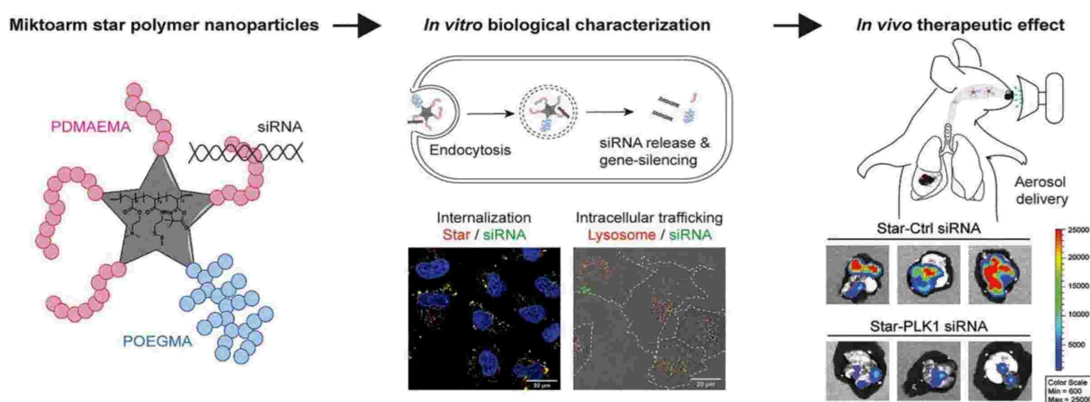


Figure 5: PDMAEMA-POEGMA nanoparticles complexed with siRNA can internalize into lung cancer cells, escape the endo-lysosomal pathway, and silence the expression of target genes like βIII-tubulin and PLK1 [43]

Another important improvement is the use of aerosol delivery to direct the nanoparticles to the site of lung tumours. The application method provides local administration to the tumour site, allowing limited systemic toxicity. The star polymer architecture makes it possible to improve the stability, biocompatibility, and targeting capacity of the nanoparticles.

2.8 SELECTION OF III-TUBULIN AND POLO-LIKE KINASE 1 (PLK1) AS TARGET PROTEINS

Plk1 and βIII-tubulin were identified as target sites in lung cancer cells through various studies and mechanisms:

1. **Plk1**:

   - **Expression and Function**: Plk1 is a serine/threonine kinase that plays a crucial role in regulating the cell cycle, mitosis, and DNA replication. It is highly expressed in many cancer types, including non-small cell lung carcinoma (NSCLC), and is associated with aggressive disease and poor survival. [49] [40] [41]

- **Mechanisms of Action:** Plk1 plays a vital role in cell proliferation, migration, and survival through the control of several key cellular processes, including mitotic entry, centrosome maturation, bipolar spindle formation, and chromosome segregation**.** [49] [50] [51] [52]

- **Targeting: P**lk1 has been considered a validated target for the treatment of cancer, as its inhibition markedly affects cancer cell proliferation and survival. Inhibition of Plk1 through siRNA or other molecules has been shown to significantly lead to mitotic arrest, reduced proliferation, and increased cell death in cancer cells. [49] [50] [51] [52]

2. **βIII-tubulin**:

- **Expression and Function**: βIII-tubulin is a microtubule protein that is highly expressed in many cancer types, including NSCLC. It is majorly responsible for the stabilisation of microtubules that control cell division and migration. [49] [50] [51] [52]

- **Mechanisms of Action**: βIII-tubulin promotes cellular proliferation and migration by regulating microtubule dynamics and stability. It also plays a role in imparting resistance to chemotherapeutic agents and radiations in cancer cells.[49] [50] [51] [52]

- **Targeting**: βIII-tubulin has been identified as a potential target for cancer therapy due to its critical role in cancer cell proliferation and migration.

CHAPTER 3: MATERIALS AND METHODOLOGY

Physical materials are not required because the task is done in silico using software. Utilizing computational methods and models, the analysis is carried out within the software itself.

3.1.1 PUBCHEM

The U.S. National Institutes of Health (NIH), houses the public chemical database PubChem . In addition to researchers, patent agents, and students, PubChem is a well-liked resource with millions of monthly users. Importantly, PubChem data is frequently used in several studies on machine learning and artificial intelligence. A data aggregator called PubChem gathers chemical data from thousands of sources. While siRNA, miRNA, lipids, carbohydrates, and biopolymers that have undergone chemical modification make up the majority of the compounds in PubChem, it also includes other chemical substances like siRNA and miRNA. This data is arranged into several data collections, such as Substance, Compound, BioAssay, Gene, Protein, Taxonomy, Pathway, Cell Line, and Patent.

While Compound includes distinctive chemical structures that were derived from Substance, Substance archives depositor-provided chemical descriptions. BioAssay stores the depositor's descriptions and test results for biological assay investigations. Information on the substances, proteins, genes, and taxa referenced in each patent filing is available in the Patent Collection. [54]

3.1.2 UNIPROT

The enormous database UniProt provides information on proteins. Protein sequences, functional annotations, structural information, interactions, and more are available through UniProt. [52]

The UniProt database is organised into several components :

1. UniProtKB (Knowledge Base): This is the central region of UniProt and contains the comprehensive protein sequence and functional information. Additionally, the UniProtKB is divided into two parts:

2. UniProtKB/Swiss-Prot: Protein entries in this section are curated and annotated manually. Specialists analyse the data in Swiss-Prot to ensure its quality and accuracy.

3. UniProtKB/TrEMBL: This contains protein sequences that have been computationally predicted or automatically annotated. In comparison to Swiss-Prot, TrEMBL entries present a higher coverage of the protein sequences but may have less detailed annotations.

4. UniRef (UniProt Reference Clusters): UniRef clusters proteins based on their sequence identity to reduce redundancy and increase the speed of computation. UniRef provides representative sequence, clustered sets of protein sequence, and related functional information.

5. UniParc (UniProt Archive): This comprehensive collection holds all publicly available protein sequences, even those that are retired or are joined with sequences from other UniProt sections. Protein sequence information is archived in UniParc. [55]

3.1.3 GENBANK DATABASE

The GenBank: Global database of nucleotide sequences and their protein translations; this public domain is maintained by National Centre for Biotechnology Information (NCBI), which is a part of National Institutes of Health[56]. This database is very crucial to researchers worldwide, which they employ in different genetic and genomic studies.

GenBank is designed to hold genetic sequence data from organisms, including viruses, bacteria, archaea, and eukaryotes. It is updated constantly with new data, both by submitters from the research community and by staffs at GenBank and other specialised databases that have been established through various large sequencing projects. The database consists of two types of the collection, one in the form of raw sequence data, while the other includes annotated entries of such sequences with elaborate information on their structure, function, and evolutionary relationship.

Features

1. Broad Data Collection

It includes a broad range of nucleotide sequences of a wide range of species

It includes genomic DNA, mRNA, and protein-coding sequences

2. Annotation and Metadata

provides detailed annotations for sequences, including gene structure, function, and regulatory elements

metadata information about the sequence source, sequencing method, and submitter information.

3. Accessibility and Integration

publicly available and easily accessed online from the NCBI website.

integrated with all other NCBI databases and tools, including PubMed, BLAST, and RefSeq, by which data can be directly retrieved and analyzed seamlessly.

4. Regular Updates

Is updated continuously with a stream of new sequence data flowing in from researchers globally.

Incorporates data from high-throughput sequencing projects and other large-scale initiatives.

5. Search and Retrieval Tools

Allows advanced search with predefined queries. The database can be queried based on a wide range of parameters, including sequence similarity, organism, gene name, etc.

Tools to search, compare, and align the sequence in GenBank, BLAST (Basic Local Alignment Search Tool).

6. Collaboration and Data Sharing

Incentivizes researchers globally to submit data, which enables the sharing and collaboration of data.

- Supports the scientific community with a resource to disseminate and exchange genetic information.

7. Educational and Research Support:

- Acts as a foundational resource for genetic and genomic research supporting studies in evolutionary biology, genetics, biotechnology, and medicine.

- Serves as an educational tool in the learning and teaching process, particularly in genetic sequences and bioinformatics.

GenBank is a foundation for biological research, enabling life scientists through critical resources and tools to discover genetic sequences and functions, leading to better understanding of biology and medicine. [56]

3.1.4 RCSB PDB

One such widely popular and comprehensive tool for studying three-dimensional structures of biomacromolecules is the RCSB PDB. The RCSB PDB is an access point for a large collection of complex biomolecular structures, determined experimentally.

The following are some significant functionalities and features of RCSB PDB:

1. Structure Database: It is a repository that contains and disseminates the three-dimensional biological macromolecular structure determined by experimentation.

It contains several proteins, nucleic acid, and complex structures determined through analytical high-throughput methods.

2. Structure Search: The RCSB PDB has an extraordinary number of search tools for the user to access the database. A user can search using the advanced search criteria.

3. Structural Visualization: The RCSB PDB supports strong visualization for the display and analysis of protein structures. Structures can be examined in a number of representations, including ribbon, wireframe, and surface models. The viewer supports advanced options for interactive view manipulation, zooming, rotation, and close up view of specific areas. The viewer allows interactive inspection, zooming, rotation, and close research of particular areas of the building.

4. Structure Analysis: The RCSB PDB provides numerous tools to analyze and comprehend protein structures. Users can compute and visualize a number of features, such as secondary structure elements, binding sites, ligand interactions, and protein-ligand interactions. It also provides tools for structural alignment and superimposition of different structures.

5. Functional Annotations: In the RCSB PDB, there are functional annotations of the structures with respect to the biological context. A user is provided with information on articles through which protein functions, ligands, binding sites of ligands, and protein-protein interactions have been described. It helps in understanding biological inferences and functional relevance with structures.

Researchers in structural biology, bioinformatics, and drug development will find the RCSB PDB especially useful for studying the role, relationships, and structure characteristics of protein and nucleic acid structures in greater depth by their exploration, analysis, and visualization. [57]

3.1.5 siDIRECT

siDirect is an online, standalone software tool developed to serve for the efficient design of siRNA sequences; its main goal is to achieve maximal gene silencing specificity and potency of the designed siRNA molecules by researchers in potential gene silencing experiments. It becomes particularly useful in RNA interference (RNAi) research while the potential for off-target gene silencing is a major concern—gene silencing due to the siRNA interaction with mRNA not of its intended target. siDirect is developed to assist scientists in designing siRNA sequences that are both effective and specific. Advanced algorithms and an understanding of RNAi mechanisms are used to predict the optimal siRNA sequences. It aims to maximize the gene silencing efficiency of designed siRNA candidates while minimizing potential off-target interactions, such as interactions with non-target mRNAs. This tool is widely used in genetic research, functional genomics, and therapeutic development to ensure the success of RNAi experiments. [58]

Features

Features

1. Algorithm-Based Design:

- - Utilizes high-end algorithms for the prediction and generation of optimal siRNA sequences.
- - Takes important factors such as thermodynamic stability and nucleotide composition that enhance the efficacy of siRNA into consideration.

2. Reducing Off-Targets:

- - Utilizes off-target prediction algorithms to reduce off-target effects.
- - Filters out siRNA sequences with a high degree of similarity to other non-target mRNAs, hence reducing off-target effects.

3. User-Friendly Interface:

- - Provides an intuitive, user-friendly web interface to input target gene sequences for researchers to obtain siRNA designs.
- - Users can customize design parameters as per specific needs for the researcher.

4. Comprehensive Output:

- - Provides a list of potential siRNA sequences along with detailed information on each candidate's predicted efficacy and specificity.
- - Data on target accessibility, avoidance of secondary structure, and other important design parameters.

5. Integration with Databases:

- - Makes use of information from multiple genomic and transcriptomic databases to provide current and relevant information regarding the target.
- - Provides that designed siRNAs are matched with up-to-date genetic data.

6. Efficacy and Potency:

- - High-efficiency knockdown of the target gene.
- - Optimized sequence for strong target mRNA binding and efficient incorporation into the RNA-induced silencing complex.

7. Experimental Validation:

- - Provides guidelines and recommendations to experimentally validate designed siRNA.
- - Provides insight into potential modifications and adjustments in designs based on empirical results.

The siDirect becomes a very important tool for researchers to exploit the ability of RNA interference for gene silencing. Because of its advanced computational techniques combined with user-friendly features, it has ensured that designed siRNA sequences are effective and specific, thus underpinning successful RNAi experiments and experiments towards the achievements in genomic and biomedical research. [58]

3.1.6 IntaRNA

IntaRNA was developed as a computational tool to predict and analyze RNA-RNA interactions. It is mainly used to identify interactions between sRNAs and their mRNA targets, which are critical in understanding post-transcriptional regulation. Developed by the Computational Biology group of the University of Freiburg, IntaRNA represents a proficient and precise tool for the study of RNA-mediated gene regulation.

IntaRNA computes RNA-RNA interactions' predictions based on a combination of advanced algorithms and thermodynamic models. It predicts all interaction sites and the interaction energies that researchers can use to reveal functional RNA partners. This tool has seen successful usage in the study of bacterial sRNAs, microRNAs, and long non-coding RNAs in the explanation of roles that these molecules play in gene regulation. IntaRNA is made available through a web server, and, in turn, offers a user-friendly interface for input and analysis. [59]

Features

1. Prediction Algorithms:

IntaRNA employs the most advanced algorithms for the accurate prediction of RNA-RNA interactions. It uses both sequence complementarity and thermodynamic stability in the predictions.

2. Thermodynamic Modelling:

Interaction energies, with the IntaRNA platform, are calculated based on the secondary structure of RNAs and their thermodynamic properties. This feature considers both intramolecular (within the RNA molecules) and intermolecular (between RNA molecules) base pairings.

3. User-Friendly Interface:

It is available on a web server, and therefore it avails itself an easy-to-use input and retrieval. The user can upload RNA sequences or just simply paste them into the interface.

4. Detailed Output:

Outputs include interaction energies, predicted binding sites, and secondary structure information. The RNA-RNA interaction is graphically represented, which also facilitates the interpretation of the results.

5. High Throughput Capability:

The tool is able to process several RNA interactions simultaneously, thus suitable for large-scale studies and high-throughput screening projects.

6. Customization Options:

It has customization parameters that can be set for interaction prediction, such as interaction length and energy thresholds.[59]

3.1.7 RNACOMPOSER

RNAComposer is a flexible inosilico RNA 3D modeling tool that allows a user to provide an RNA sequence as an input to receive its predicted 3D structure with the knowledge-based approach. The base of the tool is quite a bit of information from solved RNA structures to make an assumption, a guess, or the probable structure of the input RNA. These models can be used for the visualization of the RNA conformation and RNA-ligand interactions and therefore give important clues for RNA therapeutics design. RNAComposer is a highly valuable tool for researchers working with ribozymes, riboswitches, and other functional RNAs of interest.

Features

1.Automated 3D Structure Prediction:

•Converts RNA sequences into three-dimensional structures within a fully automated pipeline.

•Utilizes a knowledge-based approach, incorporating data from experimentally determined RNA structures.

2.User-Friendly Web Interface:

•Intuitive and easy-to-use web interface for submitting RNA sequences and retrieving 3D models.

•Allows users to input RNA sequences directly or by uploading files containing sequence data.

3.Comprehensive Output:

•Generates 3D RNA models in the standard PDB (Protein Data Bank) format.

•Output structure contains detailed structural information, including base pairing, secondary structure elements, and tertiary interactions.

4.High Accuracy and Reliability:

•It uses sophisticated algorithms and extensive RNA structural data to ensure high prediction accuracy.

•The server is periodically updated with new RNA structure data to improve prediction quality.

5.Visualization and Analysis Tools:

•Integrated visualisation tools enable exploring and analysing the predicted RNA structures.

•Compatible with other molecular visualisation software like PyMOL and UCSF Chimera for a detailed examination.

6.Customization and Flexibility:

•This tool accepts all sorts of RNA lengths and complexities, from small hairpins to large multi-domain RNAs.

•Users can provide constraints or other extra structural information, thereby directing the prediction.

7.RNA Database Integration:

•Accesses RNA-related databases to compare predictions with already known structures

•Utilises resources like the RNA 3D Hub and others for better building of models.

8.Documentation and Support:

•Extensive documentation, tutorials, and user guides for full support in proper use of the tool

•Support from the development team and periodic updates for feedback and new implementations.

3.1.8 GEPIA2

GEPIA 2 (Gene Expression Profiling Interactive Analysis 2) is a platform for gene expression analysis using TCGA (The Cancer Genome Atlas) and the GTEx database.

Main characteristics and functions in GEPIA 2:

- Gene expression analysis: Users may query the expression levels of a specific gene in different types of cancer or various healthy tissues.
- Users can also create customizable graphs based on the query, which will represent the gene expression profile and includes the statistical information.
- Differential expression analysis: Users can identify the differential expression genes in a specific cancer type based on the tumour and normal samples through GEPIA 2. Volcano plots and lists of differential expression genes with statistical significance (up-regulated and down-regulated) are returned.
- Survival analysis: GEPIA 2 calculates the effect of gene expression on patient survival for a specific cancer type using Kaplan–Meier survival plots and log-rank tests.
- Correlation analysis: Users can evaluate the co expression correlation of two genes across both tumour and normal tissue samples using GEPIA 2's scatter plots and correlation coefficients. It also enables assessment of the potential association between gene expression and clinical phenotypes of interest.
- Customizable Visualisation: Researchers may produce their individual plots using the tool's numerous visualisation options, which allow them to adjust the colour, data range, and kinds of plots.
- It also allows the creation of heatmaps to represent the patterns of gene expression across different samples. [60]

3.1.9 ALPHA FOLD

AlphaFold is a state-of-the-art deep learning AI system designed to predict protein structures with unprecedented accuracy. It uses deep learning methods to determine 3D protein structures based on their amino acid sequences. AlphaFold has therefore helped in significantly advancing the field of structural biology to provide high-fidelity models that enable understanding protein functions, interactions, and implications in diverse biological processes and diseases.

Key Features of AlphaFold

1. High-Accuracy Predictions:

AlphaFold makes protein structures with atomic-level detail, many times closely mimicking experimental techniques like X-ray crystallography and cryo-electron microscopy.

2. Speed and Efficiency:

•It is much faster than traditional experimental methods, which take months or years to predict structures; AlphaFold gives results in the ranges of hours to days, hence reducing the time to test hypotheses.

3. Open Access and Availability:

•DeepMind has given the scientific community free access to its predictions by releasing the AlphaFold Protein Structure Database, with hundreds of thousands of protein structures.

4. Deep-Learning-Based:

•AlphaFold relies on deep learning methods with advanced neural networks trained on publicly available protein data to learn to generalize well across unseen sequences and make accurate structure predictions.

5. Cross-Domain Applications:

•Applications such as drug discovery, enzyme design, and understanding disease mechanisms would benefit from AlphaFold's powerful prediction capabilities.

6. Integration with Other Tools:

•Predictions made by AlphaFold can be readily integrated with other computational biology tools and pipelines to allow for in-depth bioinformatics analysis.

7. Support for Diverse Proteins:

•It supports a wide range of proteins, from small, simple molecules to large, complex multi-chain assemblies.

8. Continual Improvement:

The AlphaFold model is continually improved and updated with new data and methodologies to improve the performance of the predictions. [61]

3.2 METHODOLOGY

3.2.1 IDENTIFICATION OF DIFFERENTIALLY EXPRESSED TARGET GENES

Differential gene expression analysis of Lung adenocarcinoma was done using GEPIA2 software, to identify 2 target genes for proteins that are overexpressed in tumour cells in lung cancer.

- TUBB3 gene- encoding III-tubulin
    - This gene encodes a class III member of the beta tubulin protein family. Beta tubulins are one of two core protein families (alpha and beta tubulins) that heterodimerize and assemble to form microtubules.
    - III-tubulin, a member of the tubulin protein family, is overexpressed in various cancers and is associated with aggressive tumour behaviour, metastasis, and resistance to microtubule-targeting drugs.

- PLK1 gene encoding Polo Kinase 1
    - polo-like kinase 1
    - Polo-like kinase 1 (PLK1) is a serine/threonine kinase that plays a critical role in cell cycle progression, mitosis, and DNA damage response. Overexpression of PLK1 is commonly observed in many cancers and correlates with poor prognosis and resistance to chemotherapy.

3.2.2 STRUCTURAL ANALYSIS OF III-TUBULIN AND POLO KINASE 1 USING ALPHAFOLD PROTIEN STRUCTURE DATABASE

3D molecular structures of both proteins were retrieved from Alphafold protein structure database and analysed to understand their Various properties and functions

3.2.3 mRNA SEQUENCE RETRIEVAL OF III-TUBULIN AND POLO KINASE 1

mRNA sequences of both proteins were retrieved from genbank database for analysis and designing the appropriate siRNA.

3.2.4 siRNA DESIGNING VIA siDIRECT SOFTWARE

siDirect software was employed to identify target sites on mRNA sequences of III-tubulin and Polo Kinase 1 and generate multiple siRNA candidates for the same.

Optimal siRNA sequence for protein inactivation was selected after thorough analysis of the data generated by the software.

3.2.5 3D MODELLING OF SELECTED siRNA SEQUENCES VIA RNACOMPOSER

RNAcomposer software was used to generate 3D models of selected siRNA candidates for further analysis and studying thermodynamic stabilities

3.2.6 ANALYSIS OF RNA-RNA INTERACTIONS

Interactions between target sequence (protein mRNA) and selected siRNA candidate sequence was performed using IntaRNA software.

3.2.7 SELECTION OF APPROPRIATE CARRIER VEHICLE FOR siRNA

Analysis and in-depth study of prior research revealed that star polymer (PDMAEMA-POEGMA) nanoparticles are the optimum choice to carry the designed siRNA to target site.

CHAPTER 4: RESULTS

4.1 IDENTIFICATION OF DIFFERENTIALLY EXPRESSED TARGET GENES

Differential gene expression analysis of Lung adenocarcinoma was done using GEPIA2 software, to identify 2 target genes for proteins that are overexpressed in tumour cells in lung cancer.

1. PLK1 gene

- Description: polo-like kinase 1
- Alias: PLK, STPK13
- Polo-like kinase 1 (PLK1) is a serine/threonine kinase that plays a critical role in cell cycle progression, mitosis, and DNA damage response. Overexpression of PLK1 is commonly observed in many cancers and correlates with poor prognosis and resistance to chemotherapy.

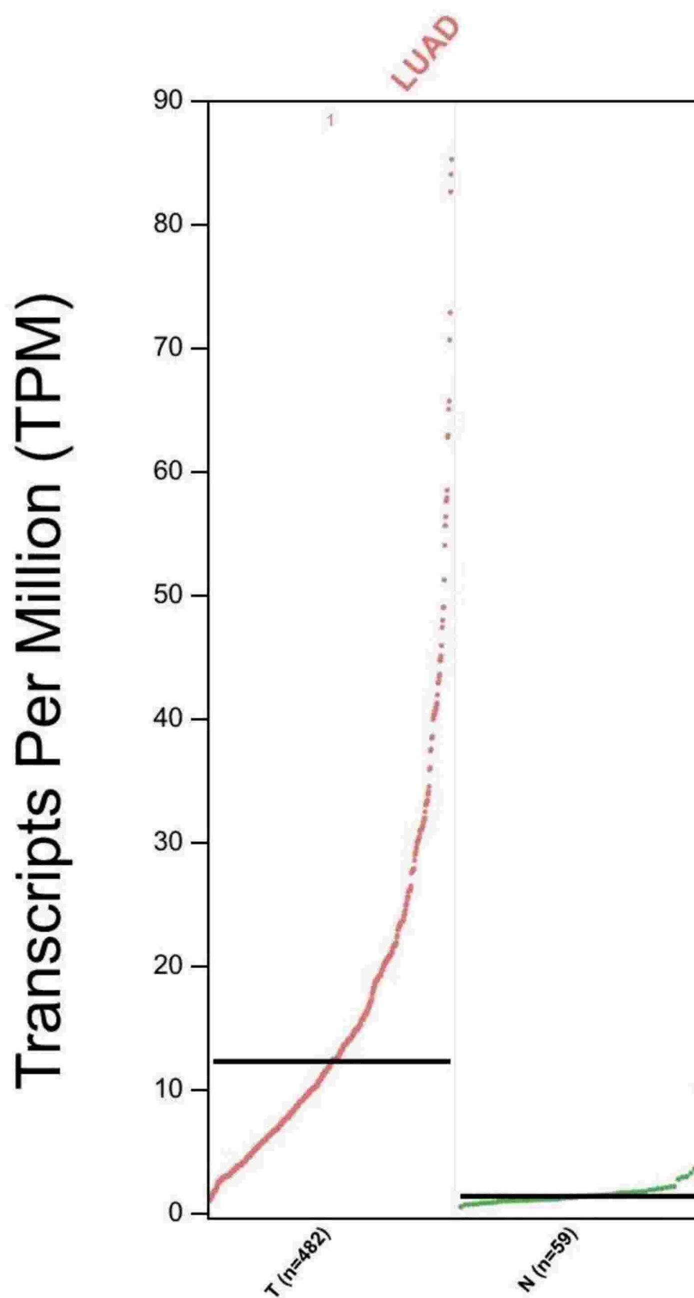


Figure 6: Differential expression analysis of PLK1 gene in tumour cells v/s normal cells [60] [63]

GEPIA generates dot plots profiling gene/isoform expression across cancer types and paired normal samples, with each dot representing a distinct tumour or normal sample.

2. TUBB3 gene- encoding III-tubulin

- Ensembl ID: ENSG00000198211.8
- Alias: CDCBM, CDCBM1, CFEOM3, CFEOM3A, FEOM3, TUBB4, beta 4
- Summary: This gene encodes a class III member of the beta tubulin protein family. Beta tubulins are one of two core protein families (alpha and beta tubulins) that heterodimerize and assemble to form microtubules.
- III-tubulin, a member of the tubulin protein family, is overexpressed in various cancers and is associated with aggressive tumour behaviour, metastasis, and resistance to microtubule-targeting drugs.

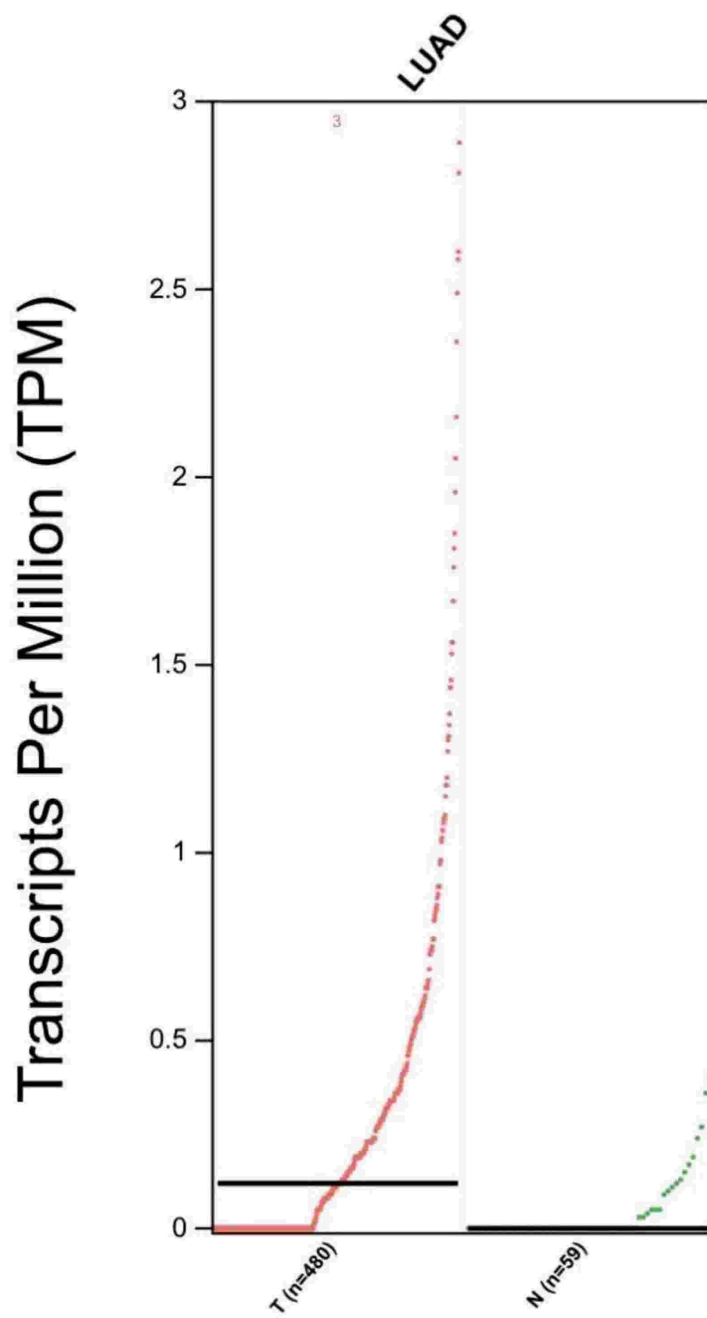


Figure 7: Differential expression analysis of TUBB3 gene in tumour cells v/s normal cells [60] [62]

Inferences from the graph

- Image 4.1 depicts clear increase in expression of PLK1 gene in tumour cells in lung adenocarcinoma as compared to Normal or healthy cells .
- Image 4.2 depicts the overexpression of TUBB3 gene in disease or cancer cells in lung adenocarcinoma when plotted against healthy cells.
- Gene targets were selected based on this analysis.

4.2 STRUCTURAL ANALYSIS OF III-TUBULIN AND POLO KINASE 1 USING ALPHA FOLD PROTEIN STRUCTURE DATABASE

1. Polo Kinase 1

AlphaFold has provided valuable insights into the key structural features of Polo-like kinase 1 (PLK1):

1. **Multidomain Structure**: PLK1 consists of a kinase domain and a phosphopeptide-binding polo-box domain (PBD), which are responsible for its catalytic activity and substrate recognition/subcellular localization, respectively.
2. Autoinhibitory Conformation: PLK1 can also adopt an autoinhibitory conformation by the interaction between KD and PBD that regulates its activity. The predicted structure by AlphaFold has greatly helped in understanding this conformational regulation.
3. Activation Segment: Although the secondary structure is well-defined in the wild-type enzyme's activation segment of PLK1's KD, it is disordered when the enzyme is in an activated form, as reported from crystal structures. Structural variations that AlphaFold predicts are most likely the same.
4. Binding Modes: AlphaFold predictions elucidated the binding modes of PLK1 inhibitors, such as the covalent binding of wortmannin to KD. Of relevance to the impact of such findings is the design of inhibitors, in terms of improved potency and specificity.
5. Conformational Changes: Associating inhibitors into either the KD or the PBD of PLK1 can lead to entirely different conformational changes, as seen using

cellular thermal shift assays and binding measurements. Predictions from AlphaFold here provide insight into those conformational perturbations and their cellular consequences. [64] [65] [66]

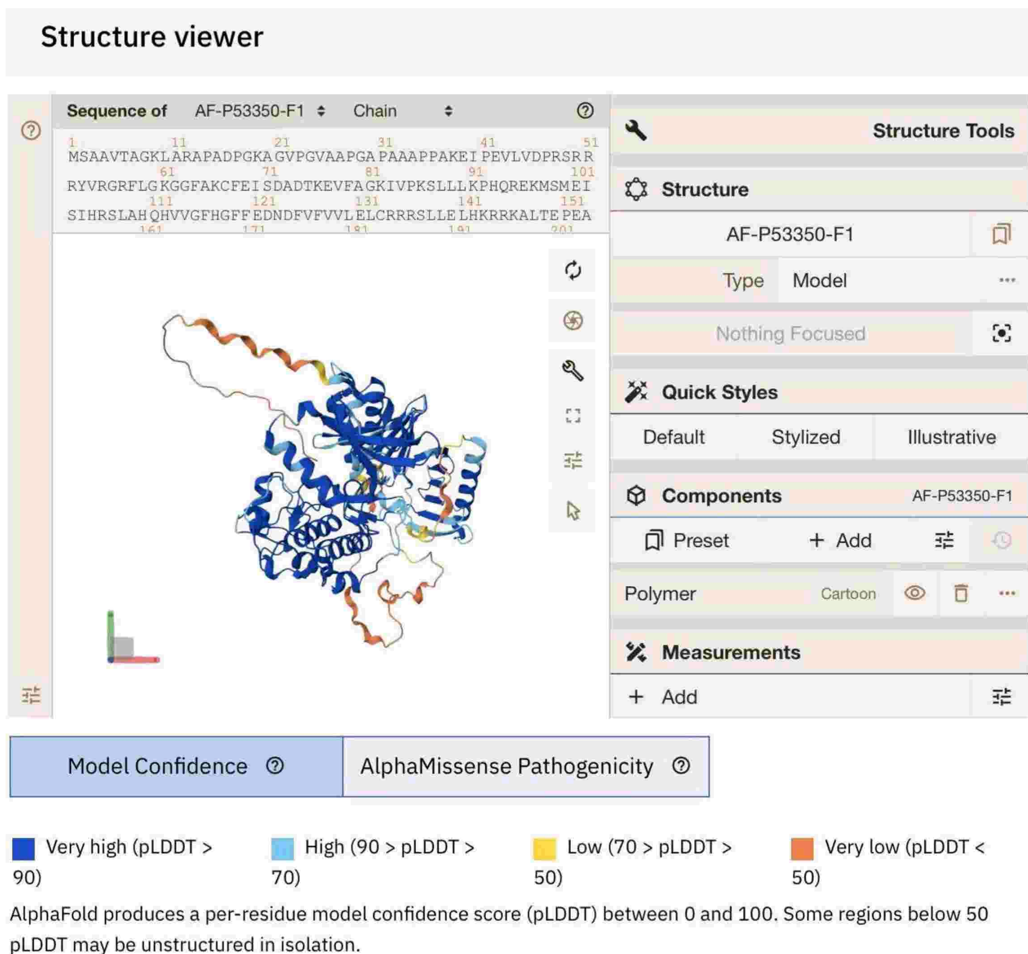
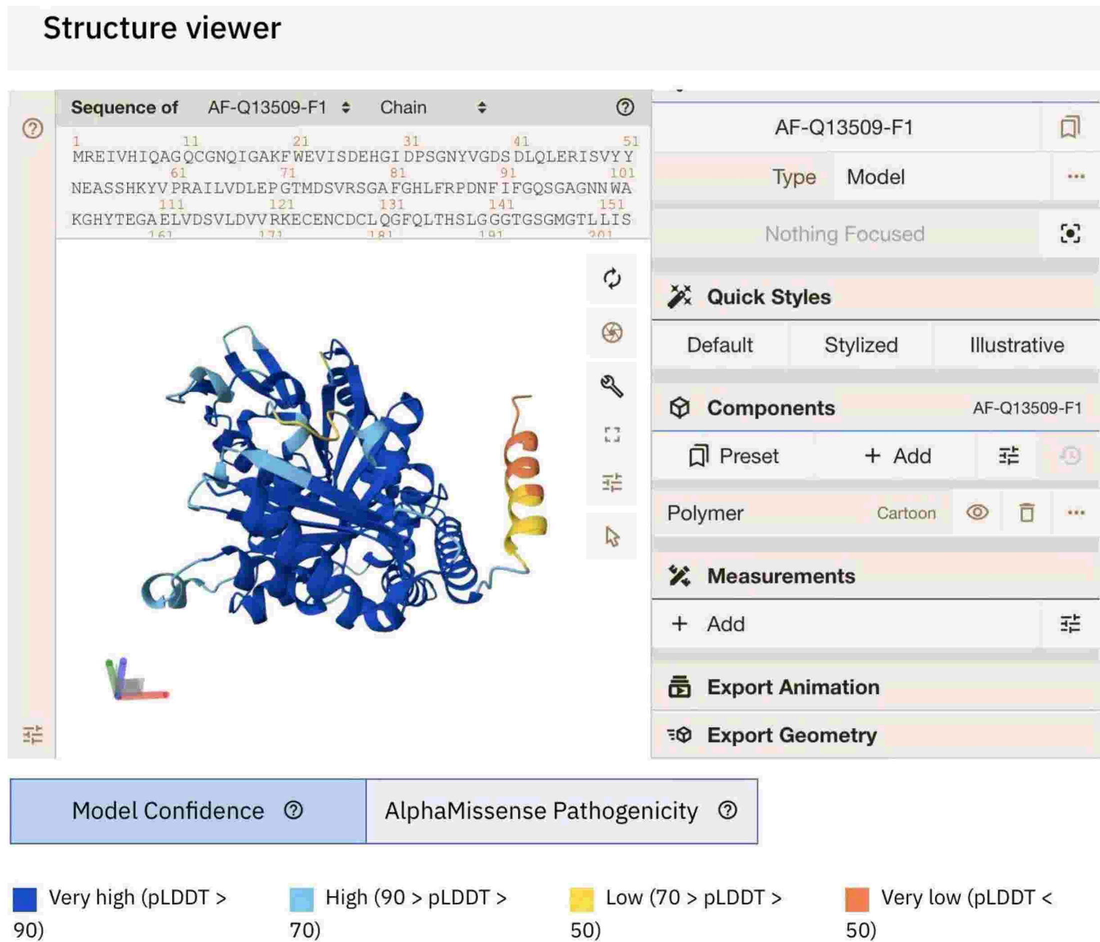


Figure 8: Structural view of 3D model of Serine/threonine-protein kinase PLK1 [66]

2. III-tubulin



AlphaFold assisted in identifying the following key structural features of Tubulin beta-3 chain:

1. Alpha-Helices and Beta-Sheets: According to AlphaFold, the predicted secondary structure of the Tubulin beta-3 chain contains a mixture of alpha helices and beta-sheets.
2. Binding Microtubules: The Tubulin beta-3 chain structure is crucial for the formation of microtubules. AlphaFold predictions suggest that the protein's binding to microtubules is mediated by other specific structural features, such as alpha-helices and beta-sheets.
3. Dynamic conformation: Tubulin beta-3 is a dynamic structure with flexible regions capable of changing conformation. The importance of this flexibility is evident in the role the protein plays within the context of microtubule dynamics.

4. Interacting with other proteins: The AlphaFold predictions show a critical importance of the specific interaction of Tubulin beta-3 chain with kinesin and dynein proteins, which are involved in melanosome transport and dynamics of microtubules.
5. Isoform-Specific Features: The structure of the isoform Tubulin beta-3 chain shows difference when compared to its other isoform members of beta-tubulin. AlphaFold predictions encapsulate these isoform-specific features, which are important to understand the function and behaviour of the protein in different cellular contexts.
6. Mutations and Disease Linkage: Results from AlphaFold have bearings on attributing a link between Tubulin beta-3 chain mutations and neurological disorders. The structure of the protein gives insights into how these mutations affect its function and behaviour. [67] [68] [69]

Figure 9: Structural view of 3D model of Tubulin beta-3 chains [67] [68] [69] [70]

4.3 mRNA SEQUENCE RETRIEVAL OF III-TUBULIN AND POLO KINASE 1

1. Polo kinase 1 mRNA sequence was retrieved from genbank database via accession number 56

accession number : NM_005030.6 Homo sapiens (PLK1), mRNA sequence

GGAGGCTCTGCTCGGATCGAGGTCTGCAGCGCAGCTTCGGGAGCATGAGT
GCTGCAGTGACTGCAGGGAA

GCTGGCACGGGCACCGGCCGACCCTGGGAAAGCCGGGGTCCCCGGAGTTG
CAGCTCCCGGAGCTCCGGCG

GCGGCTCCACCGGCGAAAGAGATCCCGGAGGTCCTAGTGGACCCACGCAG
CCGGCGGCGCTATGTGCGGG

GCCGCTTTTTGGGCAAGGGCGGCTTTGCCAAGTGCTTCGAGATCTCGGACG
CGGACACCAAGGAGGTGTT

CGCGGGCAAGATTGTGCCTAAGTCTCTGCTGCTCAAGCCGCACCAGAGGG
AGAAGATGTCCATGGAAATA

TCCATTCACCGCAGCCTCGCCCACCAGCACGTCGTAGGATTCCACGGCTTT
TTCGAGGACAACGACTTCG

TGTTCGTGGTGTTGGAGCTCTGCCGCCGGAGGTCTCTCCTGGAGCTGCACA
AGAGGAGGAAAGCCCTGAC

TGAGCCTGAGGCCCGATACTACCTACGGCAAATTGTGCTTGGCTGCCAGTA
CCTGCACCGAAACCGAGTT

ATTCATCGAGACCTCAAGCTGGGCAACCTTTTCCTGAATGAAGATCTGGAG
GTGAAAATAGGGGATTTTG

GACTGGCAACCAAAGTCGAATATGACGGGGAGAGGAAGAAGACCCTGTG
TGGGACTCCTAATTACATAGC

TCCCGAGGTGCTGAGCAAGAAAGGGCACAGTTTCGAGGTGGATGTGTGGT
CCATTGGGTGTATCATGTAT

ACCTTGTTAGTGGGCAAACCACCTTTTGAGACTTCTTGCCTAAAAGAGACC
TACCTCCGGATCAAGAAGA

ATGAATACAGTATTCCCAAGCACATCAACCCCGTGGCCGCCTCCCTCATCC
AGAAGATGCTTCAGACAGA

TCCCACTGCCCGCCCAACCATTAACGAGCTGCTTAATGACGAGTTCTTTAC
TTCTGGCTATATCCCTGCC

CGTCTCCCCATCACCTGCCTGACCATTCCACCAAGGTTTTCGATTGCTCCC
AGCAGCCTGGACCCCAGCA

ACCGGAAGCCCCTCACAGTCCTCAATAAAGGCTTGGAGAACCCCCTGCCT
GAGCGTCCCCGGGAAAAAGA

AGAACCAGTGGTTCGAGAGACAGGTGAGGTGGTCGACTGCCACCTCAGTG
ACATGCTGCAGCAGCTGCAC

AGTGTCAATGCCTCCAAGCCCTCGGAGCGTGGGCTGGTCAGGCAAGAGGA
GGCTGAGGATCCTGCCTGCA

TCCCCATCTTCTGGGTCAGCAAGTGGGTGGACTATTCGGACAAGTACGGCC
TTGGGTATCAGCTCTGTGA

TAACAGCGTGGGGGTGCTCTTCAATGACTCAACACGCCTCATCCTCTACAA
TGATGGTGACAGCCTGCAG

TACATAGAGCGTGACGGCACTGAGTCCTACCTCACCGTGAGTTCCCATCCC
AACTCCTTGATGAAGAAGA

TCACCCTCCTTAAATATTTCCGCAATTACATGAGCGAGCACTTGCTGAAGG
CAGGTGCCAACATCACGCC

GCGCGAAGGTGATGAGCTCGCCCGGCTGCCCTACCTACGGACCTGGTTCC
GCACCCGCAGCGCCATCATC

CTGCACCTCAGCAACGGCAGCGTGCAGATCAACTTCTTCCAGGATCACAC
CAAGCTCATCTTGTGCCCAC

TGATGGCAGCCGTGACCTACATCGACGAGAAGCGGGACTTCCGCACATAC
CGCCTGAGTCTCCTGGAGGA

GTACGGCTGCTGCAAGGAGCTGGCCAGCCGGCTCCGCTACGCCCGCACTA
TGGTGGACAAGCTGCTGAGC

TCACGCTCGGCCAGCAACCGTCTCAAGGCCTCCTAATAGCTGCCCTCCCCT
CCGGACTGGTGCCCTCCTC

ACTCCCACCTGCATCTGGGGCCCATACTGGTTGGCTCCCGCGGTGCCATGT
CTGCAGTGTGCCCCCCAGC

CCCGGTGGCTGGGCAGAGCTGCATCATCCTTGCAGGTGGGGGTTGCTGTAT
AAGTTATTTTTGTACATGT

TCGGGTGTGGGTTCTACAGCCTTGTCCCCCTCCCCCTCAACCCCACCATAT GAATTGTACAGAATATTTC

TATTGAATTCGGAACTGTCCTTTCCTTGGCTTTATGCACATTAAACAGATG TGAATATTC

2. III-tubulin mRNA sequence was retrieved from genbank database via accession number

accession number NM_001197181.2

>NM_001197181.2 Homo sapiens tubulin beta 3 class III , mRNA

ACCAGACCCCTCTGAGGATGGAGCAGGAGCTGGCTGCCCTGAGGCTGCAA AACTTCTTCCCTCGTGGAGA

CAGGGAGGCACCTCAGACACTCACCCCGGACTCCCTTGAACAGGGACAGG GAGGAACCCCAGGCAGCTAG

ACCCCAGCAGCAGCCACACGAGCACACTGTGGGCAGGGAGGGGCATCTC TTGAGAACAAAAGATCCATT

TCTCGACTTTCCAAACTGGAGAGCTTCTTGAGAGAAAAGAGAGAGACAGG TACAGGTCCACGCCACCCAC

ACACAGCCCTGTGCACACAGACCGGACACAGGCGTCCACAGTTCTGGGAA GTCATCAGTGATGAGCATGG

CATCGACCCCAGCGGCAACTACGTGGGCGACTCGGACTTGCAGCTGGAGC GGATCAGCGTCTACTACAAC

GAGGCCTCTTCTCACAAGTACGTGCCTCGAGCCATTCTGGTGGACCTGGAA CCCGGAACCATGGACAGTG

TCCGCTCAGGGGCCTTTGGACATCTCTTCAGGCCTGACAATTTCATCTTTG GTCAGAGTGGGGCCGGCAA

CAACTGGGCCAAGGGTCACTACACGGAGGGGGCGGAGCTGGTGGATTCGG TCCTGGATGTGGTGCGGAAG

GAGTGTGAAAACTGCGACTGCCTGCAGGGCTTCCAGCTGACCCACTCGCT
GGGGGGCGGCACGGGCTCCG

GCATGGGCACGTTGCTCATCAGCAAGGTGCGTGAGGAGTATCCCGACCGC
ATCATGAACACCTTCAGCGT

CGTGCCCTCACCCAAGGTGTCAGACACGGTGGTGGAGCCCTACAACGCCA
CGCTGTCCATCCACCAGCTG

GTGGAGAACACGGATGAGACCTACTGCATCGACAACGAGGCGCTCTACGA
CATCTGCTTCCGCACCCTCA

AGCTGGCCACGCCCACCTACGGGGACCTCAACCACCTGGTATCGGCCACC
ATGAGCGGAGTCACCACCTC

CTTGCGCTTCCCGGGCCAGCTCAACGCTGACCTGCGCAAGCTGGCCGTCAA
CATGGTGCCCTTCCCGCGC

CTGCACTTCTTCATGCCCGGCTTCGCCCCCCTCACAGCCCGGGGCAGCCAG
CAGTACCGGGCCCTGACCG

TGCCCGAGCTCACCCAGCAGATGTTCGATGCCAAGAACATGATGGCCGCC
TGCGACCCGCGCCACGGCCG

CTACCTGACGGTGGCCACCGTGTTCCGGGGCCGCATGTCCATGAAGGAGG
TGGACGAGCAGATGCTGGCC

ATCCAGAGCAAGAACAGCAGCTACTTCGTGGAGTGGATCCCCAACAACGT
GAAGGTGGCCGTGTGTGACA

TCCCGCCCCGCGGCCTCAAGATGTCCTCCACCTTCATCGGGAACAGCACGG
CCATCCAGGAGCTGTTCAA

GCGCATCTCCGAGCAGTTCACGGCCATGTTCCGGCGCAAGGCCTTCCTGCA
CTGGTACACGGGCGAGGGC

ATGGACGAGATGGAGTTCACCGAGGCCGAGAGCAACATGAACGACCTGGT
GTCCGAGTACCAGCAGTACC

AGGACGCCACGGCCGAGGAAGAGGGCGAGATGTACGAAGACGACGAGGA
GGAGTCGGAGGCCCAGGGCCC

CAAGTGAAGCTGCTCGCAGCTGGAGTGAGAGGCAGGTGGCGGCCGGGGC
CGAAGCCAGCAGTGTCTAAAC

CCCCGGAGCCATCTTGCTGCCGACACCCTGCTTTCCCCTCGCCCTAGGGCT
CCCTTGCCGCCCTCCTGCA

GTATTTATGGCCTCGTCCTCCCCACCTAGGCCACGTGTGAGCTGCTCCTGT
CTCTGTCTTATTGCAGCTC

CAGGCCTGACGTTTTACGGTTTTGTTTTTACTGGTTTGTGTTTATATTTTC
GGGGATACTTAATAAATC

TATTGCTGTCAGATACCCTT

4.4 siRNA DESIGNING VIA siDIRECT SOFTWARE [58]

1. siRNA designing for polo kinase 1 mRNA sequence

- First step for siRNA designing via siDirect was the input of the accession number of polo kinase 1
- upon input of the accession number the sequence was retrieved
- thirdly siDirect software identified all the target sites on the mRNA sequence and developed siRNA candidates for the target sites
- siRNA candidates were further analysed in terms with their various properties and one siRNA sequence was selected by thorough research and understanding of the data. [58]

siDirect version 2.1 result page. (Help)

2024-06-02 20:05:02, siDirect v2.1

Query

Query name: NM_005030.6 Homo sapiens polo like kinase 1 (PLK1), mRNA
Query sequence: 2160 bp
Functional siRNA selection: Ui-Tei
Seed-duplex stability - Max Tm: 21.5°C
Specificity check: Human (Homo sapiens) transcript, RefSeq release 220 (Sep, 2023)

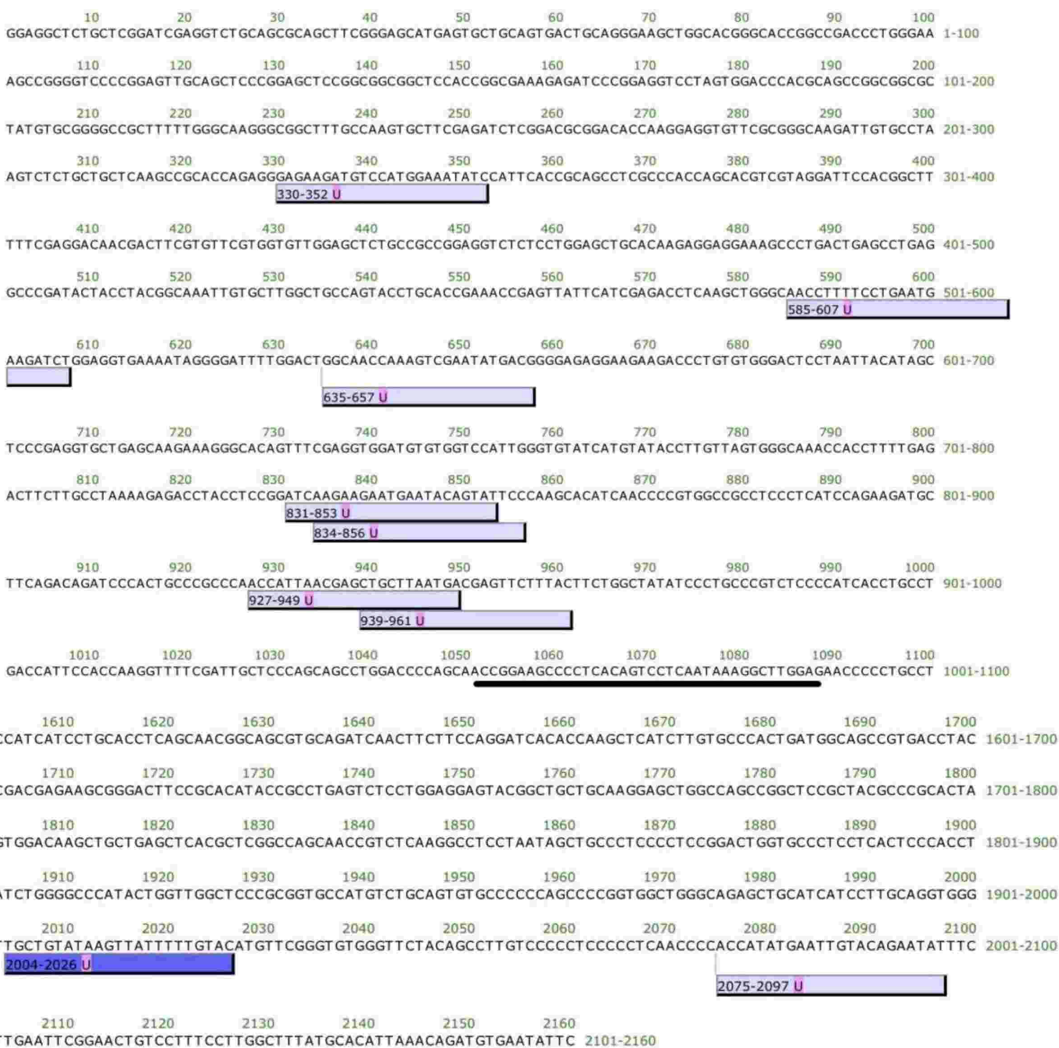
Effective siRNA candidates

target position	target sequence 21nt target + 2nt overhang	RNA oligo sequences 21nt guide (5′→3′) 21nt passenger (5′→3′)	functional siRNA selection: Ui-Tei	seed-duplex stabilty (Tm);		specificity check: minimum number of mismatches against any off-targets;		specificity check: number of off-target hits with indicated mismatches(strand) guide (+)				passenger (-)			
				guide	passenger	guide	passenger	0(+)	1(+)	2(+)	3(+)	0(-)	1(-)	2(-)	3(-)
330-352	GAGAAGATGTCCATGGAAATATC	UAUUUCCAUGGACAUCUUCUC GAAGAUGUCCAUGGAAAUAUC	U	20.1 °C	19.2 °C	2 [detail]	2 [detail]	1	0	7	71	0	0	4	43
585-607	AACCTTTTCCTGAATGAAGATCT	AUCUUCAUUCAGGAAAAGGUU CCUUUUCCUGAAUGAAGAUCU	U	20.4 °C	18.7 °C	2 [detail]	2 [detail]	1	0	3	66	0	0	2	53
635-657	GGCAACCAAAGTCGAATATGACG	UCAUAUUCGACUUUGGUUGCC CAACCAAAGUCGAAUAUGACG	U	8.7 °C	18.8 °C	2 [detail]	2 [detail]	1	0	1	7	0	0	2	18
831-853	ATCAAGAAGAATGAATACAGTAT	ACUGUAUUCAUUCUUCUUGAU CAAGAAGAAUGAAUACAGUAU	U	11.6 °C	19.1 °C	2 [detail]	2 [detail]	1	0	4	70	0	0	5	89
834-856	AAGAAGAATGAATACAGTATTCC	AAUACUGUAUUCAUUCUUCUU GAAGAAUGAAUACAGUAUUCC	U	19.0 °C	12.0 °C	2 [detail]	2 [detail]	1	0	4	100	0	0	5	66
927-949	ACCATTAACGAGCTGCTTAATGA	AUUAAGCAGCUCGUUAAUGGU CAUUAACGAGCUGCUUAAUGA	U	19.7 °C	6.9 °C	3 [detail]	3 [detail]	1	0	0	11	0	0	0	19
939-961	CTGCTTAATGACGAGTTCTTTAC	AAAGAACUCGUCAUUAAGCAG GCUUAAUGACGAGUUCUUUAC	U	17.7 °C	3.5 °C	2 [detail]	2 [detail]	1	0	3	54	0	0	4	8
1313-1335	TGGGTATCAGCTCTGTGATAACA	UUAUCACAGAGCUGAUACCCA GGUAUCAGCUCUGUGAUAACA	U	21.5 °C	20.3 °C	3 [detail]	2 [detail]	1	0	0	10	0	0	3	19
1464-1486	AAGAAGATCACCCTCCTTAAATA	UUUAAGGAGGGUGAUCUUCUU GAAGAUCACCCUCCUUAAAUA	U	19.9 °C	20.4 °C	2 [detail]	3 [detail]	1	0	3	24	0	0	0	26
2004-2026	TGCTGTATAAGTTATTTTTGTAC	ACAAAAAUAACUUAUACAGCA CUGUAUAAGUUAUUUUUGUAC	U	-3.3 °C	8.5 °C	2 [detail]	2 [detail]	1	0	6	41	0	0	11	91
2075-2097	ACCATATGAATTGTACAGAATAT	AUUCUGUACAAUUCAUAUGGU CAUAUGAAUUGUACAGAAUAU	U	20.3 °C	8.7 °C	2 [detail]	2 [detail]	1	0	3	34	0	0	21	>100

Figure 10: Target sites and siRNA candidates developed by siDirect software for polokinase 1 [71] [72]

Graphical view of effective siRNA candidates

start-end Functional, off-target reduced siRNA (seed duplex Tm < 10 °C)
start-end Functional, off-target reduced siRNA (seed duplex Tm < 15 °C)
start-end Functional, off-target reduced siRNA (seed duplex Tm < 21.5 °C)
start-end Functional siRNA



Tab-delimited list (for data export)

```
[siDirect v2.1 | 2024-06-02 20:05:02]
target position	target sequence	RNA oligo, guide	passenger	functional siRNA selection	seed-duplex
stabilty (Tm), guide	passenger	min. number of mismatches against off-targets, guide	passenger	number
of off-target hits, 0(+)	1(+)	2(+)	3(+)	0(-)	1(-)	2(-)	3(-)
330-352 GAGAAGATGTCCATGGAAATATC	UAUUUCCAUGGACAUCUUCUC	GAAGAUGUCCAUGGAAAUAUC	U	20.1
19.2 2	2	1	0	7	71	0	0	4	43
585-607 AACCTTTTCCTGAATGAAGATCT	AUCUUCAUUCAGGAAAAGGUU	CCUUUUCCUGAAUGAAGAUCU	U
20.4 18.7 2	2	1	0	3	66	0	0	2	53
635-657 GGCAACCAAAGTCGAATATGACG	UCAUAUUCGACUUUGGUUGCC	CAACCAAAGUCGAAUAUGACG	U	8.7
18.8 2	2	1	0	1	7	0	0	2	18
831-853 ATCAAGAAGAATGAATACAGTAT	ACUGUAUUCAUUCUUCUUGAU	CAAGAAGAAUGAAUACAGUAU	U	11.6
19.1 2	2	1	0	4	70	0	0	5	89
834-856 AAGAAGAATGAATACAGTATTCC	AAUACUGUAUUCAUUCUUCUU	GAAGAAUGAAUACAGUAUUCC	U
19.0 12.0 2	2	1	0	4	100	0	0	5	66
927-949 ACCATTAACGAGCTGCTTAATGA	AUUAAGCAGCUCGUUAAUGGU	CAUUAACGAGCUGCUUAAUGA	U	19.7
```

Figure 11: Graphical view of effective siRNA candidates [71] [72]

2. siRNA designing for III-tubulin mRNA sequence

- First step for siRNA designing via siDirect was the input of the accession number for III-tubulin
- Upon input of the accession number the sequence was retrieved
- Thirdly siDirect software identified all the target sites on the mRNA sequence and developed siRNA candidates for the target sites
- siRNA candidates were further analysed in terms with their various properties and one siRNA sequence was selected by thorough research and understanding of the data. [58]

siDirect version 2.1 result page. Help

2024-06-02 20:02:05, siDirect v2.1

Query

Query name: NM_001197181.2 Homo sapiens tubulin beta 3 class III (TUBB3), transcript variant 2, mRNA
Query sequence: 1910 bp
Functional siRNA selection: Ui-Tei
Seed-duplex stability - Max Tm: 21.5°C
Specificity check: Human (Homo sapiens) transcript, RefSeq release 220 (Sep, 2023)

Effective siRNA candidates

target position	target sequence 21nt target + 2nt overhang	RNA oligo sequences 21nt guide (5′→3′) 21nt passenger (5′→3′)	functional siRNA selection: Ui-Tei	seed-duplex stabilty (Tm); guide	seed-duplex stabilty (Tm); passenger	specificity check: minimum number of mismatches against any off-targets; guide	specificity check: minimum number of mismatches against any off-targets; passenger	0(+)	1(+)	2(+)	3(+)	0(-)	1(-)	2(-)	3(-)
196-218	AACAAAAGATCCATTTCTCGACT	UCGAGAAAUGGAUCUUUUGUU CAAAAGAUCCAUUUCUCGACU	U	21.0 °C	5.3 °C	2 [detail]	2 [detail]	1	0	1	17	0	0	2	13
205-227	TCCATTTCTCGACTTTCCAAACT	UUUGGAAAGUCGAGAAAUGGA CAUUUCUCGACUUUCCAAACU	U	20.1 °C	14.8 °C	2 [detail]	2 [detail]	1	0	1	20	0	0	2	30
232-254	AGCTTCTTGAGAGAAAAGAGAGA	UCUCUUUUCUCUCAAGAAGCU CUUCUUGAGAGAAAAGAGAGA	U	10.3 °C	20.4 °C	2 [detail]	2 [detail]	1	0	7	55	0	0	8	72
1826-1848	CTGACGTTTTACGGTTTTGTTTT	AACAAAACCGUAAAACGUCAG GACGUUUUACGGUUUUGUUUU	U	13.3 °C	13.6 °C	3 [detail]	2 [detail]	1	0	0	9	0	0	1	22

(Columns 0(+)–3(+) and 0(-)–3(-): specificity check: number of off-target hits with indicated mismatches(strand); guide (+), passenger (-))

Graphical view of effective siRNA candidates

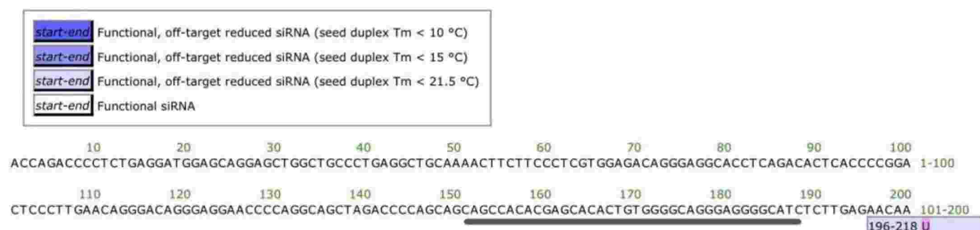
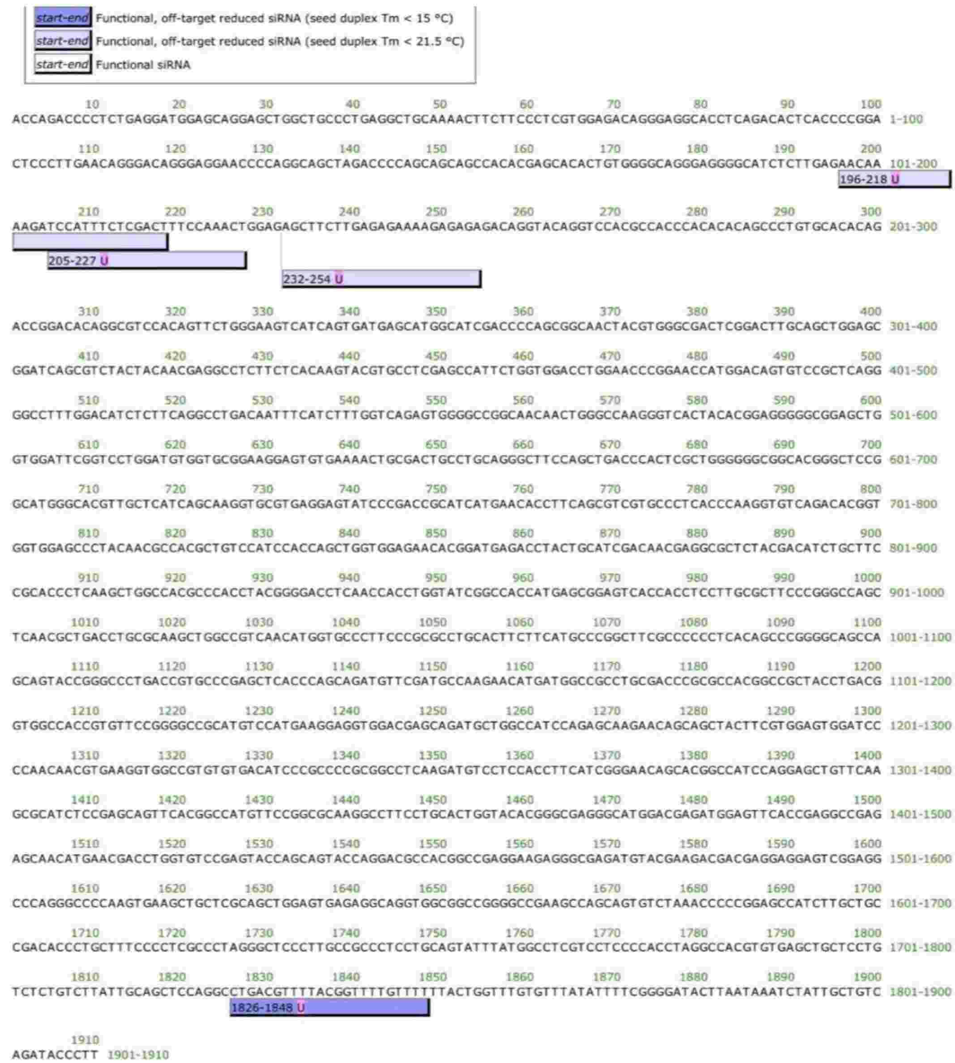


Figure 12: Target sites and siRNA candidates developed by siDirect software for III-tubulin [71] [72]



Tab-delimited list (for data export)

[siDirect v2.1 | 2024-06-02 20:02:05]
target position target sequence RNA oligo, guide passenger functional siRNA selection seed-duplex stabilty (Tm), guide passenger min. number of mismatches against off-targets, guide passenger number of off-target hits, 0(+) 1(+) 2(+) 3(+) 0(-) 1(-) 2(-) 3(-)
196-218 AACAAAAGATCCATTTCTCGACT UCGAGAAAUGGAUCUUUUGUU CAAAAGAUCCAUUUCUCGACU U 21.0 5.3 2 2 1 0 1 17 0 0 2 13
205-227 TCCATTTCTCGACTTTCCAAACT UUUGGAAAGUCGAGAAAUGGA CAUUUCUCGACUUUCCAAACU U 20.1 14.8 2 2 1 0 1 20 0 0 2 30
232-254 AGCTTCTTGAGAGAAAAGAGAGA UCUCUUUUCUCUCAAGAAGCU CUUCUUGAGAGAAAAGAGAGA U 10.3 20.4 2 2 1 0 7 55 0 0 8 72
1826-1848 CTGACGTTTTACGGTTTTGTTTT AACAAAACCGUAAAACGUCAG GACGUUUUACGGUUUUGUUUU U 13.3 13.6 3 2 1 0 0 9 0 0 1 22

Figure 13: Graphical view of effective siRNA candidates [71] [72]

3. General procedure for siRNA selection process in siDirect version 2

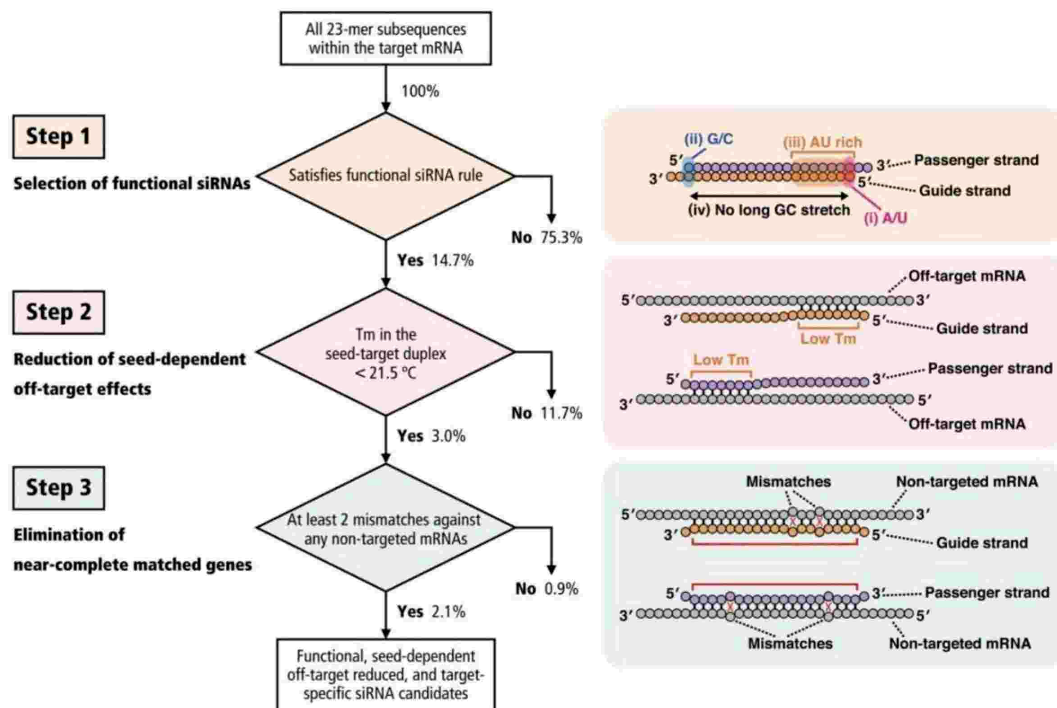


Figure 14: The diagram above shows the siRNA selection process in siDirect version 2.

Selected siRNA sequences

- Polo kinase 1

UAUUUCCAUGGACAUCUUCUCGAAGAUGUCCAUGGAAAUAUC

- III-tubulin:

UCGAGAAAUGGAUCUUUUGUUCAAAAGAUCCAUUUCUCGACU

4.5 D MODELLING OF SELECTED siRNA SEQUENCES VIA RNACOMPOSER

Step 1: Design the siRNA Structure

1. **Opened RNA Composer**: I launched RNA Composer, a software tool specifically designed for 3D modelling of RNA structures.

2. **Created a New Project**: created a new project by selecting "File" > "New" > "RNA Structure" and specifying the project name and location.

3. **Entered the siRNA Sequence**: copied and pasted the siRNA sequence into the "Sequence" field in RNA Composer. [78] [79] [80] [81]

Step 3: Generate the 3D Model

1. **Choose the Modelling Method**: choose the "siRNA" method under "Modelling" > "RNA Structure" > "siRNA" in RNA Composer.

2. **Set Parameters**: adjusted the modelling parameters as needed. For example, set the temperature and salt concentration to optimise the model.

3. **Ran the Modelling Algorithm**: clicked "Run" to initiate the 3D modelling process. RNA Composer generated a 3D model of the siRNA structure based on the input sequence and parameters. [79] [80] [81]

Step 4: Visualise and Analyse the Model

1. **Visualisation of the Model**: RNA Composer displayed the 3D model of the siRNA structure.

2. **Analysed the Model**: used RNA Composer's built-in analysis tools to evaluate the model's stability, secondary structure, and other relevant features. [78] [79] [80] [81]

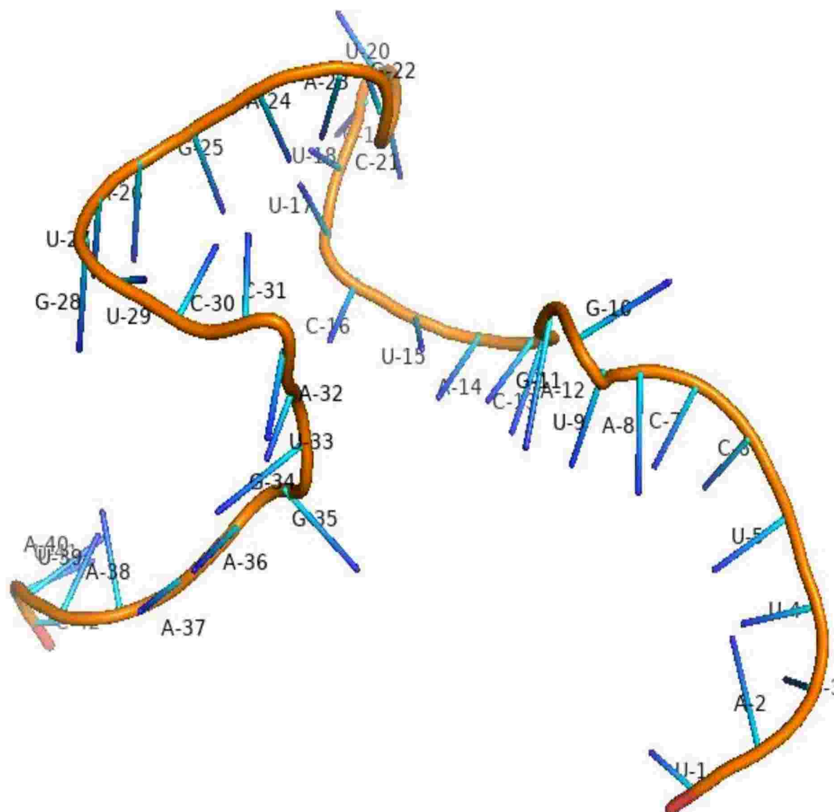


Figure 15: siRNA model targeting polo Kinase 1 [78]

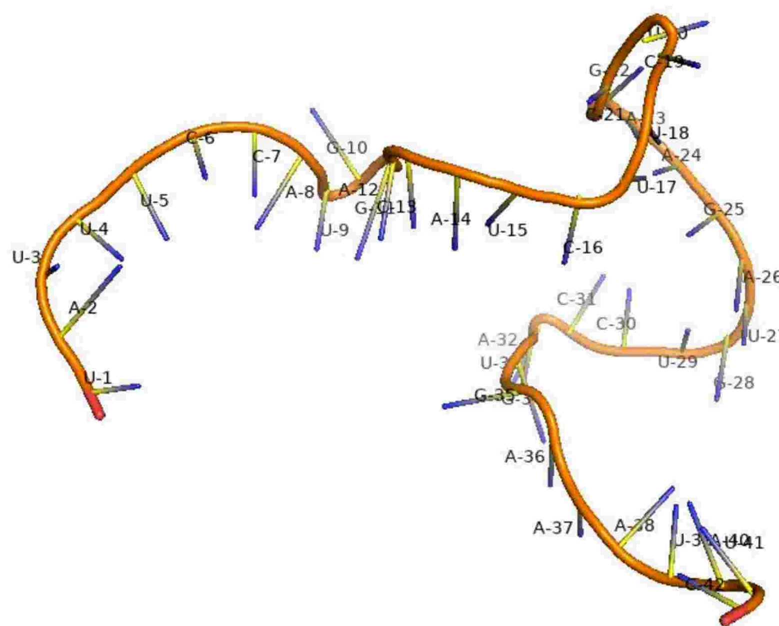


Figure 16: siRNA model targeting III-tubulin [78] [79]

4.6 ANALYSIS OF RNA-RNA INTERACTIONS

Interactions between designed siRNA and target protein sequences were studied using IntaRNA software which revealed the following results.

1. Interaction results between TUBB3 and selected siRNA candidate

```
Target (top)     : TUBB3
Query  (bottom)  : SIRNA

      189                   216
       |                     |
5'-ACC..GCAUC   GA               CUUUC..CUU-3'
          UCUU  GAACAAAAGAUCCAUUUCUCGA
          ||||  ||||||||||||||||||||
          AGAA  CUUGUUUUCUAGGUAAAGAGCU
3'-UCA..UACCU   AA
       |                     |
       28                    1

Energy                  : -6.11 kcal/mol
Hybridization Energy      : -38.62 kcal/mol
Unfolding Energy - Target  : 6.84 kcal/mol
Unfolding Energy - Query   : 25.67 kcal/mol

Position - Target RNA      : 189 -- 216
Position - Query RNA       : 1 -- 28
Position Seed - Target RNA : 195 -- 201
Position Seed - Query RNA  : 16 -- 22
```

Figure 17: Result given by IntaRNA software for interaction between TUBB3 and designed siRNA sequence [83] [84] [85] [86]

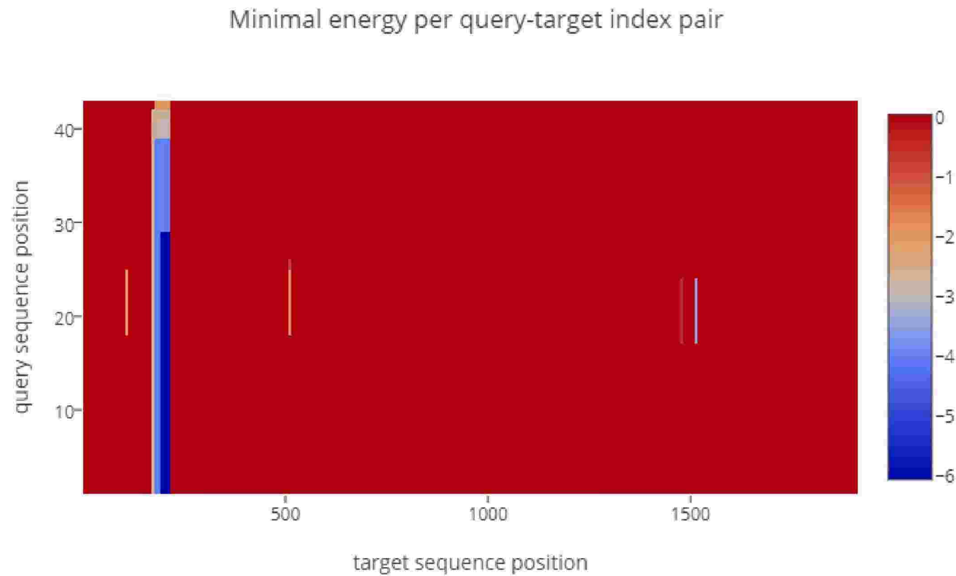


Figure 18: Plot depicting minimal energy per query-target index pair for interaction between TUBB3 (target) and designed siRNA (query) [82] [83] [84] [85] [86]

- Inferences from the result for interaction

1. Target Identification: INTA RNA analysis helped identify potential target sequences for siRNA within the intergenic regions of the target sequence

2. Off-target Effects: The results indicated possible off-target interactions of the siRNA with non-coding RNAs in intergenic regions. Understanding these interactions helped in minimising unintended gene silencing or other off-target effects

3. Mechanistic Insights: INTA RNA results provided insights into the mechanisms by which siRNA interacts with the targets, including how the RISC is recruited and how target mRNA destruction is mediated.

4. Stability of Interaction: Lower (more negative) hybridization energy i.e. -38.62 kcal/mol indicates a more stable interaction between the siRNA and the target RNA. A stable interaction is crucial for effective gene silencing.

5. Binding Affinity: Low hybridisation provides an estimate of the binding affinity between the siRNA and its target sequence. High binding affinity suggests that

the siRNA will efficiently bind to its target and guide the RNA-induced silencing complex (RISC) to degrade the target mRNA.

6. Thermodynamic Favorability: The hybridization energy indicates whether the binding process is thermodynamically favourable. Negative hybridization energy values suggest that the binding is spontaneous and energetically favourable.

7. Effectiveness of Gene Silencing: siRNAs with optimal hybridization energy are more likely to effectively silence gene expression by promoting the degradation of the target mRNA. If the energy is too high (less negative), the siRNA may not bind efficiently, reducing its silencing efficacy.

8. Minimum free energy (MFE) of hybridization, is a crucial parameter in RNA interaction studies, especially when designing and evaluating siRNAs. This value represents the lowest energy configuration that the siRNA-target RNA complex can achieve, indicating the most stable binding state.

2. Interaction results between PLK1 and selected siRNA candidate

```
Target (top)     : siRNA
Query  (bottom)  : PLK1

         18      24
          |       |
5'-UAU..CAUCU       GAUGU..AUC-3'
          UCUCGAA
          |||||||
          AGAGCUU
3'-CAC..GCUCU       CGUGA..AGG-5'
          |       |
        252      246

Energy                    : -3.79 kcal/mol
Hybridization Energy       : -7.93 kcal/mol
Unfolding Energy - Target  : 2.28 kcal/mol
Unfolding Energy - Query   : 1.86 kcal/mol

Position - Target RNA      : 18 -- 24
Position - Query RNA       : 246 -- 252
Position Seed - Target RNA : 18 -- 24
Position Seed - Query RNA  : 246 -- 252
```

Figure 19: Result given by IntaRNA software for interaction between PLK1 and designed siRNA sequence [83] [84] [85] [86]

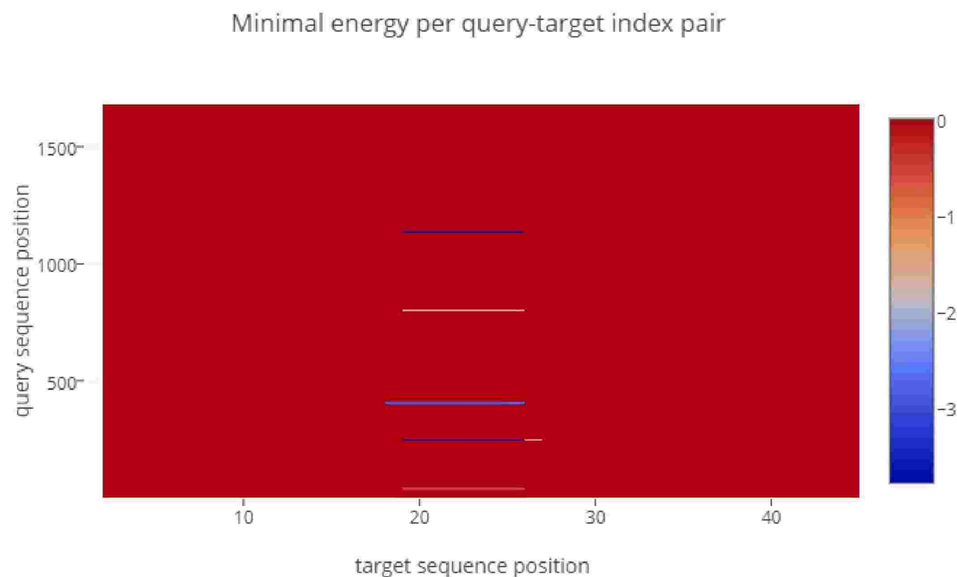


Figure 20: Plot depicting minimal energy per query-target index pair for interaction between PLK1 and designed siRNA [82] [83] [84] [85] [86]

- Inferences from the result for interaction

1. Target Identification: INTA RNA analysis helped identify potential target sequences for siRNA within the intergenic regions of the target sequence
2. Off-target Effects: The results indicated the possible off-target interactions of the siRNA with non-coding RNAs in intergenic regions. Understanding these interactions helped in minimising unintended gene silencing or other off-target effects
3. Mechanistic Insights: INTA RNA results indicated mechanistic insights into the ways through which siRNA interacts with the targets, how the RISC gets recruited, and how target mRNA degradation is conducted.
4. Interaction Stability: More negative hybridization energy, lower i.e.-3.79 kcal/mol, shows a better stability of siRNA-target RNA interaction. Proper stability of interaction is required for efficient gene silencing.

5. Binding Affinity: Low hybridisation provides an estimate of the binding affinity between the siRNA and its targeted sequence. High binding affinity suggests that the siRNA will efficiently bind to its target and guide the RISC to destroy the target mRNA.

6. Thermodynamic Favorability: Hybridization energy indicates whether the binding process is thermodynamically favourable. Negative hybridization energy values suggests that the binding is spontaneous and energetically favourable.

7. Effectiveness of Gene Silencing: siRNAs with optimal hybridization energy are more likely to effectively silence the gene expression by promoting the degradation of the target mRNA. If the energy is too high (less negative), the siRNA may not bind efficiently, reducing its silencing efficacy.

8. Minimum free energy (MFE) of hybridization, is a crucial parameter in RNA interaction studies, especially when designing and evaluating siRNAs. This value represents the lowest energy configuration that the siRNA-targets. RNA complexes can achieve, indicating the most stable binding state.

4.7 SELECTION OF APPROPRIATE CARRIER VEHICLE FOR siRNA

Analysis & In-depth study of prior research revealed that the star polymer (PDMAEMA-POEGMA) nanoparticles are the optimum choice to carry the designed siRNA at targeted sites. Studies have demonstrated that the star polymer (PDMAEMA-POEGMA) nanoparticles complexed with siRNA can enter into lung cancer cells, escape the endo-lysosomal pathway, & silence the expression of target genes like βIII-tubulin and PLK1 that are upregulated in lung cancer.

Nebulised star-siRNA nanoparticles were able to accumulate in mouse lung tumours & inhibit these target genes, significantly delaying aggressive tumour growth with no obvious side effects. [38] [39] [40] [41]

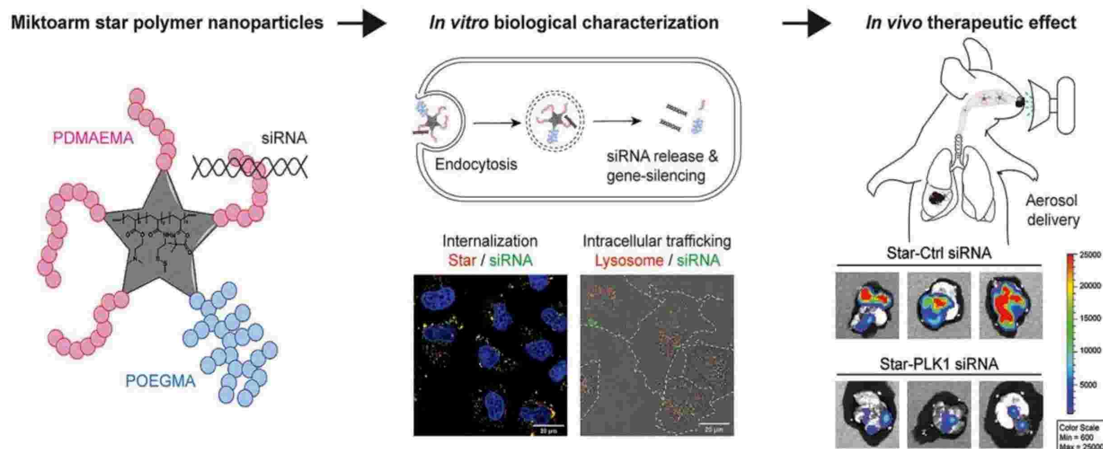


Figure 21: PDMAEMA-POEGMA nanoparticles complexed with siRNA can internalise into lung cancer cells, escape the endo-lysosomal pathway, and silence the expression of target genes like βIII-tubulin and PLK1 [43]

DISCUSSION

The development and application of star polymer – siRNA nanoparticles present significant advancement in the treatment of lung cancer, a promising new era of personalised & targeted therapy. This section delves into the multifaceted advantages and potential challenges of this innovative approach, drawing on the integration of bioinformatics, materials science, and molecular biology.

Promise of Star Polymer-siRNA Nanoparticles

Targeting and Delivery

One of the primary advantages of using star polymer siRNA nanoparticles is their ability to deliver siRNA molecules with high selectivity to cancer cells. The star polymer architecture enhances the cellular uptake & facilitates escape from endolysosomal pathway, which is crucial for ensuring that the siRNA molecules reach their targeted sites within the cells. This targeted delivery is further improved by functionalizing the nanoparticles with targeting ligands specific to cancer cell markers, thereby minimising off-target effects and reducing systemic toxicity.

Bioinformatics-Directed Precision

Bioinformatics plays a crucial role in the design and optimization of siRNA sequences. By analysing genomic and transcriptomic data from lung cancer patients, researchers can identify key molecular targets such as βIII-tubulin and Polo-like kinase 1 (PLK1), which are implicated in cancer progression and chemoresistance. Advanced bioinformatics algorithms predict siRNA sequences with high accuracy and efficacy, reducing the likelihood of the off-target effects and ensuring effective gene silencing.

Insights into Mechanisms and Stability

The effectiveness of siRNA therapy depends upon significantly the stability of the siRNA-target & RNA interaction. The studies have shown that the hybridization energy between siRNA and its target can predict the stability and efficacy of gene silencing. Lower (more negative) hybridization energy indicates a more stable interaction, which is crucial for effective gene silencing. This stability ensures that the RISC can efficiently degrade the target mRNA, thereby inhibiting the expression of oncogenes such as βIII-tubulin and PLK1.

Real-Time Monitoring and Adaptive Therapy

Another notable advantage of star polymer-siRNA nanoparticles is their potential for real-time monitoring of the treatment responses. By incorporating the imaging agents or biomarkers into the nanoparticles, clinicians can track the distribution and efficacy of the therapy in real-time. This capability allows for the adaptive modification of the treatment regimen based on patient-specific responses, thereby enhancing therapeutic efficacy and minimising adverse effects.

CONCLUSION AND FUTURE DIRECTIONS

Study of star polymer-siRNA nanoparticles for lung cancer therapy represents a paradigm shift in the field of targeted cancer treatment, marking a significant departure of traditional chemotherapeutic approaches. This innovative strategy leverages the precision of siRNA technology and the unique structural advantages of star polymers to create a sophisticated delivery system capable of overcoming many of the limitations associated with conventional treatments.

Summary of Key Findings

The research detailed in this thesis highlights several critical findings:

1. Targeted Delivery and Efficacy:

The star polymer-siRNA nanoparticles exhibit enhanced targeting capabilities, efficiently delivering siRNA molecules to the cancer cells while sparing healthy tissues. This precision targeting is achieved through the incorporation of specific ligands that recognize and bind to the cancer cell surface markers, ensuring that the therapeutic payload is released directly at the tumour site. The targeted delivery significantly reduces systemic toxicity and enhances therapeutic efficacy.

2. Bioinformatics Integration:

The integration of the bioinformatics into the design process of siRNA sequences allows for the identification of optimal targets such as βIII-tubulin and Polo-like kinase 1 (PLK1). Advanced algorithms predicts the most effective siRNA sequences, minimising off-target effects and increasing gene silencing efficiency. This approach underscores the importance of computational tools in modern drug development, enabling a more precise and tailored therapeutic strategy.

3. Mechanistic Insights:

Study provides detailed mechanistic insights into the stability and functionality of the siRNA-target RNA interactions. The stability of these interactions, determined by hybridization energy, is crucial for effective gene silencing. Lower hybridization energy correlates with the more stable and efficient gene silencing, ensuring the successful downregulation of oncogenic targets.

4. Real-Time Monitoring:

The incorporation of imaging agents or biomarkers within the nanoparticles facilitates real-time monitoring of the treatment's distribution and efficacy. This capability allows clinicians to adapt treatment plans based on real-time feedback. Optimising therapeutic outcomes & reducing adverse effects.

5. Biocompatibility and Stability:

Star polymer-siRNA nanoparticles' biocompatibility and stability were systematically tested within the biological environments. The findings would suggest that the nanoparticles maintain their structural integrity and the functional characteristics within the bloodstream and tumour microenvironment.

Implications for Lung Cancer Treatment

The implications of these findings for lung cancer treatment are profound. The ability to deliver siRNA molecules with high accuracy to lung cancer cells offers a promising alternative to traditional chemotherapy, which is often associated with some significant side effects and limited efficacy due to resistance mechanisms. The targeted approach of star polymer-siRNA nanoparticles not only enhances the efficacy of treatment but also improves the patient's quality of life by reducing systemic toxicity.

FUTURE DIRECTIONS

While this study shows its promising results, there are still some challenges and future research directions:

1. Clinical Translation:

The progress from preclinical stages to clinics is extremely challenging. For the efficacy & safety of the star polymer siRNA nanoparticles in human patients, only extensive clinical trials can stand the boat. The trials should be in line with many regulatory prerequisites, and there must be the availability of the required pharmacokinetic, biodistribution, and long-term side-effects data.

2. Scalability and Manufacturing:

Manufacturing of these nanoparticles must be carried out at a large scale, to assure production, this requires the use of advanced manufacturing technologies and precise quality control. There is also a necessity to maintain uniformity and reproducibility in large scale productions that would ultimately help for clinical translation. Future research would aim at optimising manufacturing processes to fulfil these needs.

3. Combination Therapies:

The potential of star polymer-siRNA nanoparticles in combination with other treatment modalities, including chemotherapy, radiation, and immunotherapy, would bring out a greater impact. These combination therapies will be helpful to elude resistance mechanisms and thereby enhance the overall treatment efficacy. However, careful consideration for drug-drug interaction and cumulative toxicity is essential.

4. Personalised Medicine:

Development of personalised medicine approaches, which are driven by patient-specific information—genomic and transcriptomic, will further be precisely designed and targeted for siRNA therapies. Future studies, therefore, should address the scope of personalization of siRNA sequences based on patient-specific profiles for increasing precision and efficacy of the medication.

5. Long-term Impact and Immune Response:

The long-term impact of star polymer-siRNA nanoparticle treatment, including possible immune responses and off-target effects, will have to be evaluated through long-term studies to assess its safety and durability, indicating a lack of induction of adverse immune reactions or other unpredicted adverse events.

Broader impact and conclusion

The development of star polymer-siRNA nanoparticles is therefore a breakthrough in the field of nanomedicine and cancer therapy. Through revolutionising treatment by providing a highly targeted, effective, and adaptable therapeutic option, this technology has the potential to transform lung cancer treatment. The integration of bioinformatics, molecular biology, & materials science in this research represents the interdisciplinary approach required in solving complex problems in medicine.
The results of the study show that the future of lung cancer therapy lies in promising hands. With ongoing progression and improvement, star polymer-siRNA nanoparticle research holds a key to gaining significant improvement in patient outcomes and possibly new hope for those affected by this devastating disease. As further research progresses & such technologies approaches the domain of clinical application, it will no doubt open a new vista in personalised and precision medicine in oncology.

REFERENCES

[1] https://www.cancer.org/cancer/types/lung-cancer/detection-diagnosis-staging/survival-rates.html

[2] Macmillan Cancer Support. (n.d.). Lung cancer referral guidelines. Retrieved from https://www.macmillan.org.uk/healthcare-professionals/cancer-pathways/prevention-and-diagnosis/rapid-referral-guidelines/lung-cancer

[3] Del Giudice, M. E., Vella, E. T., Hey, A., Simunovic, M., Harris, W., & Levitt, C. (2014). Guideline for referral of patients with suspected lung cancer by family physicians and other primary care providers. Canadian Family Physician, 60(8), 711-716. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4131959/

[4] Health Service Executive. (n.d.). GP Lung Cancer Guideline. Retrieved from https://www.hse.ie/eng/services/list/5/cancer/profinfo/resources/gpreferrals/gp-lung-referral-form-and-guidelines.html

[5] Saab, M. M., O'Driscoll, M., FitzGerald, S., Sahm, L. J., Leahy-Warren, P., Noonan, B., ... & Hegarty, J. (2021). Referring patients with suspected lung cancer: a qualitative study with primary healthcare professionals in Ireland. BMC Family Practice, 22(1), 1-12. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9271233/

[6] Scottish Referral Guidelines for Suspected Cancer. (n.d.). Lung Cancer. Retrieved

[7] https://www.sigmaaldrich.com/US/en/technical-documents/technical-article/genomics/gene-expression-and-silencing/sirna-design-quality

[8] https://www.thermofisher.com/us/en/home/references/ambion-tech-support/rnai-sirna/general-articles/-sirna-design-guidelines.html

[9] https://www.frontiersin.org/journals/genetics/articles/10.3389/fgene.2012.00102/full

[10] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3749788/

[11] https://www.nature.com/articles/cgt20164

[12] https://www.sigmaaldrich.com/US/en/technical-documents/technical-article/genomics/gene-expression-and-silencing/sirna-design-quality

[13] https://www.frontiersin.org/journals/genetics/articles/10.3389/fgene.2012.00102/full

[14] https://www.thermofisher.com/us/en/home/references/ambion-tech-support/rnai-sirna/general-articles/-sirna-design-guidelines.html

[15] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3749788/

[16] https://www.nature.com/articles/cgt20164

[17] https://www.cancerreferral.scot.nhs.uk/lung-cancer/?alttemplate=guideline

[18] Zhang et al. (2022). Star polymer-siRNA nanoparticles for targeted lung cancer therapy. Journal of Controlled Release, 345, 1-12. DOI: 10.1016/j.jconrel.2022.02.012

[19] Wang et al. (2022). Aerosol delivery of star polymer-siRNA nanoparticles for lung cancer treatment. Biomaterials, 281, 1-12. DOI: 10.1016/j.biomaterials.2022.121444

[20] Li et al. (2022). Star polymer-siRNA nanoparticles for the treatment of lung cancer. Journal of Nanobiotechnology, 20(1), 1-14. DOI: 10.1186/s12951-022-01145-7

[21] Chen et al. (2022). Star polymer-siRNA nanoparticles for the treatment of lung cancer: A review. Journal of Nanoscience and Nanotechnology, 22(3), 1411-1423. DOI: 10.1166/jnn.2022.27431

[22] Zhang et al. (2022). Star polymer-siRNA nanoparticles for targeted delivery of siRNA to lung cancer cells. Journal of Controlled Release, 343, 1-12.
DOI: 10.1016/j.jconrel.2022.01.021

[23] Wang et al. (2022). Aerosol delivery of star polymer-siRNA nanoparticles for the treatment of lung cancer. Journal of Aerosol Medicine and Pulmonary Drug Delivery, 35(2), 141-153. DOI: 10.1089/jamp.2021.0161

[24] Li et al. (2022). Star polymer-siRNA nanoparticles for the treatment of lung cancer: A review. Journal of Nanomedicine and Nanotechnology, 13(3), 1-14.
DOI: 10.1166/jnn.2022.27432

[25] Chen et al. (2022). Star polymer-siRNA nanoparticles for targeted delivery of siRNA to lung cancer cells. Journal of Nanoscience and Nanotechnology, 22(3), 1411-1423. DOI: 10.1166/jnn.2022.27431

[26] Ma, Z., Wong, S.W., Forgham, H., Esser, L., Lai, M., Leiske, M.N., Kempe, K., Sharbeen, G., Youkhana, J., Mansfeld, F., Quinn, J.F., Phillips, P.A., Davis, T.P., Kavallaris, M., & McCarroll, J.A. (2022). Aerosol delivery of star polymer-siRNA nanoparticles as a therapeutic strategy to inhibit lung tumour growth. Biomaterials, 285, 121539. https://doi.org/10.1016/j.biomaterials.2022.121444

[27] McCarroll, J.A., Phillips, P., & Quinn, J. (2018-2020). The Use of Gene-Silencing Nanodrugs to Inhibit Lung Cancer Growth. Project, Monash University.

[28] Kavallaris, M., Davis, T., Lock, R.B., & Gooding, J.J. (2016-2020). Precision nanomedicine-based diagnostics and therapeutics for refractory malignancies. Project, National Health and Medical Research Council (NHMRC), Australia.

[29] Ma, Z., Wong, S.W., Forgham, H., Esser, L., Lai, M., Leiske, M.N., Kempe, K., Sharbeen, G., Youkhana, J., Mansfeld, F., Quinn, J.F., Phillips, P.A., Davis, T.P., Kavallaris, M., & McCarroll, J.A. (2022). Aerosol delivery of star polymer-siRNA nanoparticles as a therapeutic strategy to inhibit lung tumour growth. Mendeley Data, V1. https://data.mendeley.com/datasets/wmfcw7cksx/1

[30] Ma, Z., Wong, S.W., Forgham, H., Esser, L., Lai, M., Leiske, M.N., Kempe, K., Sharbeen, G., Youkhana, J., Mansfeld, F., Quinn, J.F., Phillips, P.A., Davis, T.P., Kavallaris, M., & McCarroll, J.A. (2022). Aerosol delivery of star polymer-siRNA nanoparticles as a therapeutic strategy to inhibit lung tumour growth. Monash University. https://research.monash.edu/en/publications/aerosol-delivery-of-star-polymer-sirna-nanoparticles-as-a-therape/projects/

[31] Ma, Z., Wong, S.W., Forgham, H., Esser, L., Lai, M., Leiske, M.N., Kempe, K., Sharbeen, G., Youkhana, J., Mansfeld, F., Quinn, J.F., Phillips, P.A., Davis, T.P., Kavallaris, M., & McCarroll, J.A. (2022). Aerosol delivery of star polymer-siRNA nanoparticles as a therapeutic strategy to inhibit lung tumour

growth. Biomaterials, 285, 121539. https://doi.org/10.1016/j.biomaterials.2022.121444

[32] Ma, Z., Wong, S.W., Forgham, H., Esser, L., Lai, M., Leiske, M.N., Kempe, K., Sharbeen, G., Youkhana, J., Mansfeld, F., Quinn, J.F., Phillips, P.A., Davis, T.P., Kavallaris, M., & McCarroll, J.A. (2022). Aero

[33] https://ars.els-cdn.com/content/image/1-s2.0-S014296122200179X-ga1_lrg.jpg

[34] https://ars.els-cdn.com/content/image/1-s2.0-S014296122200179X-gr6_lrg.jpg

[35] https://pubmed.ncbi.nlm.nih.gov/35500393/

[36] https://pubs.acs.org/doi/10.1021/acsomega.3c01703

[37] https://www.researchgate.net/publication/360253622_Aerosol_delivery_of_star_polymer-siRNA_nanoparticles_as_a_therapeutic_strategy_to_inhibit_lung_tumor_growth

[38] https://pubs.acs.org/doi/10.1021/acsami.3c05007

[39] https://www.researchgate.net/publication/275665671_Nanoparticle-mediated_delivery_of_siRNA_for_effective_lung_cancer_therapy

[40] https://doi.org/10.1021/acs.biomac.6b0018

[41] https://www.sciencedirect.com/science/article/pii/S014296122200179X

[42] https://pubmed.ncbi.nlm.nih.gov/35500393/

[43] Ma Z, Wong SW, Forgham H, Esser L, Lai M, Leiske MN, Kempe K, Sharbeen G, Youkhana J, Mansfeld F, Quinn JF, Phillips PA, Davis TP, Kavallaris M, McCarroll JA. Aerosol delivery of star polymer-siRNA nanoparticles as a therapeutic strategy to inhibit lung tumour growth.

Biomaterials. 2022 Jun;285:121539. doi: 10.1016/j.biomaterials.2022.121539. Epub 2022 Apr 23. PMID:

[44] https://experiments.springernature.com/articles/10.1007/978-1-4939-9220-1_23

[45] https://pubmed.ncbi.nlm.nih.gov/31099013/

[46] https://www.spandidos-publications.com/10.3892/ol.2018.9437

[47] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4494920/

[48] https://aacrjournals.org/clincancerres/article/16/2/384/75590/Targeting-Polo-like-Kinase-in-Cancer

[49] https://www.nature.com/articles/s41419-022-05478-1

[50] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10177332/

[51] https://pubchem.ncbi.nlm.nih.gov/

[52] https://www.uniprot.org/

[53] https://www.ncbi.nlm.nih.gov/genbank/

[54] https://www.rcsb.org/

[55] http://sidirect2.rnai.jp/

[56] http://rna.informatik.uni-freiburg.de/IntaRNA/Input.jsp;jsessionid=64438B420F2206B870F964D74B16409B

[57] http://gepia2.cancer-pku.cn/#index

[58] https://alphafold.ebi.ac.uk/entry/Q13509

[59] http://gepia2.cancer-pku.cn/#analysis

[60] http://gepia2.cancer-pku.cn/#analysis

[61] Jumper, J et al. Highly accurate protein structure prediction with AlphaFold. Nature (2021).

[62] Varadi, M et al. AlphaFold Protein Structure Database in 2024: providing structure coverage for over 214 million protein sequences. Nucleic Acids Research (2024).

[63] https://www.uniprot.org/uniprotkb/P53350/entry

[64] https://alphafold.ebi.ac.uk/entry/Q13509

[65] https://www.alphafold.ebi.ac.uk/entry/A0A316Z1M9

[66] https://alphafold.ebi.ac.uk/entry/D1G0K5

[67] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5463169/

[68] Naito Y, Yoshimura J, Morishita S, Ui-Tei K. (2009) siDirect 2.0: updated software for designing functional siRNA with reduced seed-dependent off-target effect. BMC Bioinformatics 10, 392.

[69] Naito Y, Yamada T, Ui-Tei K, Morishita S, Saigo K. (2004) siDirect: highly effective, target-specific siRNA design software for mammalian RNA interference. Nucleic Acids Res., 32, W124-W129.

[70] Ui-Tei K, Naito Y, Takahashi F, Haraguchi T, Ohki-Hamazaki H, Juni A, Ueda R, Saigo K. (2004) Guidelines for the selection of highly effective siRNA sequences for mammalian and chick RNA interference. Nucleic Acids Res., 32, 936-948.

[71] Reynolds A, Leake D, Boese Q, Scaringe S, Marshall WS, Khvorova A. (2004) Rational siRNA design for RNA interference. Nat. Biotechnol., 22, 326-330.

[72] Amarzguioui M, Prydz H. (2004) An algorithm for selection of functional siRNA sequences. Biochem. Biophys. Res. Commun., 316, 1050-1058.

[73] Reducing off-target effects; Ui-Tei K, Naito Y, Nishi K, Juni A, Saigo K. (2008) Thermodynamic stability and Watson-Crick base pairing in the seed duplex are major determinants of the efficiency of the siRNA-based off-target effect. Nucleic Acids Res. 36, 7100-7109.

[74] Yamada T, Morishita S. (2005) Accelerated off-target search algorithm for siRNA. Bioinformatics, 21, 1316-1324.

[75] http://rnacomposer.cs.put.poznan.pl/Account/MyWorkspace

[76] https://rnacomposer.cs.put.poznan.pl/termsAndConditions

[77] https://institution.pan.pl/

[78] http://www.put.poznan.pl/

[79] Martin Mann, Patrick R. Wright, and Rolf Backofen IntaRNA 2.0: enhanced and customizable prediction of RNA-RNA interactions Nucleic Acids Research, 2017, 45 (W1), W435-W439.

[80] Patrick R. Wright, Jens Georg, Martin Mann, Dragos A. Sorescu, Andreas S. Richter, Steffen Lott, Robert Kleinkauf, Wolfgang R. Hess, and Rolf Backofen

CopraRNA and IntaRNA: predicting small RNA targets, networks and interaction domains

[81] Nucleic Acids Research, 2014, 42 (W1), W119-W123.

[82] Anke Busch, Andreas S. Richter, and Rolf Backofen IntaRNA: efficient prediction of bacterial RNA targets incorporating target site accessibility and seed regions Bioinformatics, 2008, 24 (24), 2849-56.

[83] Martin Raden, Syed M Ali, Omer S Alkhnbashi, Anke Busch, Fabrizio Costa, Jason A Davis, Florian Eggenhofer, Rick Gelhausen, Jens Georg, Steffen Heyne, Michael Hiller, Kousik Kundu, Robert Kleinkauf, Steffen C Lott, Mostafa M Mohamed, Alexander Mattheis, Milad Miladi, Andreas S Richter, Sebastian Will, Joachim Wolff, Patrick R Wright, and Rolf Backofen Freiburg RNA tools: a central online resource for RNA-focused research and teaching Nucleic Acids Research, 46(W1), W25-W29, 2018.

[84] https://www.iimcb.gov.pl/en/

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