

T.C.
YEDİTEPE UNIVERSITY
INSTITUTE OF HEALTH SCIENCES
DEPARTMENT OF MOLECULAR MEDICINE

**INVESTIGATION OF VARIATIONS IN DNA
REPAIR GENES ON Lu-177 PROSTATE
SPECIFIC MEMBRANE ANTIGEN (PSMA)
LIGANDS TREATMENT RESPONSE IN
PATIENTS WITH PROSTATE CANCER**

DOCTOR OF PHILOSOPHY THESIS

NALAN ALAN SELCUK, M.D.

İstanbul-2023

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THESIS APPROVAL FORM

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This thesis has been deemed by the jury in accordance with the relevant articles of Yeditepe University Graduate Education and Examinations Regulation and has been approved by Administrative Board of Institute with decision dated and numbered

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DECLARATION

I hereby declare that this thesis is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which has been accepted for an award of any other degree except where due acknowledgment has been made in the text.

17.08.2023

Nalan ALAN SELCUK



DEDICATION

I dedicate my thesis to my lovely Family

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LIST OF SYMBOLS AND ABBREVIATIONS

BER	Base excision repair
CT	Computed tomography
Ct	Cycle threshold
DNA	Deoxyribonucleic acid
DRE	Digital rectal exam
ERCC	Excision repair cross-complement
ERCC2	Excision repair cross-complement 2
Lu-177	Lutetium-177
miRNA	microRNA
mCi	Millicurie
mCRPC	Metastatic castrate-resistant prostate cancer
MMR	DNA mismatch repair
MRI	Magnetic resonance imaging
NCBI	National Center for Biotechnology Information
NER	Nucleotide excision repair
NHEJ	Non-homologous end joining
OD	Optical Density
PSA	Prostate specific antigen
PSMA	Prostate-specific membrane antigen
ROS	Reactive oxygen species
SNP	Single nucleotide polymorphisms
TRUS	Transrectal ultrasound

ABSTRACT

Alan Selcuk, N. (2023). Investigation of Variations in DNA Repair Genes on Lu-177 Prostate Specific Membrane Antigen (PSMA) Ligands Treatment Response in Patients with Prostate Cancer. Yeditepe University, Institute of Health Sciences, Department of Molecular Medicine, PhD Thesis, İstanbul.

The main objective of this study was to investigate the relationship between Lu-177 PSMA, a potential treatment for advanced prostate cancer, and ERCC2 gene variants, miRNA-182 and miRNA-187 expression levels. In this study, 50 of 57 patients were evaluated for treatment status, and 7 were evaluated for changes before and after treatment. It was found that the expression levels of miRNA-182 ($p < 0.001$) and miRNA-187 were significantly higher in patients who received treatment, including Lu-177 PSMA, than in patients who did not receive any treatment ($p = 0.000$). A significant relationship was found between the post-treatment expression level of miRNA-182 and the change in post-treatment DNA concentration ($p = 0.028$). Additionally, it was found that the rs13181 variant was present in both heterozygote mutant and wild type genotypes in patients who received and did not receive treatment. For the rs1799793 variant, it was determined that patients who received treatment had wild-type and homozygous mutant genotypes, while patients who did not receive treatment had homozygous mutant and heterozygous mutant genotypes. According to the pre- and post-treatment results, it was determined that 50% of the patients with PSA response after treatment had heterozygous mutant for ERCC2 rs13181 variant; 66.66% of the patients who did not have PSA response had wild-type genotype. For ERCC2 rs1799793, it was determined that 100% of those with PSA response were heterozygous mutant, while 50% of those without PSA response had wild-type and 50% had homozygous mutant genotype. It was found that patients with high post-treatment miRNA-182 levels were more likely to have heterozygous mutant genotype for ERCC2 rs13181 ($p = 0.028$).

In conclusion, it was found that miRNA expressions showed a bimodal feature and that they could vary depending on the dose of treatment, the stage and course of the disease. Monitoring of miRNA-182 expression and analysis of ERCC2 rs13181 and rs1799793 variants can help in the correct planning of personalized Lu-177 PSMA treatment strategies.

Key Words: Lu-77 PSMA, Prostate Cancer, miRNA-182, miRNA-187, ERCC2

ÖZET

Alan Selcuk, N. (2023). Lu-177 Prostat Spesifik Membran Antijeni (PSMA) Ligantları ile tedavi edilen Prostat Kanserli Hastalarda DNA Tamir Genlerindeki Varyasyonların Tedavi Cevabının Üzerine Etkisinin İncelenmesi. Yeditepe Üniversitesi Sağlık Bilimleri Enstitüsü, Moleküler Tıp ABD., Doktora Tezi. İstanbul.

Bu çalışmanın ana amacı, ileri evre prostat kanseri için çok etkili bir tedavi olma potansiyeline sahip Lu-177 PSMA ile ERCC2 gen varyasyonları, miRNA-182 ve miRNA-187 ekspresyon seviyeleri arasındaki bağlantının araştırılmasıdır. Bu çalışmada 57 hastanın 50'sinde tedaviyi alıp almama durumu ve 7'sinde ise tedavi öncesindeki ve sonrasındaki değişimler incelenmiştir. Lu-177 PSMA dâhil olmak üzere tedavi alan hastaların, herhangi bir tedavi almayan hasta grubuna göre miRNA-182 ($p<0.001$) ve miRNA-187 ekspresyon seviyeleri anlamlı olarak yüksek bulunmuştur ($p=0.000$). miRNA-182'nin tedavi sonrası ekspresyon seviyesi ile tedavi sonrası DNA konsantrasyonundaki değişim arasında anlamlı bir ilişki bulunmuştur ($p=0.028$). Lu-177 PSMA tedavisi ile ERCC2 gen varyantları arasındaki ilişki çalışma gruplarında incelenmiştir. rs13181 varyantının, tedaviyi alan ve almayan hastalarda heterozigot mutant ve wild tip genotipte olduğu; rs1799793 varyantı için ise, tedaviyi alan hastaların wild-tip ve homozigot mutant genotipte, tedaviyi almayan hastaların ise homozigot mutant ve heterozigot mutant genotipte olduğu tespit edilmiştir. Tedavi öncesi-sonrası sonuçlarına göre; tedavi sonrasında PSA yanıtı olan hastaların ERCC2 rs13181 varyantı için %50'sinin heterozigot mutant; PSA yanıtı olmayan hastaların ise %66.66'sının wild-tip genotipte; ERCC2 rs1799793 için ise PSA yanıtı olanların %100'ünün heterozigot mutant, PSA yanıtı olmayanların ise %50'sinin wild-tip ve %50'sinin homozigot mutant genotipte oldukları bulunmuştur. Ayrıca, tedavi sonrası miRNA-182 seviyeleri yüksek olan hastaların ERCC2 rs13181 heterozigot mutant genotipe sahip olma arasında ilişki saptanmıştır ($p=0.028$). Sonuç olarak, miRNA ekspresyonlarının bimodal bir özellik gösterdiği ve tedavinin uygulanan dozuna, hastalığın derecesine ve seyrine göre değişebildiği belirlenmiştir. miRNA-182 ekspresyonunun takibi ve ERCC2 rs13181 ile rs1799793 varyantlarının analizi, kişiye özel Lu-177 PSMA tedavi stratejilerinin doğru olarak planlanmasına yardımcı olabilir.

Anahtar Kelimeler: Lu 177 PSMA, Prostate Kanseri, miRNA-182, miRNA-187, ERCC2

1. STATEMENT AND PURPOSE

The prostate gland, a walnut-sized organ in men that generates fluid for semen, is where prostate cancer develops when cells proliferate malignantly (1). It is a global health issue, with an estimated 1.41 million new cases diagnosed worldwide in 2020 (2). The highest rates of prostate cancer are found in North America, Europe, and Australia, while the lowest rates are found in Africa and Asia (2).

Surgery, radiation therapy, hormone therapy, and chemotherapy are all common treatments for prostate cancer. The specific treatment that is best for each individual depends on the stage of the cancer, the patient's age and overall health, and other factors (3).

A new therapeutic option for prostate cancer is Lutetium-177 (Lu-177) Prostate-specific membrane antigen (PSMA). The PSMA protein is a target for Lu-177, a radioactive isotope. Several prostate cancer cells have PSMA on their surfaces. When LU-177 PSMA is injected into the body, it travels to the prostate cancer cells and delivers radiation to them; this can kill the cancer cells (4). Lu-177 PSMA is still under investigation, but it has the potential to be a very effective treatment for advanced forms of the disease.

Individuals or tumors with different genetic profiles may be more or less sensitive to radiotherapy (5).

Cells are constantly exposed to Deoxyribonucleic acid (DNA)-damaging agents, both from the environment and from within the body. Normal cells have a DNA damage response network that repairs these damages. However, cancer cells have accumulated genetic alterations that disrupt this network, leading to genomic instability. Cancer is characterized by genomic instability and can lead to a variety of problems, including uncontrolled cell growth, metastasis, and resistance to therapy (6).

Variations in DNA repair genes have also been linked to a higher risk of cancer development. They may also influence how well cancer treatments work and how well DNA damage is repaired (7). Patients with prostate cancer who have variations in DNA repair genes may be more sensitive to radiotherapy and may be less likely to respond to treatment as a consequence of overexpression of these genes (7, 8).

Over time, humans have evolved advanced DNA repair mechanisms to protect their genomes from damage caused by carcinogens as mentioned above. These mechanisms are essential for maintaining genetic stability and preventing cancer (9).

There are several ways that cells repair DNA damage. Base excision repair removes small changes to DNA, such as single-strand breaks, oxidative damage, and some types of chemical damage. Nucleotide excision repair (NER) removes larger changes to DNA, which can be caused by environmental factors such as smoking, UV radiation, or exposure to carcinogens (7).

NER is a critical DNA repair pathway. It involves a number of proteins that work together to recognize and repair damaged DNA (10, 11). NER has been implicated in the development of tumors and their response to treatment (12).

The ERCC gene family is involved in DNA repair. Mutations in ERCC genes can lead to defects in DNA repair, which can increase the risk of cancer (9).

In the context of prostate cancer risk elevation, numerous studies have shown a connection between ERCC gene polymorphisms (9, 13, 14). These results suggest that ERCC genes may be used to create new treatment strategies that target specific mutations in this type of cancer cells. To find DNA repair genes that may affect how the body reacts to radiation therapy and to create novel therapies that specifically target these genes, further research is required.

Like genetic variations, microRNAs (miRNAs), which are gene expression regulators, are known to regulate the response to radiotherapy treatments of cancer (15).

miRNAs are small molecules that control the activity of genes. They can also contribute to the development of cancer. Examining miRNAs, whose expression is reported to change in prostate cancer, is important for molecular investigation and clarification of prostate cancer, as well as understanding how treatment response alterations occur (16). To understand the molecular causes of prostate cancer and develop new therapies, it is essential to study the dysregulation of miRNAs in this disease.

Numerous cancers, including prostate cancer, have been related to miRNAs such as miRNA-182 and miRNA-187. These two have been shown to be altered in prostate cancer cells (17). They can be used as biomarkers to diagnose and monitor prostate cancer. According to studies, those with prostate cancer had significantly higher levels of miRNA-182 compared to those without the disease. miRNA-187 levels, on the other hand, are either reduced or unchanged in men with prostate cancer (17-19).

Moreover, oncogenic miRNA-182-5p makes cells more vulnerable to radiation by stopping the production of protective substances that are typically created in response to radiation (20).

These findings suggest that DNA repair genes and miRNAs may be potential targets for the effective and personalized application of prostate cancer therapies such as Lu-177 PSMA. Our study was designed to contribute to the clarification of the molecular basis of prostate cancer treatment with Lu-177 PSMA.

Our study was designed to contribute to the clarification of the molecular basis of prostate cancer treatment with Lu-177 PSMA.

The aim of our study was to investigate the effect of miRNA-182 and miRNA-187 expression levels and ERCC2 gene variants on the treatment response to Lu-177 PSMA therapy in patients with advanced prostate cancer, and to identify the possible relationship between them.

2. LITERATURE REVIEW

2.1. Prostate Cancer Overview from Nuclear Medicine Perspective

The second-leading cause of cancer-related mortality in men is prostate cancer, which is the most prevalent cancer among males (2, 21). Prostate cancer can be either contained within the prostate gland (local) or spread to include more bodily regions (advanced). Advanced type of this cancer can metastasize to other organs and bones (22, 23). New imaging methods and treatment options have improved survival rates for prostate cancer, especially in early-stage cases. However, the tumor's molecular complexity and heterogeneity continue to make it difficult to diagnose, treat, and predict treatment outcomes (22).

Advances in the identification and characterization of molecular markers that underlie the different stages of prostate cancer tumorigenesis have contributed to the understanding of the genomic landscape of these tumors, which can be translated into clinical practice (24).

Nuclear medicine techniques can be used to identify prostate cancer, determine the extent of the disease, and guide treatment (25).

In addition to nuclear medicine methods, the following imaging techniques are often used to identify prostate cancer:

- Transrectal ultrasound (TRUS): This is a common way to image the prostate gland. It can be used to measure the size of the prostate, show the zonal anatomy, and identify cancer. Cancerous areas usually appear darker than normal tissue on TRUS images (26).
- Magnetic resonance imaging (MRI): This technique can provide a more detailed view of the prostate gland than TRUS, making it a more accurate tool for assessing the extent of the disease. The potential of a cancer recurrence may also be determined using this method (27).
- Computed tomography (CT): This is also a common way to diagnose and monitor many types of cancer, but it is not very effective for imaging prostate cancer because it has low soft tissue contrast resolution, which prevents clear visualization of the prostate's internal and external anatomy (26).

- Bone scan: Nuclear medicine tests like a bone scan can be used to detect bone metastases, which could signify a spread of cancer from the prostate to the bones (28).

A number of criteria, including an elevated PSA level, a digital rectal exam (DRE), and a biopsy, are used to make the diagnosis of the disease (29).

Prostate cancer is classified into stages to assess its extent and severity. Staging helps to determine the best course of treatment. The TNM system is one of the standards for staging. The TNM system considers the size of the tumor (T), the extent of spread to lymph nodes (N), and the presence or lack thereof metastases (M) (30).

The stage of a patient's prostate cancer, their age, general health, and personal preferences all affect the type of prostate cancer therapy they receive (31).

The most common treatments for prostate cancer are active surveillance, surgery, radiotherapy, and hormone therapy (32).

Radiation therapy is a therapeutic option for males with localized prostate cancer. Surgery may be combined with radiation treatment or performed alone (33). Systemic treatment has come a long way, but there is still potential for further advancements to be made.

2.2. PSMA-Targeted Radioligand Therapy

Prostate cancer is susceptible to radiotherapy, which means that it can be treated with high-energy rays that kill cancer cells. For localized prostate cancer, external beam radiotherapy and brachytherapy are standard treatments. Stereotactic body radiotherapy is currently being investigated for patients with oligometastatic type of the disease. Radium-223, a calcium-mimicking alpha-emitting radionuclide, has been shown to be effective in treating metastatic prostate cancer that has spread to the bone (34).

For the treatment of advanced prostate cancer, a number of PSMA-targeted radiopharmaceuticals have been created that enable radiation delivery to both bone and soft tissue tumor locations. Clinical trials are underway to evaluate the efficacy of these radiopharmaceuticals in treating advanced prostate cancer (35).

2.2.1. Lutetium-177 (Lu-177) PSMA Radioligand Therapy

Lu-177 is a radioactive isotope that emits beta radiation with a mean energy of 133.6 keV, a maximum penetration depth of 2 mm, and a half-life of 6.7 days (36) (Table 1). Due to its appealing physicochemical characteristics, it has become the preferred radionuclide for therapeutic purposes. The ideal therapeutic radionuclide for a particular lesion will have emission characteristics that are matched to the size of the lesion in order to maximize the therapeutic effect and minimize the risk of side effects (36).

Table 1: Radionuclides used in targeted therapy (36).

Radionuclide	Physical $T_{1/2}$ (days)	Radiation type (MeV)	Particle range (mm)
^{131}I	8	β (0.6), γ (0.364)	2
^{90}Y	2.67	β (2.28)	12
^{67}Cu	2.58	β (0.54), γ (0.185)	1.8
^{186}Re	3.77	β (1.08), γ (0.131)	5
^{177}Lu	6.7	β (0.497), γ (0.208)	1.5

Iodine-131 (^{131}I , I-131), Yttrium-90 (^{90}Y), Copper-67 (^{67}Cu), Rhenium-186 (^{186}Re), Lutetium-177 (^{177}Lu)

PSMA is a glycoprotein with 750 amino acids in its molecular architecture. It is involved in a variety of biological processes, from nutrient absorption (especially folate), to cellular activities (growth, survival, migration), but its exact mechanism of action is not fully understood (37).

Specifically in prostate epithelial cells, the cell-surface protein PSMA is expressed. However, it is also expressed in other tissues, such as the brain, liver, and kidneys (38). PSMA is a promising target for cancer treatment because it is expressed more often in prostate cancer cells than in normal cells.

Numerous cellular activities, including the following, have been demonstrated to include PSMA (39, 40):

- Nutrient uptake: PSMA is an enzyme that is also known as glutamate carboxypeptidase II. Folate, which is necessary for cell development, is broken down by this enzyme.
- Cell migration matrix.
- Cell survival
- Cell proliferation

The role of PSMA in prostate cancer is still being investigated. However, there is evidence showing that it may play a role in promoting cancer cell growth and spread. PSMA may accomplish this by releasing glutamate, a neurotransmitter that acts as a messenger molecule (41).

PSMA is not a perfect target for prostate cancer, but it is a useful one because it is a relatively specific target and has low toxicity towards healthy tissues (42).

PSMA-targeted therapies are designed to bind to PSMA and deliver a toxic payload to cancer cells. Prostate cancer and other cancers are now being treated with targeted therapies that specifically target the PSMA protein (43).

The radioactive isotope Lu-177 is used in Lu-177 PSMA, a radionuclide treatment, to treat prostate cancer (44). Lu-177, a radioactive isotope, can bind to PSMA, a protein that is found on the surface of prostate cancer cells (Figure 1).

When Lu-177 PSMA is injected into the body, it binds to PSMA on the cancer cells and delivers a high dose of radiation, thus killing the cells.

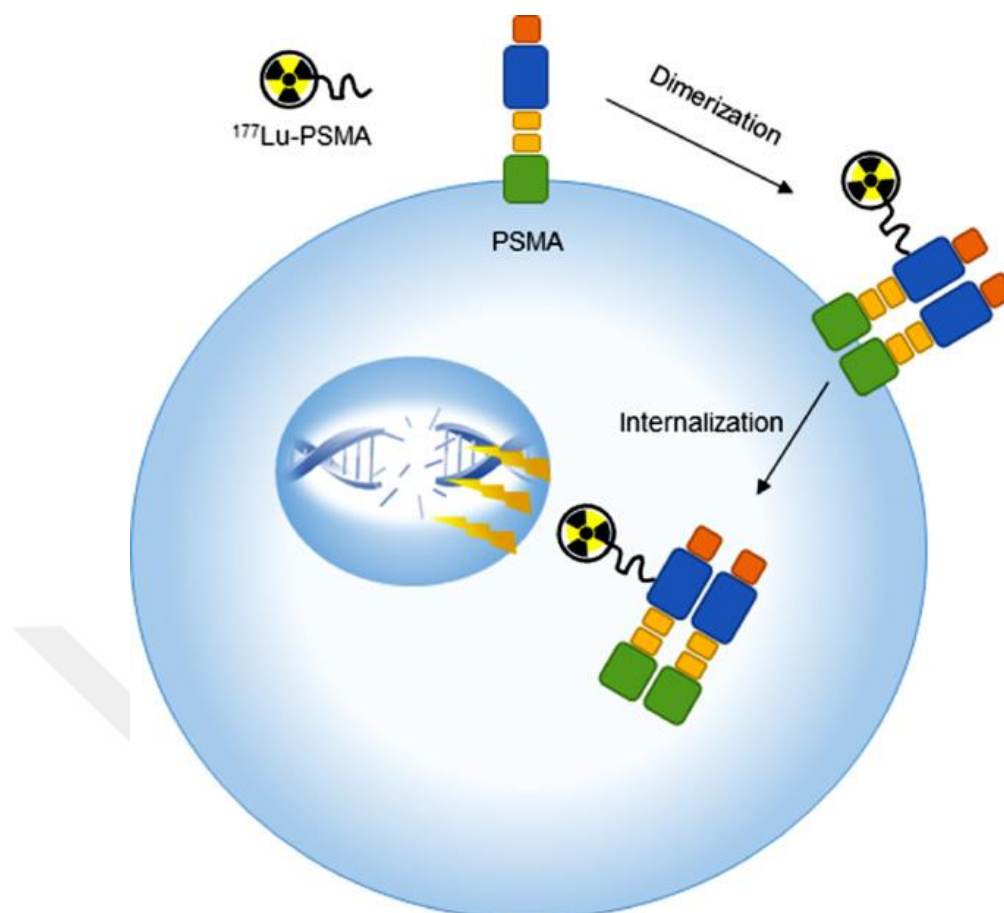


Figure 1: The way that ^{177}Lu -PSMA radioligand treatment works (44).

Lu-177 PSMA therapy works by using low-molecular weight ligands, PSMA-617 and PSMA-I&T, to bind to PSMA receptors on the surface of prostate cancer cells. This binding triggers the receptors to pair up, or dimerize, which causes the receptor-ligand complex to be internalized into the cell. Once inside the cell, the radionuclide ^{177}Lu is concentrated and emits ionizing radiation, which damages the cell's DNA. This damage can cause the cell to die or stop dividing. The ionizing radiation can also damage DNA in surrounding cells, within a radius of about 2 millimeters.

Lu-177 PSMA is a relatively novel therapy for prostate cancer, as it has only just begun to be properly investigated, yet it has showed promise in clinical studies. It is most effective in treating patients with late-stage prostate cancer, or prostate cancer that has progressed to the point where it has spread to other parts of the body (36, 45). Clinical trials have shown that Lu-177 PSMA can be an effective treatment for the locally advanced type, even in patients who have not responded to other treatments, such as surgery or radiation therapy (36). For males with metastatic castrate-resistant prostate cancer (mCRPC), numerous PSMA peptides and antibodies labeled with ^{177}Lu have been used in clinical investigations and as therapy alternatives (36).

¹⁷⁷Lu-PSMA-I&T and ¹⁷⁷Lu-PSMA-617 are two of the most promising ¹⁷⁷Lu-labeled PSMA radioligands that have been studied. ¹⁷⁷Lu-PSMA-617 is preferred because it has reduced kidney uptake, which can lead to fewer side effects.

Since 2015, a growing number of studies have shown that ¹⁷⁷Lu-PSMA radioligand treatment is both safe and effective (46).

Lu-177 PSMA is typically administered as an outpatient procedure. The patient is given a small amount of the therapy intravenously. The radioactive tracer then circulates throughout the body and specifically targets PSMA.

The isotope in the Lu-177 PSMA decays, emitting radiation that kills the cancer cells (36).

The standard activity of ¹⁷⁷Lu PSMA has not been established, as different research groups have used different methods to evaluate the medication's safety and toxicity (47-52). Men with prostate cancer have received up to six doses of radioactive material, ranging from 3 to 8 GBq each. The radioactive doses were given at intervals of at least six weeks, and the amount of material given to each patient was tailored to the individual's needs, considering the extent of the cancer, their weight, and their kidney function (36).

Lu-177 PSMA is a targeted radioligand therapy that has shown promise in the treatment of mCRPC. It has been shown to be effective in reducing PSA levels in 30% to 70% of men, which is similar to the efficacy of chemotherapy agents used in mCRPC (53). However, 10% to 32% of men have been shown to resist therapy and their disease progresses. A majority of men (80%) who participated in a recent study responded favorably to PSA therapy (47).

The safety profile of this treatment is favorable, and it is effective in treating advanced prostate cancer patients. Lu-177 PSMA is generally well-tolerated, but it can cause some side effects, such as haematological toxicity (54). Long-term effectiveness and safety of this relatively new treatment still need to be determined through further research (36).

Treatment with ¹⁷⁷Lu PSMA is a promising new option for mCRPC, but it is not a cure-all. While it can be effective in reducing PSA levels in many men, up to a third of patients treated with ¹⁷⁷Lu PSMA experience disease progression. This is likely due to a number of factors, including the heterogeneity of PSMA receptor activity within the tumor population (47).

^{177}Lu PSMA therapy is a new approach to treating mCRPC that has shown promise in early studies. However, more research is needed to determine its long-term efficacy and safety (36).

2.3. DNA Repair in Radiation-Induced Damage

Radiation can damage DNA by creating free radicals, which can cause single-stranded or double-stranded breaks (55). This harmful effects of ionizing radiation that is used for radiotherapy is DNA damage of exposed tumor tissue, which results in cell death (55). The effectiveness of radiation treatment and the toxicity to healthy tissue can be affected by internal and external factors, such as mutations in DNA damage recognition and repair. DNA repair disorders can increase sensitivity to cancer treatments (56). To maintain genetic stability and integrity, DNA repair pathways are activated when mammalian cells are exposed to DNA-damaging agents. Cells that are unable to repair DNA damage are more likely to become cancerous. The DNA damage response is often dysregulated in cancer cells, and this can lead to either hypersensitivity or resistance to genotoxic agents. Targeting the repair pathways can increase tumor sensitivity to cancer therapies, making it a potential therapeutic approach for cancer treatment (57).

2.3.1. DNA Repair Pathways and Genes

DNA repair genes are a group of genes that are responsible for repairing damaged DNA. DNA damage can occur from a variety of sources, including environmental factors such as ultraviolet radiation and chemicals, as well as from normal cellular processes such as replication. If DNA damage is not repaired, it can lead to mutations, which can cause cancer and other diseases (58).

Cells have a variety of tools to mend DNA damage. A variety of DNA repair pathways are activated to repair DNA damage caused by radiation. These pathways are important for repairing DNA damage in normal tissue that is adjacent to a tumor. The risk of developing severe toxicity after exposure to radiation may be increased by SNPs in these genes (59).

Base lesions that are caused by deamination, oxidation, or alkylation are removed by base excision repair. These lesions are small and do not warp the DNA helix. Mismatch repair and nucleotide excision repair (NER) are two DNA repair processes that protect

the genome's integrity. In contrast to Mismatch repair, which fixes insertion, deletion, and misincorporation mistakes in DNA replication, NER removes larger, helix-warping nucleotide lesions. Homologous recombination and non-homologous end joining are two mechanisms involved in double-strand break repair (60-62) (Figure 2).

The effectiveness of radiotherapy in cancer patients is limited by the damage it causes to normal tissues. DNA repair and apoptosis are two important processes that play a role in these adverse effects.

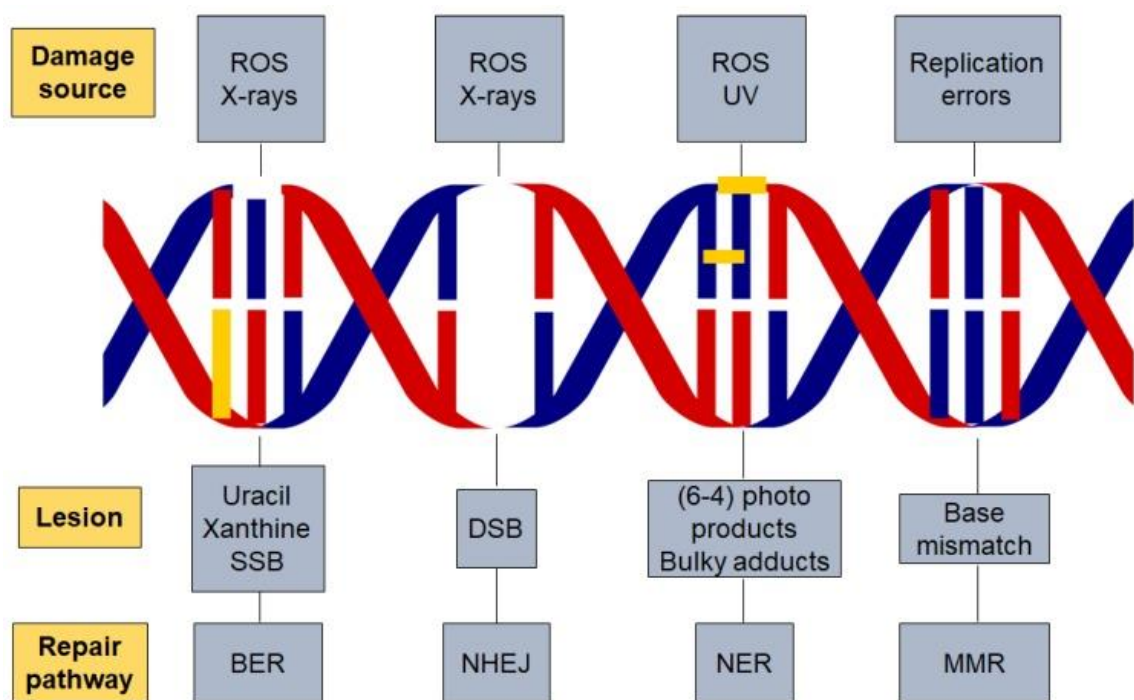


Figure 2: Major DNA repair pathways (63).

DNA damage can be caused by a variety of factors, including radiation and reactive oxygen species (ROS). These factors can cause several types of lesions in the DNA double helix, which can lead to mutations and cancer. The repair pathway involved in the process is dependent on the damaging agent and lesion generated. Base excision repair (BER), nucleotide excision repair (NER), non-homologous end joining (NHEJ), reactive oxygen species (ROS) and DNA mismatch repair (MMR).

DNA repair genes are important for preventing prostate cancer, but mutations in these genes can increase the risk of developing the disease and make it more likely to be an aggressive form (64).

The specific types of DNA damage that are repaired by DNA repair genes are many and varied. The fate of prostate cancer biology is significantly impacted by some of the most important DNA repair genes, which include:

- **BRCA1 and BRCA2:** Homologous recombination is a type of DNA repair that is crucial for patching up double-strand breaks in DNA. The BRCA1 and BRCA2 genes mediate this process. Prostate cancer is more likely to affect men who have BRCA1 or BRCA2 gene mutations (65).
- **ATM:** ATM is a gene that is involved in detecting and repairing DNA damage. Prostate cancer risk is enhanced by ATM mutations, and these mutations can also increase the aggressiveness of prostate cancer (66).
- **CHEK2:** CHEK2 is a gene that is involved in the checkpoint pathway, which is a pathway that helps cells to stop dividing if they have DNA damage. Prostate cancer risk is elevated in the presence of CHEK2 mutations (67).

The ERCC protein family is essential for repairing DNA damage brought on by bulky molecules, such as those created by UV light. This process is carried out by the NER pathway, as mentioned above (68).

2.3.1.1. NER Pathway and ERCC Genes

Over time, humans have evolved complex DNA repair mechanisms to protect against cancer-causing agents. These mechanisms are made up of many different pathways, and the ability to maintain DNA stability is essential for preventing cancer, controlling its growth, and responding to treatment. (69).

Damaged DNA is removed by NER, which is one important DNA repair mechanism. NER is carried out by a group of proteins that work together to recognize and repair damaged DNA (70).

Cells have a sophisticated system for repairing DNA damage, called NER. NER is especially effective at removing bulky DNA lesions, which are caused by UV light, radiation, and other mutagens. In eukaryotic cells, NER removes damaged DNA segments up to 32 nucleotides long.

Using the original strand as a model, the repair process creates a new segment of DNA after ligase repairs the break. NER is a complicated mechanism in which roughly 30 proteins work in unison. (71). NER is a sophisticated DNA repair system that employs two distinct pathways to detect and eliminate bulky DNA lesions, either globally or in response to transcription. Cooperating to protect cells from substantial DNA damage, TC-NER and GG-NER are two DNA repair pathways. GG-NER detects and removes damage to both silent chromatin and untranscribed portions of the genome. TC-NER is triggered when damage to a transcribed DNA strand prevents transcription. The XPC protein factor regulates GG-NER, while RNA polymerase II inhibition at the damaged location activates TC-NER (72- 74) (Figure 3). Xeroderma pigmentosum, the Cockayne syndrome, and trichothiodystrophy are all caused by abnormalities in NER activity, which can lead to a variety of neurodegenerative symptoms (71).

The nucleotide excision repair (NER) process is controlled by a complex interplay of protein-DNA and protein-protein interactions. In a eukaryotic cell, NER is performed by a complex called the reparasome, which is a dynamic assembly of proteins that can vary in composition depending on the type of DNA damage. The individual subunits of the reparasome are not able to recognize and remove damaged DNA on their own. However, when they assemble at the site of damage, they form a stable structure that has the necessary affinity and selectivity for the DNA substrate. This allows the reparasome to recognize and remove the damaged DNA. The structure of the reparasome also allows for specific protein-protein interactions that are essential for the repair process. Changes in these interactions can fine-tune the repair machinery and ensure that high-precision nucleotide excision repair is carried out. (71).

Here is a more detailed description of the NER DNA pathway (75) (Figure 3):

1. Damage recognition: The first step in NER is damage recognition. This is accomplished by a protein called XPC, which binds to damaged DNA.
2. Excision: Once damage has been recognized, a group of proteins called the XPA complex unwinds the DNA around the damaged site. Another protein, called TFIIH, then recruits a group of enzymes that cleave the DNA on either side of the damaged site.
3. Recombination: The final step in NER is recombination. This is a process by which the DNA is repaired by using a homologous template. A homologous template is a piece of DNA that is identical to the damaged DNA, except for the damaged site.

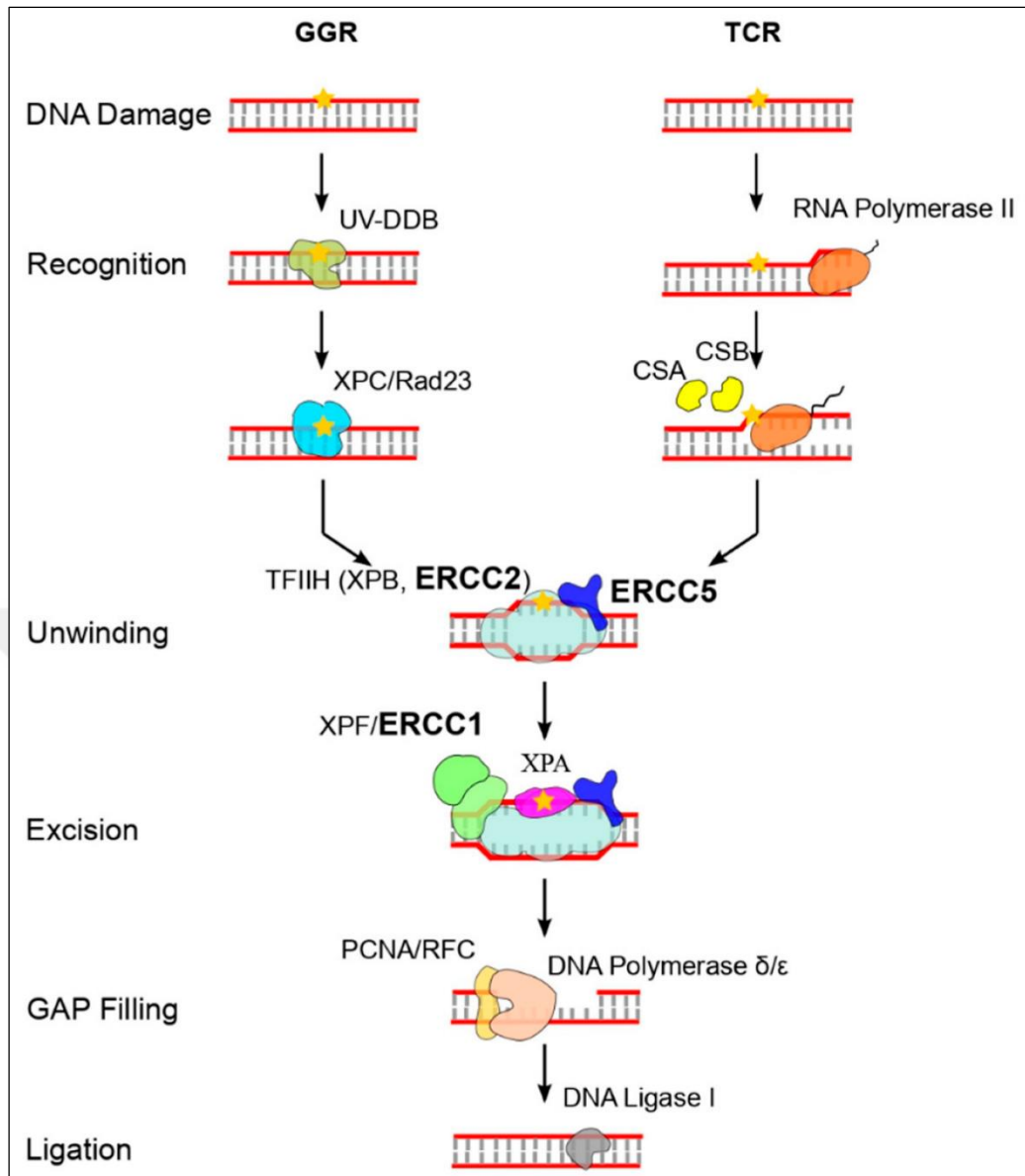


Figure 3: Nucleotide excision repair pathway (NER) (12).

GGR, global genomic NER; TCR, transcription coupled NER.

As of right now, the NER pathway requires the participation of at least eight crucial genes (XP A–G and ERCC1) to operate.

NER is a critical DNA repair pathway, and defects in the ERCC genes can compromise this pathway, leading to cancer. For example, the ERCC1 gene is a key enzyme in NER, and the ERCC5 gene encodes a protein that helps to repair damaged DNA. The ERCC2 gene is involved in DNA unwinding and identifying damaged DNA.

Variants in the ERCC genes can lead to defects in DNA repair, genomic instability, and the failure to regulate genetic transcription. These defects can increase the risk of cancer (9).

NER is a nucleotide excision repair process that uses ERCC1, a DNA repair endonuclease, to catalyze the 5' incision (76). ERCC1 is a DNA repair enzyme that catalyzes the first step of the NER process, which is the removal of a damaged section of DNA. ERCC1 is expressed in a variety of tissues, including skin, breast, colon, testis, and ovary (77). Head and neck squamous cell carcinoma, non-small cell lung cancer, and other cancers have all been shown to have overexpression of ERCC1. ERCC1 overexpression has been suggested to be a prognostic and/or predictive tumor marker for some cancers (78).

Prostate cancer research has been focusing on ERCC1. Experimental findings in an animal model indicated that an altered ERCC1 function may be a contributing factor to the invasive prostate cancer phenotype (79). Additionally, a particular nucleotide variant of the ERCC1 gene was associated with the aggressiveness of prostate cancer (80).

A protein called XPD, also known as ERCC2, aids in repairing DNA damage caused by ultraviolet light (81). A helicase protein with 761 amino acids is encoded by the gene on chromosome 19q13.3 (82). The XPD protein is involved in the NER process, which is facilitated by the general transcription factor IIH complex. The complex activates the DNA double helix when XPC-RAD23B detects damage (83). Single nucleotide polymorphisms (SNPs) in the XPD gene can affect how well DNA can be repaired. These SNPs may be used to predict how well a treatment would work.

Xeroderma pigmentosum is a rare genetic disorder that causes excessive photosensitivity and a high risk of skin cancer. It can be caused by mutations in the ERCC2 gene (81).

Due to their potential prognostic usefulness in the progression and therapy of prostate cancer, substantial research has been conducted on SNPs affecting codons 156, 312, and 751 (rs238406, rs1799793, and rs13181, respectively) (9).

ERCC5, or the XPG gene, is one of the eight critical genes that perform NER pathway functions. This gene produces a protein that cleaves the 3' end of the damaged nucleotide during NER. It is found on chromosome 13q32-33. This protein is a structure-specific endonuclease (84). Studies have shown that ovarian cancer patients with low levels of XPG expression may be less responsive to treatment (85, 86). Two SNPs, rs1047768 and rs17655, are among those reported to be significantly associated with the outcome of cancer treatment (9, 87).

2.4. miRNAs as Biomarkers for Cancer Progression and Response to Radiation Therapy

The expression of genes is controlled by miRNAs, which are generally 22 nucleotides long. These small, non-coding RNA molecules are predicted to interact with at least 30% of the human genome's protein-coding genes. In 1993, the first miRNA was discovered, which according to recent literature can function as tumor suppressors and oncogenes (88-90).

It has been shown that they hold crucial roles in the control of a number of cellular and metabolic processes, as well as the expression of genes. In the majority of organisms with multiple cells, they are also an essential component of gene silencing by targeting particular genes and preventing their expression (91, 92).

Recent evidence points to the biological involvement of miRNAs in many various cancer types. Researchers have suggested that enhancing certain cancer therapeutic methods may reactivate or inhibit miRNA activity (93).

Oncogenes, tumor suppressor genes, or both, may make up the regulatory components that have been demonstrated to have a direct impact on the cell cycle, differentiation, and even death. They have influence over the elements in the environment that affect how cancer develops.

Also, they have shown to be effective biomarkers for determining radiation response and creating patient-specific treatments. Finally, miRNAs may provide patients with prostate cancer with a fresh therapeutic avenue for individualized treatment (94-96).

These RNA molecules can aid in determining treatment strategies and therapeutic sensitivity, reduce the risk of various adverse drug responses and toxicity, and even assist with treatment monitoring (Figure 4). As increasing evidence demonstrates miRNAs' substantial association with the disease, they are becoming a crucial target for therapy in the fight against cancer (97).

Dysregulated miRNA expression is a hallmark of prostate cancer, and miRNAs may also influence the production of oncogenes and tumor suppressor genes. miRNA modification therapy, which may be used to assess therapeutic response and predict the effectiveness of therapies like chemotherapy and radiation, may be helpful for cancer patients. But there is still a big problem with resistance to therapy (97-100).

Examples of miRNA treatment strategies include oligonucleotides, synthetic small molecules, and viral or non-viral transfection. It is possible to interfere with the activity of miRNAs, indirectly disrupt other transcription factors, or target genes that are involved in the regulation of miRNAs by using these treatment strategies (101, 102).

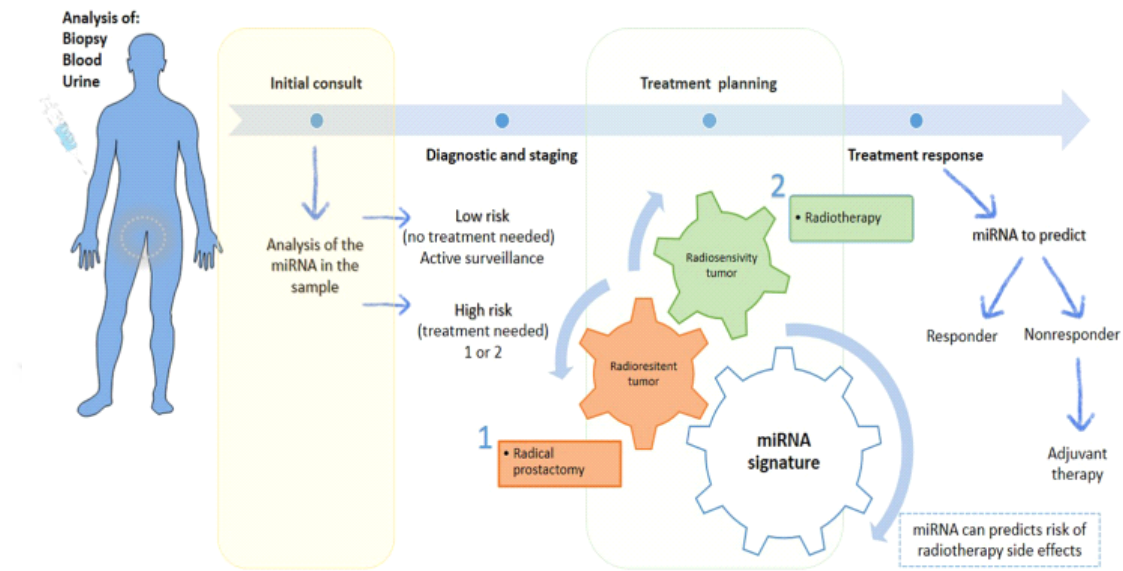


Figure 4: Potential scheme of miRNAs in prostate cancer (15).

Small molecules known as miRNAs are present in biological samples. In the initial consultation, they may be utilized to identify and stage cancer, which enables precise risk assessment. Depending on the data gathered and whether the tumour is radiosensitive or radioresistant, the treatment strategy can be tailored. Radical prostatectomy is the most suitable course of treatment if the malignancy is radioresistant. In all other cases, radiation therapy is the best option for treating radiosensitive malignancies. Additionally, miRNA fingerprints can tell us if a patient is responding to therapy or at danger of experiencing adverse effects. This can result in less side effects and better tumor management, which improves the patient's quality of life.

Personalized medicine is being used to influence how cells react to ionizing radiation. This can make cells more radiosensitive or radioresistant through a number of mechanisms, including modifying the tumor microenvironment, DNA repair mechanisms, and apoptosis. Some miRNAs regulate these processes, which could be used to develop personalized cancer treatments (103).

There is a lot of interest in miRNAs because of concerns about the microenvironment of tumors. The epithelial-mesenchymal transition, the tumor radiation response, and the DNA repair process are all under their control (104).

The microenvironment of a tumor, as well as the type and dose of radiation, the stage and grade of the tumor, and the presence or lack thereof other health conditions, all influence how cancer responds to radiation. Radioresistance is a challenging topic that is being studied actively (105, 106).

Gene expression can now be mimicked or decreased by miRNAs in therapeutic procedures (107). The first technique for increasing miRNA expression is to provide more miRNA. The second technique uses synthetic anti-miRNAs to inhibit miRNA expression when miRNAs are overexpressed.

miRNA use is generally tolerated and is not associated with any adverse effects that are significant, according to preliminary research. However, it is critical to establish the security and effectiveness of miRNA therapy for the treatment of cancer in order to provide patients with the best care possible (108).

2.4.1. miRNA-182 and miRNA-187 in prostate cancer

miRNAs are small but mighty molecules that can have a big impact on gene expression, acting as either brakes or accelerators, depending on their target. A wide range of biological processes, from cell proliferation to cell death, are involved with miRNAs, which are emerging as promising therapeutic targets for a variety of diseases. The study of miRNAs is a rapidly growing field, with new discoveries being made all the time, making them a fascinating area of research (109).

Prostate cancer diagnosis is a growing field of research, with miRNAs emerging as a promising new biomarker (110).

miRNAs could be used to diagnose, predict, or monitor prostate cancer. They can be used to detect the disease early, track its progression, and assess the effectiveness of treatment (110). The effects of these genes on tumor growth depend on their target. They can either promote or suppress tumor growth, which can contribute to the progression of cancer, including prostate cancer (110). miRNAs, once thought to be mere bystanders in the body, have emerged as promising biomarkers for prostate cancer diagnosis and staging. However, the clinical utility of miRNAs is limited by the lack of research on their role in prostate cancer (111).

Measuring miRNAs 182 and 187 expression levels has been suggested as a way to predict the clinicopathological staging and development of prostate cancer.

miRNA-182 is overexpressed in prostate cancer cells, while miRNA-187 is underexpressed (112-114). One key area of focus in medical research is miRNA-182 and the miRNA cluster 7q31–34 includes this miRNA (115) (Figure 5). The ability to control cell differentiation, proliferation, and the expression of several target genes is one of its most notable features (116).

Numerous solid malignancies, from breast to prostate cancer, have been shown to express miRNA-182 abnormally, according to various studies. These findings imply that it could function in these cancerous tumors in a manner similar to that of an oncogene (117).

Prostate cancer tissues have been found to overexpress miRNA-182 (114), but extensive functional investigation of it has not yet been accomplished (118). Several miRNAs, including miRNA-182, have been found to be encoded in regions of genes associated with cancer. This suggests that changes in miRNA expression may be causally linked to carcinogenesis, aggressive disease, and therapy resistance (119).

In prostate cancer tissues, overexpression of a particular miRNA, miRNA-182-5p, has been linked to tumor development and a decline in survival rates. In prostate cancer cell types, miRNA-182-5p dysregulation has been shown to have a strong impact on the proliferation, formation of colonies, migration, and invasion of cancer cells (120).

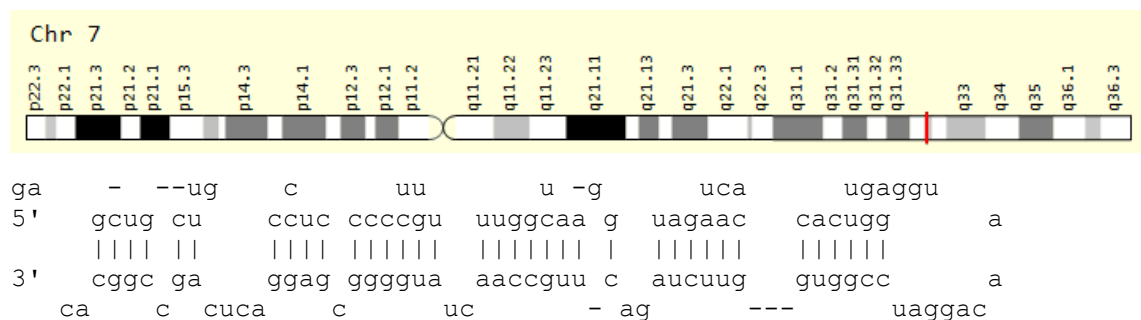
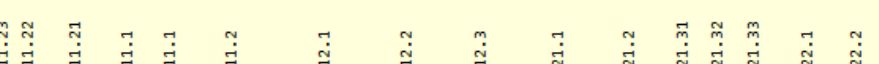


Figure 5: miRNA-182 Gene in genomic location (121).

The miRNA-182/96/183 cluster was found to be significantly upregulated by radiotherapy, with miRNA-182 being the most affected gene, according to a study by the Cancer Genome Atlas (TCGA) (20).

Chr 18



g u caccaugacacag aga g a c - c - ug

5' g cgggcu ugug ccuc ggcu caacacaggac cg gg g c c

 | | | | | | | | | | | | | | | | | | | | | | | | |

3' c gccugg acgc ggag ccga guuguguucug gc cc c g u

 a - ----- -ag g c u u c a uc

Chemo and radiation resistance in tumor cells is still a significant obstacle for modern medical treatment (124). The specific mechanism is still being researched, although many researchers are looking at the involvement of miRNAs in therapeutic insensitivity (125, 126).

NER family of proteins includes ERCC (excision repair cross-complement), and various earlier studies has shown that ERCC is linked to resistance to specific drugs that have an impact on cancer patients' chances of surviving. It is understood that miRNA-187 can promote drug resistance by improving DNA damage repair capability through upregulating the expression of ERCC family members like ERCC3 and ERCC4 in the NER pathway (19).

It has also been shown that protein levels of the ERCC family members, ERCC3 and ERCC4, as well as proteins such as TGF- β 1 and p-Smad4, are significantly reduced after miRNA-187 overexpression (127).



3. MATERIAL AND METHOD

3.1. Study Population and Aim

With approval from the Clinical Research Ethics Committee (No:1155, 30.01.2020), blood and serum samples were collected from prostate cancer patients at the Yeditepe University Hospital Nuclear Medicine Department. This thesis study was conducted using samples from 57 patients diagnosed with mCRPC. The study examined both 25 patients who received Lu-177 PSMA treatment and 25 patients who did not receive treatment, as well as the changes in 7 patients before and after receiving Lu-177 PSMA treatment. Two separate study groups of 50 patients and 7 patients were formed for this purpose and the results were evaluated according to these criteria (Figure 7).

The aim of our study was to investigate the effect of miRNA-182 and miRNA-187 expression levels and ERCC2 gene variants on the treatment response to Lu-177 PSMA therapy in patients with advanced prostate cancer.

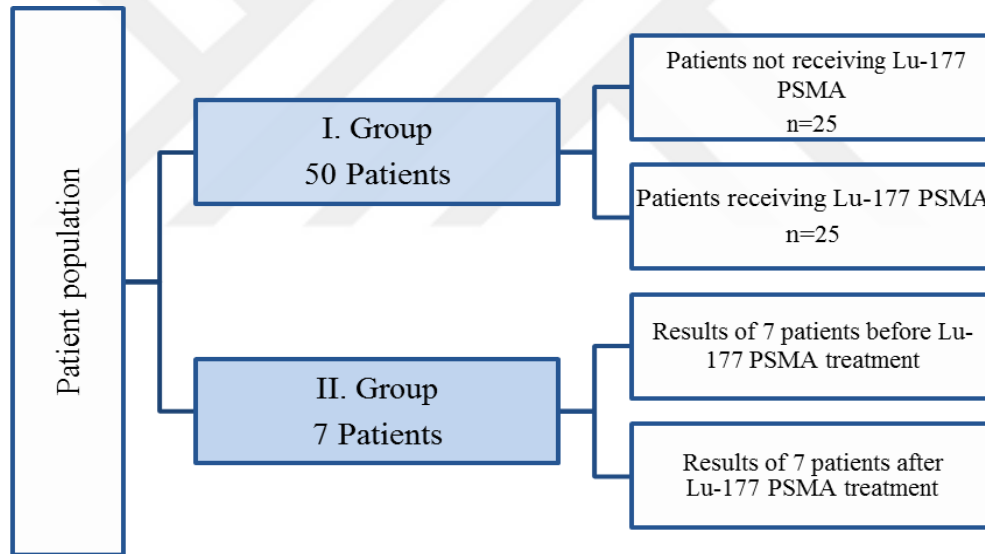


Figure 7: Study population and research design

3.2. Selection of the Samples and HPLC Technique for Radiochemical Purity

High-performance liquid chromatography (HPLC) was used to check radiochemical purity and validate treatment quality using the 1260 Infinity II Prime LC System before and after radioisotope treatment.

- Before radioisotope treatment: HPLC systems can be used to confirm the identity and purity of the radioisotope to be used in treatment. This is important to ensure that the patient is receiving the correct treatment and that there are no impurities that could cause adverse reactions.

- After radioisotope treatment: HPLC systems can also be used to monitor the patient's body for the presence of the radioisotope. This can help to determine how well the treatment is working and to identify any potential side effects.

HPLC is a popular analytical method in chemistry and biology for separating, identifying, and quantifying mixtures' constituent parts. It is based on chromatography, which separates components depending on how they interact via two phases: stationary and mobile. The main components and working principles of the HPLC system are:

- Mobile phase: A liquid solvent or solvent mixture that carries the mixture throughout the chromatographic system. The choice of mobile phase depends on the properties of the sample and the properties of the analytes of interest.
- Stationary phase: A solid or liquid phase that is immobilized on the chromatography column. The components that make up the stationary phase might include polymers, bound phases, or silica.
Depending on the needs for separation, the stationary phase is chosen.
- Chromatography column: The tube in which the stationary phase is placed. The column is a critical component where separation of components takes place. The length and diameter of the column can vary depending on the application and desired separation efficiency.
- Sample injection: The sample is introduced into the HPLC system using the injection valve or autosampler. The sample may be presented in liquid form or dissolved in a suitable solvent.
- Pump: The system's mobile phase is pumped through at a steady flow rate by a high-pressure pump. For efficient separation, the pump enables the mobile phase to move through the column at high pressure.
- Detector: HPLC detectors monitor the components passing through the column and generate a signal that can be used for qualitative and quantitative analysis. These detectors include UV-visible detectors, fluorescent detectors, refractive index detectors, and mass spectrometers.
- Data system: HPLC systems are usually equipped with a computer or data acquisition system.

The 1260 Infinity II Prime LC System is a powerful and easy-to-use HPLC instrument that can handle a wide range of applications. It can operate at pressures up to 800 bar and flow rates up to 5 mL/min, making it ideal for both analytical and quaternary UHPLC. The system also features Agilent Intelligent System Emulation Technology (ISET), which allows you to seamlessly transfer methods from other HPLC systems.

The Agilent 1260 Infinity II Prime LC System's flexible pump can be used with BlendAssist software to quickly optimize and simplify the modification of solvent composition. BlendAssist streamlines LC processes on the UHPLC system and expedites method development by automatically mixing additive or buffer amounts without operator intervention. Only the concentrated stock solution and pure solvent need to be prepared. When the stock concentration is entered, the high-performance liquid chromatography equipment provides the necessary mobile phase composition for each analysis.

3.3. Protocol for Genomic DNA Isolation from Blood

The DNA isolation process began with the collection of venous blood samples from the patient and control groups into 5cc tubes containing EDTA. The samples were stored at +4°C until the process began. The iPrep DNA extraction robot and iPrep gDNA Blood Kit were used to isolate DNA from the samples. The iPrep DNA extraction robot uses CST®, which binds to nucleic acid structures due to its negative charge. Positively charged proteins and other deposits do not bind to the CST® beads, so they can be discarded by utilizing washing steps. The DNA is then eluted from the CST® beads using an elution buffer. The entire process took 45 minutes and yielded 150 µl of DNA from each sample. The DNA samples were stored at +4°C.

3.4. Purity Determination of Genomic DNA by NanoDrop

The NanoDrop 2000 device was used to determine the purity and concentration of isolated DNA samples. The device measures the absorbance of DNA at two wavelengths, 260 and 280 nanometers. The purity of the DNA is assessed using the ratio of these absorbance values, OD₂₆₀/OD₂₈₀. A pure DNA sample is expected to have an aspect ratio of 1.7 to 1.9. The DNA content was determined by measuring the absorbance at 260 nanometers and using a standard curve. The DNA concentrations were expressed in ng/L units.

3.5. miRNA Isolation from Serum Samples

A number of chemicals and buffers were used to separate miRNA from serum. This process was performed using the miRNeasy Serum/Plasma Kit from Qiagen. The protocol involves the following steps:

1. Lysis and homogenization: The serum or plasma sample is lysed using a buffer that breaks down the cells. The sample is then homogenized using a vortex mixer to ensure that the miRNA is evenly distributed.
2. Ethanol addition: The miRNA is precipitated from the sample by adding ethanol. The precipitated miRNA is then pelleted by centrifugation.
3. Washing: The liquid portion of the solution is removed, leaving the solid pellet behind. The pellet is then washed with a buffer to remove any residual ethanol.
4. Elution: The miRNA is eluted from the pellet using a buffer. The eluted miRNA is then ready for analysis.

Blood samples were taken from volunteer prostate cancer patients (Lu-177 PSMA treated/not treated) and placed into tubes. The serum was obtained by spinning the blood at high speed for 15 minutes. The samples were stored at -80°C until needed. Before isolating the miRNA, the serum was heated to 37°C to break down the lipids and proteins. This step released the miRNA from the cells. In a sterile tube, 200 microliters (µL) of serum were mixed with 1,000 µL of Qiazol Lysis Solution. The mixture was incubated at room temperature for 5 minutes. Then, 3.5 µL of miReasy Spike in control were added. Under a fume hood, 200 µL of chloroform were added to each sample and the mixture was incubated for 3 minutes. The mixture was then vortexed for 5 minutes. To isolate the miRNA, the mixture was centrifuged at 4 °C and 12,000g for 15 minutes. This step separated the mixture into two phases: a top phase and a bottom phase. The top phase contained the miRNA. Six hundred microliters of the top phase were transferred to a clean tube. Nine hundred microliters of 100% ethanol were added and the mixture was vortexed. This step precipitated the miRNA. The precipitated miRNA was washed by passing it through a column. The supernatant was discarded and the miRNA was eluted from the column using RWT buffer. The eluted miRNA was then concentrated by passing it through a column containing RPE buffer. The concentrated miRNA was now ready for the next step.

After the miRNA was precipitated and washed, the supernatant was discarded. The columns were placed in new tubes and centrifuged at high speed for 5 minutes to

remove any remaining ethanol. Finally, 14 μ L of RNA-free water were added to the columns and the miRNA was eluted by centrifugation at high speed for 1 minute. The miRNA isolate was now ready for the next step.

3.5.1. cDNA Synthesis

By using Qiagen's miScript II Kit and miRCURY LNA RT Kit, the miRNA isolates were transformed into cDNA. The reverse transcription process converts miRNA into a single-stranded DNA copy called cDNA. This is done using an enzyme called reverse transcriptase. The components of reverse transcription were as follows: 5 μ l, RNAase free water; 2 μ l, reaction buffer; 1 μ l, reverse transcriptase enzyme; and 2 μ l, template RNA. Reverse transcription was performed with incubation at 42°C for 60 min and 95°C for 5 min. The cDNA samples were then kept at -20 °C until the date of the experiment.

3.5.2. Measurement of miRNA Purity

The NanoDrop 2000 instrument was used to assess the purity of the miRNA samples. The OD260/OD280 ratio was used as a marker of purity, with samples with a ratio greater than 2 considered pure.

3.5.3. Determination of miRNA Levels by Fluorometer

The levels of miRNAs were determined using a fluorescent method. The method involved adding 199 μ L of miRNA buffer and 1 μ L of miRNA reagent to a sample tube. The volume was then adjusted to 200 μ L. Two standard solutions were prepared for the device's calibration. Standard 1 was prepared by mixing 10 μ L of standard solution with 190 μ L of buffer. Standard 2 was prepared similarly. miRNA sample measurement was performed by combining 198 μ L of working solution with 2 μ L from each sample.

3.5.4. Detection of miRNA Expression Levels by Real-Time Polymerase Chain Reaction (RT-PCR)

The miRNAs selected for this study were identified using the MirBase / TargetScan databases. These databases were used to analyze miRNAs and their targets in relation to the molecular mechanisms of prostate cancer. miRNA-182 and miRNA-187 were chosen as the target miRNAs for this project.

A real-time PCR machine (Rotor Gene-Q, Qiagen) was used to analyze miRNA expression levels. The amount of fluorescent signal emitted by the machine was determined by the binding of miRNA primer sequences (miRNA-182 and miRNA-187, Qiagen), which were labeled with Syber Green dye (miScript SYBR Green PCR, Qiagen), to cDNA sequences and a housekeeping primer (RNU6, Qiagen). The components of RT-PCR were as follows: 5 μ L, miRCURY SYBR Green Master Mix; 2 μ L, RNase-free water; 1 μ L, miRNA-182 or miRNA-187 /housekeeping gene (RNU6); and 2 μ L, cDNA template.

The expression levels of miRNAs were determined using the delta CT (Δ CT) method. An internal control (housekeeping assay, RNU6) was used to calculate the fold change. The expression level determinations were accomplished using the delta delta CT ($\Delta\Delta$ CT) and fold change equations.

3.6. Variation Analysis by Sanger Sequencing

To validate the variant found in patients, target-specific primers have been designed and synthesized by experts in this field based on their rs numbers for Sanger sequencing.

3.6.1. Designing Primers for Sanger Sequencing

It is crucial to consider the absence of previously reported single-nucleotide variants in the chosen primers when designing primers for Sanger sequencing.

In addition, there shouldn't be any repeating sequences in these areas. The primers' binding temperatures in the areas for which they are intended shouldn't be too close to one another. While the primers were designed, care was taken to ensure that purines and pyrimidines were evenly distributed. The sequencing primers and data from NCBI Blast are shown in Table 2.

Table 2: Designed Primer Sequences

Gene	Forward primer sequence (5'-3')	Reverse primer sequence (5'-3')
ERCC2 Asp312Asn c.934G>A (rs1799793)	TCGACTCCATGAGCGTCAAC	AGGAAGCCCAGGAAATGCTC
ERCC2 Lys751Gln c.2251A>C (rs13181)	GCCCGCTCTGGATTATACG	GGTGAGAGGGGGTCTATCAT

3.6.2. Polymerase Chain Reaction (PCR)

While amplifying the candidate novel variants in the ERCC genes, site-specific primer sequences were used in PCR. Details of PCR with a total volume of 25 μ l are given in Table 3.

Table 3: The Reaction Mix Prepared for Each Sample

Component	Reaction
1X Dream Taq Master Mix*	12.5 μ l (2X)
200 nM Forward Primer (10 pmol/ μ l)	0.5 μ l
200 nM Reverse Primer (10 pmol/ μ l)	0.5 μ l
dH ₂ O	-
DNA (100-150 ng)	-
Total	25 μL

*DreamTaq PCR Master Mix (2X) Thermo Scientific™ Catalog number: K1072

25 μ l of the reaction mixture was added for each sample. The thermal cycler (Applied Biosystems, Veriti 96 Well) was used to perform PCR-based amplification. The PCR program in Table 4 was applied.

Table 4: Thermal Cycle Program

Step	Temperature	Time	Cycle
Denaturation	95°C	2 minutes	35 cycle
	95°C	30 seconds	
Annealing	60°C	30 seconds	
	72°C	60 seconds	
Final Extension	72°C	5 minutes	
	4°C	10 minutes	
	10°C	∞	

3.6.3. Agarose gel electrophoresis, PCR purification and Sequence PCR

After the PCR step, the Agarose gel electrophoresis step was started. 2% Agarose gel was used in this step. It was operated at 90 Volts for 60 minutes with 1X TBE (Tris-borate-EDTA). After the procedure, gel image was recorded.

After Agarose Gel Electrophoresis, PCR Purification (Exosap) was performed Table 5 and Table 6.

Table 5: PCR Purification (Exosap) Components

PCR Component	1x Reaction
Exonuclease I (20U/ μ l)	0.5 μ l
Shrimp Alkaline Phosphatase (1U/ μ l)	1 μ l
Total	1.5 μL

Table 6. Thermal Profile

Temperature	Time
37°C	30 minutes
80°C	15 minutes
10°C	∞

Following the Exosap stage sequence PCR step was performed. The preparation of the sequence PCR mix can be seen in the Table 7 and Table 8.

Table 7: Sequence PCR

PCR Component	1x Reaction
BigDye v.3.1	2 μ l
5X Reaksiyon Buffer	2 μ l
Forward or Reverse (2pmol/ μ l) primer	2 μ l
All of the exosap purified product	2 μ l
dH ₂ O	2 μ l
Total	1.5 μL

Table 8: Sequence PCR Thermal Profile

Temperature	Time	Cycle
96°C	1 minutes	25 Cycles
96°C	10 seconds	
50°C	5 seconds	
60°C	4 minutes	
4°C	∞	

After sequence PCR, the study was completed by Ethanol Purification and loading the samples into the ABI 3500 Genetic Analyzer device. Sequence analysis results of the loaded samples were obtained.

3.6.4. Sequence Analysis

The PCR amplification products were obtained using the "Cycle Sequence" PCR. This PCR uses site-specific primers and labeled dideoxynucleotides from the "Bigdye Terminator v3.1 Cycle Sequencing kit" (Applied Biosystems, Foster City, CA, USA). After electrophoresis of the reaction products on the "ABI PRISM 3130XL Genetic Analyzer" (Applied Biosystems, Foster City, CA, USA) automated DNA sequence analysis instrument, chromatograms representing DNA sequence analysis data were obtained. Chromatography analyzes the color/base relation for each reaction's waveform, enabling the secure recording and storing of sequence analysis data. After the sequence analysis, the chromatogram wave peak lengths were compared. Corrections were made where necessary. CLUSTALX and GENEDOC multiple sequence analysis tools were used to analyze the chromatographic results.

In a CLUSTAL X alignment, the two chains were placed facing each other (version 1.83). The ends of the closely matched sequences were trimmed using the GENEDOC (version 2.6.002) software, and the final consensus sequence was recorded.

3.7. Statistical Analysis Method

Microsoft Excel, GraphPad Prism version 9 (GraphPad Software, La Jolla, CA, USA) and MedCalc software (NY, USA) were used for statistical analysis of the study findings. Descriptive statistical techniques (number, percentage, median, etc.) were employed while analyzing the study data.

The $\Delta\Delta C_t$ method was used to calculate the expression of the miRNAs. The data were then analyzed using parametric methods, as most of the miRNA values followed a normal distribution. Data analysis was performed using SPSS for Windows (16.0, SPSS Inc., Chicago, Illinois). All values are presented as mean $\pm$ standard deviation ($M \pm SD$). P values <0.05 were considered statistically significant. Continuous variables were compared using Student's t-test.

The choice of statistical test was based on the normality of the distribution. The Student t-test or Mann-Whitney test was used for unpaired samples, and the Wilcoxon test was used for paired samples.

4. RESULTS

4.1. Demographic and Clinical Results of the Study Groups

This study examined the treatment response of prostate cancer to Lu-177 PSMA treatment in two groups of 57 patients. The study also evaluated the effect of treatment on miRNA-182 and miRNA-187 levels and DNA repair gene variations (ERCC2 gene). The clinical and pathological features of 57 males with prostate cancer who were diagnosed and followed-up by the Department of Nuclear Medicine in collaboration with the Department of Urology at Yeditepe University Faculty of Medicine are listed in Table 9 and Table 10.

To compare the expression levels of miRNA-182 and miRNA-187 in prostate cancer patients who received Lu-177 PSMA therapy and those who did not, we analyzed data from 25 patients in each group. The mean age of the Lu-177 PSMA treatment group was 70.88 years, while the mean age of the untreated group was 67.68 years. This was based on the 50 patient samples that were utilized in the research. In the Lu-177 PSMA group, 60% of patients had received radiotherapy before our therapy, while 40% had not received any treatment prior to ours. In our patient group, of which we did not apply Lu-177 PSMA, no patients had received any treatment before us. Therefore, the parameter for whether patients had received treatment differed significantly between the two groups ($p = 0.000$).

When the prostate lodge ($p = 0.135$), lymph node ($p = 0.842$), and visceral organ ($p = 0.180$) parameters were examined, there was no significant difference between the two groups. Between the patient groups, a difference in the bone metastasis parameter was noted ($p < 0.000$).

A total of 25 patients with advanced prostate cancer were treated with Lu-177 PSMA. There was no significant difference in PSA levels before (27.60 ± 30.44 ng/ml) and after (24.87 ± 34.58 ng/ml) treatment ($p = 0.801$). However, it was observed that PSA levels decreased in 9 of the 25 patients, increased in 9 of the 25 patients, and remained the same in 1 of the 25 patients.

Table 9: The demographic and clinical characteristics of 50 patients who received Lu-177 PSMA treatment and did not receive treatment.

Parameters	Prostate Cancer Patients treated with Lu-177 PSMA (n=25)	Not-treated Prostate Cancer Patients (n=25)	P value
Age (yr)	70.88 ±9.83	67.54 ±8.78	0.216
Received Treatment Before			
Yes	15	0	0.000
No	10	25	
Prostate Lodge			
Yes	15	21	0.135
No	9	3	
N/A	1	1	
Lenf Node			
Yes	13	15	0.842
No	11	9	
N/A	1	1	
Bone Metastasis			
Yes	23	6	<0.000
No	1	18	
N/A	1	1	
Visceral Organ			
Yes	7	2	0.180
No	17	22	
N/A	1	1	
PSA Level (ng/ml)			
Before Treatment	27.60 ±30.44		0.801
After Treatment	24.87 ±34.58		

N/A: not available

The bolded p-values are significant (< 0.05)

Additionally, seven patients in all were enrolled in the research. They constituted another study group and received 150 millicurie (mCi), 200 mCi, and 250 mCi Lu-177 PSMA treatments, respectively. The 7 patients' ages averaged out to 65.85 years. Both miRNA-182 and miRNA-187 expression levels of these patients were examined, and the presence of variation in DNA repair genes was investigated by Sanger sequencing. While 42.85% of our study population received 200 mCi of Lu-177 PSMA treatment, 42.85% received 250 mCi, and just 14.28% received 150 mCi.

One of the seven patients received 150 mCi Lu-177 PSMA and had a Gleason score of 9. This patient had lung metastases and a post-treatment PSA response.

Three of the seven patients received 200 mCi Lu-177 PSMA. Two of these patients had a Gleason score of 9, and one had a Gleason score of 10. All three patients had metastases limited to the bone, and they all had PSA responses after treatment.

Three out of seven patients received 250 mCi Lu-177 PSMA. One patient had a Gleason score of 10, another had a 6, and another had a 9. Of the three patients, two had widespread cancer spread, and one of them also had cancer in the bones and lymph nodes. All three patients had no PSA response after treatment.

Table 10: The demographic and clinical characteristics of 7 patients who received Lu-177 PSMA treatment

Patient ID	Age	Lu-177 PSMA Activity	PSA Response	Gleason Score	Type of Metastasis
Patient 1	67	150 mCi	Yes	4+5	Lung (Oligometastasis)
Patient 2	73	200 mCi	Yes	4+5	Limited Bone
Patient 3	64	200 mCi	Yes	5+5	Limited Bone
Patient 4	60	200 mCi	Yes	4+5	Limited Bone
Patient 5	68	250 mCi	No	4+6	Bone + Lymph Nodes
Patient 6	60	250 mCi	No	3+3	Widespread Bone
Patient 7	69	250 mCi	No	4+5	Widespread Bone

The physicochemical tests (radioactivity, radionuclidic identity, radionuclidic purity, chemical purity, and radiochemical purity), the biological purity tests (sterility, apyrogenicity, and toxicity), and the pharmaceutical property-dependent tests (pH, particle size, and isotonicity) were done.

For this purpose, quality controls of the radiopharmaceuticals prepared in the HPLC device were performed before the bound radioactivities were injected into the patients. Radioactivities with a binding rate of at least 95% in the results obtained after HPLC were injected into the patients. Reversed phase high pressure liquid chromatography (RP-HPLC) samples of the incubation solution were used to evaluate the *in vitro* stability of Lu-177. The stability was evaluated from the reaction vial, the incubation solution at the 6th hour, the blood sample taken from the patient at the 3rd hour, and the activity in the urine at the 3-5th hour (Figure 8).

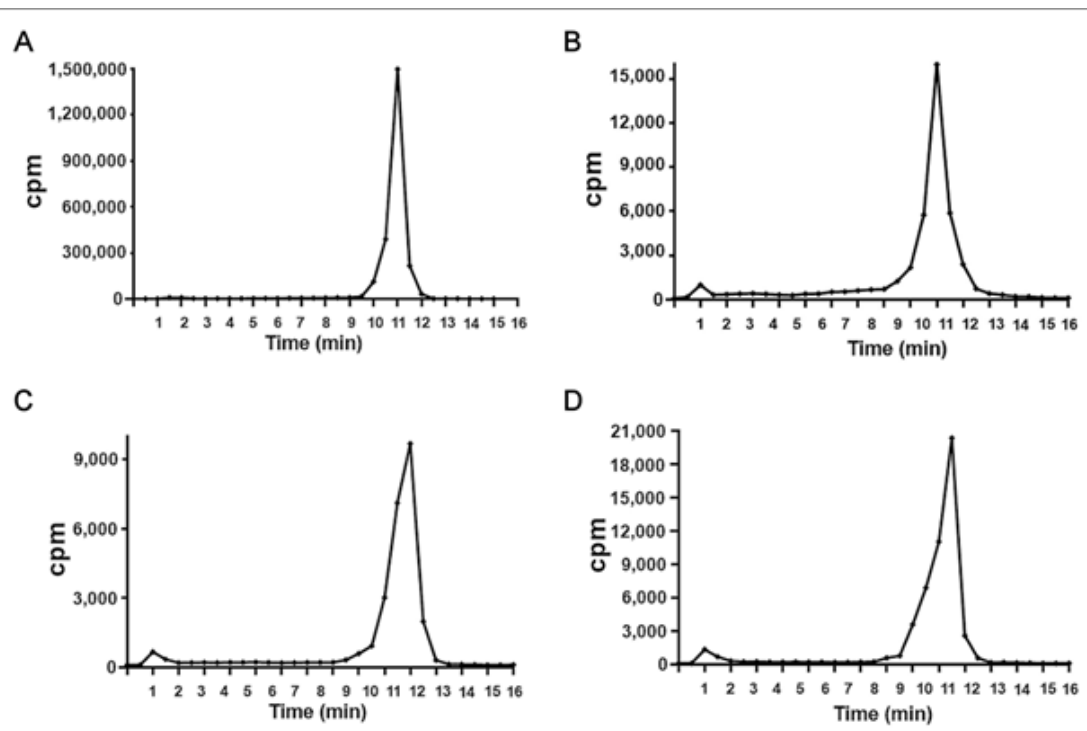


Figure 8: Activity stability graphs according to blood and urine samples.

A) From the reaction vial, B) From 6 hr incubation solution, C) Lu-177 PSMA to the patient from blood 3 hours after administration, D) activity stability in urine samples taken 3 hours after the activity is given to the patient.

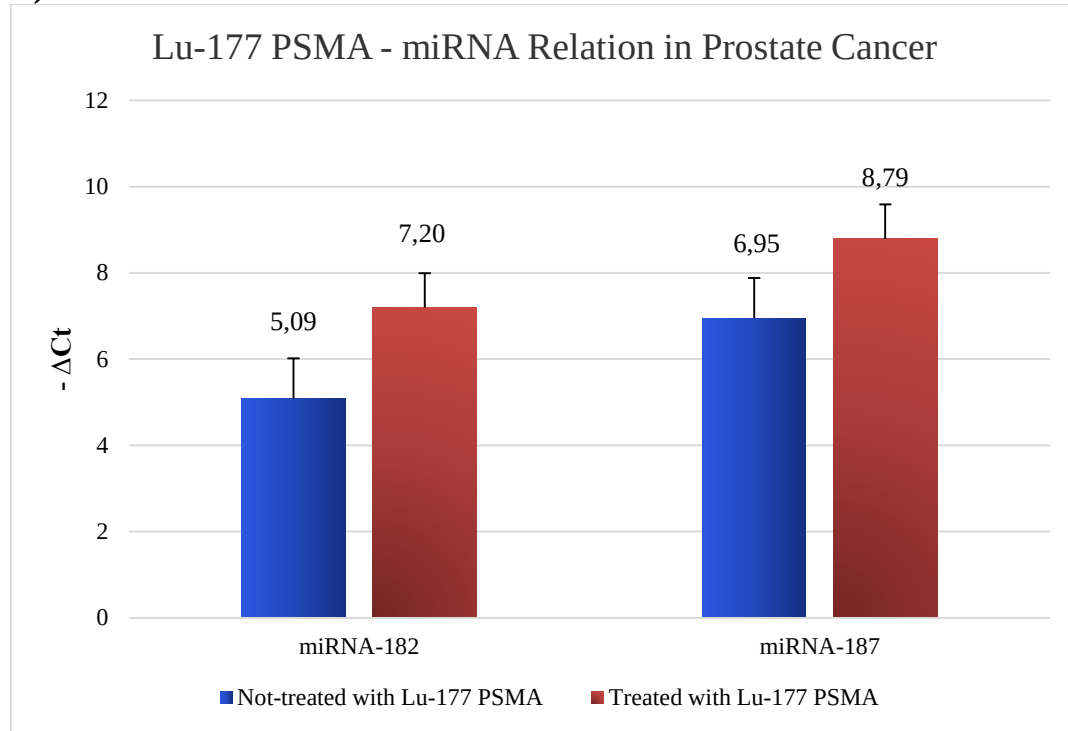
4.2. miRNA Results

4.2.1. miRNA expression levels in prostate cancer patients who received and did not receive Lu-177 PSMA treatment

miRNA-182 expression levels (CT values) of the patient group, which included patients treated with Lu-173 PSMA, were higher than those of the control group, which included patients with non-treated with Lu-173 PSMA. On the otherhand, there was no significant relationship between miRNA-187 expression levels (CT values) of the patient group, which included patients treated with Lu-173 PSMA, were higher than other group.

In our study, Lu-177 PSMA treatment was associated with a significant increase in the levels of miRNA-182 and miRNA-187 in patients with advanced castration-resistant metastatic prostate cancer (Figure 9).

A)



B)

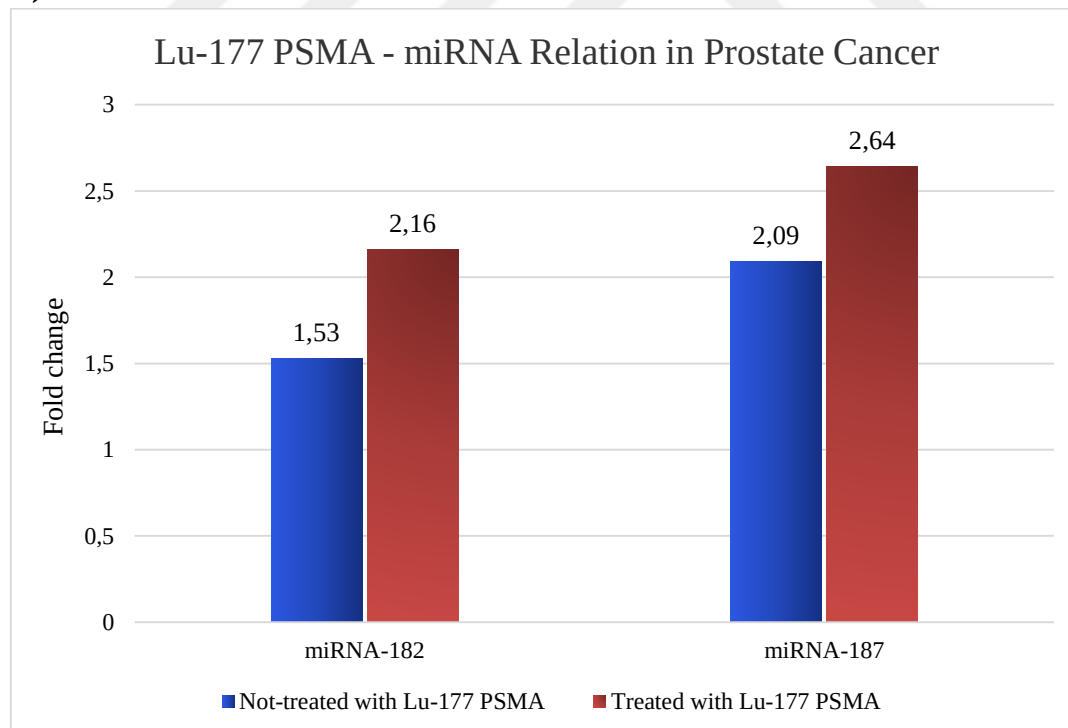


Figure 9: Lu-177 PSMA treatment and miRNA relation in prostate cancer

In serum of prostate cancer patients at before and after treatment with Lu-177 PSMA miRNA-182 ($p < 0.00001$) and miRNA-187 ($p = 0.00016$) expression levels significantly changed. (A) - ΔC_t values before and after Lu-177 PSMA therapy (B) Expression fold change before and after treatment.

4.2.2. miRNA Expression Levels According to Lu-177 PSMA mCi Dosage

miRNA analyses of the results of the study examining also the changes in these target miRNAs in 7 prostate cancer patients before and after treatment showed that these miRNAs cannot be associated with different doses of Lu-177 PSMA in patients.

As a result of this study, some participants reported up-regulation of miRNA expression, while others reported down-regulation or no change after treatment. The results were preferred to be interpreted according to the individual patients for this reason.

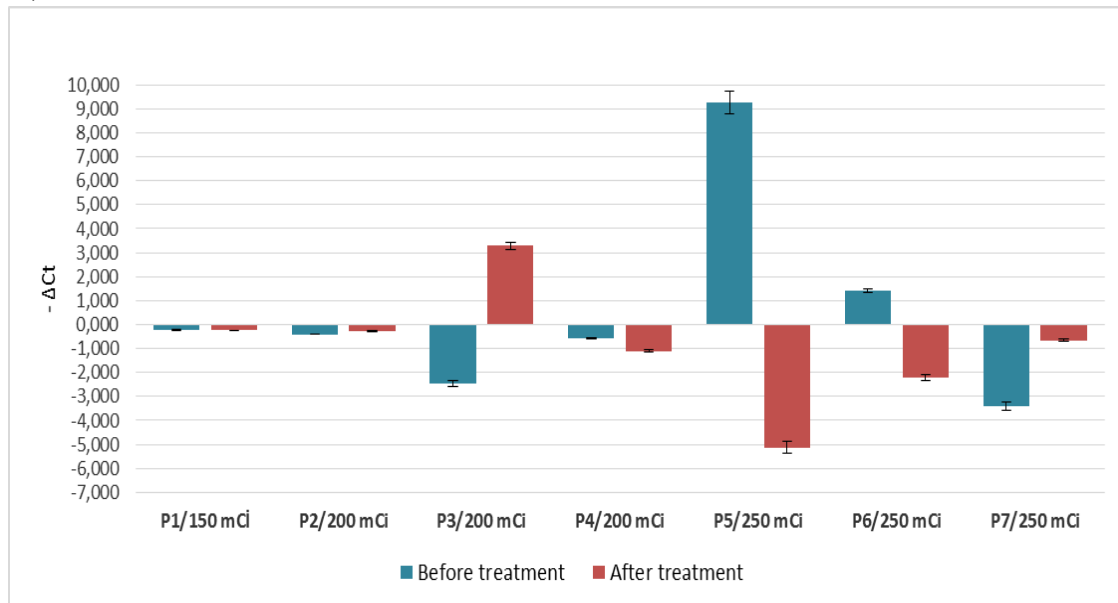
There was no difference between the expression profile before and after the 150 mCi Lu-177 PSMA therapy in the first patient. This patient had down-regulated miRNA-182 before treatment and remained down-regulated after the treatment.

The second patient, who was treated with 200 mCi, exhibited a different expression pattern than the first patient. This patient's miRNA-182 expression level was down-regulated before treatment and up-regulated after the treatment. The third patient exhibited the same expression pattern as the second patient. The fourth patient was the last member of the 200 mCi treatment group. This patient exhibited different expression levels than the other two patients in the same dosage group. The fourth patient's miRNA-182 expression level was down-regulated before and after the treatment. The fifth to seventh patients were members of the 250 mCi treatment group. We could not find the same expression pattern for the 250 mCi treatment group as for the 200 mCi treatment group. The fifth patient had an up-regulated miRNA-182 level before treatment. After treatment, we observed that this miRNA's expression level was down-regulated. The sixth patient had the same results as the fifth patient, and miRNA-182 decreased after treatment. The seventh patient had a decreased miRNA-182 expression level and after treatment it decreased even further.

Patients who received a higher dose of 150 or 200 mCi had a better chance of a positive PSA response. These patients' miRNA-182 expression levels were down-regulated before the treatment. After treatment, miRNA-182 expression levels were changed or remained down-regulated. In the patient group receiving 250 millicuries (mCi), there was no PSA response. When miRNA-182 expression levels were assessed, two patients had increased miRNA-182 levels, and their expression levels decreased after treatment. The other patient had no change in terms of miRNA-182 expression level, but increased after the treatment (Figure 10).

The results of the study imply that in patients with advanced castration-resistant metastatic prostate cancer, miRNA-182 levels should be evaluated in the context of therapy response. These levels may be used to determine the optimal dose of Lu-177 PSMA treatment.

A)



B)

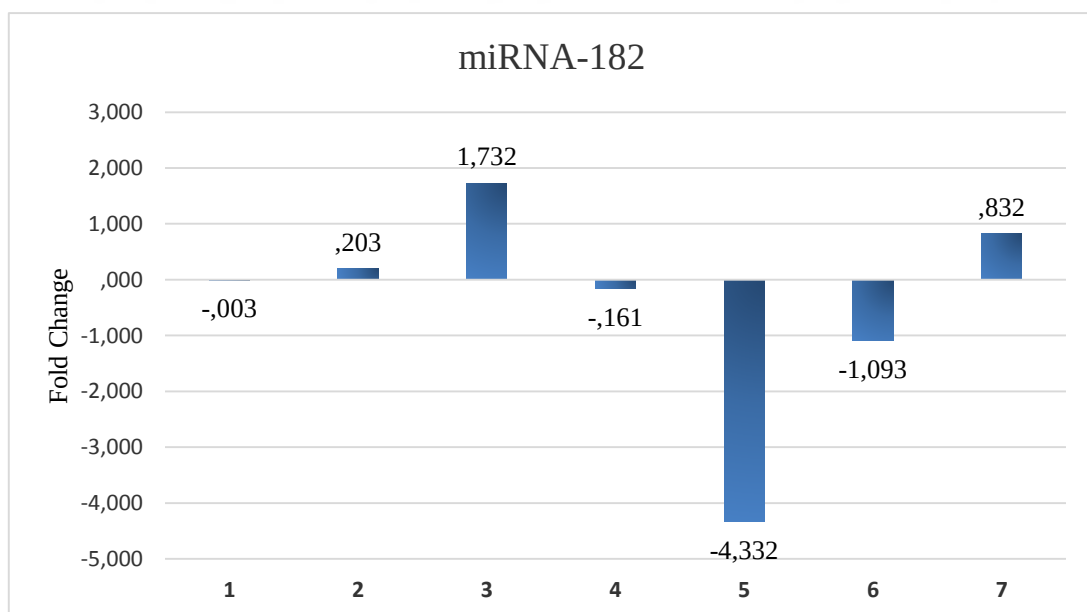


Figure 10: Lu-177 PSMA treatment and miRNA-182 relation in prostate cancer. In serum of prostate cancer patients at before and after treatment with Lu-177 PSMA miRNA-182 expression levels. (A) - ΔC_t values before and after Lu-177 PSMA therapy (B) Expression fold change before and after treatment.

The results of miRNA-187 treatment were mixed, with some patients showing an increase in miRNA expression, while others showed a decrease or no change. This suggests that miRNA-187 expression may vary depending on the individual patient (Figure 11).

The first patient's expression profile remained unchanged before and after receiving 150 mCi Lu-177 PSMA therapy. This patient had the same expression levels before and after treatment, but they increased after the treatment.

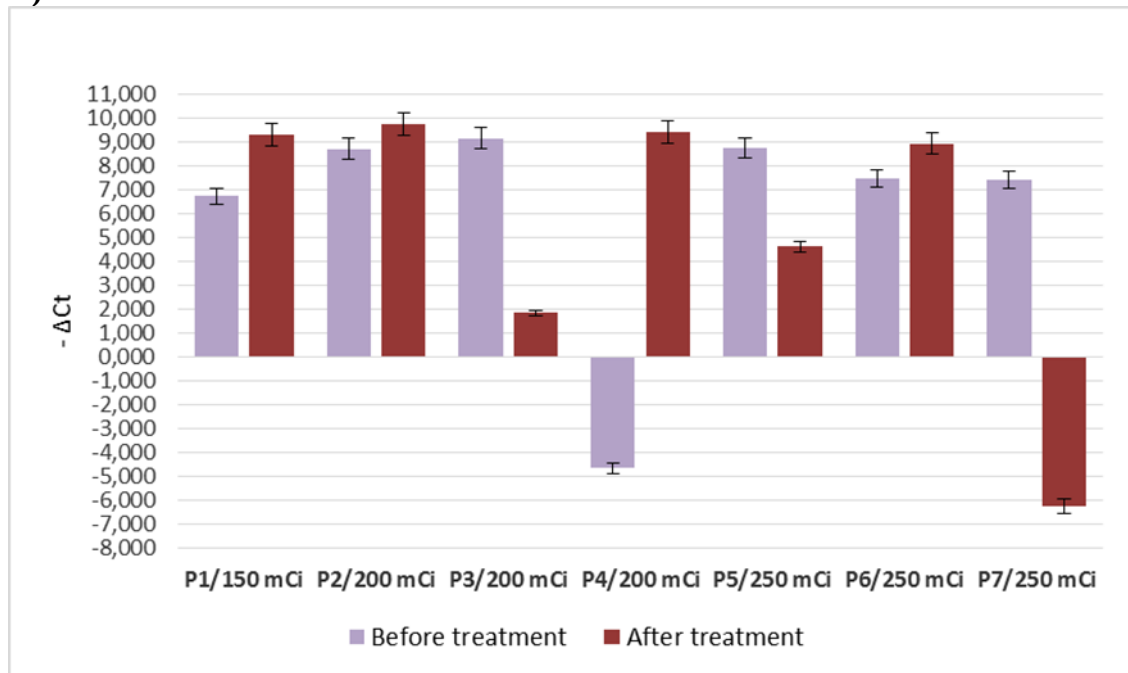
The second, third, and fourth patients received 200 mCi treatment. The second patient, who was treated with 200 mCi, exhibited the same expression pattern as the 150 mCi treated patient. This patient's miRNA-187 expression level was up-regulated before and after the treatment. The third patient also had the same expression levels before and after treatment, but they decreased after the treatment.

The fourth patient was the last member of the 200 mCi treatment group. This patient exhibited different expression levels than the other two patients in the same dosage group. The fourth patient's miRNA-187 expression level was down-regulated before and up-regulated after the treatment.

The fifth to seventh patients were members of the 250 mCi treatment group. We could not find the same expression pattern for the 250 mCi treatment group as for the 200 mCi treatment group.

The fifth patient had the same expression levels before and after treatment, but they decreased after the treatment. The sixth patient had the same expression levels before and after treatment, but they increased after the treatment. The seventh patient's miRNA-187 expression level was up-regulated before and decreased after the treatment. The PSA responses of the patients who received 150 and 200 mCi were better. However, the miRNA change could not reach a clear result before and after the treatment, depending on the dose applied.

A)



B)

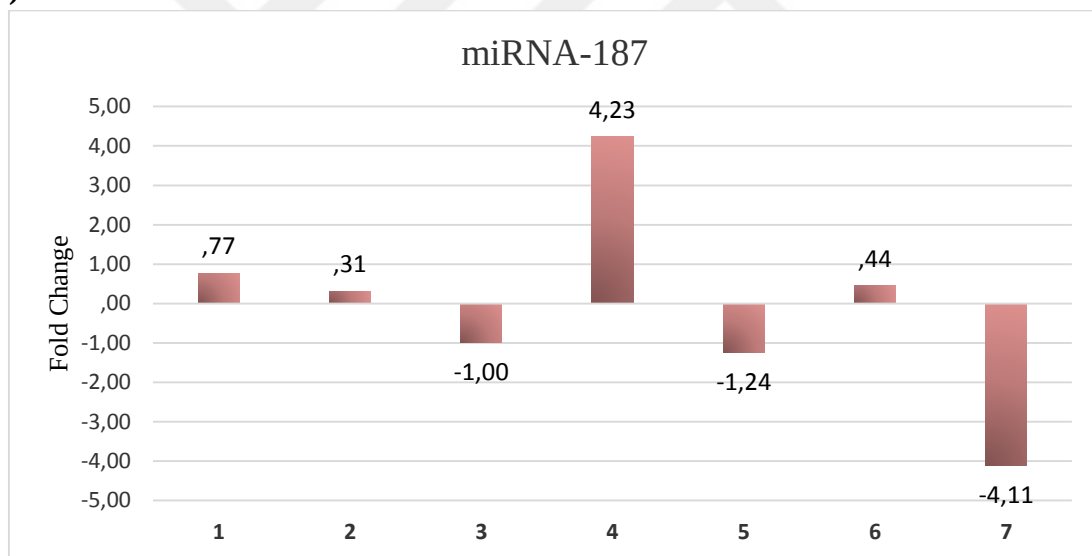


Figure 11: Lu-177 PSMA treatment and miRNA-187 relation in prostate cancer
In serum of prostate cancer patients at before and after treatment with Lu-177 PSMA miRNA-187 expression levels. (A) - ΔC_t values before and after Lu-177 PSMA therapy
(B) Expression fold change before and after treatment.

4.2.3. Results of ROC analysis of miRNA-182 and miRNA-187 in patients who received and did not receive Lu-177 PSMA therapy

ROC analyses were performed for both of our research groups. The study found that the target miRNAs were significantly associated with the treatment response of Lu-177 PSMA in prostate cancer patients. According to the analysis results of the overall miRNAs, the specificity of miRNA182 is 100.0, with a sensitivity of 92.0% (95% CI = 0.897–0.996, AUC = 0.982, $p = 0.0001$). The specificity of miRNA187 is 96.0, with a sensitivity of 76.0% (95% CI = 0.793–0.971, AUC = 0.909, $p = 0.0001$) (Figure 12).

The specificity of miRNA182 was 100.0%, with a sensitivity of 92.0%. This means that miRNA182 was able to correctly identify 100% of the samples that did not have the disease, and it was able to correctly identify 92.0% of the samples that did have the disease. The p -value for miRNA182 was < 0.0001 , which indicates that the results are statistically significant. The AUC value for miRNA182 was 0.982, which indicates that it is a good predictor of the disease. The 95% CI for miRNA182 was 0.897–0.996, which indicates that the true value for the specificity is likely to be between 89.7% and 99.6%, and the true value for the sensitivity is likely to be between 92.0% and 99.6%.

The specificity of miRNA187 was 96.0%, with a sensitivity of 76.0%. This means that miRNA187 was able to correctly identify 96.0% of the samples that did not have the disease, and it was able to correctly identify 76.0% of the samples that did have the disease. The p -value for miRNA187 was < 0.0001 , which indicates that the results are statistically significant. The AUC value for miRNA187 was 0.909, which indicates that it is a good predictor of the disease. The 95% CI for miRNA187 was 0.793–0.971, which indicates that the true value for the specificity is likely to be between 79.3% and 97.1%, and the true value for the sensitivity is likely to be between 76.0% and 97.1% (Figure 12).

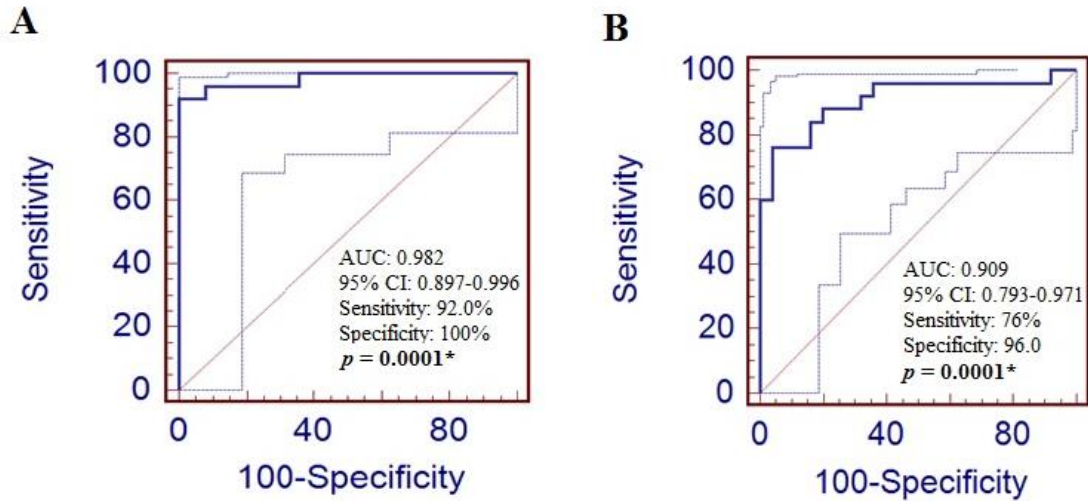


Figure 12: Receiver operator characteristic curves of expression levels of miRNA182 (A) and miRNA187 (B).

However, a study of 7 prostate cancer patients found that changes in target miRNAs before and after treatment could not be reliably associated with different doses of Lu-177 PSMA.

According to the analysis results of the overall miRNAs, the specificity of miRNA182 is 71.4%, with a sensitivity of 57.1% (95% CI = 0.289–0.822, AUC = 0.571, $p = 0.650$). The specificity of miRNA187 is 100.0%, with a sensitivity of 57.1% (95% CI = 0.324–0.851, AUC = 0.612, $p = 0.468$). Additionally, ROC analyses were performed using Lu-177 PSMA dosage (150 mCi, 200 mCi, and 250 mCi) differences. These results also have no statistical significance in this context.

4.3. DNA Concentration Results

DNA concentrations of all individuals included in the study were determined. Table 11 represents the effect of exposure to Lu-177 PSMA therapy on blood DNA concentration (ng/ μ L) measured by spectrophotometer in comparison to the not-treated controls.

There is highly significant increase in the quantity of blood DNA extracted from blood exposed to Lu-177 PSMA with mean value $\pm$ SD (73.68 $\pm$ 21.86) compared to not-treated controls (39.04 $\pm$ 13.36) (p = 0.0069).

Table 11: Effect of Lu-177 PSMA Therapy on Blood DNA Concentration (ng/ μ L)

	Blood DNA concentration (ng/ μ L)		p value
	Mean	$\pm$ SD	
Non-treated	39.04	$\pm$ 13.36	0.0069
Treated	73.68	$\pm$ 21.86	

Abbreviation: SD, standard deviation.
 $p < 0.005$ significant.

Figure 13 represents the comparison between the level of DNA concentration in blood after exposure to Lu-177 PSMA therapy. There was no significant difference in the concentration of DNA between before and after treatment, when exposed to Lu-177 PSMA grades 150 mCi, 200 mCi and 250 mCi. However, a change was detected before and after the treatment.

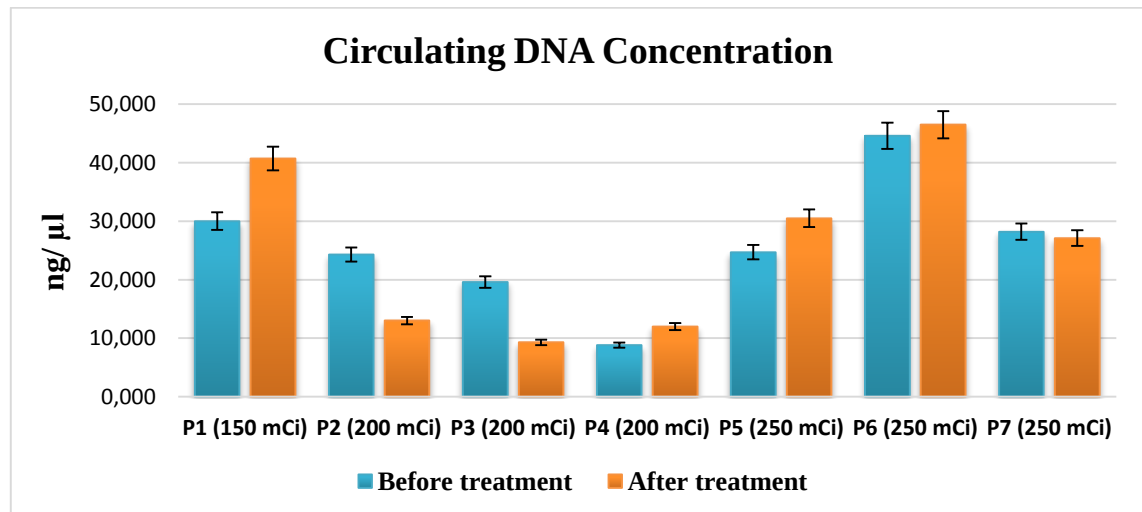


Figure 13: Comparison between mean concentration level of DNA in blood before and after exposure to different grades of Lu-177 PSMA therapy.

P1. After 150 mCi Lu-177 PSMA radionuclide therapy, the patient has PSA response, with increase DNA concentration. The patient was found to have decrease miRNA-182 and increased miRNA-187 expression levels.

P2. After 200 mCi Lu-177 PSMA radionuclide therapy, the patient has PSA response, with decreased DNA concentration. The patient was found to have increased miRNA-182 and increased miRNA-187 expression levels.

P3. After 200 mCi Lu-177 PSMA radionuclide therapy, the patient has PSA response, with decreased DNA concentration. The patient was found to have increased miRNA-182 and decreased miRNA-187 expression levels.

P4. After 200 mCi Lu-177 PSMA radionuclide therapy, the patient has PSA response, with increased DNA concentration. The patient was found to have decreased miRNA-182 and increased miRNA-187 expression levels.

P5. After 250 mCi Lu-177 PSMA radionuclide therapy, the patient has not PSA response, with increased DNA concentration. The patient was found to have decreased miRNA-182 and decreased miRNA-187 (up) expression levels.

P6. After 250 mCi Lu-177 PSMA radionuclide therapy, the patient has not PSA response, with increased DNA concentration. The patient was found to have decreased miRNA-182 and increased miRNA-187 (up) expression levels.

P7. After 250 mCi Lu-177 PSMA radionuclide therapy, the patient has not PSA response, with decreased DNA concentration. The patient was found to have increased miRNA-182 (down) and decreased miRNA-187 expression levels.

Our study found a significant relation with expression levels of miRNA-182 after Lu-177 PSMA treatment and circulating DNA concentration ($p = 0.028$).

4.4. Sanger Sequencing Results

4.4.1. ERCC2 gene variant analyses of patients receiving and not receiving Lu-177 PSMA Treatment with Sanger sequencing

At the same time, Sanger sequencing was performed on blood samples. Sanger sequences of patients who received and did not receive Lu-177 PSMA treatment, which constituted our first study group, were performed only on 10 randomly selected prostate cancer patients, 5 who received the treatment and 5 who did not.

The presence of ERCC2 Lys751Gln c.2251A>C (rs13181) and ERCC2 Asp312Asn c.934G>A (rs1799793) in prostate cancer patients who received and did not receive Lu-177 PSMA treatment was performed by agarose gel electrophoresis (Figure 14).

It was determined that 3 of the non-treated prostate cancer patients were heterozygous mutants, and 2 were wild-type for the ERCC2 Lys751Gln c.2251A>C (rs13181). Of the patients who received treatment, 3 were heterozygous for the ERCC2 Lys751Gln variation, 1 was wild-type, and 1 was not detected (Table 12; Figure 15-23).

Of the patients who received treatment, 4 were homozygous for the ERCC2 Asp312Asn (rs1799793) mutation, and 1 was wild-type. It was determined that 3 of the non-treated prostate cancer patients were heterozygous mutant, and 2 were homozygous mutant (Table 12; Figure 24-33).

Table 12: Sanger sequencing results for treated and non-treated patients of ERCC2 rs13181 and rs1799793 variants.

Patient	Lu-177 PSMA	ERCC2 Lys751Gln c.2251A>C (rs13181)	ERCC2 Asp312Asn c.934G>A (rs1799793)
	Yes/No		
P1 (Treated)	Yes*	-	Wild-type
P4 (Treated)	Yes*	Heterozygous Mutant	Homozygous Mutant
P9 (Treated)	Yes*	Wild Type	Homozygous Mutant
P11 (Treated)	Yes*	Heterozygous Mutant	Homozygous Mutant
P17 (Treated)	Yes*	Heterozygous Mutant	Homozygous Mutant
NT3 (Non-treated)	No ⁺	Heterozygous Mutant	Homozygous Mutant
NT6 (Non-treated)	No ⁺	Heterozygous Mutant	Homozygous Mutant
NT10 (Non-treated)	No ⁺	Wild Type	Heterozygous Mutant
NT14 (Non-treated)	No ⁺	Heterozygous Mutant	Heterozygous Mutant
NT21 (Non-treated)	No ⁺	Wild Type	Heterozygous Mutant

These prostate cancer patients were selected from among 25 patients who received treatment and 25 patients who did not receive treatment.

*Yes: Patients treated with Lu-177 PSMA

+No: Patients non-treated with Lu-177 PSMA

Results of ERCC2 Lys751Gln c.2251A>C (rs13181) Sanger sequencing in patients who received and did not receive Lu-177 PSMA therapy

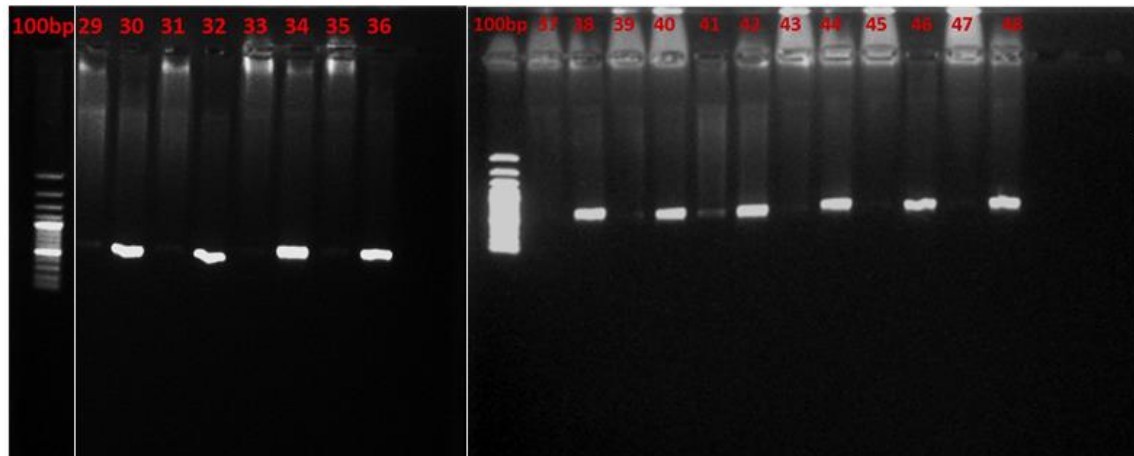


Figure 14: Agarose gel electrophoresis results of ERCC2 rs13181 and rs1799793 gene variants in treated and non-treated patients with prostate cancer.

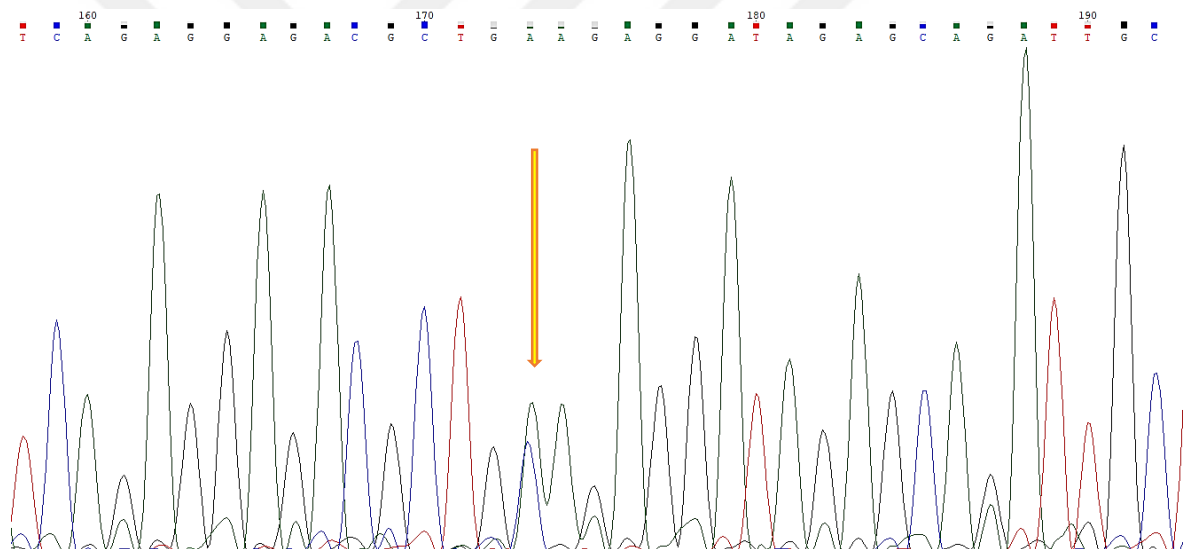


Figure 15: Heterozygous Mutant ERCC2 Lys751Gln c.2251A>C (rs13181) (non-treated C3)

*Forward image of Sanger Sequencing.

Our Sanger sequencing study displays A in green fluorescence, T in red, G in black, and C in blue, as is standard.

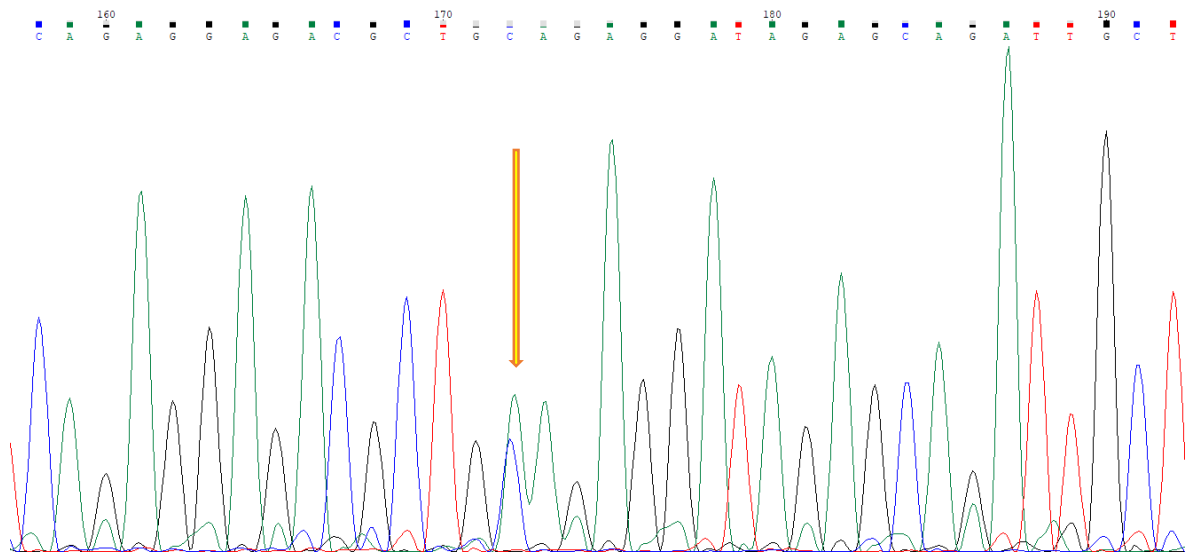


Figure 16: Heterozygous Mutant ERCC2 Lys751Gln c.2251A>C (rs13181) (non-treated C6)
 *Forward image of Sanger Sequencing.

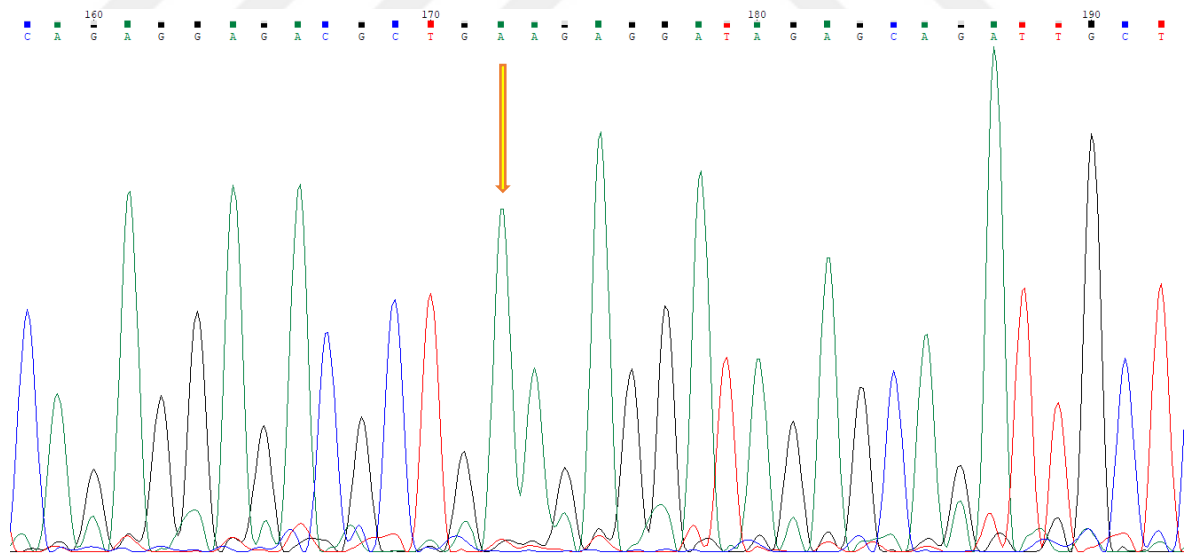


Figure 17: Wild-type ERCC2 Lys751Gln c.2251A>C (rs13181) (non-treated C10)
 *Forward image of Sanger Sequencing.

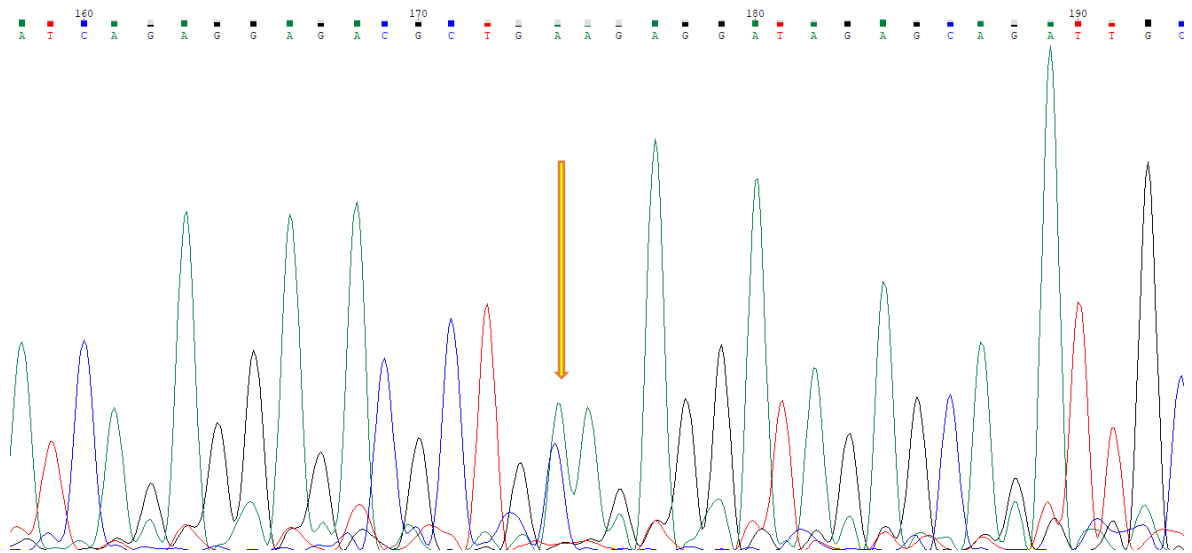


Figure 18: Heterozygous Mutant ERCC2 Lys751Gln c.2251A>C (rs13181) (non-treated C14)
 *Forward image of Sanger Sequencing.

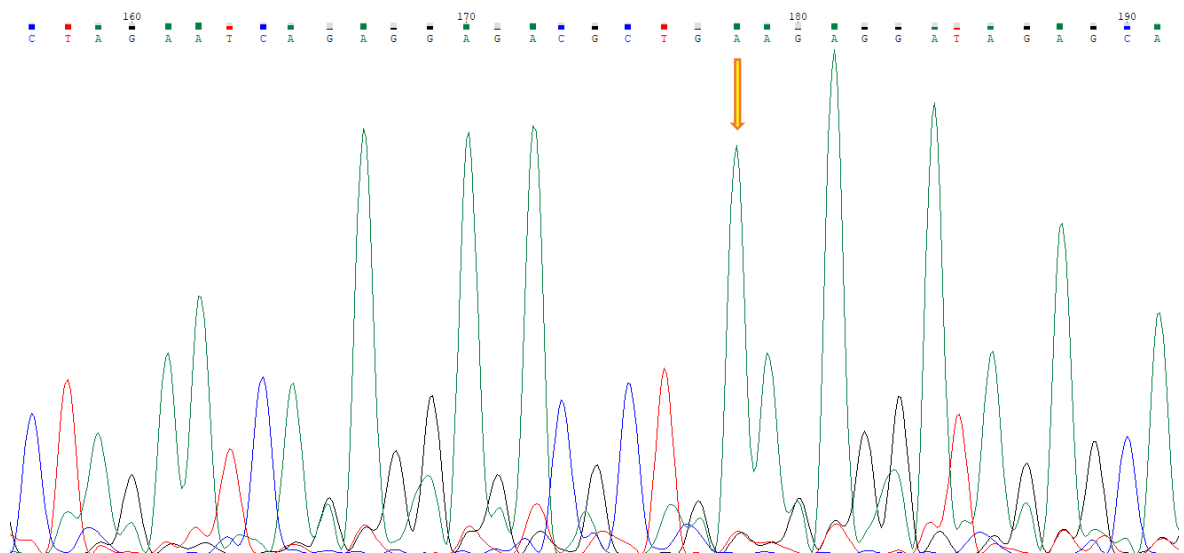


Figure 19: Wild-type ERCC2 Lys751Gln c.2251A>C (rs13181) (non-treated C21)
 *Forward image of Sanger Sequencing.

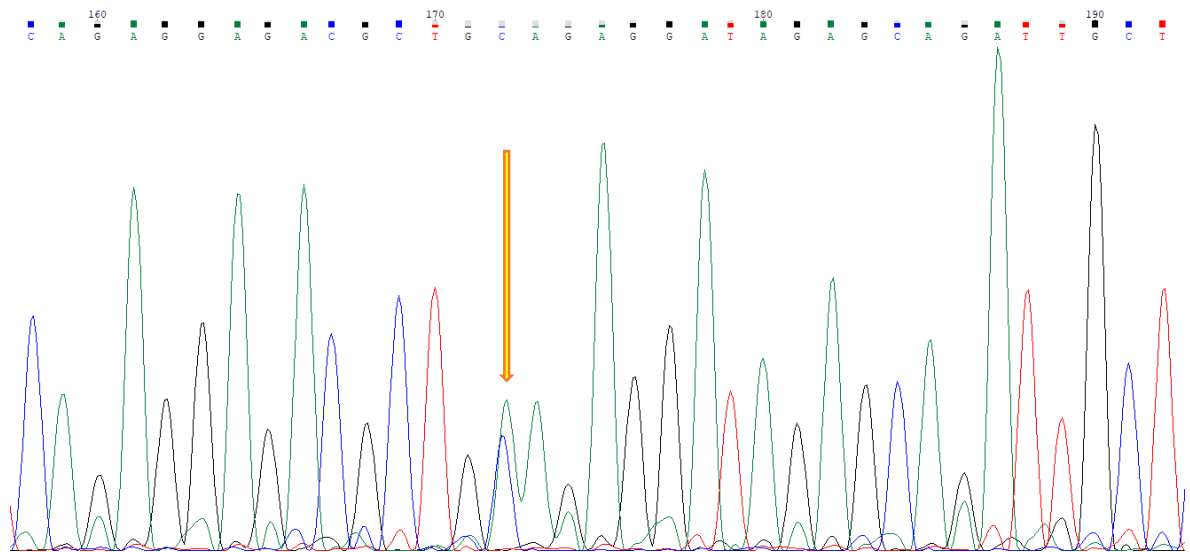


Figure 20: Heterozygous Mutant ERCC2 Lys751Gln c.2251A>C (rs13181) (treated P4)
 *Forward image of Sanger Sequencing.

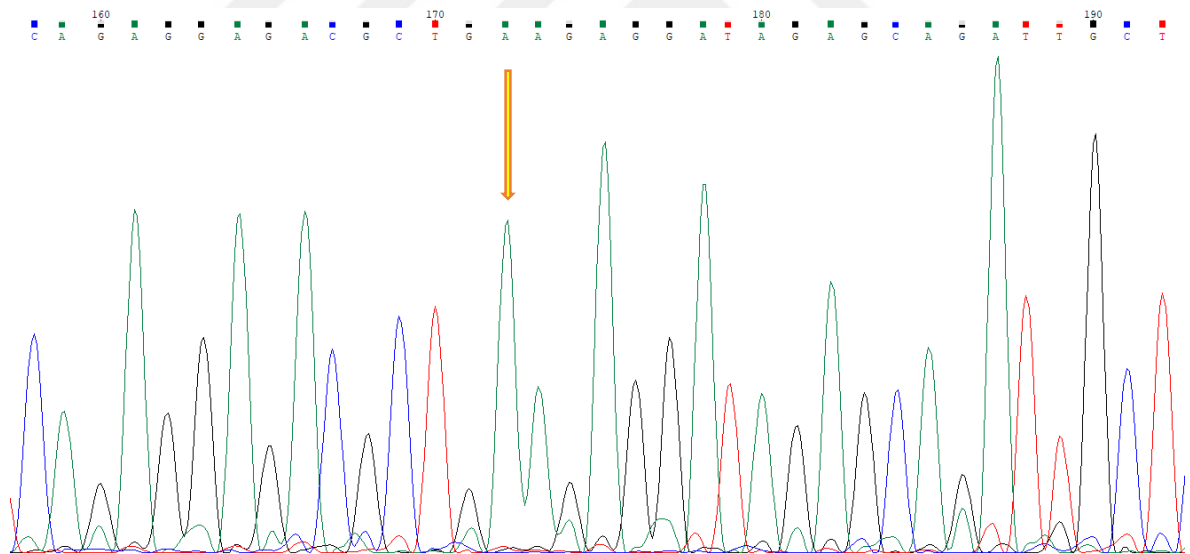
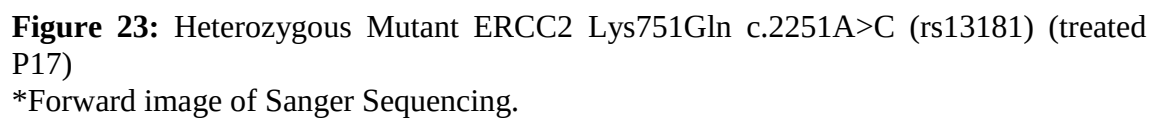
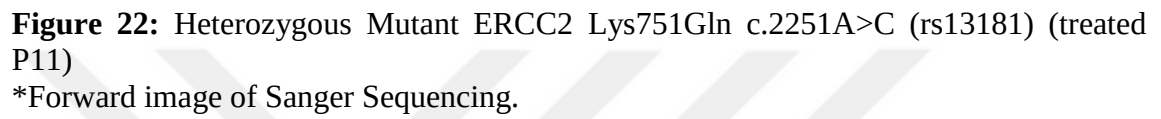


Figure 21: Wild-type ERCC2 Lys751Gln c.2251A>C (rs13181) (treated P9)
 *Forward image of Sanger Sequencing.



Results of Sanger sequencing of the ERCC2 Asp312Asn c.934G>A gene variant in patients who received and did not receive Lu-177 PSMA therapy

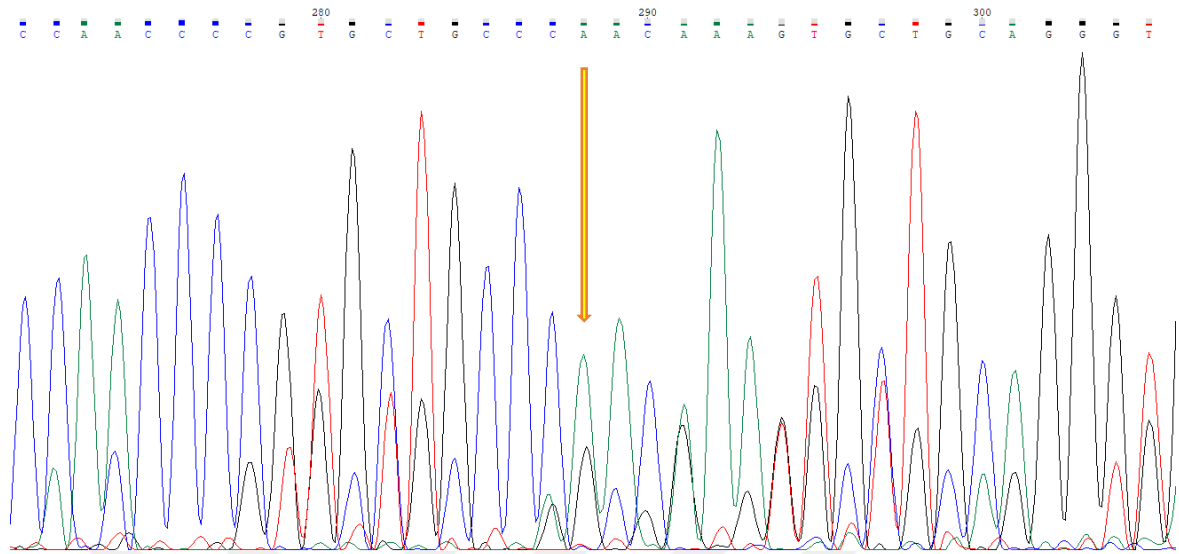


Figure 24: Homozygous Mutant ERCC2 Asp312Asn c.934G>A (non-treated C3)
*Forward image of Sanger Sequencing.

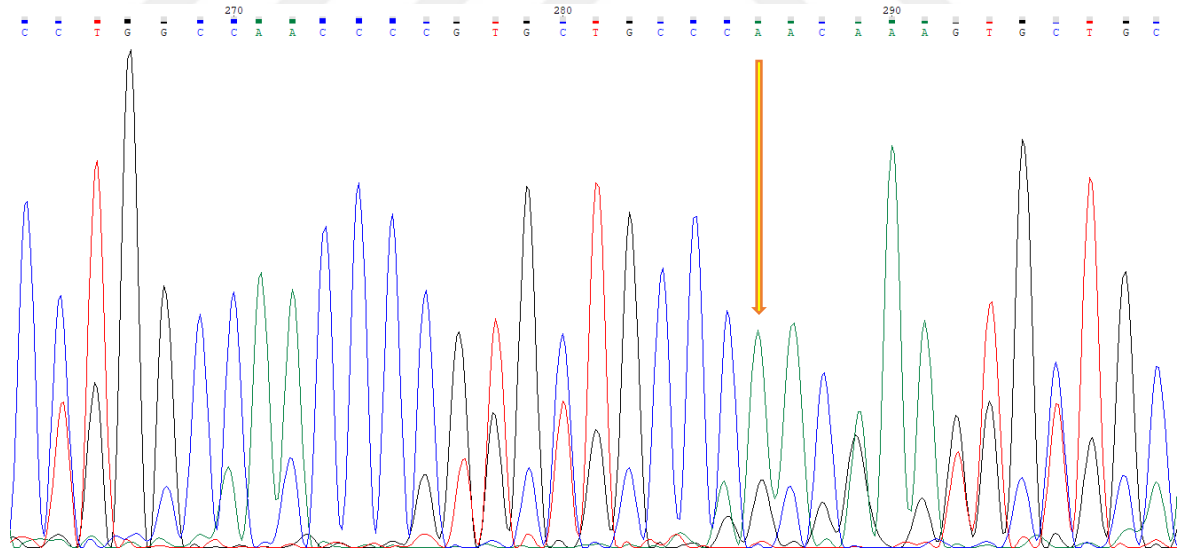


Figure 25: Homozygous Mutant ERCC2 Asp312Asn c.934G>A (non-treated C6)
*Forward image of Sanger Sequencing.

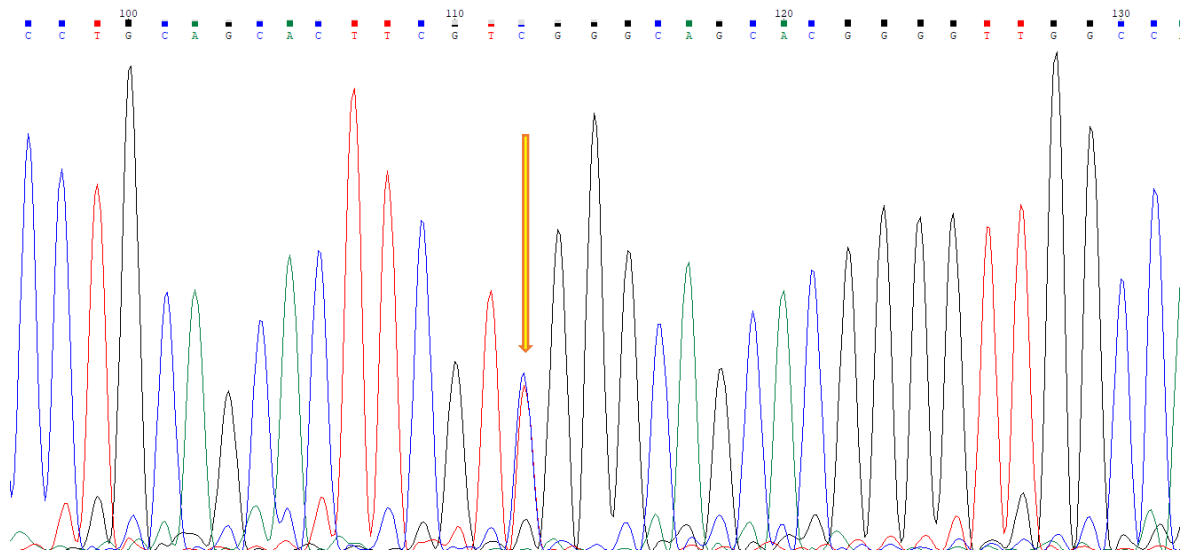


Figure 26: Heterozygous Mutant ERCC2 Asp312Asn c.934G>A (non-treated C10)
*Reverse image of Sanger Sequencing.

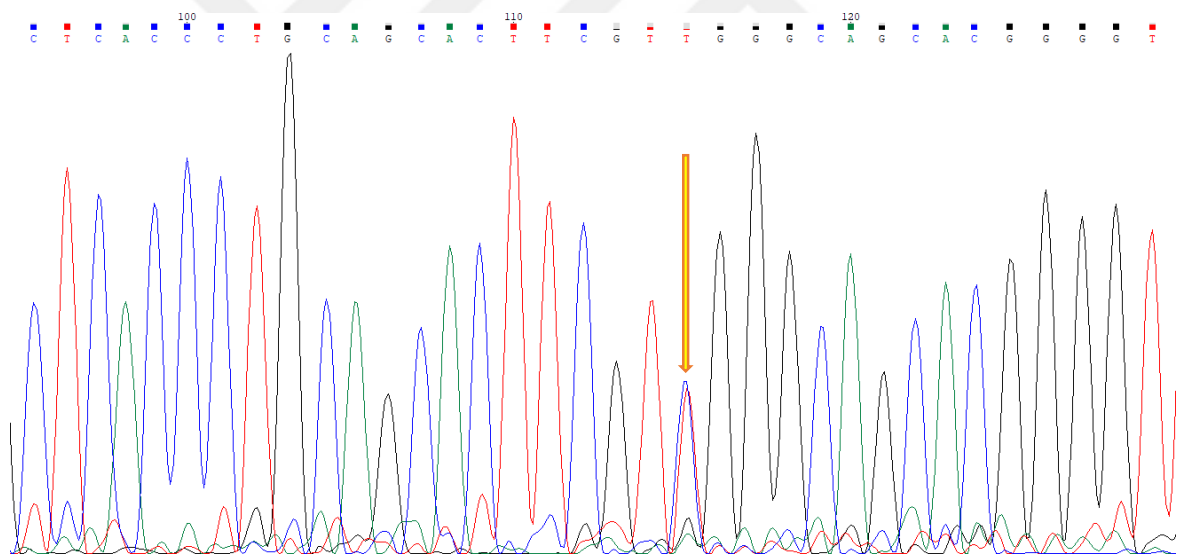


Figure 27: Heterozygous Mutant ERCC2 Asp312Asn c.934G>A (non-treated C14)
*Reverse image of Sanger Sequencing.

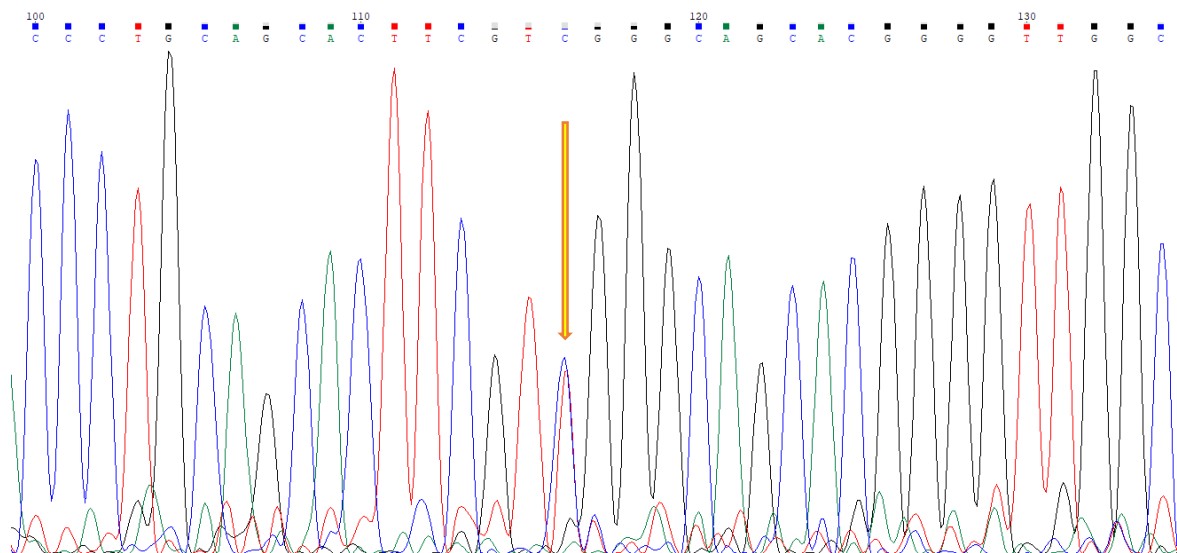


Figure 28: Heterozygous Mutant ERCC2 Asp312Asn c.934G>A (non-treated C21)
*Reverse image of Sanger Sequencing.

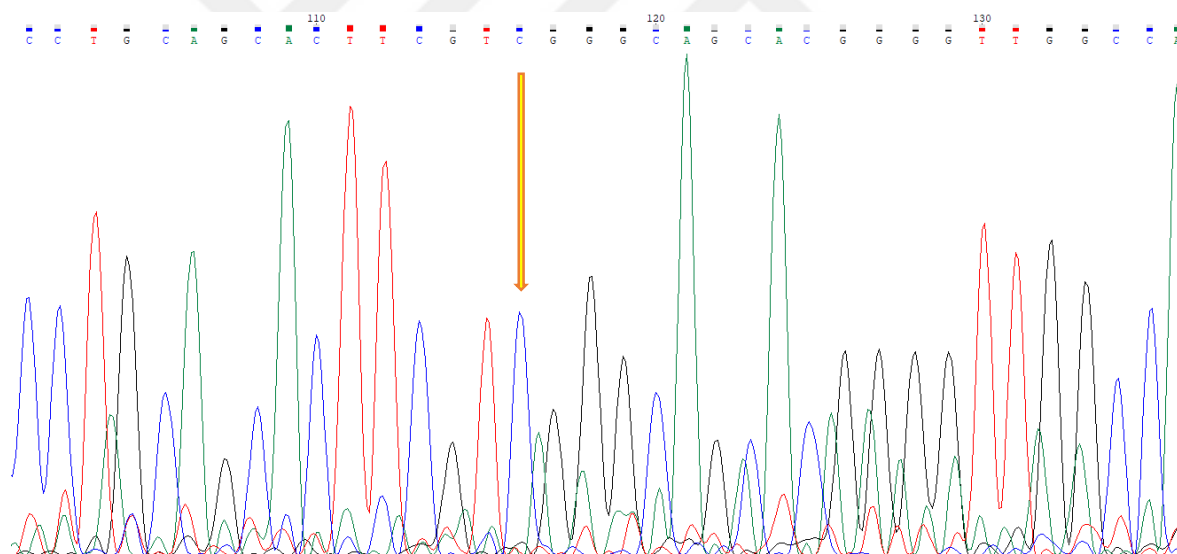


Figure 29: Wild-type ERCC2 Asp312Asn c.934G>A (treated P1)
*Reverse image of Sanger Sequencing.

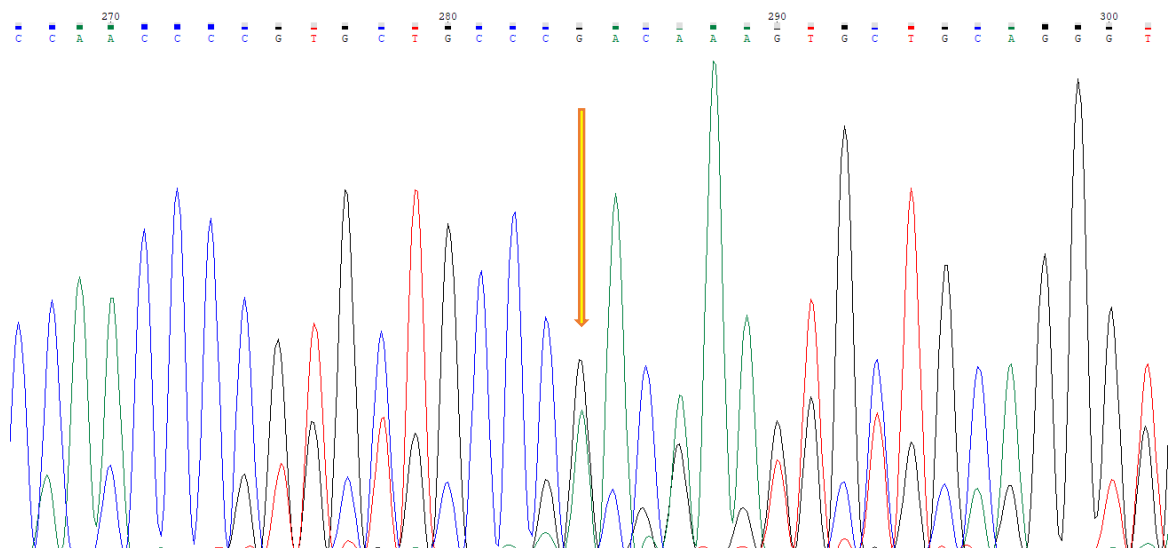


Figure 30: Homozygous mutant ERCC2 Asp312Asn c.934G>A (treated P4)
*Forward image of Sanger Sequencing.

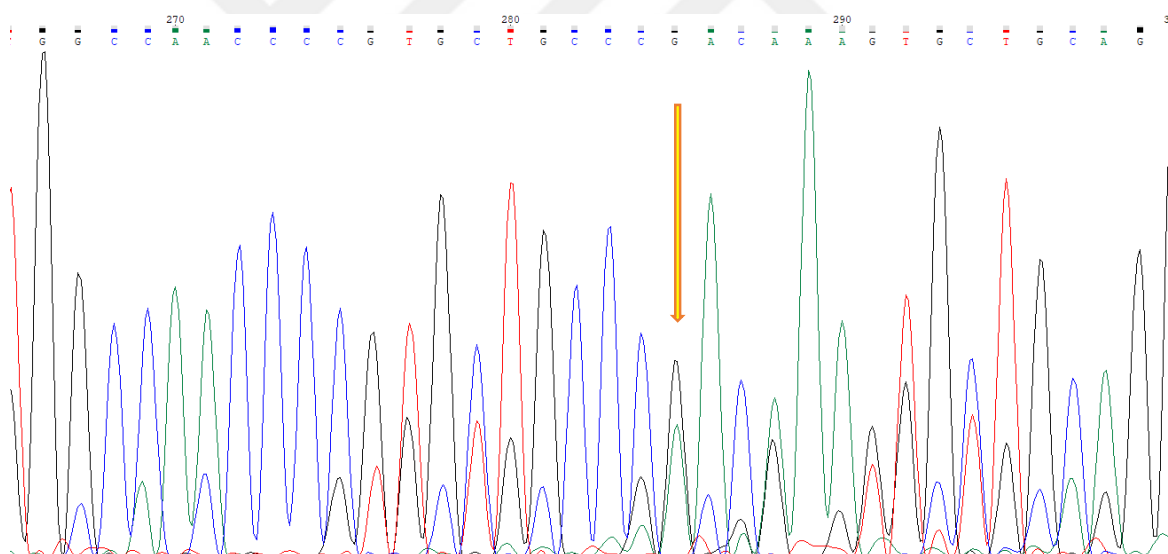


Figure 31: Homozygous mutant ERCC2 Asp312Asn c.934G>A (treated P9)
*Forward image of Sanger Sequencing.

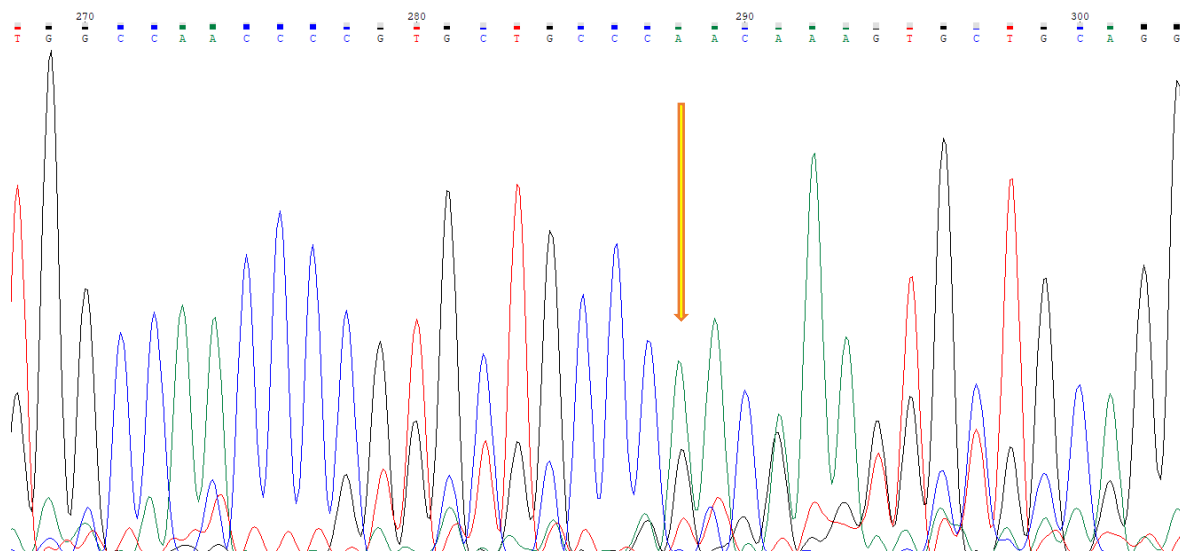


Figure 32: Homozygous mutant ERCC2 Asp312Asn c.934G>A (treated P11)
*Forward image of Sanger Sequencing.

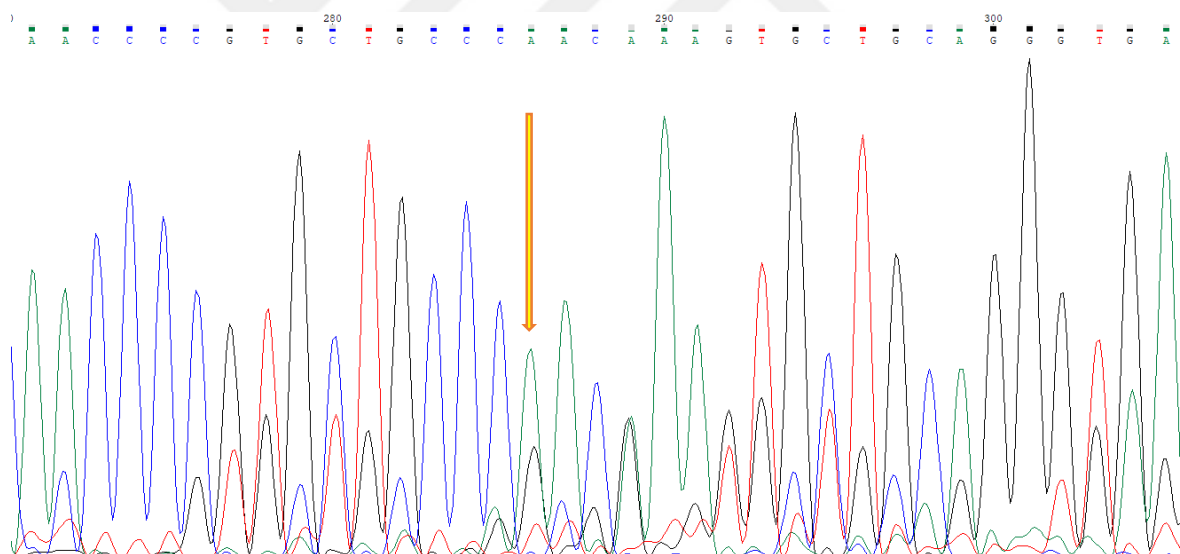


Figure 33: Homozygous mutant ERCC2 Asp312Asn c.934G>A (treated P17)
*Forward image of Sanger Sequencing.

4.4.2. Analysis of ERCC gene variants in 7 prostate cancer patients before and after treatment using Sanger sequencing

Our second study group consisted of prostate cancer patients who were examined before and after the treatment of 7 patients. Sanger sequencing was performed both before and after treatment (Figure 34, Table 13-24).

The patient 1 who received 150 mCi Lu-177 PSMA treatment was determined to be homozygous mutant for ERCC2 rs13181 and heterozygous mutant for ERCC2 rs1799793 both before and after treatment (Table 13, Table 14, Table 21). The patient 2 who received 200 mCi Lu-177 PSMA treatment was determined to be heterozygous mutant for ERCC2 rs13181 both before and after treatment. Analysis could not be performed for ERCC2 rs1799793 (Table 13, Table 15). The patient 3 who received 200 mCi Lu-177 PSMA treatment was determined to be heterozygous mutant for ERCC rs13181 and ERCC2 rs1799793 both before and after treatment (Table 13, Table 16, Table 22). The patient 4 who received 200 mCi Lu-177 PSMA treatment was determined to be wild-type for ERCC rs13181 both before and after treatment. Analysis could not be performed for ERCC2 rs1799793 (Table 13, Table 17). The patient 5 who received 250 mCi Lu-177 PSMA treatment was determined to be wild-type for ERCC rs13181 both before and after treatment. Analysis could not be performed for ERCC2 rs1799793 (Table 13, Table 18). The patient 6, who received 250 mCi Lu-177 PSMA treatment was determined to be wild-type for ERCC2 rs13181 and ERCC2 rs1799793 both before and after treatment (Table 13, Table 19, Table 23). Patient 7, who received 250 mCi Lu-177 PSMA treatment, was determined to be heterozygous mutant for ERCC rs13181 and homozygous mutant for ERCC2 rs1799793 both before and after treatment (Table 13, Table 20, Table 24).

When the rs13181 variant was examined in prostate cancer patients who responded to Lu-177 PSMA treatment, 1 homozygous mutant, 2 heterozygous mutants, and 1 wild-type character were determined. When the rs13181 variant was examined in prostate cancer patients who did not respond to Lu-177 PSMA treatment, it was determined that 1 of them was a heterozygous mutant and 2 of them were wild-type characters.

This examination was also performed in ERCC2 rs1799793. For this variant in prostate cancer patients who did not have a Lu-177 PSMA treatment response, it was concluded that it has 2 heterozygous mutant genotypes. Wild-type genotype was detected in the patient who did not respond to treatment.

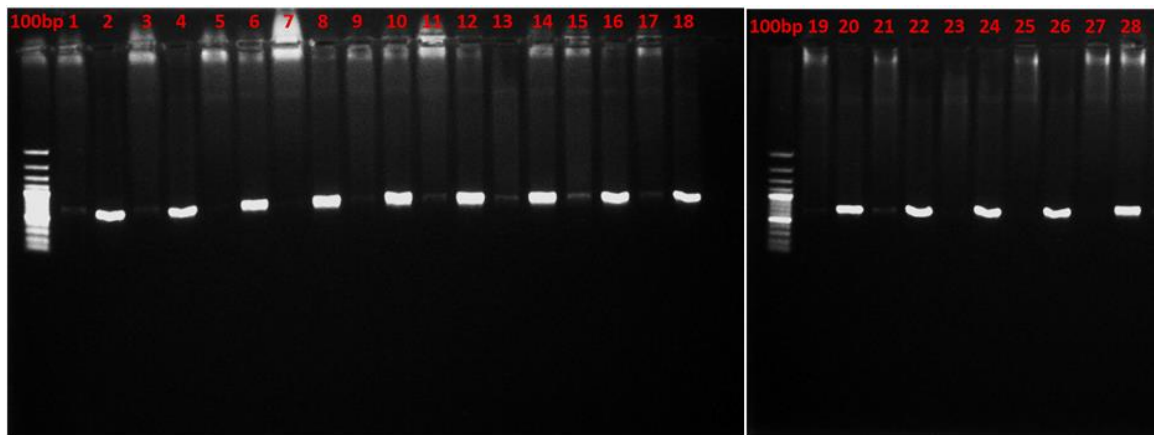


Figure 34: ERCC2 rs13181 and rs1799793 gene variants agarose gel electrophoresis results before and after Lu-177 PSMA treatment.

The relationship of this variant to treatment response and miRNA expression is being questioned.

This study found that people with higher levels of miRNA-182 and decreased DNA concentration after Lu-177 PSMA treatment are more likely to have a heterozygous mutant genotype in the ERCC2 Lys751Gln c.2251A>C (rs13181) variant ($p = 0.028$).

Table 13: Clinical data and Sanger sequencing results before and after treatment for 7 patients who received Lu-177 PSMA treatment.

Patient ID	Age	Lu-177 PSMA Activity	PSA Response	miR-182	miR-187	ERCC2 Lys751Gln c.2251A>C (rs13181)		ERCC2 Asp312Asn c.934G>A (rs1799793)		Gleason Score	Type of Metastasis
				After treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment		
Patient 1	67	150 mCi	Yes	Down	Up	Homozygous Mutant	Homozygous Mutant	Heterozygous Mutant	Heterozygous Mutant	4+5	Lung (Oligometastasis)
Patient 2	73	200 mCi	Yes	Up	Up	Heterozygous Mutant	Heterozygous Mutant	-	-	4+5	Limited Bone
Patient 3	64	200 mCi	Yes	Up	Down	Heterozygous Mutant	Heterozygous Mutant	Heterozygous Mutant	Heterozygous Mutant	5+5	Limited Bone
Patient 4	60	200 mCi	Yes	Down	Up	Wild Type	Wild Type	-	-	4+5	Limited Bone
Patient 5	68	250 mCi	No	Down	Down	Wild Type	Wild Type	-	-	4+6	Bone + Lymph Nodes
Patient 6	60	250 mCi	No	Down	Up	Wild Type	Wild Type	Wild Type	Wild Type	3+3	Widespread Bone
Patient 7	69	250 mCi	No	Up	Down	Heterozygous Mutant	Heterozygous Mutant	Homozygous Mutant	Homozygous Mutant	4+5	Widespread Bone

Table 14: The Sanger sequence results of patient 1 who received 150 mCi Lu-177 PSMA treatment showed a variation in the ERCC2 gene, c.2251A>C (p.Lys751Gln).

Patient ID	Age	Lu-177 PSMA Activity	PSA Response	miR-182	miR-187	ERCC2 Lys751Gln c.2251A>C (rs13181)		Gleason Score	Type of Metastasis
				After	After	Before	After		
Patient 1	67	150 mCi	Yes	Down	Up	Homozygous Mutant	Homozygous Mutant	4+5	Lung (Oligometastasis)
SANGER SEQUENCING RESULTS									
Before Treatment					After Treatment				

Table 15: The Sanger sequence results of patient 2 who received 200 mCi Lu-177 PSMA treatment showed a variation in the ERCC2 gene, c.2251A>C (p.Lys751Gln).

Patient ID	Age	Lu-177 PSMA Activity	PSA Response	miR-182	miR-187	ERCC2 Lys751Gln c.2251A>C (rs13181)		Gleason Score	Type of Metastasis
				After	After	Before	After		
Patient 2	73	200 mCi	Yes	Up	Up	Heterozygous Mutant	Heterozygous Mutant	4+5	Limited Bone
SANGER SEQUENCING RESULTS									
Before Treatment					After Treatment				

Table 16: The Sanger sequence results of patient 3 who received 200 mCi Lu-177 PSMA treatment showed a variation in the ERCC2 gene, c.2251A>C (p.Lys751Gln).

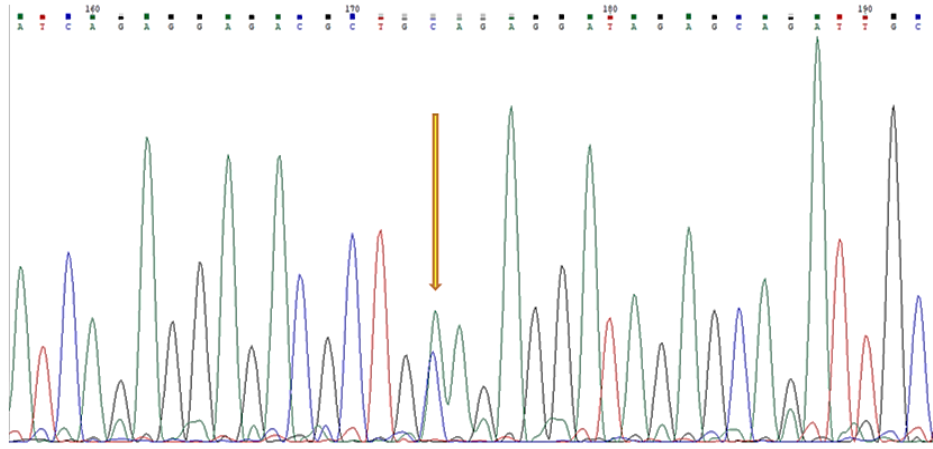
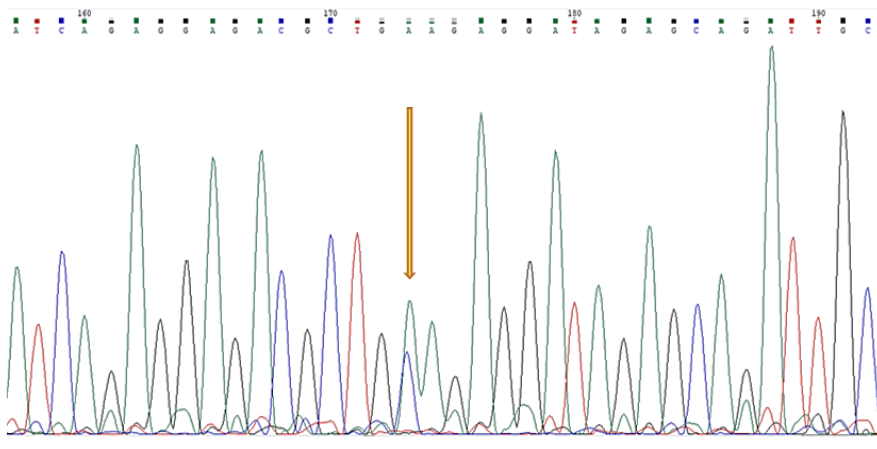
Patient ID	Age	Lu-177 PSMA Activity	PSA Response	miR-182	miR-187	ERCC2 Lys751Gln c.2251A>C (rs13181)		Gleason Score	Type of Metastasis
				After	After	Before	After		
Patient 3	64	200 mCi	Yes	Up	Down	Heterozygous Mutant	Heterozygous Mutant	5+5	Limited Bone
SANGER SEQUENCING RESULTS									
Before Treatment					After Treatment				
									

Table 17: The Sanger sequence results of patient 4 who received 200 mCi Lu-177 PSMA treatment showed a variation in the ERCC2 gene, c.2251A>C (p.Lys751Gln).

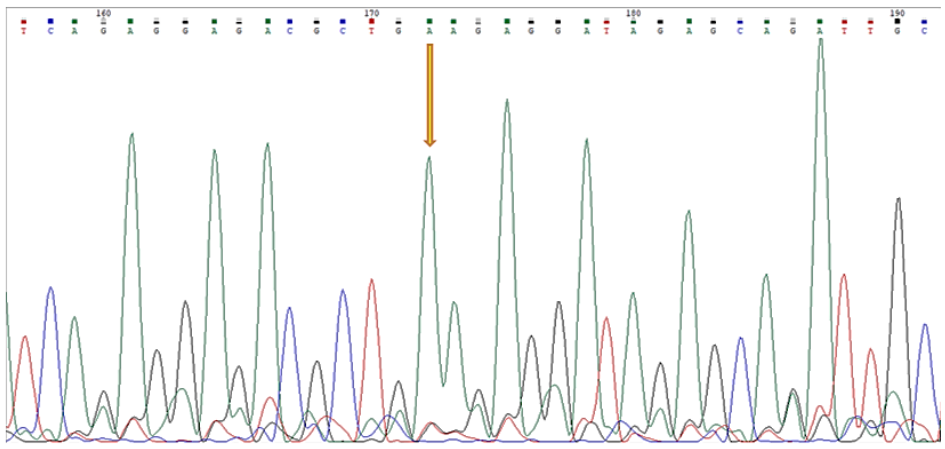
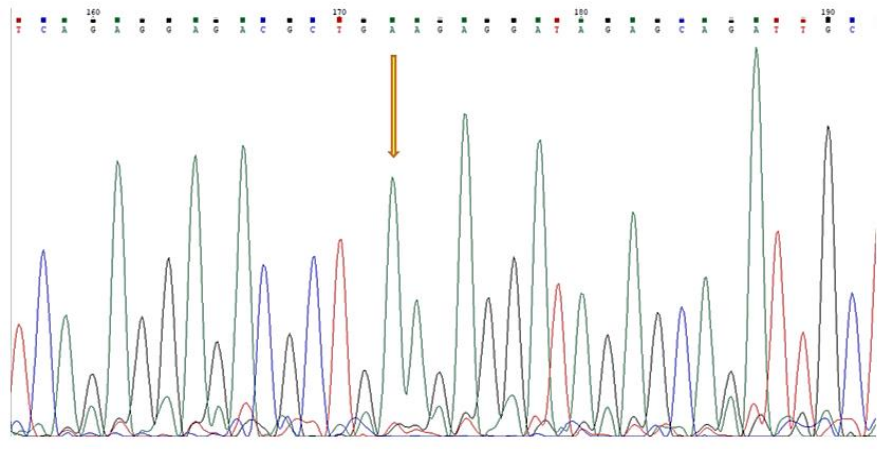
Patient ID	Age	Lu-177 PSMA Activity	PSA Response	miR-182	miR-187	ERCC2 Lys751Gln c.2251A>C (rs13181)		Gleason Score	Type of Metastasis
				After	After	Before	After		
Patient 4	60	200 mCi	Yes	Down	Up	Wild type	Wild type	4+5	Limited Bone
SANGER SEQUENCING RESULTS									
Before Treatment					After Treatment				
									

Table 18: The Sanger sequence results of patient 5 who received 250 mCi Lu-177 PSMA treatment showed a variation in the ERCC2 gene, c.2251A>C (p.Lys751Gln).

Patient ID	Age	Lu-177 PSMA Activity	PSA Response	miR-182	miR-187	ERCC2 Lys751Gln c.2251A>C (rs13181)		Gleason Score	Type of Metastasis
				After	After	Before	After		
Patient 5	68	250 mCi	No	Down	Down	Wild type	Wild type	4+6	Bone + Lymph Nodes
SANGER SEQUENCING RESULTS									
Before Treatment					After Treatment				

Table 19: The Sanger sequence results of patient 6 who received 250 mCi Lu-177 PSMA treatment showed a variation in the ERCC2 gene, c.2251A>C (p.Lys751Gln).

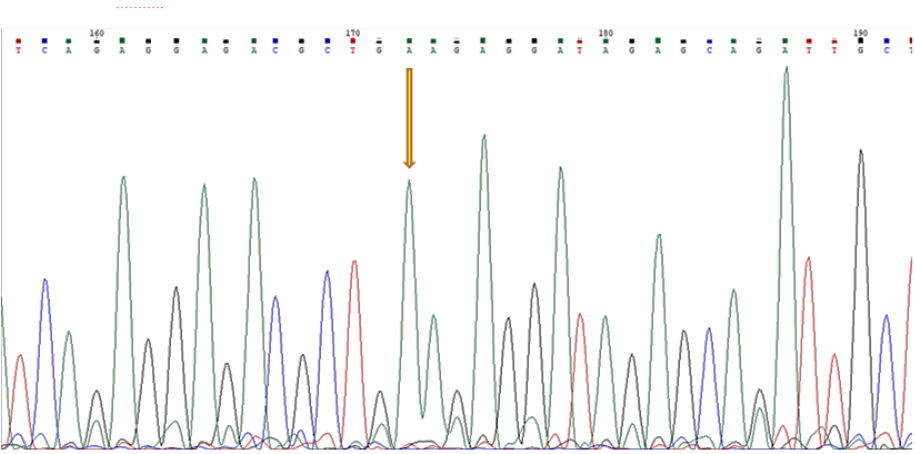
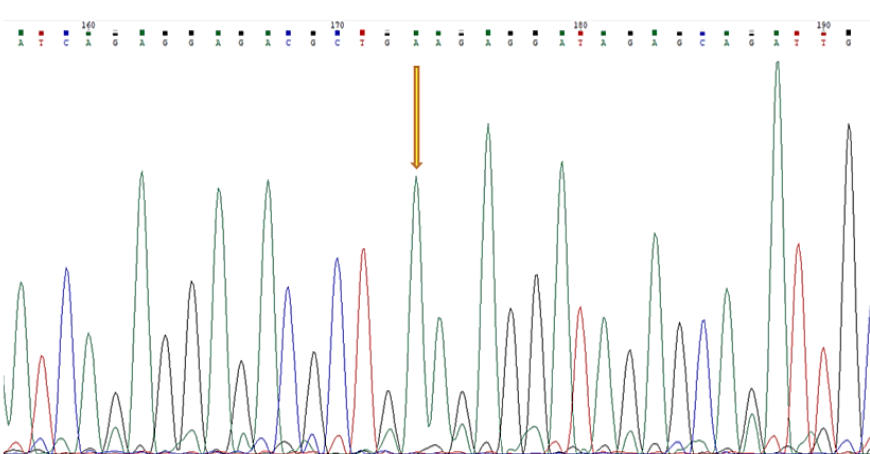
Patient ID	Age	Lu-177 PSMA Activity	PSA Response	miR-182	miR-187	ERCC2 Lys751Gln c.2251A>C (rs13181)		Gleason Score	Type of Metastasis
				After	After	Before	After		
Patient 6	60	250 mCi	No	Down	Up	Wild type	Wild type	3+3	Widespread Bone
SANGER SEQUENCING RESULTS									
Before Treatment					After Treatment				
									

Table 20: The Sanger sequence results of patient 7 who received 250 mCi Lu-177 PSMA treatment showed a variation in the ERCC2 gene, c.2251A>C (p.Lys751Gln).

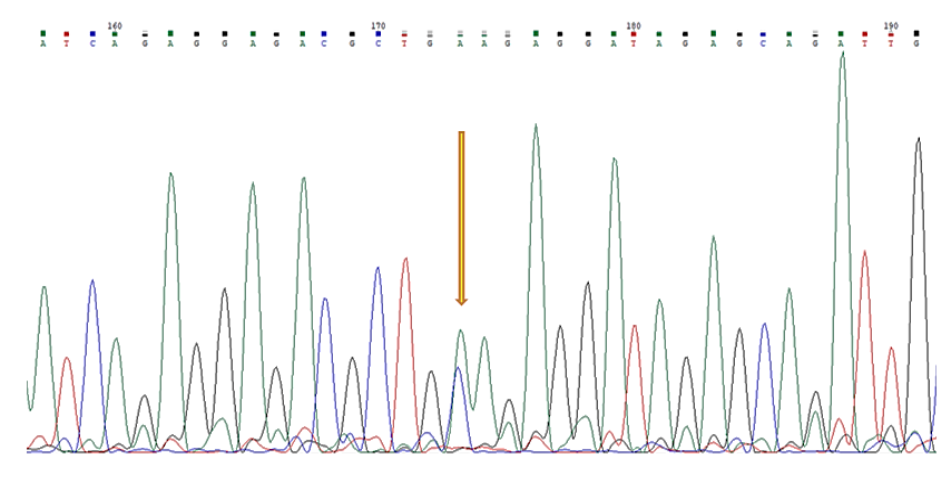
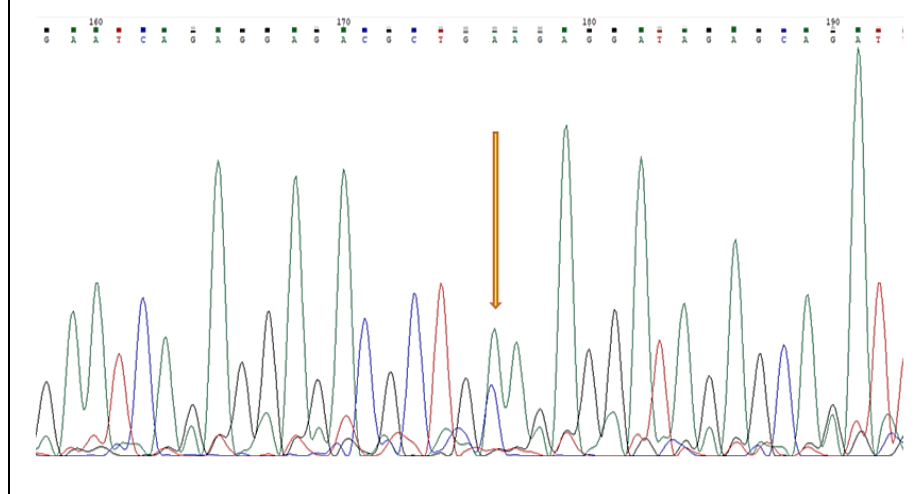
Patient ID	Age	Lu-177 PSMA Activity	PSA Response	miR-182	miR-187	ERCC2 Lys751Gln c.2251A>C (rs13181)		Gleason Score	Type of Metastasis
				After	After	Before	After		
Patient 7	69	250 mCi	No	Up	Down	Heterozygous Mutant	Heterozygous Mutant	4+5	Widespread Bone
SANGER SEQUENCING RESULTS									
Before Treatment					After Treatment				
									

Table 21: The Sanger sequence results of patient 1 who received 150 mCi Lu-177 PSMA treatment showed a variation in the ERCC2 Asp312Asn c.934G>A (rs1799793).

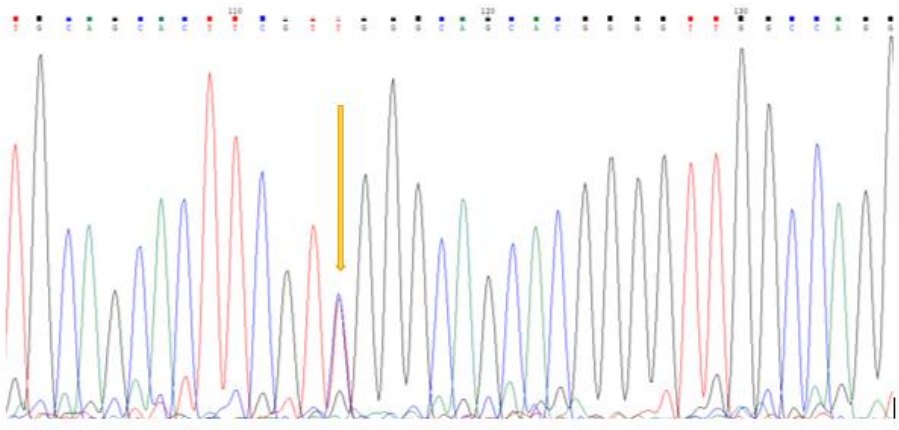
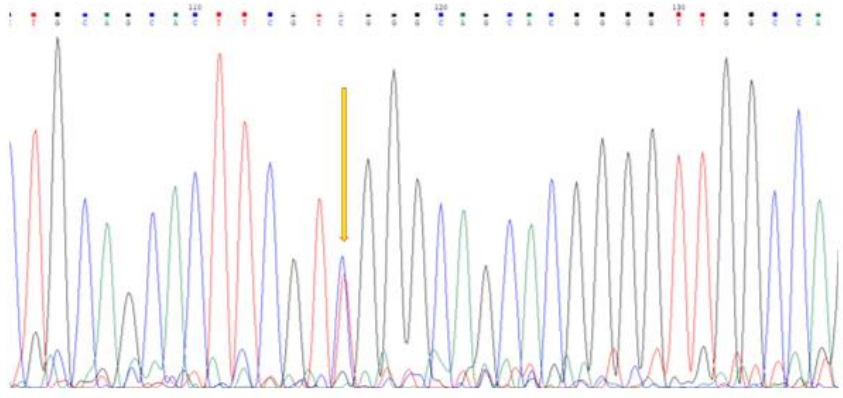
Patient ID	Age	Lu-177 PSMA Activity	PSA Response	miR-182	miR-187	ERCC2 Asp312Asn (rs1799793)		Gleason Score	Type of Metastasis
				After	After	Before	After		
Patient 1	67	150 mCi	Yes	Down	Up	Heterozygous Mutant	Heterozygous Mutant	4+5	Lung (Oligometastasis)
SANGER SEQUENCING RESULTS									
Before Treatment					After Treatment				
									

Table 22: The Sanger sequence results of patient 3 who received 200 mCi Lu-177 PSMA treatment showed a variation in the ERCC2 Asp312Asn c.934G>A (rs1799793).

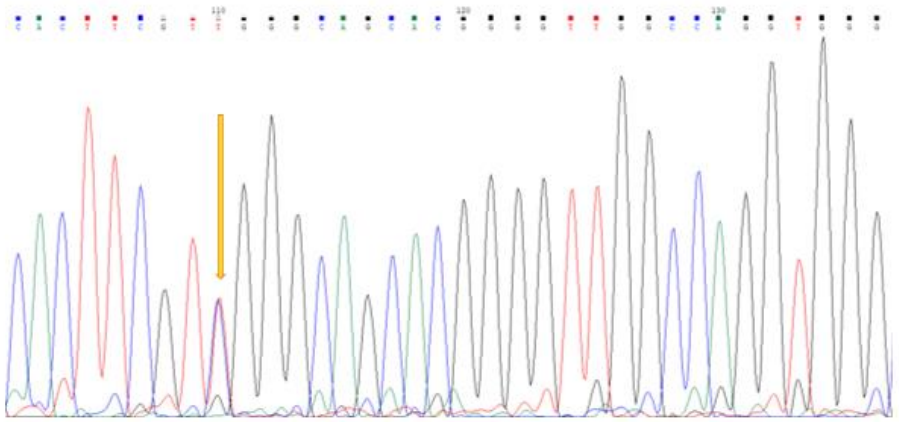
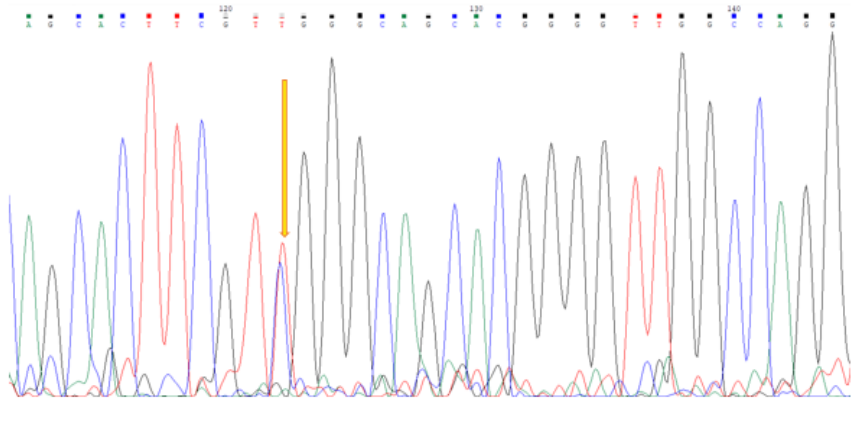
Patient ID	Age	Lu-177 PSMA Activity	PSA Response	miR-182	miR-187	ERCC2 Asp312Asn (rs1799793)		Gleason Score	Type of Metastasis
				After	After	Before	After		
Patient 3	64	200 mCi	Yes	Up	Down	Heterozygous Mutant	Heterozygous Mutant	5+5	Limited Bone
SANGER SEQUENCING RESULTS									
Before Treatment					After Treatment				
									

Table 23: The Sanger sequence results of patient 6 who received 250 mCi Lu-177 PSMA treatment showed a variation in the ERCC2 Asp312Asn c.934G>A (rs1799793).

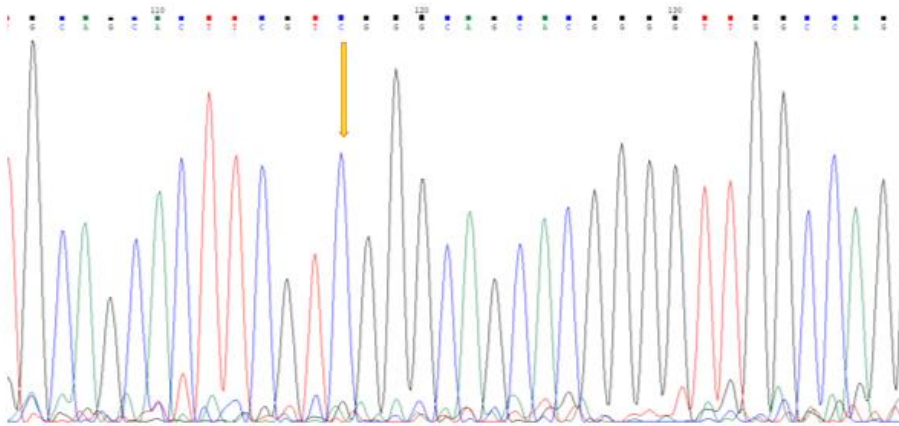
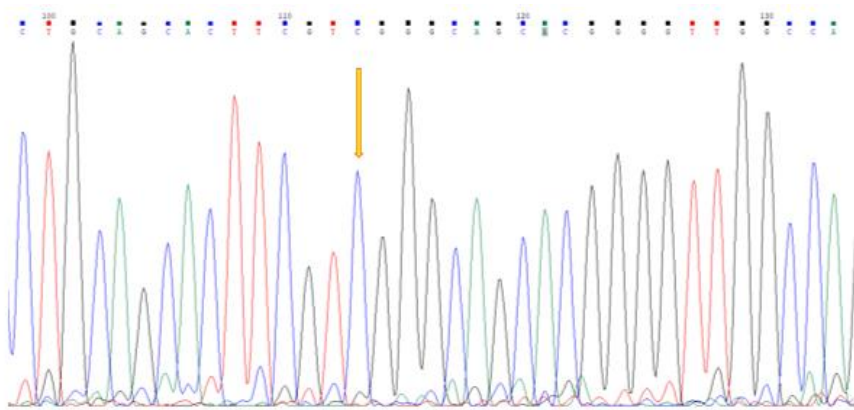
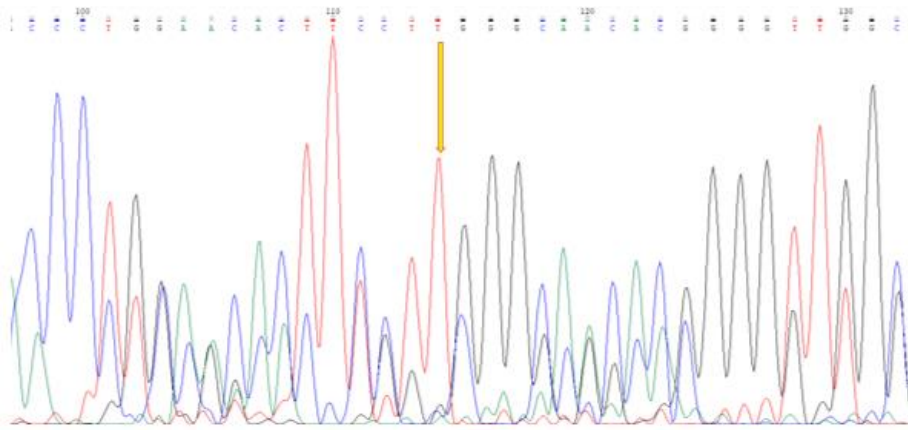
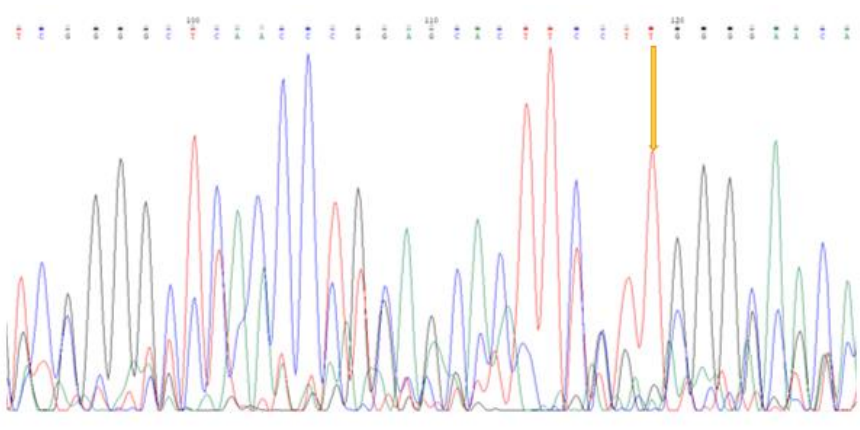
Patient ID	Age	Lu-177 PSMA Activity	PSA Response	miR-182	miR-187	ERCC2 Asp312Asn (rs1799793)		Gleason Score	Type of Metastasis
				After	After	Before	After		
Patient 6	60	250 mCi	No	Down	Up	Wild-type	Wild-type	3+3	Widespread Bone
SANGER SEQUENCING RESULTS									
Before Treatment					After Treatment				
									

Table 24: The Sanger sequence results of patient 7 who received 250 mCi Lu-177 PSMA treatment showed a variation in the ERCC2 Asp312Asn c.934G>A (rs1799793).

Patient ID	Age	Lu-177 PSMA Activity	PSA Response	miR-182	miR-187	ERCC2 Asp312Asn (rs1799793)		Gleason Score	Type of Metastasis
				After	After	Before	After		
Patient 7	69	250 mCi	No	Up	Down	Homozygous Mutant	Homozygous Mutant	4+5	Widespread Bone
SANGER SEQUENCING RESULTS									
Before Treatment					After Treatment				
									

5. DISCUSSION AND CONCLUSION

Nuclear medicine is a rapidly evolving field, and personalized treatments are becoming increasingly important. One such treatment is PSMA-labeled Lu-177 therapy, which is a targeted therapy for advanced prostate cancer.

The Lu-177 isotope binds to the PSMA ligand, which is overexpressed in prostate cancer cells. PSMA ligands enter cells by binding to receptors on the cell surface. Once inside the cell, the Lu-177 isotope emits beta radiation, which damages the DNA of the cancer cells. This damage can lead to the death of the cancer cells.

PSMA-labeled Lu-177 therapy is a promising new treatment for advanced prostate cancer. This type of therapy is called targeted therapy because it targets the cancer cells specifically, minimizing damage to the healthy tissue around them.

This could have a positive impact on the outcomes of patients with prostate cancer. Thus, Lu-177 beta radiation that enters the cell causes chain breaks in the cell's DNA or ionization in water molecules in the cytoplasm, leading to cell death.

An international multicenter phase III study, VISION, demonstrated prolonged overall survival in advanced PSMA-positive mCRPC patients with Lu-PSMA-617. The study was approved by the European Medicines Agency and the U.S. Food and Drug Administration.

Although Lu-177 PSMA treatment has achieved significant results in advanced prostate cancer, approximately 30% of patients become resistant to Lu-177 PSMA treatment. The causes of radioresistance in prostate cancer are not fully understood, but miRNAs are thought to play a role in cell signaling pathways. Identifying a panel of miRNAs that could be used to diagnose prostate cancer could be a valuable tool, as radioresistance biomarkers are currently lacking (128, 129).

This thesis investigates the potential of miRNAs as biomarkers and therapeutic targets for prostate cancer. miRNAs are small RNA molecules that can be used to identify and track cancer progression. They can also be used to develop new treatments for cancer (88-90).

Several physiological and metabolic processes, notably in controlling gene expression, have been shown to be significantly influenced. It is also essential for gene silencing in the majority of multicellular animals, as it silences particular genes. (91, 92).

Recent research suggests that miRNAs are implicated in the biological processes of several types of cancer. Researchers have proposed that restoring or suppressing

miRNA function can enhance various cancer therapy approaches (93). Resistance and progression are still frequent, even though radionuclide treatment dosages are standardized for each individual. Regulatory elements and genes can influence the cell cycle, differentiation, and death. Tumor suppressor genes and oncogenes are two examples of regulatory elements that can control the environmental variables that contribute to cancer (94).

This makes miRNAs attractive biomarkers for determining radiation sensitivity and creating patient-specific treatments. In conclusion, miRNAs may provide a novel treatment avenue for individuals with prostate cancer who want individualized care.

Using miRNAs in cancer diagnosis can help to plan treatment, improve therapeutic sensitivity, reduce the likelihood of many adverse medication reactions and toxicity, and even help with treatment monitoring. miRNAs are becoming a key therapeutic target in the treatment of cancer as more data shows their strong correlation with the disease (97).

Prostate cancer is characterized by dysregulated miRNA expression, and oncogene and tumor suppressor gene expression may also be controlled by miRNAs. Cancer patients may benefit from miRNA modification therapy, which may be used to gauge therapeutic response and forecast the efficacy of treatments like chemotherapy and radiation. Resistance to therapy is still a significant issue, though (97-100).

Examples of miRNA therapeutic approaches include oligonucleotides, synthetic small compounds, and viral or non-viral transfection. Oligonucleotides and other small molecules can be used to interfere with the function of miRNAs. This can be done by either directly binding to the miRNA and preventing it from binding to its target gene, or by indirectly interfering with the regulation of miRNAs by other transcription factors (101, 102).

As underlined earlier, personalized medicine is increasingly being used and promoted nowadays, which is remarkable. There is growing evidence that miRNA can influence how cells react to ionizing radiation, making them more or less radiosensitive through a number of distinct mechanisms. In order to change the activation of cell cycle checkpoints, this also entails altering the tumor microenvironment, DNA repair processes, and apoptosis.

Some miRNAs regulate the tumor microenvironment, cell cycle progression, apoptosis, and signaling pathways associated with radioactivity (103).

Concern about the tumor microenvironment has generated a lot of interest in miRNAs. They control the stem cell properties in the tumor radiation response, the epithelial-mesenchymal transition, and the DNA repair process (104).

Additional investigation is necessary to completely comprehend these systems. The modality (or way of administering the radiation), radiation dose, tumor stage/grade, presence or lack thereof various medical conditions, and inherent malignancy microenvironment all have an impact on radiation response rates. Radioactive resistance is a complex issue (105, 106).

As possible indicators for the existence or for the prognosis of the disease, dysregulated miRNAs in this disease have been postulated to contribute to the disease's development (130). The miRNA expression patterns caused by radiation treatment in patients with prostate cancer have very seldom been studied in clinical research. Zedan et al. investigated the effects of radiation therapy on miRNA expression in prostate cancer patients (131). They found that the levels of miRNA-221 and miRNA-93 were decreased, and they suggested that these miRNAs may be radiosensitive (131, 132).

The cell cycle and the repair of DNA damage may be indirectly impacted by ionizing radiation's ability to change the expression of miRNAs (15). miRNA-99a and miRNA-100 are two miRNAs that play a role in DNA repair. The inhibition of them can lead to impaired DNA repair and increased cancer risk. This is because these miRNAs play a role in preventing cell death and promoting DNA repair. When these miRNAs are inhibited, cells are more likely to die from DNA damage, or to accumulate DNA damage that can lead to cancer (133, 134).

miRNA-521 could be a promising target for the development of novel radiation therapies for LNCaP cells. By restoring DNA repair protein, miRNA-521 can make LNCaP cells more sensitive to radiation, which could lead to improved treatment outcomes (135).

miRNA-191 is a promising target for new cancer therapies. By targeting miRNA-191, we may be able to make prostate cancer cells more sensitive to radiation and other cancer treatments.

When compared to normal prostate cells, the miRNA miR-107 is more often downregulated in prostate cancer cells and tissues. Granulin, a protein that encourages cell growth and survival, is prevented from being produced in PC3 cells when miR-107 is overexpressed. It is possible to treat prostate cancer by increasing the radiation sensitivity of PC3 cells by inhibiting granulin expression (136).

miRNA-498 has been associated with prostate cancer cell proliferation, radiosensitivity, invasion, and migration, according to research by Duan et al (137). In addition, researchers found that miRNA-498 is underexpressed in LNCaP and DU145 cells after exposure to ionizing radiation. This causes radiation resistance in these cells. miRNA-498 reduces radiation-induced apoptosis by regulating the expression of BAX and Bcl-2, which results in radiation resistance (137).

In the same prostate cancer cell lines, miRNA-18a is upregulated, and Hsu et al. suggest that this miRNA may have an oncomiRNA role in the growth of cancer (138). Another finding made by Yang et al. is that miRNA-18a is controlled by a protein that, when produced at low levels, both protects cells against radiation and induces apoptosis (139). The radiation response in prostate cancer tissue samples and cell lines has recently been studied using miRNA-541-3p. When exposed to radiotherapy, a variety of prostate cancer cells exhibit an overexpression of miRNA-541-3p despite its low expression in prostate cancer tissues. Thus, miRNA-541-3p directly interacts with a protein by the mimic method, enhancing apoptosis and boosting radiosensitivity (140).

Iinuma et al. found that miRNA-93 levels in extracellular vesicles in the serum of patients who underwent low-dose rapid prostate brachytherapy were significantly lower than those of patients who did not undergo the therapy. This suggests that miRNA-93 may be a biomarker for response to the therapy in patients with prostate cancer (141).

The expression of miRNA-145 in the tumor tissue of 30 individuals with prostate cancer was examined in a research study. According to the study, miRNA-145 may enhance the effectiveness of radiation treatment by lowering the rate at which radiation-induced DNA double-strand breaks may be repaired (142).

Numerous cancers, including prostate cancer, have been related to miRNAs such as miRNA-182 and miRNA-187. These two have been shown to be altered in prostate cancer cells (17). They can be used as biomarkers to diagnose and monitor prostate cancer. According to studies, those with prostate cancer had significantly higher levels of miRNA-182 compared to those without the disease. miRNA-187 levels, on the other hand, are either reduced or unchanged in men with prostate cancer (17-19).

The miRNA-182 that we targeted is also one of the miRNAs that are associated with a variety of cellular processes like DNA repair pathways (143). According to Baumann et al., miRNA-182's function alters during prostate cancer, possibly supporting early oncogenic alterations, but switching to a protective state in high-risk prostate cancer (130). It has been reported that miRNA-182 has a complicated, bimodal role in prostate cancer (130). In low-grade, localized prostate cancer, increased expression of miRNA-182 may promote tumor growth and progression. However, in high-grade, aggressive prostate cancer, higher magnitude changes in miRNA-182 expression may actually protect against disease recurrence or not be selected for by the tumor. Their investigation suggests that miRNA-182 may have different effects on prostate cancer depending on the stage of disease, and that further research is needed to understand its full role in this cancer (130).

Studies have shown that miRNA-187-3p can be used to treat cancer by targeting genes that are involved in cancer cell growth and survival (144).

However, despite the mounting evidence, a study of the literature shows that miRNA expression levels can change following prostate cancer irradiation (145, 146). The unpredictable variations in miRNA expression patterns within the same cell line have drawn much attention due to their strong dependence on the radiation dose applied to the cells and the time it takes for the cells to recover (147).

Although there are few publications on the relationship between miRNA-182 and prostate cancer, as mentioned above, none are associated with Lu-177 PSMA treatment. The molecular mechanisms underlying the effects of radiotherapy on miRNA-182 and the cluster to which it belongs remain largely unknown, despite the widespread use of radiotherapy as a treatment for prostate cancer treatment. Radiation therapy is a common treatment for the disease, but its effectiveness can be reduced by the development of radiation resistance and the recurrence of cancer in the same area (20).

Our main goals were to investigate the relationship between Lu-177 PSMA treatment and the expression levels of miRNA-182 and 187 in prostate cancer patients with and without radionuclide therapy, as well as the difference in expression levels before and after therapy.

To compare the levels of miRNA-182 and miRNA-187 in patients with prostate cancer who received Lu-177 PSMA treatment with those who did not receive treatment, we measured the levels of these miRNAs in both groups. We also measured miRNA-182 and miRNA-187 levels in patients who received Lu-177 PSMA treatment at three different activity amounts: 150 mCi, 200 mCi, and 250 mCi.

The results showed that the miRNA-182 and miRNA-187 levels were significantly higher in patients who received Lu-177 PSMA treatment compared to patients who did not receive it ($p < 0.0001$).

Nayak et al. reported that miRNA-182 expression is often high in prostate cancer, while miRNA-187 is decreased (17). Our study results are consistent with Nayak et al.'s studies (17) for miRNA-182 but opposite for miRNA-187. In our study, we also found that both miRNA-182 and miRNA-187 expression levels were high in prostate cancer patients who did not receive any treatment, including Lu-177 PSMA therapy. This result confirms that miRNA-182 is a candidate oncological miRNA and also shows miRNA-187 as a candidate oncological miRNA for prostate cancer. Additionally, it has been determined that the expression levels of miRNA-182 and miRNA-187 are even higher in prostate cancer patients who receive different treatments, including Lu-177 PSMA. As a result of this, it may be possible that miRNA-182 and miRNA-187 have a role in the prostate cancer cells' radiation response. This result is consistent with Lin et al.'s studies (20). They also showed that the expression of miRNA-182 increased after radiotherapy and thus made the cells radiosensitive. They suggested that miRNA-182 could alter radiation-induced oxidative stress, thereby affecting the radiosensitivity of HNSCC cells (20).

The most important point to consider when evaluating this study group is that the 25 prostate cancer patients who received treatment were also treated with different treatments before receiving Lu-177 PSMA therapy. Therefore, it is clear that these results cannot be attributed solely to Lu-177 PSMA therapy, even though blood samples were taken after Lu-177 PSMA therapy. Radiotherapy treatment has significantly increased the expression levels of miRNA-182 and miRNA-187.

These results need to be confirmed, and more study is required to determine exactly how miRNA-182 and miRNA-187 contribute to the radiation response of advanced prostate cancer patients. However, the research findings suggest that miRNA-182 and miRNA-187 could be possible targets for new prostate cancer treatments and monitoring response to Lu-177 PSMA treatment.

Our first study, the results of which we presented above, investigated the relationship between the levels of miRNA-182 and miRNA-187 expression and the application of radiotherapy, including Lu-177 PSMA. The second stage of the study includes the evaluation of the levels of miRNA-182 and miRNA-187 expression in patients who received only Lu-177 PSMA treatment, as well as their treatment responses before and after treatment.

When looking at the relationship between the expression levels of target miRNAs and Lu-177 treatment in different dosages (150, 200, and 250 mCi), miRNAs have different expression patterns before and after treatment, depending on the dose and patient.

According to the medical literature, as mentioned previously, miRNA-182 is known to exhibit bimodal activity (130). Our results confirm this. In three different dose groups, miRNA-182 levels were found to be up-regulated, down-regulated, or did not change. Although miRNA-187 expression also differed between pre- and post-treatment, as with miRNA-182, miRNA-187 showed a more stable profile.

The change in miRNA expression was used to determine the response to Lu-177 PSMA treatment by examining at whether there was a PSA response and DNA concentration after the treatment.

When these patients' PSA responses were analyzed, it was shown that the PSA responses of the 150 and 200 mCi recipients were better. In the patient group receiving 250 mCi, there was no PSA response.

No association was found between patients' PSA response and alterations in miRNA expressions. This could be because of a variety of things, including the specific type of cancer, where it is in the cancer's progression, or the patient's unique genetic profile. According to Nayak et al., miRNA-187 expression and PSA levels in prostate cancer were inversely correlated (17).

More research is needed to understand the role of miRNAs in prostate cancer and how they can be used to predict PSA response.

As a result of our study, we observed changes in total circulating DNA concentration depending on the treatment and even the dose of the treatment. We compared the DNA concentrations of patients who received radiotherapy, including Lu-177, with those who did not receive treatment before. We determined that the DNA concentration increased after the treatment. In addition, we determined that 7 patients showed changes before and after Lu-177 treatment.

Leon et al. has showed that in lymphoma, lung, ovary, uterus, and cervical tumors, radiation therapy was associated with a decrease in DNA levels in 66 to 90% of patients (148). In contrast, only 16 to 33% of patients with glioma, breast, colon, and rectal tumors experienced a decrease in DNA levels after radiation therapy. A decrease in DNA levels was generally associated with a reduction in tumor size and pain relief. However, an increase or lack of change in DNA levels was associated with a lack of response to treatment (148). Also, Jahr et al. explain that dead cells are normally cleared by phagocytes, but this process may not be efficient in and around tumors (149). As a result, some of the DNA released from degenerating cells may escape clearance by phagocytes and enter the bloodstream. The fate of this DNA is determined by a number of complex processes, which may vary depending on a number of factors. This may explain the large variation in the amounts and composition of DNA found in the circulation of cancer patients (149). Additionally, Peled's study found that a reduction in tumor burden through surgery and chemotherapy was associated with a reduction in cfDNA levels (150). However, in a minority of patients, an increase in cfDNA levels after treatment may indicate the presence of a residual tumor and a higher risk of recurrence. The study authors suggest that further follow-up is needed to assess the long-term outcomes of these patients (150).

Our study showed parallelism with the results of these studies. While the DNA concentrations of patients with treatment response tended to decrease after radiotherapy application, an increase in DNA concentration was observed in patients who did not respond to treatment compared to their pre-treatment concentrations.

It was found that the DNA concentration decreased in 50% of prostate cancer patients with PSA response after treatment, while the DNA concentration decreased in only 33.3% of patients without PSA response after treatment. An increase in the post-treatment blood DNA level in patients who do not respond to treatment, and a decrease in the DNA level in patients who respond to treatment may be indicative of the efficacy of Lu-177 PSMA therapy.

Therefore, measuring circulating DNA before and after treatment may be beneficial for individual management of prostate cancer patients. Our findings suggest that circulating free DNA measured using our simple method may be a potential biomarker for assessing treatment response. However, the amount of circulating DNA that is from cancer cells versus noncancer cells is unknown.

Apart from the relationship between PSA response and DNA concentration, a significant relationship was found between the change in miRNA expression after treatment and DNA concentration. When miRNA-182 expression level increases after treatment, DNA concentration decreases, while when miRNA-182 level decreases, it increases ($p = 0.028$).

Variations in DNA repair genes can influence the clinical outcome of anticancer therapy that damages DNA, as well as individual DNA repair capacity, DNA damage levels, and cancer risk (151).

Our research aimed to better understand the molecular biology of prostate cancer and identify the key players involved in the response to radiotherapy. To this end, we examined variations in the ERCC2 gene in blood samples from prostate cancer patients. We used Sanger sequencing, a gold standard method, to validate the existence of the detected variants.

We investigated the relationship between two common non-synonymous single-nucleotide variants in the ERCC2 gene (rs13181 and rs1799793) and Lu-177 PSMA therapy in patients with prostate cancer.

It was determined that 3 of the untreated prostate cancer patients were heterozygous mutants, and 2 were wild-type. Of the patients who received treatment, 3 were heterozygous for the ERCC2 Lys751Gln c.2251A>C (rs13181) mutation, 1 was wild-type, and 1 was not detected. Of the patients who received treatment, 4 were homozygous for the ERCC2 Asp312Asn (rs1799793) mutation, and 1 was wild-type. It was determined that 3 of the untreated prostate cancer patients were heterozygous mutants, and 2 were homozygous mutant.

These variants were also examined before and after Lu-177 PSMA treatment. No change in genotype was detected in any of the 7 patients after treatment. Our study found that prostate cancer patients had both the rs1799793 variant and the rs13181 wild-type, heterozygous mutant, and homozygous mutant genotypes. Patients who responded to PSA after treatment had homozygous mutant (25%), heterozygous mutant (50%), and

wild-type (25%) genotypes for the ERCC2 rs13181 variant; patients who did not respond to PSA had wild-type (66.66%) and heterozygous mutant (33.34%) genotypes. For ERCC2 rs1799793, only heterozygous mutants (100%) had PSA response, while wild-type (50%) and homozygous mutants (50%) had no PSA response.

Due to their potential prognostic usefulness in the progression and therapy of prostate cancer, substantial research has been conducted on SNPs affecting codons 312, and 751 (rs1799793, and rs13181, respectively) (9). A study conducted in Saudi Arabia found that genetic variants of ERCC2 gene were associated with an increased risk of prostate cancer. In their study, Abdulmonem et al. showed that ERCC2 rs13181 is not associated with prostate cancer risk, but that individuals who carry the ERCC2-rs1799793 variant in a recessive manner are at an increased risk of prostate cancer in their study population (81). This is consistent with our findings. In addition, a study conducted in the Asian population reported that the homozygous genotype of ERCC2-rs1799793 variant is associated with prostate cancer (9). Our research group also includes individuals with homozygous mutant genotypes. According to the pre- and post-treatment analyses of this variant, it can be suggested that it is a genotype that can be associated with radioresistance.

Zanusso et al. showed that the ERCC2-rs1799793 variant is one of the genetic changes that are significantly associated with biochemical recurrence in prostate cancer patients (152). It has been reported that people who are homozygous for ERCC2-rs1799793 variant have lower NER activity than people who have wild-type allele (152). The results of this study contradicted the previous findings. On the contrary, it was observed that the patient with homozygous mutant genotype for rs1799793 was resistant to treatment. It can be concluded that DNA repair, which is dependent on the activation of NER activity, is the underlying cause of this resistance. Zhang et al. investigated the relationship between cisplatin resistance and ERCC2 Lys751Gln (rs13181) (153). They emphasized that this variant could be a valid biomarker candidate to predict cisplatin resistance (153). They suggested that individuals with the C allele of the ERCC2 rs13181 gene are less able to repair DNA damage, which can lead to cell death. This is also related with prolonged S-phase. The S phase is the phase of the cell cycle during which DNA is replicated. A prolonged S phase means that the cell takes longer to replicate its DNA, which can lead to errors in DNA replication. Also, individuals with the C allele of the ERCC2 rs13181 gene are more likely to experience serious DNA damage when they are treated with cisplatin (153).

Our study is consistent with these findings. Individuals with heterozygous mutant and homozygous mutant genotypes were observed to respond to Lu-177 PSMA therapy. Tejasvi et al. worked on oral squamous cell carcinoma and they found that people with heterozygous mutant genotypes of the ERCC2 rs12181 and rs1799793 were more likely to develop the disease (154). They also suggested that these these genotypes may be associated with sensitivity to chemotherapy (154). This result is consistent with our findings. 50% of the patients who responded to Lu-177 PSMA therapy are heterozygous mutants, and their response to treatment suggests that they are not resistant to this treatment.

It has been reported that NER was significantly defective in people with the ERCC2 751(rs13181) AC/CC genotype (154). This result is consistent with our study. It allows us to conclude that individuals carrying the rs13181 variant as heterozygous mutants and homozygous mutants showed treatment response, and this was mediated by the inhibitory effect on NER activity.

As we can see above, our study found both different and similar results from previous studies. The most likely reason for the different results is that our patients had advanced prostate cancer, were treated with Lu-177 PSMA, and ethnic differences.

To sum up, considering rs13181 variant, it is suggested that the heterozygous mutant and homozygous mutant genotypes may inhibit the activity of the NER. It can be interpreted that these genotypes make cells sensitive to Lu-177 PSMA therapy and provides a basis for treatment response. Additionally, considering rs1799793 variant, wild-type and homozygous mutant genotypes may contribute to resistance to Lu-177 PSMA therapy by stimulating NER pathways and activating DNA repair mechanisms.

ERCC2 variants were also evaluated in terms of miRNA expression, treatment response, and DNA concentration.

This study found that people with higher levels of miRNA-182 and decreased DNA concentration after Lu-177 PSMA treatment are more likely to have a heterozygous mutation in the ERCC2 rs13181variant ($p = 0.028$). This finding could have implications for the development of new treatments for cancer. The mechanism behind this finding is not fully understood, but it is thought that miRNA-182 may play a role in regulating the expression of the ERCC2 gene. The ERCC2 gene is involved in DNA repair, and mutations in this gene can lead to an increased risk of cancer.

It is possible that miRNA-182 up-regulation may lead to decreased expression of the ERCC2 gene, which could make individuals more sensitive to Lu-177 PSMA treatment. This is just one possible explanation for the finding, and more research is needed to confirm this mechanism.

This study, conducted at a single institution, suggests that certain variations in the ERCC2 gene may be predictive of radiosensitivity and radioresistance after Lu-177 PSMA treatment. These findings warrant further investigation in larger studies to confirm their clinical significance.

Here are some questions that future studies need to answer in order to pave the way for the results of this study to be incorporated into clinical practice:

- What is the mechanism by which miRNA-182 and miRNA-187 affects the response to Lu-177 PSMA treatment?
- What are the molecular mechanisms underlying the expression differences in miRNA-182 and miRNA-187 expression levels that were observed due to the application of Lu-177 PSMA therapy alone or in combination with other treatment methods?
- Can miRNA-182 levels be used to predict the risk of developing prostate cancer?
- Can miRNA-182 levels be used to monitor the effectiveness of treatment for prostate cancer?

These are just a few of the questions that could be explored in future research on miRNA-182 and miRNA-187 and prostate cancer treatment. The results of this study provide a promising starting point for this research and could lead to new advances in the prevention, diagnosis, and treatment of this disease.

Conclusion

The results of this study are very promising. Our study results suggest that miRNA-182 may be a significant predictor of treatment response in patients with advanced castration-resistant metastatic prostate cancer. This could lead to the development of new personalized treatment strategies for these patients.

Additionally, the study's findings suggest that miRNA-182 levels may be used to determine the optimal dose of Lu-177 PSMA treatment. This could help to improve the efficacy and safety of this treatment.

Of course, more research is needed to confirm these findings and to determine the clinical utility of miRNA-182 as a biomarker for prostate cancer treatment. However, these results are a significant step forward in the understanding of the role of miRNAs in prostate cancer and their potential for clinical applications.

Some highlights are as follows:

- miRNA-182 and miRNA-187 expression levels have increased by Lu-177 PSMA in addition to other radiotherapy application than non-treated prostate cancer patients. PSA levels not changed and DNA concentration is increased.
- Patients with higher levels of miRNA-182 after treatment had better PSA responses and decreased DNA concentration. This suggests that miRNA-182 may be a prognostic indicator for radiosensitivity. In other words, patients with higher levels of miRNA-182 are more likely to respond well to Lu-177 PSMA treatment. Additionally, ERCC2 rs13181 heterozygous genotype may linked with increased miRNA-182 expression level and decreased DNA concentration after Lu-177 PSMA treatment.
- Optimal dose of Lu-177 PSMA treatment may vary depending on miRNA-182 levels. Patients with higher levels of miRNA-182 before the treatment may require dose optimization before treatment to achieve a good response.
- 50% of ERCC2 rs13181 heterozygous mutant genotype carriers and 50% of ERCC2 1799793 heterozygous mutant carriers have PSA response to Lu-177 PSMA treatment.

The study was conducted on a relatively small number of patients, single-institutional, and single-population based, so more research is needed to confirm these findings. However, this is a good model for personalized medicine. The results of this study are promising and suggest that miRNA-182 and miRNA-187 may be useful biomarkers for prostate cancer treatment with Lu-177 PSMA.

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7. APPENDICES

7.1. Ethical Approval



Sayı : 37068608-6100-15- 1814
Konu: Klinik Araştırmalar
Etik kurul Başvurusu hk.

30/01/2020

İlgili Makama (Nalan Alan Selçuk)

Yeditepe Üniversitesi Sağlık Bilimleri Fakültesi Moleküler Tıp ABD Prof. Dr. Turgay İsbir'in sorumlu araştırmacı olduğu "Lu-177 Prostat Spesifik Membran Antijeni (PSMA) Ligantları ile Tedavi Edilen Prostat Kanseri Hastalarda DNA Tamir Genlerindeki Varyasyonların Tedavi Cevabının Üzerine Etkisinin İncelenmesi" isimli araştırma projesine ait Klinik Araştırmalar Etik Kurulu (KA EK) Başvuru Dosyası (1796) kayıt Numaralı KA EK Başvuru Dosyası, Yeditepe Üniversitesi Klinik Araştırmalar Etik Kurulu tarafından 29.01.2020 tarihli toplantıda incelenmiştir.

Kurul tarafından yapılan inceleme sonucu, yukarıdaki isimi belirtilen çalışmanın yapılmasının etik ve bilimsel açıdan uygun olduğuna karar verilmiştir (KA EK Karar No: 1155).

Prof. Dr. Turgay ÇELİK
Yeditepe Üniversitesi
Klinik Araştırmalar Etik Kurulu Başkanı

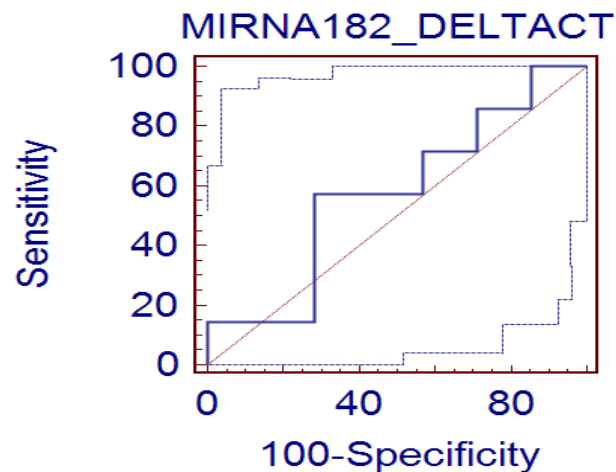
7.2. Patient Data

ROC Analysis

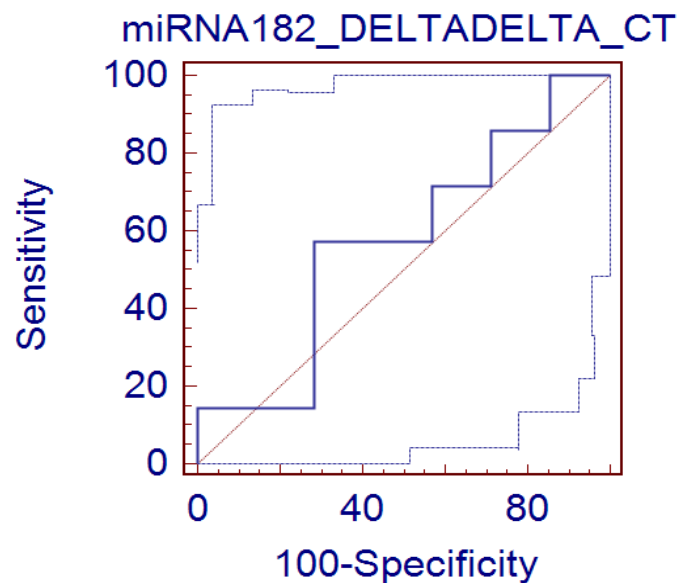
Variable	MIRNA182_DELTACT
Classification variable	DIAGNOSIS
Positive group	
DIAGNOSIS	= 1
Sample size	7
Negative group	
DIAGNOSIS	= 0
Sample size	7
Disease prevalence (%)	50,0
Area under the ROC curve (AUC)	0,571
Standard Error	0,158
95% Confidence Interval	0,289 to 0,822
z statistic	0,453
Significance level P (Area=0.5)	0,6508

Criterion values and coordinates of the ROC curve [Hide]

Criterion	Sensitivity	95% CI	Specificity	95% CI	+LR	95% CI	-LR	95% CI	+PV	95% CI	-PV	95% CI
>=-9,26	100,00	58,9 - 100,0	0,00	0,0 - 41,1	1,00				50,0	23,1 - 76,9		
>-9,26	100,00	58,9 - 100,0	14,29	2,4 - 57,8	1,17	0,2 - 7,2	0,00		53,8	25,2 - 80,7	100,0	16,5 - 100,0
>-3,285	85,71	42,2 - 97,6	14,29	2,4 - 57,8	1,00	0,2 - 6,3	1,00	0,2 - 6,3	50,0	21,2 - 78,8	50,0	8,2 - 91,8
>-1,425	85,71	42,2 - 97,6	28,57	4,5 - 70,7	1,20	0,4 - 4,0	0,50	0,08 - 3,3	54,5	23,5 - 83,1	66,7	11,6 - 94,5
>-0,275	71,43	29,3 - 95,5	28,57	4,5 - 70,7	1,00	0,3 - 3,5	1,00	0,3 - 3,5	50,0	18,9 - 81,1	50,0	8,3 - 91,7
>0,23	71,43	29,3 - 95,5	42,86	10,4 - 81,2	1,25	0,5 - 3,3	0,67	0,2 - 2,5	55,6	21,4 - 86,0	60,0	15,4 - 93,5
>0,24	57,14	18,8 - 89,6	42,86	10,4 - 81,2	1,00	0,3 - 2,9	1,00	0,3 - 2,9	50,0	16,0 - 84,0	50,0	12,4 - 87,6
>0,4	57,14	18,8 - 89,6	57,14	18,8 - 89,6	1,33	0,5 - 3,3	0,75	0,2 - 2,5	57,1	18,8 - 89,6	57,1	18,8 - 89,6
>0,56 *	57,14	18,8 - 89,6	71,43	29,3 - 95,5	2,00	0,9 - 4,4	0,60	0,1 - 2,6	66,7	22,7 - 94,7	62,5	24,7 - 91,0
>0,65	42,86	10,4 - 81,2	71,43	29,3 - 95,5	1,50	0,6 - 4,0	0,80	0,2 - 3,0	60,0	15,4 - 93,5	55,6	21,4 - 86,0
>1,095	28,57	4,5 - 70,7	71,43	29,3 - 95,5	1,00	0,3 - 3,5	1,00	0,3 - 3,5	50,0	8,3 - 91,7	50,0	18,9 - 81,1
>2,205	14,29	2,4 - 57,8	71,43	29,3 - 95,5	0,50	0,08 - 3,3	1,20	0,4 - 4,0	33,3	5,5 - 88,4	45,5	16,9 - 76,5
>2,47	14,29	2,4 - 57,8	85,71	42,2 - 97,6	1,00	0,2 - 6,3	1,00	0,2 - 6,3	50,0	8,2 - 91,8	50,0	21,2 - 78,8
>3,415	14,29	2,4 - 57,8	100,00	58,9 - 100,0			0,86		100,0	16,5 - 100,0	53,8	25,2 - 80,7
>5,13	0,00	0,0 - 41,1	100,00	58,9 - 100,0			1,00				50,0	23,1 - 76,9



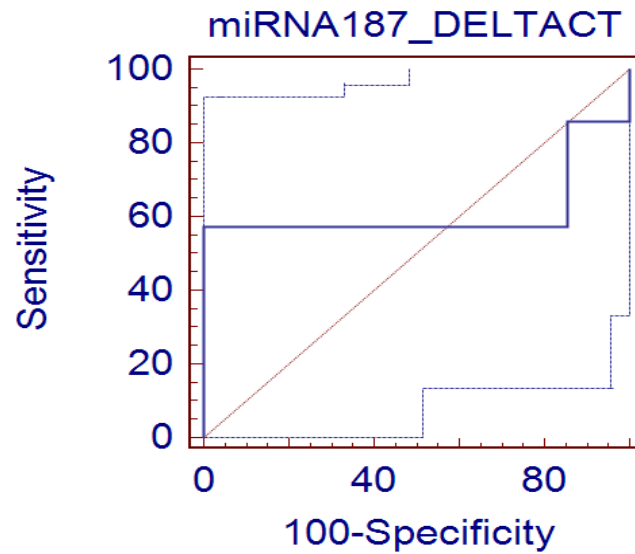
Sensitivity: 57,1
Specificity: 71,4
Criterion : >0,56



Variable	miRNA182_DELTADelta_CT
Classification variable	DIAGNOSIS
Positive group	
DIAGNOSIS	= 1
Sample size	7
Negative group	
DIAGNOSIS	= 0
Sample size	7
Disease prevalence (%)	50,0
Area under the ROC curve (AUC)	0,571
Standard Error	0,158
95% Confidence Interval	0,289 to 0,822
z statistic	0,453
Significance level P (Area=0.5)	0,6508

Criterion values and coordinates of the ROC curve [\[Hide\]](#)

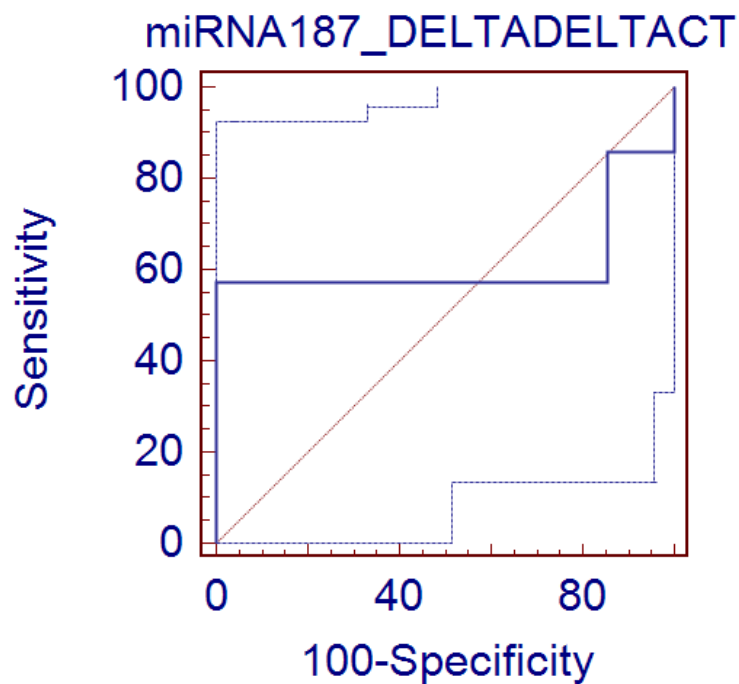
Criterion	Sensitivity	95% CI	Specificity	95% CI	+LR	95% CI	-LR	95% CI	+PV	95% CI	-PV	95% CI
>=-9,03	100,00	58,9 - 100,0	0,00	0,0 - 41,1	1,00				50,0	23,1 - 76,9		
>-9,03	100,00	58,9 - 100,0	14,29	2,4 - 57,8	1,17	0,2 - 7,2	0,00		53,8	25,2 - 80,7	100,0	16,5 - 100,0
>-3,055	85,71	42,2 - 97,6	14,29	2,4 - 57,8	1,00	0,2 - 6,3	1,00	0,2 - 6,3	50,0	21,2 - 78,8	50,0	8,2 - 91,8
>-1,195	85,71	42,2 - 97,6	28,57	4,5 - 70,7	1,20	0,4 - 4,0	0,50	0,08 - 3,3	54,5	23,5 - 83,1	66,7	11,6 - 94,5
>-0,045	71,43	29,3 - 95,5	28,57	4,5 - 70,7	1,00	0,3 - 3,5	1,00	0,3 - 3,5	50,0	18,9 - 81,1	50,0	8,3 - 91,7
>0,46	71,43	29,3 - 95,5	42,86	10,4 - 81,2	1,25	0,5 - 3,3	0,67	0,2 - 2,5	55,6	21,4 - 86,0	60,0	15,4 - 93,5
>0,47	57,14	18,8 - 89,6	42,86	10,4 - 81,2	1,00	0,3 - 2,9	1,00	0,3 - 2,9	50,0	16,0 - 84,0	50,0	12,4 - 87,6
>0,63	57,14	18,8 - 89,6	57,14	18,8 - 89,6	1,33	0,5 - 3,3	0,75	0,2 - 2,5	57,1	18,8 - 89,6	57,1	18,8 - 89,6
>0,79 *	57,14	18,8 - 89,6	71,43	29,3 - 95,5	2,00	0,9 - 4,4	0,60	0,1 - 2,6	66,7	22,7 - 94,7	62,5	24,7 - 91,0
>0,88	42,86	10,4 - 81,2	71,43	29,3 - 95,5	1,50	0,6 - 4,0	0,80	0,2 - 3,0	60,0	15,4 - 93,5	55,6	21,4 - 86,0
>1,325	28,57	4,5 - 70,7	71,43	29,3 - 95,5	1,00	0,3 - 3,5	1,00	0,3 - 3,5	50,0	8,3 - 91,7	50,0	18,9 - 81,1
>2,435	14,29	2,4 - 57,8	71,43	29,3 - 95,5	0,50	0,08 - 3,3	1,20	0,4 - 4,0	33,3	5,5 - 88,4	45,5	16,9 - 76,5
>2,7	14,29	2,4 - 57,8	85,71	42,2 - 97,6	1,00	0,2 - 6,3	1,00	0,2 - 6,3	50,0	8,2 - 91,8	50,0	21,2 - 78,8
>3,645	14,29	2,4 - 57,8	100,00	58,9 - 100,0			0,86		100,0	16,5 - 100,0	53,8	25,2 - 80,7
>5,36	0,00	0,0 - 41,1	100,00	58,9 - 100,0			1,00				50,0	23,1 - 76,9



Variable	miRNA187_DELTACT
Classification variable	DIAGNOSIS
Positive group	
DIAGNOSIS	= 1
Sample size	7
Negative group	
DIAGNOSIS	= 0
Sample size	7
Disease prevalence (%)	50,0
Area under the ROC curve (AUC)	0,612
Standard Error	0,155
95% Confidence Interval	0,324 to 0,851
z statistic	0,724
Significance level P (Area=0.5)	0,4688

Criterion values and coordinates of the ROC curve [\[Hide\]](#)

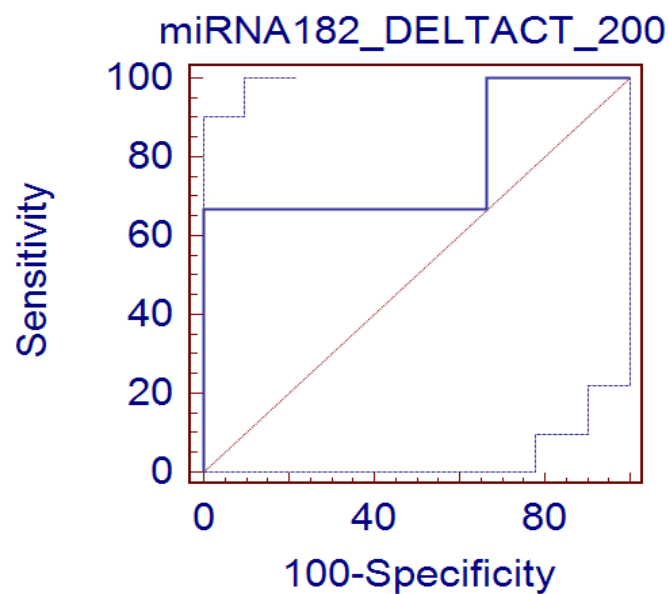
Criterion	Sensitivity	95% CI	Specificity	95% CI	+LR	95% CI	-LR	95% CI	+PV	95% CI	-PV	95% CI
< -9,745	0,00	0,0 - 41,1	100,00	58,9 - 100,0			1,00				50,0	23,1 - 76,9
<= -9,745	14,29	2,4 - 57,8	100,00	58,9 - 100,0			0,86		100,0	16,5 - 100,0	53,8	25,2 - 80,7
<= -9,385	28,57	4,5 - 70,7	100,00	58,9 - 100,0			0,71		100,0	19,3 - 100,0	58,3	27,8 - 84,7
<= -9,28	42,86	10,4 - 81,2	100,00	58,9 - 100,0			0,57		100,0	30,5 - 100,0	63,6	30,9 - 88,8
<= -8,93 *	57,14	18,8 - 89,6	100,00	58,9 - 100,0			0,43		100,0	40,2 - 100,0	70,0	34,8 - 93,0
<= -8,735	57,14	18,8 - 89,6	85,71	42,2 - 97,6	4,00	2,0 - 8,1	0,50	0,07 - 3,7	80,0	28,8 - 96,7	66,7	30,1 - 92,1
<= -8,7	57,14	18,8 - 89,6	71,43	29,3 - 95,5	2,00	0,9 - 4,4	0,60	0,1 - 2,6	66,7	22,7 - 94,7	62,5	24,7 - 91,0
<= -7,46	57,14	18,8 - 89,6	57,14	18,8 - 89,6	1,33	0,5 - 3,3	0,75	0,2 - 2,5	57,1	18,8 - 89,6	57,1	18,8 - 89,6
<= -7,385	57,14	18,8 - 89,6	42,86	10,4 - 81,2	1,00	0,3 - 2,9	1,00	0,3 - 2,9	50,0	16,0 - 84,0	50,0	12,4 - 87,6
<= -6,725	57,14	18,8 - 89,6	28,57	4,5 - 70,7	0,80	0,2 - 3,0	1,50	0,6 - 4,0	44,4	14,0 - 78,6	40,0	6,5 - 84,6
<= -5,15	57,14	18,8 - 89,6	14,29	2,4 - 57,8	0,67	0,1 - 4,6	3,00	1,2 - 7,4	40,0	12,4 - 73,6	25,0	4,1 - 79,7
<= -4,605	71,43	29,3 - 95,5	14,29	2,4 - 57,8	0,83	0,1 - 5,4	2,00	0,6 - 6,7	45,5	16,9 - 76,5	33,3	5,5 - 88,4
<= -1,825	85,71	42,2 - 97,6	14,29	2,4 - 57,8	1,00	0,2 - 6,3	1,00	0,2 - 6,3	50,0	21,2 - 78,8	50,0	8,2 - 91,8
<= 4,665	85,71	42,2 - 97,6	0,00	0,0 - 41,1	0,86				46,2	19,3 - 74,8	0,0	0,0 - 83,5
<= 6,275	100,00	58,9 - 100,0	0,00	0,0 - 41,1	1,00				50,0	23,1 - 76,9		



Variable	miRNA187_DELTADeltaTACT
Classification variable	DIAGNOSIS
Positive group	
DIAGNOSIS	= 1
Sample size	7
Negative group	
DIAGNOSIS	= 0
Sample size	7
Disease prevalence (%)	50,0
Area under the ROC curve (AUC)	0,612
Standard Error	0,155
95% Confidence Interval	0,324 to 0,851
z statistic	0,724
Significance level P (Area=0.5)	0,4688

Criterion values and coordinates of the ROC curve [\[Hide\]](#)

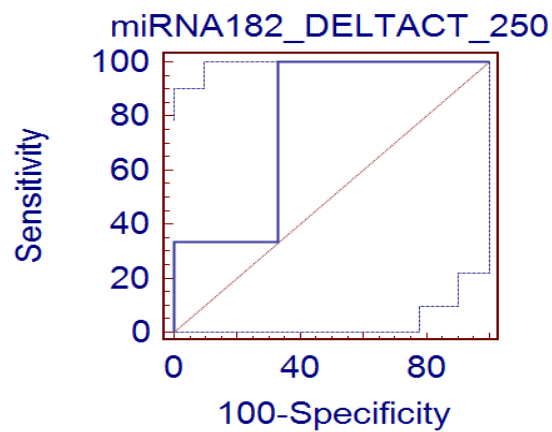
Criterion	Sensitivity	95% CI	Specificity	95% CI	+LR	95% CI	-LR	95% CI	+PV	95% CI	-PV	95% CI
< -4,144	0,00	0,0 - 41,1	100,00	58,9 - 100,0			1,00				50,0	23,1 - 76,9
<=-3,326 *	57,14	18,8 - 89,6	100,00	58,9 - 100,0			0,43		100,0	40,2 - 100,0	70,0	34,8 - 93,0
<=0,451	57,14	18,8 - 89,6	14,29	2,4 - 57,8	0,67	0,1 - 4,6	3,00	1,2 - 7,4	40,0	12,4 - 73,6	25,0	4,1 - 79,7
<=3,776	85,71	42,2 - 97,6	14,29	2,4 - 57,8	1,00	0,2 - 6,3	1,00	0,2 - 6,3	50,0	21,2 - 78,8	50,0	8,2 - 91,8
<=10,266	85,71	42,2 - 97,6	0,00	0,0 - 41,1	0,86				46,2	19,3 - 74,8	0,0	0,0 - 83,5
<=11,876	100,00	58,9 - 100,0	0,00	0,0 - 41,1	1,00				50,0	23,1 - 76,9		



Variable	miRNA182_DELTACT_200
Classification variable	DIAGNOSIS
Positive group	
DIAGNOSIS	= 1
Sample size	3
Negative group	
DIAGNOSIS	= 0
Sample size	3
Disease prevalence (%)	50,0
Area under the ROC curve (AUC)	0,778
Standard Error	0,207
95% Confidence Interval	0,312 to 0,971
z statistic	1,340
Significance level P (Area=0.5)	0,1803

Criterion values and coordinates of the ROC curve [\[Hide\]](#)

Criterion	Sensitivity	95% CI	Specificity	95% CI	+LR	95% CI	-LR	95% CI	+PV	95% CI	-PV	95% CI
< -3,285	0,00	0,0 - 69,5	100,00	30,5 - 100,0			1,00				50,0	12,4 - 87,6
<= -3,285	33,33	5,5 - 88,4	100,00	30,5 - 100,0			0,67		100,0	16,5 - 100,0	60,0	15,4 - 93,5
<= -0,275 *	66,67	11,6 - 94,5	100,00	30,5 - 100,0			0,33		100,0	19,3 - 100,0	75,0	20,3 - 95,9
<= 0,4	66,67	11,6 - 94,5	66,67	11,6 - 94,5	2,00	0,6 - 6,2	0,50	0,05 - 4,8	66,7	11,6 - 94,5	66,7	11,6 - 94,5
<= 0,56	66,67	11,6 - 94,5	33,33	5,5 - 88,4	1,00	0,2 - 6,0	1,00	0,2 - 6,0	50,0	8,3 - 91,7	50,0	8,2 - 91,8
<= 1,095	100,00	30,5 - 100,0	33,33	5,5 - 88,4	1,50	0,3 - 7,4	0,00		60,0	15,4 - 93,5	100,0	16,5 - 100,0
<= 2,47	100,00	30,5 - 100,0	0,00	0,0 - 69,5	1,00				50,0	12,4 - 87,6		



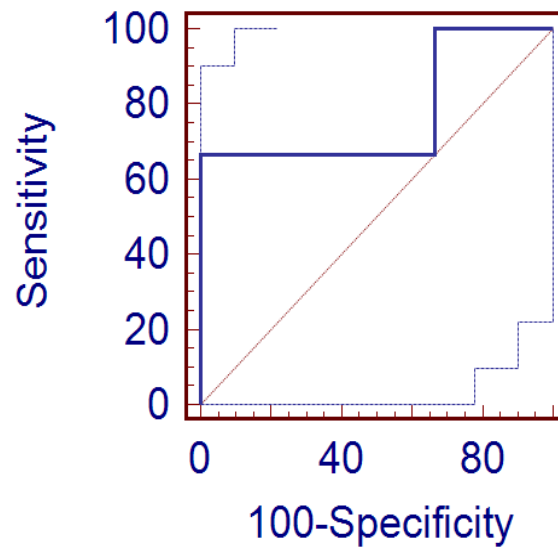
Sensitivity: 100,0
Specificity: 66,7
Criterion : >= -1,425

Variable	miRNA182_DELTACT_250
Classification variable	DIAGNOSIS
Positive group	
DIAGNOSIS	= 1
Sample size	3
Negative group	
DIAGNOSIS	= 0
Sample size	3
Disease prevalence (%)	50,0
Area under the ROC curve (AUC)	0,778
Standard Error	0,207
95% Confidence Interval	0,312 to 0,971
z statistic	1,340
Significance level P (Area=0.5)	0,1803

Criterion values and coordinates of the ROC curve [Hide]

Criterion	Sensitivity	95% CI	Specificity	95% CI	+LR	95% CI	-LR	95% CI	+PV	95% CI	-PV	95% CI
>= -9,26	100,00	30,5 - 100,0	0,00	0,0 - 69,5	1,00				50,0	12,4 - 87,6		
> -9,26	100,00	30,5 - 100,0	33,33	5,5 - 88,4	1,50	0,3 - 7,4	0,00		60,0	15,4 - 93,5	100,0	16,5 - 100,0
> -1,425 *	100,00	30,5 - 100,0	66,67	11,6 - 94,5	3,00	1,3 - 6,7	0,00		75,0	20,3 - 95,9	100,0	19,3 - 100,0
> 0,65	66,67	11,6 - 94,5	66,67	11,6 - 94,5	2,00	0,6 - 6,2	0,50	0,05 - 4,8	66,7	11,6 - 94,5	66,7	11,6 - 94,5
> 2,205	33,33	5,5 - 88,4	66,67	11,6 - 94,5	1,00	0,2 - 6,0	1,00	0,2 - 6,0	50,0	8,2 - 91,8	50,0	8,3 - 91,7
> 3,415	33,33	5,5 - 88,4	100,00	30,5 - 100,0			0,67		100,0	16,5 - 100,0	60,0	15,4 - 93,5
> 5,13	0,00	0,0 - 69,5	100,00	30,5 - 100,0			1,00				50,0	12,4 - 87,6

miRNA182_DELTADELTACT_200

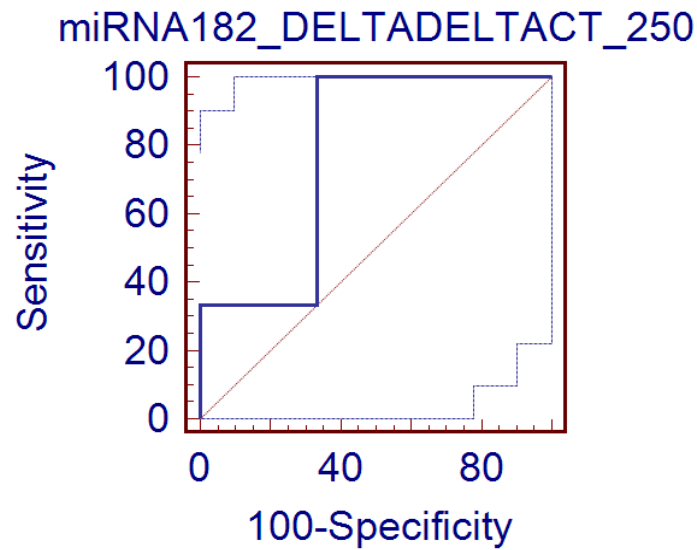


Sensitivity: 66,7
Specificity: 100,0
Criterion : <=-0,045

Variable	miRNA182_DELTADELTACT_200
Classification variable	DIAGNOSIS
Positive group	
DIAGNOSIS	= 1
Sample size	3
Negative group	
DIAGNOSIS	= 0
Sample size	3
Disease prevalence (%)	50,0
Area under the ROC curve (AUC)	0,778
Standard Error	0,207
95% Confidence Interval	0,312 to 0,971
z statistic	1,340
Significance level P (Area=0.5)	0,1803

Criterion values and coordinates of the ROC curve [\[Hide\]](#)

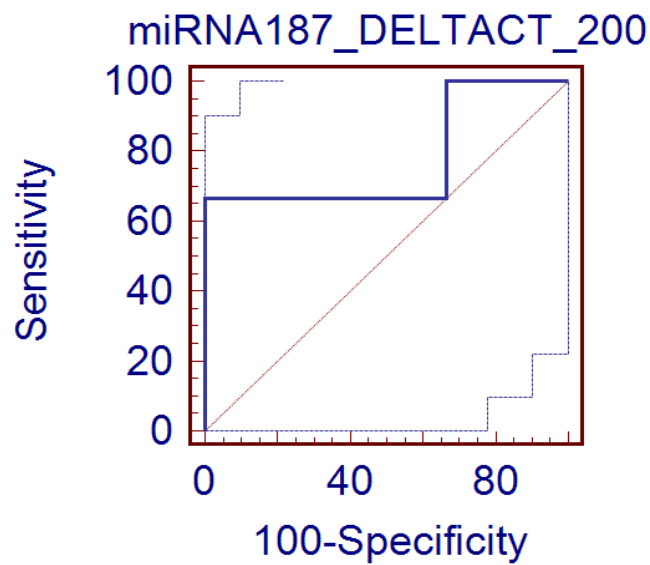
Criterion	Sensitivity	95% CI	Specificity	95% CI	+LR	95% CI	-LR	95% CI	+PV	95% CI	-PV	95% CI
< -3,055	0,00	0,0 - 69,5	100,00	30,5 - 100,0			1,00				50,0	12,4 - 87,6
<= -3,055	33,33	5,5 - 88,4	100,00	30,5 - 100,0			0,67		100,0	16,5 - 100,0	60,0	15,4 - 93,5
<= -0,045 *	66,67	11,6 - 94,5	100,00	30,5 - 100,0			0,33		100,0	19,3 - 100,0	75,0	20,3 - 95,9
<= 0,63	66,67	11,6 - 94,5	66,67	11,6 - 94,5	2,00	0,6 - 6,2	0,50	0,05 - 4,8	66,7	11,6 - 94,5	66,7	11,6 - 94,5
<= 0,79	66,67	11,6 - 94,5	33,33	5,5 - 88,4	1,00	0,2 - 6,0	1,00	0,2 - 6,0	50,0	8,3 - 91,7	50,0	8,2 - 91,8
<= 1,325	100,00	30,5 - 100,0	33,33	5,5 - 88,4	1,50	0,3 - 7,4	0,00		60,0	15,4 - 93,5	100,0	16,5 - 100,0
<= 2,7	100,00	30,5 - 100,0	0,00	0,0 - 69,5	1,00				50,0	12,4 - 87,6		



Variable	miRNA182_DELTADeltaTACT_250
Classification variable	DIAGNOSIS
Positive group	
DIAGNOSIS	= 1
Sample size	3
Negative group	
DIAGNOSIS	= 0
Sample size	3
Disease prevalence (%)	50,0
Area under the ROC curve (AUC)	0,778
Standard Error	0,207
95% Confidence Interval	0,312 to 0,971
z statistic	1,340
Significance level P (Area=0.5)	0,1803

Criterion values and coordinates of the ROC curve [\[Hide\]](#)

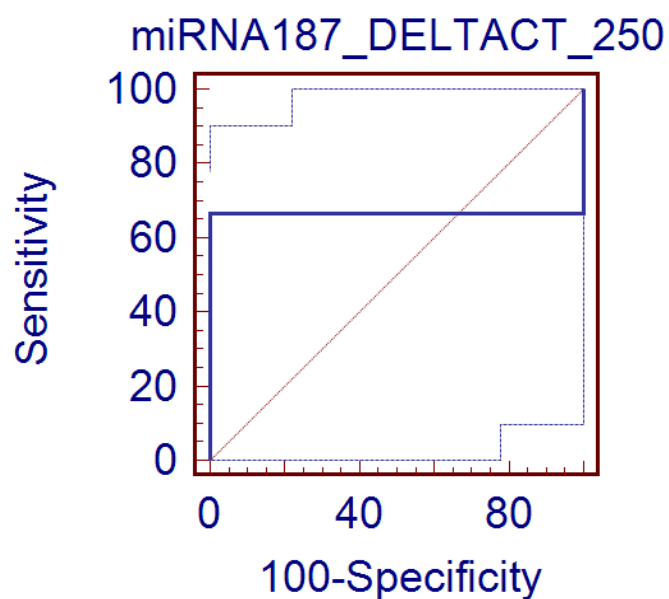
Criterion	Sensitivity	95% CI	Specificity	95% CI	+LR	95% CI	-LR	95% CI	+PV	95% CI	-PV	95% CI
>=-9,03	100,00	30,5 - 100,0	0,00	0,0 - 69,5	1,00				50,0	12,4 - 87,6		
>-9,03	100,00	30,5 - 100,0	33,33	5,5 - 88,4	1,50	0,3 - 7,4	0,00		60,0	15,4 - 93,5	100,0	16,5 - 100,0
>-1,195 *	100,00	30,5 - 100,0	66,67	11,6 - 94,5	3,00	1,3 - 6,7	0,00		75,0	20,3 - 95,9	100,0	19,3 - 100,0
>0,88	66,67	11,6 - 94,5	66,67	11,6 - 94,5	2,00	0,6 - 6,2	0,50	0,05 - 4,8	66,7	11,6 - 94,5	66,7	11,6 - 94,5
>2,435	33,33	5,5 - 88,4	66,67	11,6 - 94,5	1,00	0,2 - 6,0	1,00	0,2 - 6,0	50,0	8,2 - 91,8	50,0	8,3 - 91,7
>3,645	33,33	5,5 - 88,4	100,00	30,5 - 100,0			0,67		100,0	16,5 - 100,0	60,0	15,4 - 93,5
>5,36	0,00	0,0 - 69,5	100,00	30,5 - 100,0			1,00				50,0	12,4 - 87,6



Variable	miRNA187_DELTACT_200
Classification variable	DIAGNOSIS
Positive group	
DIAGNOSIS	= 1
Sample size	3
Negative group	
DIAGNOSIS	= 0
Sample size	3
Disease prevalence (%)	50,0
Area under the ROC curve (AUC)	0,778
Standard Error	0,207
95% Confidence Interval	0,312 to 0,971
z statistic	1,340
Significance level P (Area=0.5)	0,1803

Criterion values and coordinates of the ROC curve [Hide]

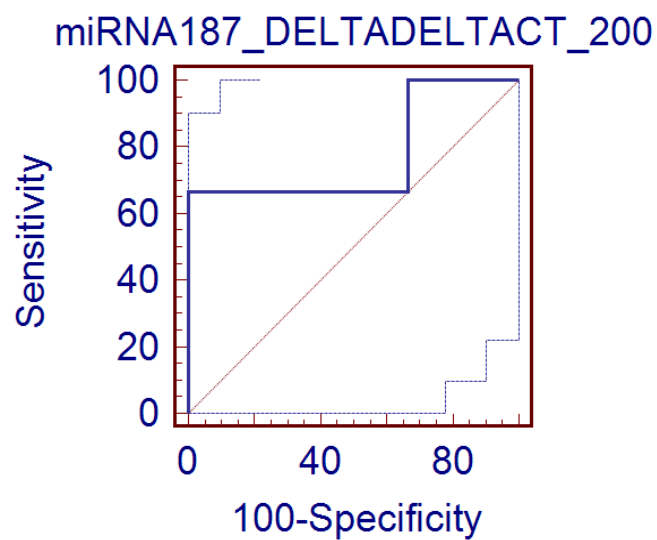
Criterion	Sensitivity	95% CI	Specificity	95% CI	+LR	95% CI	-LR	95% CI	+PV	95% CI	-PV	95% CI
< -9,745	0,00	0,0 - 69,5	100,00	30,5 - 100,0			1,00				50,0	12,4 - 87,6
<=-9,745	33,33	5,5 - 88,4	100,00	30,5 - 100,0			0,67		100,0	16,5 - 100,0	60,0	15,4 - 93,5
<=-9,385 *	66,67	11,6 - 94,5	100,00	30,5 - 100,0			0,33		100,0	19,3 - 100,0	75,0	20,3 - 95,9
<=-8,7	66,67	11,6 - 94,5	66,67	11,6 - 94,5	2,00	0,6 - 6,2	0,50	0,05 - 4,8	66,7	11,6 - 94,5	66,7	11,6 - 94,5
<=-5,15	66,67	11,6 - 94,5	33,33	5,5 - 88,4	1,00	0,2 - 6,0	1,00	0,2 - 6,0	50,0	8,3 - 91,7	50,0	8,2 - 91,8
<=-1,825	100,00	30,5 - 100,0	33,33	5,5 - 88,4	1,50	0,3 - 7,4	0,00		60,0	15,4 - 93,5	100,0	16,5 - 100,0
<=4,665	100,00	30,5 - 100,0	0,00	0,0 - 69,5	1,00				50,0	12,4 - 87,6		



Variable	miRNA187_DELTACT_250
Classification variable	DIAGNOSIS
Positive group	
DIAGNOSIS	= 1
Sample size	3
Negative group	
DIAGNOSIS	= 0
Sample size	3
Disease prevalence (%)	50,0
Area under the ROC curve (AUC)	0,667
Standard Error	0,238
95% Confidence Interval	0,227 to 0,947
z statistic	0,699
Significance level P (Area=0.5)	0,4843

Criterion values and coordinates of the ROC curve [Hide]

Criterion	Sensitivity	95% CI	Specificity	95% CI	+LR	95% CI	-LR	95% CI	+PV	95% CI	-PV	95% CI
>=-8,93	100,00	30,5 - 100,0	0,00	0,0 - 69,5	1,00				50,0	12,4 - 87,6		
>-8,93	66,67	11,6 - 94,5	0,00	0,0 - 69,5	0,67				40,0	6,5 - 84,6	0,0	0,0 - 83,5
>-8,735	66,67	11,6 - 94,5	33,33	5,5 - 88,4	1,00	0,2 - 6,0	1,00	0,2 - 6,0	50,0	8,3 - 91,7	50,0	8,2 - 91,8
>-7,46	66,67	11,6 - 94,5	66,67	11,6 - 94,5	2,00	0,6 - 6,2	0,50	0,05 - 4,8	66,7	11,6 - 94,5	66,7	11,6 - 94,5
>-7,385 *	66,67	11,6 - 94,5	100,00	30,5 - 100,0			0,33		100,0	19,3 - 100,0	75,0	20,3 - 95,9
>-4,605	33,33	5,5 - 88,4	100,00	30,5 - 100,0			0,67		100,0	16,5 - 100,0	60,0	15,4 - 93,5
>6,275	0,00	0,0 - 69,5	100,00	30,5 - 100,0			1,00				50,0	12,4 - 87,6

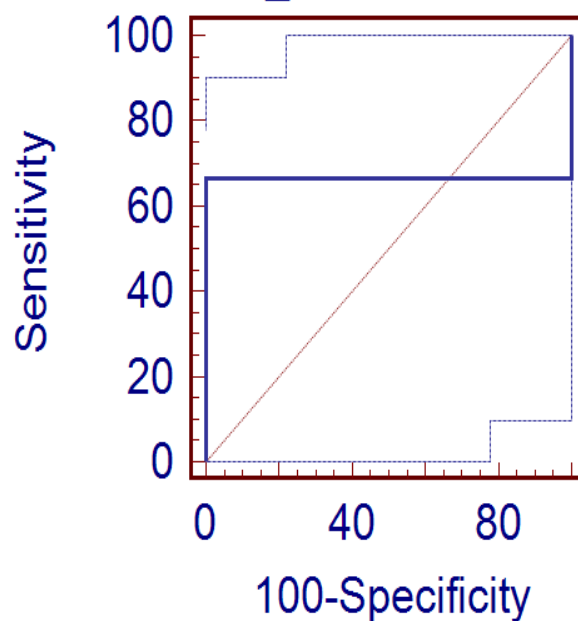


Variable	miRNA187_DELTADeltaTACT_200
Classification variable	DIAGNOSIS
Positive group	
DIAGNOSIS	= 1
Sample size	3
Negative group	
DIAGNOSIS	= 0
Sample size	3
Disease prevalence (%)	50,0
Area under the ROC curve (AUC)	0,778
Standard Error	0,207
95% Confidence Interval	0,312 to 0,971
z statistic	1,340
Significance level P (Area=0.5)	0,1803

Criterion values and coordinates of the ROC curve [\[Hide\]](#)

Criterion	Sensitivity	95% CI	Specificity	95% CI	+LR	95% CI	-LR	95% CI	+PV	95% CI	-PV	95% CI
< -4,144	0,00	0,0 - 69,5	100,00	30,5 - 100,0			1,00				50,0	12,4 - 87,6
<=-3,784 *	66,67	11,6 - 94,5	100,00	30,5 - 100,0			0,33		100,0	19,3 - 100,0	75,0	20,3 - 95,9
<=0,451	66,67	11,6 - 94,5	33,33	5,5 - 88,4	1,00	0,2 - 6,0	1,00	0,2 - 6,0	50,0	8,3 - 91,7	50,0	8,2 - 91,8
<=3,776	100,00	30,5 - 100,0	33,33	5,5 - 88,4	1,50	0,3 - 7,4	0,00		60,0	15,4 - 93,5	100,0	16,5 - 100,0
<=10,266	100,00	30,5 - 100,0	0,00	0,0 - 69,5	1,00				50,0	12,4 - 87,6		

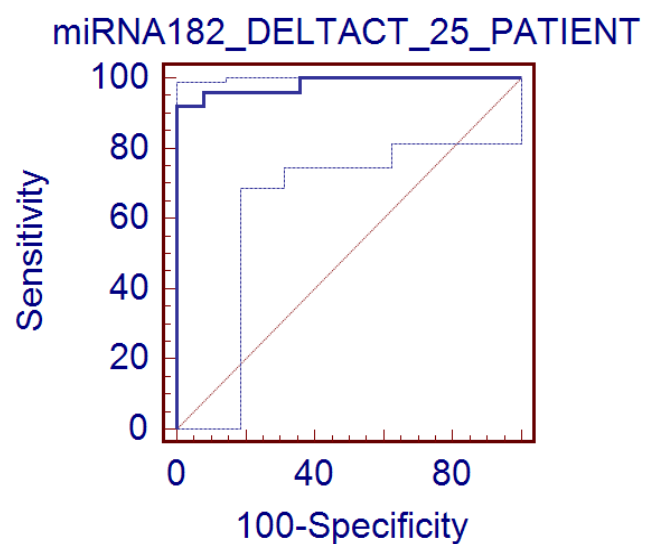
miRNA187_DELTADeltaTACT_250



Variable	miRNA187_DELTADeltaTACT_250
Classification variable	DIAGNOSIS
Positive group	
DIAGNOSIS	= 1
Sample size	3
Negative group	
DIAGNOSIS	= 0
Sample size	3
Disease prevalence (%)	50,0
Area under the ROC curve (AUC)	0,667
Standard Error	0,238
95% Confidence Interval	0,227 to 0,947
z statistic	0,699
Significance level P (Area=0.5)	0,4843

Criterion values and coordinates of the ROC curve [Hide]

Criterion	Sensitivity	95% CI	Specificity	95% CI	+LR	95% CI	-LR	95% CI	+PV	95% CI	-PV	95% CI
>=-3,326	100,00	30,5 - 100,0	0,00	0,0 - 69,5	1,00				50,0	12,4 - 87,6		
>-3,326	66,67	11,6 - 94,5	0,00	0,0 - 69,5	0,67				40,0	6,5 - 84,6	0,0	0,0 - 83,5
>-3,134	66,67	11,6 - 94,5	33,33	5,5 - 88,4	1,00	0,2 - 6,0	1,00	0,2 - 6,0	50,0	8,3 - 91,7	50,0	8,2 - 91,8
>-1,859	66,67	11,6 - 94,5	66,67	11,6 - 94,5	2,00	0,6 - 6,2	0,50	0,05 - 4,8	66,7	11,6 - 94,5	66,7	11,6 - 94,5
>-1,784 *	66,67	11,6 - 94,5	100,00	30,5 - 100,0			0,33		100,0	19,3 - 100,0	75,0	20,3 - 95,9
>0,996	33,33	5,5 - 88,4	100,00	30,5 - 100,0			0,67		100,0	16,5 - 100,0	60,0	15,4 - 93,5
>11,876	0,00	0,0 - 69,5	100,00	30,5 - 100,0			1,00				50,0	12,4 - 87,6

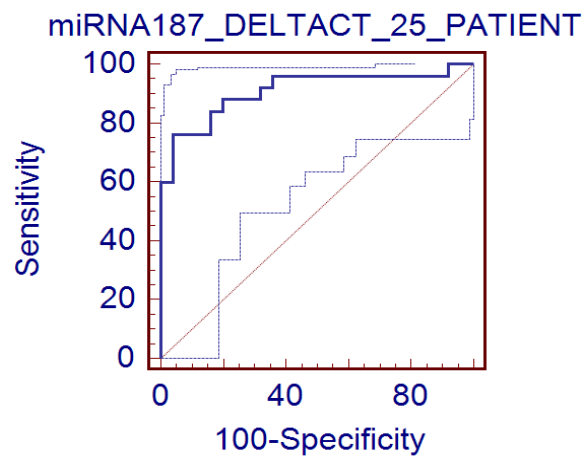


Sensitivity: 92,0
Specificity: 100,0
Criterion : <=-6,666

Variable	miRNA182_DELTACT_25_PATIENT		
Classification variable	DIAGNOSIS		
Positive group			
DIAGNOSIS	= 1		
Sample size	25		
Negative group			
DIAGNOSIS	= 0		
Sample size	25		
Disease prevalence (%)		50,0	
Area under the ROC curve (AUC)		0,982	
Standard Error		0,0192	
95% Confidence Interval		0,897 to 0,996	
z statistic		25,141	
Significance level P (Area=0.5)		0,0001	

Criterion values and coordinates of the ROC curve [\[Hide\]](#)

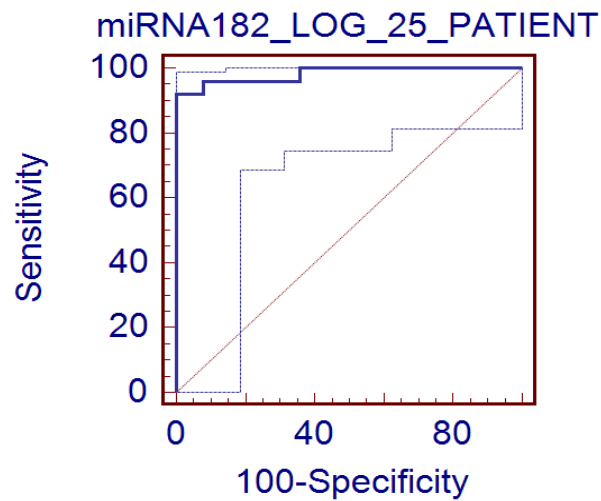
Criterion	Sensitivity	95% CI	Specificity	95% CI	+LR	95% CI	-LR	95% CI	+PV	95% CI	-PV	95% CI
< -7,966	0,00	0,0 - 13,8	100,00	86,2 - 100,0			1,00				50,0	35,5 - 64,5
<= -7,966	4,00	0,7 - 20,4	100,00	86,2 - 100,0			0,96		100,0	16,5 - 100,0	51,0	36,3 - 65,6
<= -7,876	8,00	1,2 - 26,1	100,00	86,2 - 100,0			0,92		100,0	19,3 - 100,0	52,1	37,2 - 66,7
<= -7,726	12,00	2,7 - 31,2	100,00	86,2 - 100,0			0,88		100,0	30,5 - 100,0	53,2	38,1 - 67,9
<= -7,576	16,00	4,6 - 36,1	100,00	86,2 - 100,0			0,84		100,0	40,2 - 100,0	54,3	39,0 - 69,1
<= -7,526	20,00	6,9 - 40,7	100,00	86,2 - 100,0			0,80		100,0	48,0 - 100,0	55,6	40,0 - 70,3
<= -7,516	24,00	9,4 - 45,1	100,00	86,2 - 100,0			0,76		100,0	54,1 - 100,0	56,8	41,0 - 71,6
<= -7,426	32,00	15,0 - 53,5	100,00	86,2 - 100,0			0,68		100,0	62,9 - 100,0	59,5	43,3 - 74,4
<= -7,406	36,00	18,0 - 57,5	100,00	86,2 - 100,0			0,64		100,0	66,2 - 100,0	61,0	44,5 - 75,8
<= -7,396	40,00	21,2 - 61,3	100,00	86,2 - 100,0			0,60		100,0	69,0 - 100,0	62,5	45,8 - 77,3
<= -7,386	44,00	24,4 - 65,1	100,00	86,2 - 100,0			0,56		100,0	71,3 - 100,0	64,1	47,2 - 78,8
<= -7,376	48,00	27,8 - 68,7	100,00	86,2 - 100,0			0,52		100,0	73,4 - 100,0	65,8	48,6 - 80,4
<= -7,346	52,00	31,3 - 72,2	100,00	86,2 - 100,0			0,48		100,0	75,1 - 100,0	67,6	50,2 - 82,0
<= -7,266	56,00	34,9 - 75,6	100,00	86,2 - 100,0			0,44		100,0	76,7 - 100,0	69,4	51,9 - 83,6
<= -7,236	60,00	38,7 - 78,8	100,00	86,2 - 100,0			0,40		100,0	78,0 - 100,0	71,4	53,7 - 85,3
<= -7,196	64,00	42,5 - 82,0	100,00	86,2 - 100,0			0,36		100,0	79,2 - 100,0	73,5	55,6 - 87,1
<= -7,136	68,00	46,5 - 85,0	100,00	86,2 - 100,0			0,32		100,0	80,3 - 100,0	75,8	57,7 - 88,9
<= -7,126	72,00	50,6 - 87,9	100,00	86,2 - 100,0			0,28		100,0	81,3 - 100,0	78,1	60,0 - 90,7
<= -7,056	76,00	54,9 - 90,6	100,00	86,2 - 100,0			0,24		100,0	82,2 - 100,0	80,6	62,5 - 92,5
<= -6,986	80,00	59,3 - 93,1	100,00	86,2 - 100,0			0,20		100,0	83,0 - 100,0	83,3	65,3 - 94,3
<= -6,876	84,00	63,9 - 95,4	100,00	86,2 - 100,0			0,16		100,0	83,7 - 100,0	86,2	68,3 - 96,0
<= -6,866	88,00	68,8 - 97,3	100,00	86,2 - 100,0			0,12		100,0	84,4 - 100,0	89,3	71,7 - 97,6
<= -6,666 *	92,00	73,9 - 98,8	100,00	86,2 - 100,0			0,08		100,0	85,0 - 100,0	92,6	75,7 - 98,9
<= -6,2125	92,00	73,9 - 98,8	92,00	73,9 - 98,8	11,50	9,8 - 13,5	0,087	0,01 - 0,6	92,0	73,9 - 98,8	92,0	73,9 - 98,8
<= -6,196	96,00	79,6 - 99,3	92,00	73,9 - 98,8	12,00	10,4 - 13,8	0,043	0,004 - 0,4	92,3	74,8 - 98,8	95,8	78,8 - 99,3
<= -5,9025	96,00	79,6 - 99,3	88,00	68,8 - 97,3	8,00	6,8 - 9,4	0,045	0,005 - 0,4	88,9	70,8 - 97,5	95,7	78,0 - 99,3
<= -5,8725	96,00	79,6 - 99,3	84,00	63,9 - 95,4	6,00	5,0 - 7,2	0,048	0,006 - 0,4	85,7	67,3 - 95,9	95,5	77,1 - 99,2
<= -5,7825	96,00	79,6 - 99,3	80,00	59,3 - 93,1	4,80	3,9 - 5,9	0,05	0,006 - 0,4	82,8	64,2 - 94,1	95,2	76,1 - 99,2
<= -5,7225	96,00	79,6 - 99,3	72,00	50,6 - 87,9	3,43	2,7 - 4,4	0,056	0,007 - 0,4	77,4	58,9 - 90,4	94,7	73,9 - 99,1
<= -5,6625	96,00	79,6 - 99,3	68,00	46,5 - 85,0	3,00	2,3 - 4,0	0,059	0,008 - 0,4	75,0	56,6 - 88,5	94,4	72,6 - 99,1
<= -5,6425	96,00	79,6 - 99,3	64,00	42,5 - 82,0	2,67	2,0 - 3,6	0,063	0,009 - 0,5	72,7	54,5 - 86,7	94,1	71,2 - 99,0
<= -5,626	100,00	86,2 - 100,0	64,00	42,5 - 82,0	2,78	2,1 - 3,7	0,00		73,5	55,6 - 87,1	100,0	79,2 - 100,0
<= -5,6025	100,00	86,2 - 100,0	60,00	38,7 - 78,8	2,50	1,8 - 3,4	0,00		71,4	53,7 - 85,3	100,0	78,0 - 100,0
<= -5,5725	100,00	86,2 - 100,0	56,00	34,9 - 75,6	2,27	1,6 - 3,2	0,00		69,4	51,9 - 83,6	100,0	76,7 - 100,0
<= -5,5525	100,00	86,2 - 100,0	52,00	31,3 - 72,2	2,08	1,4 - 3,0	0,00		67,6	50,2 - 82,0	100,0	75,1 - 100,0
<= -5,4625	100,00	86,2 - 100,0	48,00	27,8 - 68,7	1,92	1,3 - 2,9	0,00		65,8	48,6 - 80,4	100,0	73,4 - 100,0
<= -5,4325	100,00	86,2 - 100,0	44,00	24,4 - 65,1	1,79	1,1 - 2,8	0,00		64,1	47,2 - 78,8	100,0	71,3 - 100,0
<= -5,3125	100,00	86,2 - 100,0	40,00	21,2 - 61,3	1,67	1,0 - 2,7	0,00		62,5	45,8 - 77,3	100,0	69,0 - 100,0
<= -5,2325	100,00	86,2 - 100,0	36,00	18,0 - 57,5	1,56	0,9 - 2,6	0,00		61,0	44,5 - 75,8	100,0	66,2 - 100,0
<= -5,0825	100,00	86,2 - 100,0	32,00	15,0 - 53,5	1,47	0,8 - 2,6	0,00		59,5	43,3 - 74,4	100,0	62,9 - 100,0
<= -5,0725	100,00	86,2 - 100,0	28,00	12,1 - 49,4	1,39	0,7 - 2,6	0,00		58,1	42,1 - 73,0	100,0	58,9 - 100,0
<= -5,0525	100,00	86,2 - 100,0	24,00	9,4 - 45,1	1,32	0,7 - 2,6	0,00		56,8	41,0 - 71,6	100,0	54,1 - 100,0
<= -4,7025	100,00	86,2 - 100,0	20,00	6,9 - 40,7	1,25	0,6 - 2,7	0,00		55,6	40,0 - 70,3	100,0	48,0 - 100,0
<= -4,6725	100,00	86,2 - 100,0	16,00	4,6 - 36,1	1,19	0,5 - 2,9	0,00		54,3	39,0 - 69,1	100,0	40,2 - 100,0
<= -4,6325	100,00	86,2 - 100,0	12,00	2,7 - 31,2	1,14	0,4 - 3,3	0,00		53,2	38,1 - 67,9	100,0	30,5 - 100,0
<= -3,2825	100,00	86,2 - 100,0	8,00	1,2 - 26,1	1,09	0,3 - 4,1	0,00		52,1	37,2 - 66,7	100,0	19,3 - 100,0
<= -2,3525	100,00	86,2 - 100,0	4,00	0,7 - 20,4	1,04	0,2 - 7,1	0,00		51,0	36,3 - 65,6	100,0	16,5 - 100,0
<= -1,6825	100,00	86,2 - 100,0	0,00	0,0 - 13,8	1,00				50,0	35,5 - 64,5		



Variable	miRNA187_DELTACT_25_PATIENT		
Classification variable	DIAGNOSIS		
Positive group			
DIAGNOSIS	= 1		
Sample size	25		
Negative group			
DIAGNOSIS	= 0		
Sample size	25		
Disease prevalence (%)		50,0	
Area under the ROC curve (AUC)		0,909	
Standard Error		0,0437	
95% Confidence Interval		0,793 to 0,971	
z statistic		9,344	
Significance level P (Area=0.5)		0,0001	

Criterion values and coordinates of the ROC curve [\[Hide\]](#)

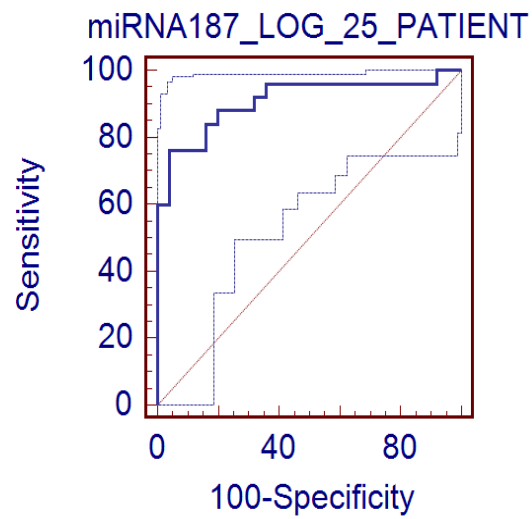
Criterion	Sensitivity	95% CI	Specificity	95% CI	+LR	95% CI	-LR	95% CI	+PV	95% CI	-PV	95% CI
< -14,296	0,00	0,0 - 13,8	100,00	86,2 - 100,0			1,00				50,0	35,5 - 64,5
<= -14,296	4,00	0,7 - 20,4	100,00	86,2 - 100,0			0,96		100,0	16,5 - 100,0	51,0	36,3 - 65,6
<= -12,296	8,00	1,2 - 26,1	100,00	86,2 - 100,0			0,92		100,0	19,3 - 100,0	52,1	37,2 - 66,7
<= -9,846	12,00	2,7 - 31,2	100,00	86,2 - 100,0			0,88		100,0	30,5 - 100,0	53,2	38,1 - 67,9
<= -9,396	16,00	4,6 - 36,1	100,00	86,2 - 100,0			0,84		100,0	40,2 - 100,0	54,3	39,0 - 69,1
<= -9,256	20,00	6,9 - 40,7	100,00	86,2 - 100,0			0,80		100,0	48,0 - 100,0	55,6	40,0 - 70,3
<= -9,156	28,00	12,1 - 49,4	100,00	86,2 - 100,0			0,72		100,0	58,9 - 100,0	58,1	42,1 - 73,0
<= -9,096	32,00	15,0 - 53,5	100,00	86,2 - 100,0			0,68		100,0	62,9 - 100,0	59,5	43,3 - 74,4
<= -9,006	36,00	18,0 - 57,5	100,00	86,2 - 100,0			0,64		100,0	66,2 - 100,0	61,0	44,5 - 75,8
<= -8,806	40,00	21,2 - 61,3	100,00	86,2 - 100,0			0,60		100,0	69,0 - 100,0	62,5	45,8 - 77,3
<= -8,766	44,00	24,4 - 65,1	100,00	86,2 - 100,0			0,56		100,0	71,3 - 100,0	64,1	47,2 - 78,8
<= -8,666	48,00	27,8 - 68,7	100,00	86,2 - 100,0			0,52		100,0	73,4 - 100,0	65,8	48,6 - 80,4
<= -8,476	52,00	31,3 - 72,2	100,00	86,2 - 100,0			0,48		100,0	75,1 - 100,0	67,6	50,2 - 82,0
<= -8,406	56,00	34,9 - 75,6	100,00	86,2 - 100,0			0,44		100,0	76,7 - 100,0	69,4	51,9 - 83,6
<= -8,346	60,00	38,7 - 78,8	100,00	86,2 - 100,0			0,40		100,0	78,0 - 100,0	71,4	53,7 - 85,3
<= -8,308	60,00	38,7 - 78,8	96,00	79,6 - 99,3	15,00	10,8 - 20,9	0,42	0,06 - 3,0	93,7	69,7 - 99,0	70,6	52,5 - 84,9
<= -8,216	64,00	42,5 - 82,0	96,00	79,6 - 99,3	16,00	11,8 - 21,7	0,37	0,05 - 2,7	94,1	71,2 - 99,0	72,7	54,5 - 86,7
<= -8,116	68,00	46,5 - 85,0	96,00	79,6 - 99,3	17,00	12,8 - 22,5	0,33	0,04 - 2,5	94,4	72,6 - 99,1	75,0	56,6 - 88,5
<= -8,086	72,00	50,6 - 87,9	96,00	79,6 - 99,3	18,00	13,9 - 23,3	0,29	0,04 - 2,2	94,7	73,9 - 99,1	77,4	58,9 - 90,4
<= -8,046 *	76,00	54,9 - 90,6	96,00	79,6 - 99,3	19,00	15,0 - 24,0	0,25	0,03 - 1,9	95,0	75,1 - 99,2	80,0	61,4 - 92,2
<= -8,008	76,00	54,9 - 90,6	92,00	73,9 - 98,8	9,50	7,4 - 12,2	0,26	0,06 - 1,2	90,5	69,6 - 98,5	79,3	60,3 - 92,0
<= -7,998	76,00	54,9 - 90,6	88,00	68,8 - 97,3	6,33	4,9 - 8,2	0,27	0,08 - 1,0	86,4	65,1 - 96,9	77,6	59,0 - 91,7
<= -7,938	76,00	54,9 - 90,6	84,00	63,9 - 95,4	4,75	3,6 - 6,3	0,29	0,09 - 0,9	82,6	61,2 - 94,9	77,8	57,7 - 91,3
<= -7,926	84,00	63,9 - 95,4	84,00	63,9 - 95,4	5,25	4,1 - 6,7	0,19	0,05 - 0,7	84,0	63,9 - 95,4	84,0	63,9 - 95,4
<= -7,918	84,00	63,9 - 95,4	80,00	59,3 - 93,1	4,20	3,2 - 5,4	0,20	0,06 - 0,7	80,8	60,6 - 93,4	83,3	62,6 - 95,2
<= -7,826	88,00	68,8 - 97,3	80,00	59,3 - 93,1	4,40	3,4 - 5,6	0,15	0,04 - 0,6	81,5	61,9 - 93,6	87,0	66,4 - 97,1
<= -7,808	88,00	68,8 - 97,3	76,00	54,9 - 90,6	3,67	2,8 - 4,8	0,16	0,04 - 0,6	78,6	59,0 - 91,7	86,4	65,1 - 96,9
<= -7,768	88,00	68,8 - 97,3	72,00	50,6 - 87,9	3,14	2,4 - 4,2	0,17	0,05 - 0,6	75,9	56,5 - 89,7	85,7	63,6 - 96,8
<= -7,748	88,00	68,8 - 97,3	68,00	46,5 - 85,0	2,75	2,0 - 3,7	0,18	0,05 - 0,6	73,3	54,1 - 87,7	85,0	62,1 - 96,6
<= -7,726	92,00	73,9 - 98,8	68,00	46,5 - 85,0	2,88	2,1 - 3,9	0,12	0,03 - 0,5	74,2	55,4 - 88,1	89,5	66,8 - 98,4
<= -7,608	92,00	73,9 - 98,8	64,00	42,5 - 82,0	2,56	1,9 - 3,5	0,12	0,03 - 0,5	71,9	53,3 - 86,2	88,9	65,2 - 98,3
<= -7,486	96,00	79,6 - 99,3	64,00	42,5 - 82,0	2,67	2,0 - 3,6	0,063	0,009 - 0,5	72,7	54,5 - 86,7	94,1	71,2 - 99,0
<= -7,328	96,00	79,6 - 99,3	60,00	38,7 - 78,8	2,40	1,7 - 3,3	0,067	0,009 - 0,5	70,6	52,5 - 84,9	93,7	69,7 - 99,0
<= -7,288	96,00	79,6 - 99,3	52,00	31,3 - 72,2	2,00	1,4 - 2,9	0,077	0,01 - 0,5	66,7	49,0 - 81,4	92,9	66,1 - 98,8
<= -7,278	96,00	79,6 - 99,3	44,00	24,4 - 65,1	1,71	1,1 - 2,7	0,091	0,01 - 0,6	63,2	46,0 - 78,2	91,7	61,5 - 98,6
<= -7,218	96,00	79,6 - 99,3	40,00	21,2 - 61,3	1,60	1,0 - 2,6	0,10	0,01 - 0,7	61,5	44,6 - 76,6	90,9	58,7 - 98,5
<= -6,968	96,00	79,6 - 99,3	36,00	18,0 - 57,5	1,50	0,9 - 2,5	0,11	0,02 - 0,8	60,0	43,3 - 75,1	90,0	55,5 - 98,3
<= -6,718	96,00	79,6 - 99,3	32,00	15,0 - 53,5	1,41	0,8 - 2,5	0,13	0,02 - 0,9	58,5	42,1 - 73,7	88,9	51,7 - 98,2
<= -6,558	96,00	79,6 - 99,3	28,00	12,1 - 49,4	1,33	0,7 - 2,5	0,14	0,02 - 1,0	57,1	41,0 - 72,3	87,5	47,4 - 97,9
<= -6,498	96,00	79,6 - 99,3	24,00	9,4 - 45,1	1,26	0,6 - 2,5	0,17	0,02 - 1,2	55,8	39,9 - 70,9	85,7	42,2 - 97,6
<= -6,488	96,00	79,6 - 99,3	20,00	6,9 - 40,7	1,20	0,5 - 2,6	0,20	0,03 - 1,4	54,5	38,9 - 69,6	83,3	36,1 - 97,2
<= -6,268	96,00	79,6 - 99,3	16,00	4,6 - 36,1	1,14	0,5 - 2,8	0,25	0,04 - 1,7	53,3	37,9 - 68,3	80,0	28,8 - 96,7
<= -6,228	96,00	79,6 - 99,3	12,00	2,7 - 31,2	1,09	0,4 - 3,2	0,33	0,05 - 2,3	52,2	36,9 - 67,1	75,0	20,3 - 95,9
<= -5,658	96,00	79,6 - 99,3	8,00	1,2 - 26,1	1,04	0,3 - 4,0	0,50	0,07 - 3,4	51,1	36,1 - 65,9	66,7	11,6 - 94,5
<= -5,596	100,00	86,2 - 100,0	8,00	1,2 - 26,1	1,09	0,3 - 4,1	0,00		52,1	37,2 - 66,7	100,0	19,3 - 100,0
<= -5,548	100,00	86,2 - 100,0	4,00	0,7 - 20,4	1,04	0,2 - 7,1	0,00		51,0	36,3 - 65,6	100,0	16,5 - 100,0
<= -2,168	100,00	86,2 - 100,0	0,00	0,0 - 13,8	1,00				50,0	35,5 - 64,5		



Variable	miRNA182_LOG_25_PATIENT		
Classification variable	DIAGNOSIS		
Positive group			
DIAGNOSIS	= 1		
Sample size	25		
Negative group			
DIAGNOSIS	= 0		
Sample size	25		
Disease prevalence (%)		50,0	
Area under the ROC curve (AUC)		0,982	
Standard Error		0,0192	
95% Confidence Interval		0,897 to 0,996	
z statistic		25,141	
Significance level P (Area=0.5)		0,0001	

Criterion values and coordinates of the ROC curve [\[Hide\]](#)

Criterion	Sensitivity	95% CI	Specificity	95% CI	+LR	95% CI	-LR	95% CI	+PV	95% CI	-PV	95% CI
>=0,506	100,00	86,2 - 100,0	0,00	0,0 - 13,8	1,00				50,0	35,5 - 64,5		
>0,506	100,00	86,2 - 100,0	4,00	0,7 - 20,4	1,04	0,2 - 7,1	0,00		51,0	36,3 - 65,6	100,0	16,5 - 100,0
>0,708	100,00	86,2 - 100,0	8,00	1,2 - 26,1	1,09	0,3 - 4,1	0,00		52,1	37,2 - 66,7	100,0	19,3 - 100,0
>0,988	100,00	86,2 - 100,0	12,00	2,7 - 31,2	1,14	0,4 - 3,3	0,00		53,2	38,1 - 67,9	100,0	30,5 - 100,0
>1,395	100,00	86,2 - 100,0	16,00	4,6 - 36,1	1,19	0,5 - 2,9	0,00		54,3	39,0 - 69,1	100,0	40,2 - 100,0
>1,407	100,00	86,2 - 100,0	20,00	6,9 - 40,7	1,25	0,6 - 2,7	0,00		55,6	40,0 - 70,3	100,0	48,0 - 100,0
>1,416	100,00	86,2 - 100,0	24,00	9,4 - 45,1	1,32	0,7 - 2,6	0,00		56,8	41,0 - 71,6	100,0	54,1 - 100,0
>1,521	100,00	86,2 - 100,0	28,00	12,1 - 49,4	1,39	0,7 - 2,6	0,00		58,1	42,1 - 73,0	100,0	58,9 - 100,0
>1,527	100,00	86,2 - 100,0	32,00	15,0 - 53,5	1,47	0,8 - 2,6	0,00		59,5	43,3 - 74,4	100,0	62,9 - 100,0
>1,53	100,00	86,2 - 100,0	36,00	18,0 - 57,5	1,56	0,9 - 2,6	0,00		61,0	44,5 - 75,8	100,0	66,2 - 100,0
>1,575	100,00	86,2 - 100,0	40,00	21,2 - 61,3	1,67	1,0 - 2,7	0,00		62,5	45,8 - 77,3	100,0	69,0 - 100,0
>1,599	100,00	86,2 - 100,0	44,00	24,4 - 65,1	1,79	1,1 - 2,8	0,00		64,1	47,2 - 78,8	100,0	71,3 - 100,0
>1,635	100,00	86,2 - 100,0	48,00	27,8 - 68,7	1,92	1,3 - 2,9	0,00		65,8	48,6 - 80,4	100,0	73,4 - 100,0
>1,644	100,00	86,2 - 100,0	52,00	31,3 - 72,2	2,08	1,4 - 3,0	0,00		67,6	50,2 - 82,0	100,0	75,1 - 100,0
>1,671	100,00	86,2 - 100,0	56,00	34,9 - 75,6	2,27	1,6 - 3,2	0,00		69,4	51,9 - 83,6	100,0	76,7 - 100,0
>1,677	100,00	86,2 - 100,0	60,00	38,7 - 78,8	2,50	1,8 - 3,4	0,00		71,4	53,7 - 85,3	100,0	78,0 - 100,0
>1,687	100,00	86,2 - 100,0	64,00	42,5 - 82,0	2,78	2,1 - 3,7	0,00		73,5	55,6 - 87,1	100,0	79,2 - 100,0
>1,694	96,00	79,6 - 99,3	64,00	42,5 - 82,0	2,67	2,0 - 3,6	0,063	0,009 - 0,5	72,7	54,5 - 86,7	94,1	71,2 - 99,0
>1,699	96,00	79,6 - 99,3	68,00	46,5 - 85,0	3,00	2,3 - 4,0	0,059	0,008 - 0,4	75,0	56,6 - 88,5	94,4	72,6 - 99,1
>1,705	96,00	79,6 - 99,3	72,00	50,6 - 87,9	3,43	2,7 - 4,4	0,056	0,007 - 0,4	77,4	58,9 - 90,4	94,7	73,9 - 99,1
>1,723	96,00	79,6 - 99,3	80,00	59,3 - 93,1	4,80	3,9 - 5,9	0,05	0,006 - 0,4	82,8	64,2 - 94,1	95,2	76,1 - 99,2
>1,741	96,00	79,6 - 99,3	84,00	63,9 - 95,4	6,00	5,0 - 7,2	0,048	0,006 - 0,4	85,7	67,3 - 95,9	95,5	77,1 - 99,2
>1,768	96,00	79,6 - 99,3	88,00	68,8 - 97,3	8,00	6,8 - 9,4	0,045	0,005 - 0,4	88,9	70,8 - 97,5	95,7	78,0 - 99,3
>1,777	96,00	79,6 - 99,3	92,00	73,9 - 98,8	12,00	10,4 - 13,8	0,043	0,004 - 0,4	92,3	74,8 - 98,8	95,8	78,8 - 99,3
>1,865	92,00	73,9 - 98,8	92,00	73,9 - 98,8	11,50	9,8 - 13,5	0,087	0,01 - 0,6	92,0	73,9 - 98,8	92,0	73,9 - 98,8
>1,87 *	92,00	73,9 - 98,8	100,00	86,2 - 100,0			0,08		100,0	85,0 - 100,0	92,6	75,7 - 98,9
>2,007	88,00	68,8 - 97,3	100,00	86,2 - 100,0			0,12		100,0	84,4 - 100,0	89,3	71,7 - 97,6
>2,067	84,00	63,9 - 95,4	100,00	86,2 - 100,0			0,16		100,0	83,7 - 100,0	86,2	68,3 - 96,0
>2,07	80,00	59,3 - 93,1	100,00	86,2 - 100,0			0,20		100,0	83,0 - 100,0	83,3	65,3 - 94,3
>2,103	76,00	54,9 - 90,6	100,00	86,2 - 100,0			0,24		100,0	82,2 - 100,0	80,6	62,5 - 92,5
>2,124	72,00	50,6 - 87,9	100,00	86,2 - 100,0			0,28		100,0	81,3 - 100,0	78,1	60,0 - 90,7
>2,145	68,00	46,5 - 85,0	100,00	86,2 - 100,0			0,32		100,0	80,3 - 100,0	75,8	57,7 - 88,9
>2,148	64,00	42,5 - 82,0	100,00	86,2 - 100,0			0,36		100,0	79,2 - 100,0	73,5	55,6 - 87,1
>2,166	60,00	38,7 - 78,8	100,00	86,2 - 100,0			0,40		100,0	78,0 - 100,0	71,4	53,7 - 85,3
>2,178	56,00	34,9 - 75,6	100,00	86,2 - 100,0			0,44		100,0	76,7 - 100,0	69,4	51,9 - 83,6
>2,187	52,00	31,3 - 72,2	100,00	86,2 - 100,0			0,48		100,0	75,1 - 100,0	67,6	50,2 - 82,0
>2,211	48,00	27,8 - 68,7	100,00	86,2 - 100,0			0,52		100,0	73,4 - 100,0	65,8	48,6 - 80,4
>2,22	44,00	24,4 - 65,1	100,00	86,2 - 100,0			0,56		100,0	71,3 - 100,0	64,1	47,2 - 78,8
>2,223	40,00	21,2 - 61,3	100,00	86,2 - 100,0			0,60		100,0	69,0 - 100,0	62,5	45,8 - 77,3
>2,226	36,00	18,0 - 57,5	100,00	86,2 - 100,0			0,64		100,0	66,2 - 100,0	61,0	44,5 - 75,8
>2,229	32,00	15,0 - 53,5	100,00	86,2 - 100,0			0,68		100,0	62,9 - 100,0	59,5	43,3 - 74,4
>2,235	24,00	9,4 - 45,1	100,00	86,2 - 100,0			0,76		100,0	54,1 - 100,0	56,8	41,0 - 71,6
>2,263	20,00	6,9 - 40,7	100,00	86,2 - 100,0			0,80		100,0	48,0 - 100,0	55,6	40,0 - 70,3
>2,266	16,00	4,6 - 36,1	100,00	86,2 - 100,0			0,84		100,0	40,2 - 100,0	54,3	39,0 - 69,1
>2,281	12,00	2,7 - 31,2	100,00	86,2 - 100,0			0,88		100,0	30,5 - 100,0	53,2	38,1 - 67,9
>2,326	8,00	1,2 - 26,1	100,00	86,2 - 100,0			0,92		100,0	19,3 - 100,0	52,1	37,2 - 66,7
>2,371	4,00	0,7 - 20,4	100,00	86,2 - 100,0			0,96		100,0	16,5 - 100,0	51,0	36,3 - 65,6
>2,398	0,00	0,0 - 13,8	100,00	86,2 - 100,0			1,00				50,0	35,5 - 64,5



Variable	miRNA187_LOG_25_PATIENT		
Classification variable	DIAGNOSIS		
Positive group			
DIAGNOSIS	= 1		
Sample size	25		
Negative group			
DIAGNOSIS	= 0		
Sample size	25		
Disease prevalence (%)		50,0	
Area under the ROC curve (AUC)		0,909	
Standard Error		0,0437	
95% Confidence Interval		0,793 to 0,971	
z statistic		9,344	
Significance level P (Area=0.5)		0,0001	

Criterion values and coordinates of the ROC curve [\[Hide\]](#)

Criterion	Sensitivity	95% CI	Specificity	95% CI	+LR	95% CI	-LR	95% CI	+PV	95% CI	-PV	95% CI
>=0,653	100,00	86,2 - 100,0	0,00	0,0 - 13,8	1,00				50,0	35,5 - 64,5		
>0,653	100,00	86,2 - 100,0	4,00	0,7 - 20,4	1,04	0,2 - 7,1	0,00		51,0	36,3 - 65,6	100,0	16,5 - 100,0
>1,67	100,00	86,2 - 100,0	8,00	1,2 - 26,1	1,09	0,3 - 4,1	0,00		52,1	37,2 - 66,7	100,0	19,3 - 100,0
>1,685	96,00	79,6 - 99,3	8,00	1,2 - 26,1	1,04	0,3 - 4,0	0,50	0,07 - 3,4	51,1	36,1 - 65,9	66,7	11,6 - 94,5
>1,703	96,00	79,6 - 99,3	12,00	2,7 - 31,2	1,09	0,4 - 3,2	0,33	0,05 - 2,3	52,2	36,9 - 67,1	75,0	20,3 - 95,9
>1,875	96,00	79,6 - 99,3	16,00	4,6 - 36,1	1,14	0,5 - 2,8	0,25	0,04 - 1,7	53,3	37,9 - 68,3	80,0	28,8 - 96,7
>1,887	96,00	79,6 - 99,3	20,00	6,9 - 40,7	1,20	0,5 - 2,6	0,20	0,03 - 1,4	54,5	38,9 - 69,6	83,3	36,1 - 97,2
>1,953	96,00	79,6 - 99,3	24,00	9,4 - 45,1	1,26	0,6 - 2,5	0,17	0,02 - 1,2	55,8	39,9 - 70,9	85,7	42,2 - 97,6
>1,956	96,00	79,6 - 99,3	28,00	12,1 - 49,4	1,33	0,7 - 2,5	0,14	0,02 - 1,0	57,1	41,0 - 72,3	87,5	47,4 - 97,9
>1,974	96,00	79,6 - 99,3	32,00	15,0 - 53,5	1,41	0,8 - 2,5	0,13	0,02 - 0,9	58,5	42,1 - 73,7	88,9	51,7 - 98,2
>2,022	96,00	79,6 - 99,3	36,00	18,0 - 57,5	1,50	0,9 - 2,5	0,11	0,02 - 0,8	60,0	43,3 - 75,1	90,0	55,5 - 98,3
>2,098	96,00	79,6 - 99,3	40,00	21,2 - 61,3	1,60	1,0 - 2,6	0,10	0,01 - 0,7	61,5	44,6 - 76,6	90,9	58,7 - 98,5
>2,173	96,00	79,6 - 99,3	44,00	24,4 - 65,1	1,71	1,1 - 2,7	0,091	0,01 - 0,6	63,2	46,0 - 78,2	91,7	61,5 - 98,6
>2,191	96,00	79,6 - 99,3	52,00	31,3 - 72,2	2,00	1,4 - 2,9	0,077	0,01 - 0,5	66,7	49,0 - 81,4	92,9	66,1 - 98,8
>2,194	96,00	79,6 - 99,3	60,00	38,7 - 78,8	2,40	1,7 - 3,3	0,067	0,009 - 0,5	70,6	52,5 - 84,9	93,7	69,7 - 99,0
>2,206	96,00	79,6 - 99,3	64,00	42,5 - 82,0	2,67	2,0 - 3,6	0,063	0,009 - 0,5	72,7	54,5 - 86,7	94,1	71,2 - 99,0
>2,254	92,00	73,9 - 98,8	64,00	42,5 - 82,0	2,56	1,9 - 3,5	0,12	0,03 - 0,5	71,9	53,3 - 86,2	88,9	65,2 - 98,3
>2,29	92,00	73,9 - 98,8	68,00	46,5 - 85,0	2,88	2,1 - 3,9	0,12	0,03 - 0,5	74,2	55,4 - 88,1	89,5	66,8 - 98,4
>2,326	88,00	68,8 - 97,3	68,00	46,5 - 85,0	2,75	2,0 - 3,7	0,18	0,05 - 0,6	73,3	54,1 - 87,7	85,0	62,1 - 96,6
>2,332	88,00	68,8 - 97,3	72,00	50,6 - 87,9	3,14	2,4 - 4,2	0,17	0,05 - 0,6	75,9	56,5 - 89,7	85,7	63,6 - 96,8
>2,339	88,00	68,8 - 97,3	76,00	54,9 - 90,6	3,67	2,8 - 4,8	0,16	0,04 - 0,6	78,6	59,0 - 91,7	86,4	65,1 - 96,9
>2,351	88,00	68,8 - 97,3	80,00	59,3 - 93,1	4,40	3,4 - 5,6	0,15	0,04 - 0,6	81,5	61,9 - 93,6	87,0	66,4 - 97,1
>2,356	84,00	63,9 - 95,4	80,00	59,3 - 93,1	4,20	3,2 - 5,4	0,20	0,06 - 0,7	80,8	60,6 - 93,4	83,3	62,6 - 95,2
>2,384	84,00	63,9 - 95,4	84,00	63,9 - 95,4	5,25	4,1 - 6,7	0,19	0,05 - 0,7	84,0	63,9 - 95,4	84,0	63,9 - 95,4
>2,386	76,00	54,9 - 90,6	84,00	63,9 - 95,4	4,75	3,6 - 6,3	0,29	0,09 - 0,9	82,6	61,2 - 94,9	77,8	57,7 - 91,3
>2,39	76,00	54,9 - 90,6	88,00	68,8 - 97,3	6,33	4,9 - 8,2	0,27	0,08 - 1,0	86,4	65,1 - 96,9	78,6	59,0 - 91,7
>2,408	76,00	54,9 - 90,6	92,00	73,9 - 98,8	9,50	7,4 - 12,2	0,26	0,06 - 1,2	90,5	69,6 - 98,5	79,3	60,3 - 92,0
>2,411 *	76,00	54,9 - 90,6	96,00	79,6 - 99,3	19,00	15,0 - 24,0	0,25	0,03 - 1,9	95,0	75,1 - 99,2	80,0	61,4 - 92,2
>2,422	72,00	50,6 - 87,9	96,00	79,6 - 99,3	18,00	13,9 - 23,3	0,29	0,04 - 2,2	94,7	73,9 - 99,1	77,4	58,9 - 90,4
>2,434	68,00	46,5 - 85,0	96,00	79,6 - 99,3	17,00	12,8 - 22,5	0,33	0,04 - 2,5	94,4	72,6 - 99,1	75,0	56,6 - 88,5
>2,443	64,00	42,5 - 82,0	96,00	79,6 - 99,3	16,00	11,8 - 21,7	0,37	0,05 - 2,7	94,1	71,2 - 99,0	72,7	54,5 - 86,7
>2,473	60,00	38,7 - 78,8	96,00	79,6 - 99,3	15,00	10,8 - 20,9	0,42	0,06 - 3,0	93,7	69,7 - 99,0	70,6	52,5 - 84,9
>2,501	60,00	38,7 - 78,8	100,00	86,2 - 100,0			0,40		100,0	78,0 - 100,0	71,4	53,7 - 85,3
>2,512	56,00	34,9 - 75,6	100,00	86,2 - 100,0			0,44		100,0	76,7 - 100,0	69,4	51,9 - 83,6
>2,53	52,00	31,3 - 72,2	100,00	86,2 - 100,0			0,48		100,0	75,1 - 100,0	67,6	50,2 - 82,0
>2,552	48,00	27,8 - 68,7	100,00	86,2 - 100,0			0,52		100,0	73,4 - 100,0	65,8	48,6 - 80,4
>2,609	44,00	24,4 - 65,1	100,00	86,2 - 100,0			0,56		100,0	71,3 - 100,0	64,1	47,2 - 78,8
>2,639	40,00	21,2 - 61,3	100,00	86,2 - 100,0			0,60		100,0	69,0 - 100,0	62,5	45,8 - 77,3
>2,651	36,00	18,0 - 57,5	100,00	86,2 - 100,0			0,64		100,0	66,2 - 100,0	61,0	44,5 - 75,8
>2,711	32,00	15,0 - 53,5	100,00	86,2 - 100,0			0,68		100,0	62,9 - 100,0	59,5	43,3 - 74,4
>2,738	28,00	12,1 - 49,4	100,00	86,2 - 100,0			0,72		100,0	58,9 - 100,0	58,1	42,1 - 73,0
>2,756	20,00	6,9 - 40,7	100,00	86,2 - 100,0			0,80		100,0	48,0 - 100,0	55,6	40,0 - 70,3
>2,786	16,00	4,6 - 36,1	100,00	86,2 - 100,0			0,84		100,0	40,2 - 100,0	54,3	39,0 - 69,1
>2,828	12,00	2,7 - 31,2	100,00	86,2 - 100,0			0,88		100,0	30,5 - 100,0	53,2	38,1 - 67,9
>2,964	8,00	1,2 - 26,1	100,00	86,2 - 100,0			0,92		100,0	19,3 - 100,0	52,1	37,2 - 66,7
>3,701	4,00	0,7 - 20,4	100,00	86,2 - 100,0			0,96		100,0	16,5 - 100,0	51,0	36,3 - 65,6
>4,304	0,00	0,0 - 13,8	100,00	86,2 - 100,0			1,00				50,0	35,5 - 64,5

7.3. Curriculum Vitae

Personal Informations

Name	Nalan	Surname	Alan Selçuk
Place of Birth	Malatya		
Nationality	TC		
E-mail			

Educational Informations

Title	Speciality	Name of Institution	Year
Doctorate	Nuclear Medicine	Yeditepe Üniversitesi Institute of Health Sciences	2023
Medical Expertise	Nuclear Medicine	İstanbul Üniversitesi Cerrahpaşa Medical School	2003
Medical School	Medicine	İnönü University	1996
High School		Sümer High School	

Foreign Languages	Foreign Language Exam Grade
İngilizce	90.00

Work Experience

Job Title	Name of Institution	Year
Nuclear Medicine	İnternational Hospital	1
Nuclear Medicine	Yeditepe University	18

Computer skills

Program	Level

Scientific Informations

Publications / Notices Certificates /

International Articles (SCI, SCI-Expanded)

42

Articles published in other journals

50

International Oral Presentations and Proceedings

200

Publications in the proceedings of refereed conferences/symposiums

10

Other (Projects Involved/Certificates/Awards)

2
