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Evaluation of Prognostic Factors, New Predictive Biomarkers, and Organ-Sparing Strategy for Rectal Cancer after Combined Neoadjuvant Therapy

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Abbreviations used in the Thesis

A	*aorta*
ADP-ribose	*Adenosine Diphosphate Ribose*
AJCC	*American Joint Committee on Cancer*
App	*appendix*
ASCO	*American Society of Clinical Oncology*
USA	*United States of America*
BRAF	*B-Raf proto-oncogene*
CA 19-9	*Carbohydrate antigen 19-9*
cCR	*complete clinical response*
CEA	*carcinoembryonic antigen*
CI	*confidence interval*
CR	*complete response*
DCC	*Deleted in Colorectal Carcinoma*
DFS	*disease free survival*
DNA	*deoxyribonucleic acid*
ECIS	*European Cancer Information System*
ESMO	*European Society for Medical Oncology*
FDG	*Fluorine−18 2-fluoro-2-deoxy-D-glucose*
FFPE	*formalin-fixed paraffin-embedded*
FOLFOX	*FOL-Folinic Acid, F-fluorouracil-5, OX-oxaliplatin*
IWWD	*International Watch and Wait Database*
KRAS	*Kirsten Ras oncogene homolog*
GR	*good response*
LAR	*low anterior resection*
LARS	*low anterior resection syndrome*
MCC	*Mutated in Colorectal Cancers or Malignant Colonic Carcinoma*
miRNA, miR	*microRNA*
MRI	*magnetic resonance imaging*
mrTRG	*magnetic resonance tumour regression grade*
NAT	*neoadjuvant therapy*
NCCN	*National Comprehensive Cancer Network*
NPTX2	*Neuronal pentraxin II*
PCR	*polymerase chain reaction*
PĒK	*Research Ethics Committee*
PET/CT	*positron emission tomography / computed tomography*

PET/MRI	*positron emission tomography / magnetic resonance imaging*
PI3K	*Phosphoinositide 3-kinase*
PSCUH	*Pauls Stradiņš Clinical University Hospital*
RECIST	*The Response Evaluation Criteria in Solid Tumours*
RT	*reverse transcription*
BR	*bad response*
SMA	*superior mesenteric artery*
SMAD3	*Mothers against decapentaplegic homolog 3*
SMAD4	*Mothers against decapentaplegic (DPP) homolog 4*
TNM	*Tumour, Node, Metastasis (staging system)*
TRG	*tumour regression grade*
TSR	*tumour-stroma ratio*
VNN1	*vascular non-inflammatory molecule 1*
VSD	*Health statistics database*
"W&W"	*"Watch and Wait"*

Introduction

Relevance of the problem

Colorectal cancer is one of the most common types of cancer in Latvia and Europe. Approximately 500 new cases are diagnosed in Latvia each year (CDPC data, 2024). Standard treatment for stage II and III rectal cancer involves preoperative radiation and chemotherapy or radiation therapy alone (neoadjuvant therapy), followed by surgical treatment – rectal resection or extirpation (*Glynne-Jones et al*., 2017). Neoadjuvant therapy (NAT) plays a significant role in reducing the size and spread of the tumour, thereby improving the radicality of surgical treatment and the patient's prognosis. In addition, it has been observed that in approximately 10–40 % of cases, NAT results in complete tumour remission – complete clinical response (cCR) is achieved *(Wei et al.,* 2018). Based on the currently accepted guidelines for the treatment of rectal cancer (ESMO 2025, NCCN 2022, ASCO 2024), surgical treatment after NAT is recommended as the primary option. However, given that for a significant proportion of patients it is associated with a significant reduction in quality of life (persistent pelvic organ dysfunction in the postoperative period or irreversible stoma creation), organ-preserving tactics have become increasingly popular in the last decade – local excision of the tumour or "Watch and Wait" ("W&W") strategy in cases when cCR is diagnosed after NAT *(Bernier et al.,* 2018). Currently, a topical issue in rectal cancer research is the identification of tumour prognostic factors. This would not only enable the prediction of tumour response to NAT (by identifying tumours for which the expected benefit of NAT is minimal and does not outweigh the side effects of therapy) but also facilitate preliminary identification of tumours that are more suitable for organ-sparing strategies or are likely to exhibit greater biological aggressiveness. Biological markers currently have the greatest potential not only in determining tumour behaviour but also as tools for the dynamic assessment of tumour response during therapy and as markers of tumour recurrence in the post-treatment period. Currently, the range of markers used in clinical practice is limited, and their specificity and sensitivity are incomplete.

Aim of the study

To determine the clinical and molecular biological prognostic factors of locally advanced rectal cancer and to evaluate disease-free survival and overall survival rates depending on the tumour response to NAT.

Tasks of the study

1 To identify cases when cCR has been achieved after NAT and evaluate the results of the "W&W" strategy in terms of disease progression and overall survival.
2 To evaluate clinical prognostic factors for rectal cancer and their relationship to post-treatment tumour pathomorphosis.
3 To evaluate the relationship between post-treatment tumour pathomorphosis and disease-free survival and overall survival rates.
4 To select potentially clinically significant altered miRNAs in rectal cancer tissues that could be used as prognostic molecular biological markers.

Hypotheses of the study

The results of the "W&W" strategy for rectal cancer patients after NAT at PSCUH are comparable to the results published by other research centres.

Clinical and radiological parameters indicative of tumour characteristics can be used to predict the response of rectal cancer to NAT.

Weaker pathomorphosis of rectal cancer in surgical material after NAT is associated with poorer local recurrence, distant metastasis and overall survival rates.

The microRNA profile of rectal cancer with good and poor response to NAT is different. The quantitative microRNA parameters in tumour tissue can serve as a predictive marker for the response of rectal cancer to NAT and as a prognostic indicator of local recurrence, distant metastasis, and overall survival in patients after radical surgical treatment.

Novelty of the study

The study design combines an assessment of the prognostic potential of clinical, morphological, and molecular biological parameters of rectal cancer in the context of the use of organ-preserving tactic ("W&W").

Cooperation partners and material technical support

The study involves patients who received treatment at the PSCUH Oncology Clinic and Surgery Clinic. The study data were compiled in files created by the researcher and available only to those involved in the study. Tissue samples from paraffin blocks for further molecular biological analysis were obtained from the PSCUH Pathology Institute. The experimental part was executed at the Institute of Oncology and Molecular Genetics of RSU. No additional funding was attracted for the study.

Author's personal contribution

The author of the Doctoral Thesis collected clinical data from patients for the study, performed surgical treatment for several cases, and conducted follow-up observations. The author organised a working group in PSCUH to develop local "W&W" guidelines and implement the strategy in the hospital, initiated cooperation with the "International Watch and Wait Database" (IWWD), and ensures data collection in local and international databases. The author has monitored all patients who have undergone organ-sparing strategy. The author has obtained tissue samples from paraffin blocks and performed all subsequent laboratory stages of molecular biological analysis of the tissue. The author has performed statistical processing and analysis of the study results, and has written this Doctoral Thesis and related publications.

Ethical aspects

Three permissions from the RSU Ethics Committee have been obtained to conduct the study: RSU PĒK decision 28.07.2016., 2-PĒK-4/494/2022 on 21 November 2022, and 2-PĒK-4/120/ 2023 on 26 January 2023 (Appendices 1, 2, and 3) – for the collection of clinical data for the study and molecular biological analysis of tissue samples from living and deceased patients.

The study was conducted in accordance with the Declaration of Helsinki and the regulations of good clinical practice. All patients involved in the study or their relatives (if the patient is deceased) have signed an informed consent form (Appendices 4 and 5) in two copies, one of which is kept by the patient/relative and the other at the PSCUH Surgery Clinic.

Conclusions

1 11 % of the patients selected for the study achieved complete clinical response after NAT. The DFS in patients who underwent the subsequent "W&W" strategy is comparable to data published worldwide.

2 A smaller rectal tumour circumference in pre-treatment pelvic MRI examination is associated with a more pronounced tumour reduction in post-treatment MRI. The mrTRG classification, based on the evaluation of post-treatment pelvic MRI findings, allows for a reliable prediction of tumour regression rate in surgical specimens according to the Dworak classification.

3 The pathomorphosis of rectal cancer according to Dworak, tumour pT stage, and mucinous component are prognostic indicators for the risk of local recurrence after surgical treatment, and pT stage is a prognostic indicator for distant metastases.

4 Altered microRNA (miR-99a-3p, miR-142-5p, and mirR-182-3p) in rectal cancer tissue have the potential to be used as biological markers for determining the response of rectal cancer to NAT and for predicting long-term oncological outcomes.

Proposals

1 The application of the "W&W" strategy in clinical practice should be based on clearly defined inclusion criteria, considering individual and tumour-related risk factors. Strict patient monitoring, adherence to the protocol, and systematic collection of clinical data are essential prerequisites for the successful application of this strategy.

2 In addition to the existing rectal cancer parameters assessed in pre-treatment MRI, it is recommended to evaluate the rectal tumour circumference, given that this indicator has prognostic significance in terms of tumour reduction after NAT.

3 It is recommended to use a unified classification of post-treatment tumour pathomorphosis, which would reduce subjectivity in the evaluation of surgical material and improve the stratification of risk factors associated with disease recurrence.

4 The creation of pre- and post-treatment risk scales that include clinical, morphological, and molecular biological parameters related to the patient and the tumour should be considered and could be used as a practical tool in clinical decision-making.

5 Tumour miRNA panels can be used as additional biological markers in predicting the response of rectal cancer to NAT, as well as in predicting long-term oncological outcomes. A study involving a larger group of patients is needed to determine validated miRNA in tissue samples and blood from rectal cancer patients.

List of publications, reports and patents on the topic of the Thesis

Scientific publications in journals included in international databases:

1. Kokaine, L., Daneberga, Z., Šatcs, M., Zvina, D., Naļivaiko, D., Nazarovs, J., Gardovskis, A., Nakazawa-Miklaševiča, M., Miklaševičs, E. 2025. The prognostic role of microRNAs 142-5p, 182-3p, and 99a-3p in locally advanced rectal cancer patients. *Anticancer research*, 45(9): 3895-3912 (PubMed, SCOPUS).
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3. Kokaine, L., Radziņa, M., Liepa, M., Geriņa-Bērziņa, A., Sīviņa, E., Nikolajeva, J., Gardovskis, A., Gardovskis, J., Miklaševičs, E. 2024. "Watch and Wait" strategy in rectal cancer patients with a complete clinical response after neoadjuvant chemoradiation therapy – a single-centre experience. *Experimental Oncology*, 46(1):53-60 (PubMed, SCOPUS).
4. Kokaine, L., Gardovskis, A., Gardovskis, J. 2021. Evaluation and Predictive Factors of Complete Response in Rectal Cancer after Neoadjuvant Chemoradiation Therapy. *Medicina*, 57:1044 (PubMed, SCOPUS).

Scientific articles published in peer-reviewed journals in Latvia:

1. Kokaine, L., Subatniece, S. 2024. "Pārmantotais vēzis"; *Ārsts.lv*, 8:50-57.
2. Kokaine, L. 2023. "Watch and Wait" jeb novērošanas taktika pacientiem ar taisnās zarnas adenokarcinomas pilnu klīnisku remisiju pēc neoadjuvantu terapijas. *Doctus*, 10:25-35.

Projects:

1. Baltiņa, D., Geriņa-Bērziņa, A., Kokaine-Šapovalova, L., Plaudis, H., Liepa, Z. 2020. Latvijā biežāk sastopamo audzēju primārā un metastāžu terapija. Kolorektālais vēzis (C18-C21). Algoritmi. Eiropas Sociālā fonda līdzfinansētais projekts Nr. 9.2.3.0/15/I/001 "Veselības tīklu attīstības vadlīniju un kvalitātes nodrošināšanas sistēmas izstrāde un ieviešana prioritāro veselības jomu ietvaros". Pieejams: https://www.spkc.gov.lv/sites/spkc/files/content/Profesionaliem/Kliniskie%20algoritmi%20un%20pacientu%20celi/Onkologija/Pacientu%20celi/11_kliniskie-celi_zarnas-terapija.pdf

Presentations at international conferences:

1. Kokaine, L., Zvina, D., Nazarovs, J., Gardovskis, A., Gardovskis, J. 2023. Disease-free survival in rectal cancer patients depends on tumour regression grade after neoadjuvant chemoradiation therapy. Baltic Congress of Oncologists and Surgeons; Tallina, Igaunija 07.-08.09.2023.;
2. Kokaine, L. 2023. "Watch and Wait" strategy in rectal cancer – the challenges and opportunities. Baltic Congress of Oncologists and Surgeons; Tallina, Igaunija 07.-08.09.2023.

Presentations at local conferences:

1. Kokaine, L. 2025. "Watch and Wait" stratēģija – pasaules un Latvijas pieredze. Latvijas Onkologu ķīmijterapeitu asociācijas (LOĶA) sēde, 27.03.2025., Rīga, Latvija.
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Annexes

Decision of the RSU Research Ethics Committee on 28.07.2016

Veidlapa Nr. E-9 (2)

RSU ĒTIKAS KOMITEJAS LĒMUMS

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Komitejas sastāvs:

1. Profesors Olafs Brūvers, EK priekšsēdētājs;
2. Profesore Vija Sīle; EK priekšsēdētāja vietniece;
3. Profesore Regīna Kleina;
4. Profesors Guntars Pupelis;
5. Asoc. profesore Santa Purviņa;
6. Asoc. profesors Viesturs Liguts;
7. Asoc. profesors Voldemārs Arnis;
8. Docente Iveta Jankovska;
9. Docents Kristaps Circenis.

Pētījuma nosaukums: **Taisnās zarnas vēža molekulārbioloģiskie marķieri pacientiem pirms neaodjuvantu terapijas kā ārstēšanas efektivitātes prognostiskie faktori**

Iesniegšanas datums: **28.07.2016.**

Pieteikuma iesniedzējs: **Dr. Linda Kokaine-Šapovalova, 4. studiju gada rezidente**
RSU struktūrvienība **Tālākizglītības fakultāte**

RSU ētikas komiteja, izskatot augstāk minētā pētījuma pieteikuma materiālus (protokolu) secina, ka pētījuma mērķis tiek sasniegts veicot pacientu medicīniskās dokumentācijas (laboratoro, radioloģisko, instrumentālo un morfoloģisko izmeklējumu datu, kā arī bioloģisko materiālu - asins paraugu, audu biopsijas materiālu) izpēti, iegūto datu apstrādi un analīzi, kā arī izsakot priekšlikumus. Personu (pacientu, dalībnieku) datu aizsardzība un konfidencialitāte tiek nodrošināta. Līdz ar to pieteikums atbilst biomedicīnas pētījuma ētikas prasībām. Ētikas komitejas lēmums ir piekrist pētījuma veikšanai. Iebildumu nav.

Ētiskā aspekta ieteikumi: **nav**

Izskaidrošanas formulārs: **nav nepieciešams**

Piekrišana piedalīties pētījumā: **nav nepieciešama**

Citi dokumenti: **saskaņots ar iestādi (iestādēm), kurā veic pētījumu**

Lēmums: **piekrist pētījumam**

Vārds, uzvārds: **Olafs Brūvers** Tituls: **Profesors**
RSU ētikas komitejas priekšsēdētājs

Paraksts _____ Datums: **28.07.2016.**

RIGAS STRADIŅA UNIVERSITĀTE ĒTIKAS KOMITEJA

Decision of the RSU Research Ethics Committee 2-PĒK-4/494/2022

Veidlapa Nr. E-9(3)
APSTIPRINĀTA
ar Rīgas Stradiņa universitātes rektora
2018. gada 26. septembra rīkojumu Nr. 5-1/238/2018

Rīgas Stradiņa universitātes
Pētījumu ētikas komitejas
LĒMUMS
Rīgā

21.11.2022 2-PĒK-4/494/2022

	Komitejas sastāvs	Kvalifikācija	Nodarbošanās
1	Profesors Jānis Vētra	Dr.habil. med.	Morfoloģijas katedra
2	Asoc. Prof. Zanda Daneberga	Dr.med.	OI Molekulārās ģenētikas laboratorijas vadītāja
3	Asoc. Prof. Anita Vētra	Dr.med.	Rehabilitācijas katedras vadītāja
4	Profesore Ingrīda Čēma	Dr.habil. med.	Mutes medicīnas katedras vadītāja
5	Docente Anna Junga	Dr.med.	Morfoloģijas laboratorijas vadītāja
6	Vadošā pētniece, docente Karina Palkova	Ph.D.	Advokāte, Doktora studiju programmas vadītāja
7	Marina Siņkovska		Datu drošības un pārvaldības nodaļas vadītāja

Pieteikuma iesniedzējs/i: **Linda Kokaine, Doktorantūras nodaļa**

Pētījuma / pētnieciskā darba nosaukums: Taisnās zarnas vēža ārstēšanas efektivitātes prognostiskie faktori

Pētījumu ētikas komitejas sēdes datums: 27.10.2022.

Pētījuma protokols: Pētījuma mērķi- noteikt iespējami prognostiskos neoadjuvantu terapijas efektivitātes klīniskos, radioloģiskos, ar ārstēšanu saistītos, morfoloģiskos un molekulārbioloģiskos faktorus pacientiem ar II un III stadijas taisnās zarnas vēzi. Minēto ir paredzēts sasniegt, veicot pētījuma dalībnieku retrospektīvo datu apkopojumu: pacienta personas dati, slimības un citu saslimšanu anamnestiskie dati; laboratoro, endoskopisko un radioloģisko izmeklējumu dati; primārās biopsijas un operācijas materiāla morfoloģiskās izmeklēšanas rezultāti; operācijas materiāla molekulārbioloģiska analizēšana (miRNS sekvenēšana); informācija par turpmāko slimības un ārstēšanas gaitu. Plānotā realizēšanai paredzēts izveidot 3 pētījuma grupas, atbilstoši iekļaušanas un izslēgšanas kritērijiem. Pētījums tiks veikts saskaņā ar Helsinku deklarāciju, Eiropas Padomes un Eiropas Savienības Tiesību aktu (Regulu), Latvijas un citiem normatīvajiem aktiem. Pacientiem, kuri piedalīsies pētnieciskajā projektā, nav gaidāms nekāds fizisks vai psiholoģisks kaitējums, riski vai apdraudējuma ārstēšanas gaitai nepastāv. Pētījumam nepieciešamā informācija tiks iegūta no jau veikto izmeklējumu rezultātiem un jau esošajiem audu paraugiem. Personu (dalībnieku) informēta brīvprātīga piekrišana piedalīties,

iegūto personu datu apstrāde un aizsardzība, to pielietošana, glabāšana, anonimitāte un konfidencialitāte ir nodrošināta. Pētījumā izvirzītais mērķis, pielietotās metodes un pētījuma sabiedrības veselības ieguvums ir samērojams. Līdz ar to pieteikums atbilst pētījuma ētikas prasībām.

Komitejas lēmums: **Piekrist** pētījuma īstenošanai

Komitejas priekšsēdētājs Jānis Vētra Tituls: Dr.habil. med., profesors.

ŠIS DOKUMENTS IR ELEKTRONISKI PARAKSTĪTS AR DROŠU ELEKTRONISKO PARAKSTU UN SATUR LAIKA ZĪMOGU

K. Ķauķe
Tālrunis: 26691306

Annex 3

Decision of the RSU Research Ethics Committee 2-PĒK-4/120/2023

Veidlapa Nr. E-9(3)
APSTIPRINĀTA
ar Rīgas Stradiņa universitātes rektora
2018. gada 26. septembra rīkojumu Nr. 5-1/238/2018

Rīgas Stradiņa universitātes
Pētījumu ētikas komitejas
LĒMUMS
Rīgā

26.01.2023 2-PĒK-4/120/2023

	Komitejas sastāvs	Kvalifikācija	Nodarbošanās
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2	Asoc. Prof. Zanda Daneberga	Dr.med.	OI Molekulārās ģenētikas laboratorijas vadītāja
3	Asoc. Prof. Anita Vētra	Dr.med.	Rehabilitācijas katedras vadītāja
4	Profesore Ingrīda Čēma	Dr.habil. med.	Mutes medicīnas katedras vadītāja
5	Docente Anna Junga	Dr.med.	Morfoloģijas laboratorijas vadītāja
6	Vadošā pētniece, docente Karina Palkova	Ph.D.	Advokāte, Doktora studiju programmas vadītāja
7	Marina Siņkovska		Datu drošības un pārvaldības nodaļas vadītāja

Pieteikuma iesniedzējs/i: **Linda Kokaine, Doktorantūras nodaļa**

Pētījuma / pētnieciskā darba nosaukums: Taisnās zarnas vēža ārstēšanas efektivitātes prognostiskie faktori

Pētījumu ētikas komitejas sēdes datums: 15.12.2022.

Pētījuma protokols: Pētījuma mērķis - noteikt iespējami prognostiskos neoadjuvantu terapijas efektivitātes klīniskos, radioloģiskos, ar ārstēšanu saistītos, morfoloģiskos un molekulārbioloģiskos faktorus pacientiem ar II un III stadijas taisnās zarnas vēzi. Attiecībā uz pētījuma daļu, kurā tiks iesaistīti pacienti, kuru piekrišanu līdzdalībai pētījumā apliecina parakstīta informētās piekrišanas anketa, RSU Pētījumu ētikas komiteja 21.11.2022. ir pieņēmusi Lēmumu 2-PĒK-4/494/2022 – *Piekrist pētījuma īstenošanai.*
Pētījumā ir paredzēts arī iekļaut datus no pacientiem, kas pētījuma uzsākšanas brīdī ir miruši un ir pauduši piekrišanu savu audu izmantošanai dzīves laikā (dokumentēts rakstiski vai pieejamajās datubāzēs, kurās apkopota iedzīvotāju personas dati un ar veselību saistītie dati; vai pacients paudis savu gribu piederīgajiem dzīves laikā mutiski) vai arī nav aizlieguši savu audu izmantošanu, kas būtu fiksēts kādā no iepriekš minētajiem informācijas avotiem.
Izskatot augstāk minētā pētījuma pieteikuma materiālus, pētījuma mērķi ir paredzēts sasniegt, veicot pacientu medicīniskās dokumentācijas (slimības vēstures un citi dati) izpēti, iegūto datu apstrādi un analīzi, kā arī publiskojot iegūtos rezultātus. Iegūto personu (pacientu) datu apstrāde un aizsardzība, to pielietošana, glabāšana, anonimitāte un konfidencialitāte ir nodrošināta. Pētījumā izvirzītais mērķis, pielietotās metodes un pētījuma sabiedrības veselības

	ieguvums ir samērojams, personas datu aizsardzība ir nodrošināta, pētījums atbilst pētījuma ētikas prasībām. Pētījuma protokols sastādīts atbilstoši Pacientu tiesību likuma 10. panta 8.[1] daļas prasībām. Medicīniskajā dokumentācijā fiksētos pacienta datus atļauts izmantot pēc atbilstošo ārstniecības iestāžu piekrišanas.
Komitejas lēmums:	Piekrist pētījuma īstenošanai.

Komitejas priekšsēdētājs Jānis Vētra Tituls: Dr.habil. med., profesors.

ŠIS DOKUMENTS IR ELEKTRONISKI PARAKSTĪTS AR DROŠU ELEKTRONISKO PARAKSTU UN SATUR LAIKA ZĪMOGU

K. Ķauķe
Tālrunis: 26691306

Annex 4

Informed consent to participate in the study

Dear Madam / Dear Sir!

We invite you to participate in the study ***"Prognostic factors for the effectiveness of rectal cancer treatment"*** conducted by Surgery Clinic of Pauls Stradiņš Clinical University Hospital (PSCUH) in collaboration with the Institute of Pathology and the Oncology Institute of Rīga Stradiņš University (RSU). We would like to inform you about the purpose, procedure and content of the study. Please read all the information carefully before signing this document. Before signing the document, you have the right to ask questions about the study and receive answers to them.

Relevance of the problem

Rectal cancer is one of the most common types of cancer in Latvia and Europe. Every year, around 500 new cases of rectal cancer are diagnosed in Latvia. Accurate preoperative staging of rectal cancer is crucial for the choice of further treatment and the patient's survival. The standard treatment method for non-metastatic (stage II and III) rectal cancer involves preoperative radiation and chemotherapy or radiation therapy alone (neoadjuvant therapy), followed by surgical treatment – rectal resection/extirpation (removal) with or without stoma creation (intestinal outlet in the front wall of the abdomen). Experience to date shows that neoadjuvant therapy plays a significant role in reducing the size and spread of the tumor, thereby improving the radicality of surgical treatment and the patient's prognosis. In addition, it has been observed that in approximately 20 % of cases, the tumor disappears completely as a result of neoadjuvant therapy. Nevertheless, even in these cases, surgical treatment is performed based on the guidelines currently accepted in Europe. However, over the last decade, an alternative treatment approach has become increasingly popular – in cases when complete tumor disappearance is observed after neoadjuvant therapy, surgery is not performed. Instead, the patient is monitored regularly and the tumor site is checked, considering that in about 25 % of patients, the tumor recurs within a few months or a year after treatment. In these cases, standard surgical treatment is performed. Recent studies have shown that the oncological safety outcome for patients undergoing observation is equivalent to that for patients undergoing surgical treatment. Overall, although watchful waiting carries a certain risk, in most cases (if the tumor has disappeared after neoadjuvant therapy) it offers the opportunity to avoid major surgery that affects quality of life. Given the current knowledge about the different tumor behaviour during neoadjuvant therapy, research on prognostic factors for rectal cancer is relevant, as it would allow predicting the possible response of the tumor before starting treatment.

Aim of the study

To evaluate the characteristics of stage II and III rectal cancer depending on its response to preoperative radiation and chemotherapy.

Study procedure

The study plans to include patients with stage II and III rectal cancer who have received preoperative radiation and chemotherapy or radiation therapy alone (neoadjuvant therapy) and who have undergone surgical treatment at the Surgery Clinic of the PSCUH between 1 October, 2015, and 1 October, 2021. Patients will be divided into 3 groups: 1. those who have had a moderate response to neoadjuvant therapy; 2. those who have had a good response to neoadjuvant therapy; 3. those whose tumour has completely disappeared under the influence of neoadjuvant therapy. By comparing these groups,

information will be compiled on pre- and post-operative examinations (endoscopic and radiological), as well as the results of microscopic evaluation of surgical material (the resected colon segment together with the tumor). The study will summarise the results of examinations performed to date and will also perform additional molecular biological examination of the surgical material. The study is retrospective, based on the summary of the results of examinations that have already been performed. For the in-depth molecular biological examination of the surgical material, it is planned to use the material that has already been prepared and evaluated, which is stored at the Pathology Institute PSCUH. The results obtained will be compared between the above-mentioned groups.

Benefits

The aim of the study is to identify molecular biological differences in tissues depending on the tumour's response to neoadjuvant therapy. The study plans to find additional markers that would allow predicting the responsiveness of rectal cancer to neoadjuvant therapy. By participating in the study, you are supporting the advancement of medicine and science in Latvia. The analysis of facts and materials related to the patient's disease and treatment is the basis for discovering new treatment methods and improving the effectiveness of existing treatments. Participation in the study will not bring immediate benefits, but will serve to gain new knowledge about the disease in the future, which may help people suffering from rectal cancer.

Risks and inconveniences

The information required for the study will be obtained from the results of examinations already performed and existing tissue samples. Participation in the study will not harm your health and will not affect your treatment.

Personal data processing

Data will be collected on age, gender, preoperative stage of the tumor, results of radiological examinations, preoperative therapy received (radiation and chemotherapy), the extent of the surgery performed, the results of morphological and molecular biological examinations of the surgical material, and postoperative therapy and the course of the disease.

Confidentiality and data security

The data obtained in the study will be stored in complete confidentiality and will only be available to a closed group of researchers involved in the study. Upon agreeing to participate in the study, the patient will be assigned an identification code, under which their data will be stored in a database created specifically for the study. Access to this database will be restricted to a closed group of researchers. The data will be stored without any time limit. We confirm that the project will comply with patient data protection and confidentiality requirements in accordance with the applicable regulations in the field of general human rights, personal data protection, and biomedicine.

Voluntary participation

Participation in this study is voluntary. Your refusal to participate in the study will not have any adverse effect on the quality of healthcare you receive.

If you have any questions about this study, please contact Dr. Linda Kokaine by calling or sending a text message on weekdays from 9:00 a.m. to 6:00 p.m. at 23273845 or by emailing linda.kokaine@stradini.lv.

This document has been drawn up in two copies, one of which is kept by the researcher and the other by the research participant.

I hereby confirm with my signature that **(mark your choice with an X):**

- ☐ I have read and understood the contents of this document;
- ☐ I AGREE to participate in this study;
- ☐ I UNDERSTAND that my participation in this study is voluntary and that refusal to participate in the study or withdrawal from the study will not result in any adverse consequences or changes to my treatment plan;
- ☐ I AGREE that during the study, in accordance with legal requirements, my personal data (age, gender) and medical examination results, data from my medical history will be collected, stored and processed;
- ☐ I AGREE that my biological material obtained during the operation will be stored in the laboratory of the RSU Oncology Institute during the study and may be used in this and/or other medical studies which have been approved by the Ethics Committee as complying with bioethical standards, by conducting laboratory, morphological, immunohistochemical or genetic examinations in Latvia and abroad;
- ☐ I AGREE that anonymous data and results obtained in the study may be published;
- ☐ I WISH to be informed about any incidental findings obtained during the study;
- ☐ I DO NOT WISH to be informed about any incidental findings obtained during the study.

_____	_____	_____
Name, family name	Signature	Date

Doctor / clinician: _____	_____	_____
Name, family name	Signature	Date

Annex 5

Informed consent to participate in the study

Information for relatives of deceased person

Dear Madam / Dear Sir!

We invite you to participate in the study ***"Prognostic factors for the effectiveness of rectal cancer treatment"*** conducted by Surgery Clinic of Pauls Stradiņš Clinical University Hospital (PSCUH) in collaboration with the Institute of Pathology and the Oncology Institute of Rīga Stradiņš University (RSU). We would like to inform you about the purpose, procedure and content of the study. Please read all the information carefully before signing this document. Before signing the document, you have the right to ask questions about the study and receive answers to them.

Relevance of the problem

Rectal cancer is one of the most common types of cancer in Latvia and Europe. Every year, around 500 new cases of rectal cancer are diagnosed in Latvia. Accurate preoperative staging of rectal cancer is crucial for the choice of further treatment and the patient's survival. The standard treatment method for non-metastatic (stage II and III) rectal cancer involves preoperative radiation and chemotherapy or radiation therapy alone (neoadjuvant therapy), followed by surgical treatment – rectal resection/extirpation (removal) with or without stoma creation (intestinal outlet in the front wall of the abdomen). Experience to date shows that neoadjuvant therapy plays a significant role in reducing the size and spread of the tumor, thereby improving the radicality of surgical treatment and the patient's prognosis. In addition, it has been observed that in approximately 20 % of cases, the tumor disappears completely as a result of neoadjuvant therapy. Nevertheless, even in these cases, surgical treatment is performed based on the guidelines currently accepted in Europe. However, over the last decade, an alternative treatment approach has become increasingly popular – in cases when complete tumour disappearance is observed after neoadjuvant therapy, surgery is not performed. Instead, the patient is monitored regularly and the tumour site is checked, considering that in about 25 % of patients, the tumour recurs within a few months or a year after treatment. In these cases, standard surgical treatment is performed. Recent studies have shown that the oncological safety outcome for patients undergoing observation is equivalent to that for patients undergoing surgical treatment. Overall, although watchful waiting carries a certain risk, in most cases (if the tumor has disappeared after neoadjuvant therapy) it offers the opportunity to avoid major surgery that affects quality of life. Given the current knowledge about the different tumor behaviour during neoadjuvant therapy, research on prognostic factors for rectal cancer is relevant, as it would allow predicting the possible response of the tumor before starting treatment.

Aim of the study

To evaluate the characteristics of stage II and III rectal cancer depending on its response to preoperative radiation and chemotherapy.

Study procedure

The study plans to include patients with stage II and III rectal cancer who have received preoperative radiation and chemotherapy or radiation therapy alone (neoadjuvant therapy) and who have undergone surgical treatment at the Surgery Clinic of the PSCUH between October 1, 2015, and October 1, 2021. Patients will be divided into 3 groups: 1. those who have had a moderate response to neoadjuvant

therapy; 2. those who have had a good response to neoadjuvant therapy; 3. those whose tumor has completely disappeared under the influence of neoadjuvant therapy. By comparing these groups, information will be compiled on pre- and post-operative examinations (endoscopic and radiological), as well as the results of microscopic evaluation of surgical material (the resected colon segment together with the tumor). The study will summarise the results of examinations performed to date and will also perform additional molecular biological examination of the surgical material. The study is retrospective, based on the summary of the results of examinations that have already been performed. For the in-depth molecular biological examination of the surgical material, it is planned to use the material that has already been prepared and evaluated, which is stored at the Pathology Institute PSCUH. The results obtained will be compared between the above-mentioned groups.

Benefits

The aim of the study is to identify molecular biological differences in tissues depending on the tumor's response to neoadjuvant therapy. The study plans to find additional markers that would allow predicting the responsiveness of rectal cancer to neoadjuvant therapy. By participating in the study, you are supporting the advancement of medicine and science in Latvia. The analysis of facts and materials related to the patient's disease and treatment is the basis for discovering new treatment methods and improving the effectiveness of existing treatments. Participation in the study will not bring immediate benefits, but will serve to gain new knowledge about the disease in the future, which may help people suffering from rectal cancer.

Risks and inconveniences

The information required for the study will be obtained from the results of examinations already performed and existing tissue samples. Participation in the study will not harm your health and will not affect your treatment.

Legal basis for the study

The study considers Article 4 of the Law of the Republic of Latvia "On the Protection of the Body of a Deceased Person and the Use of Human Tissues and Organs in Medicine," which stipulates that:

- If the health information system does not contain any information about the deceased person's wishes expressed during their lifetime to prohibit or allow the use of their body, tissues and organs after death, the medical institution (tissue and organ procurement centre) is obliged to ascertain information about the deceased person's wishes expressed during their lifetime to prohibit or allow the use of their body, tissues and organs after death, by contacting the closest relative present (spouse, parents, adult child, brother, sister, or the deceased person's contact person indicated in the health information system).
- The information provided by the next of kin shall be recorded in the deceased person's medical records.
- If there is no information in the relevant national information system about the deceased person's wishes expressed during their lifetime to prohibit or allow the use of their body, tissues and organs after death, and it has not been possible to ascertain from the next of kin in accordance with the first and second paragraphs of this section the wishes expressed during his or her lifetime to prohibit or permit the use of his or her body, tissues and organs after death, it shall be presumed that the deceased person consented during his or her lifetime to the use of his or her body, tissues and organs after death.

- If the information provided by the next of kin about the deceased person's wishes expressed during his or her lifetime to prohibit or permit the use of his or her body, tissues, and organs after death is contradictory, the use of his or her body, tissues, and organs is prohibited.

Personal data processing

Data will be collected on age, gender, preoperative stage of the tumor, results of radiological examinations, preoperative therapy received (radiation and chemotherapy), the extent of the surgery performed, the results of morphological and molecular biological examinations of the surgical material, and postoperative therapy and the course of the disease.

Confidentiality and data security

The data obtained in the study will be stored in complete confidentiality and will only be available to a closed group of researchers involved in the study. Upon agreeing to participate in the study, the patient will be assigned an identification code, under which their data will be stored in a database created specifically for the study. Access to this database will be restricted to a closed group of researchers. The data will be stored without any time limit. We confirm that the project will comply with patient data protection and confidentiality requirements in accordance with the applicable regulations in the field of general human rights, personal data protection, and biomedicine.

Voluntary participation

Participation in this study is voluntary. Your refusal to participate in the study will not have any adverse effect on the quality of healthcare you receive.

If you have any questions about this study, please contact Dr. Linda Kokaine by calling or sending a text message on weekdays from 9:00 a.m. to 6:00 p.m. at 23273845 or by emailing linda.kokaine@stradini.lv.

This document has been drawn up in two copies, one of which is kept by the researcher and the other by the research participant.

Deceased person to whom the study applies:

_____

Name, family name, ID

The following applies to the analysis of data and biological material from deceased patient, for which consent is given by a relative of the patient (i.e. spouse or first/second/third/fourth degree relative).

I hereby confirm with my signature that **(mark your choice with an X):**

□ I have read and understood the contents of this document;

□ I AGREE that my deceased relative is included in this study;

□ I AGREE that during the study, in accordance with legal requirements, the deceased person's data (age, gender) and medical examination results, as well as data from their medical history, will be collected, stored, and processed;

□ I AGREE that the biological material of the deceased person obtained during the operation will be stored in the laboratory of the RSU Oncology Institute during the study and may be used in this and/or other medical studies which have been approved by the Ethics Committee as complying with bioethical standards, by conducting laboratory, morphological, immunohistochemical or genetic examinations in Latvia and abroad;

□ AGREE that anonymous data and results obtained in the study may be published.

The relative of
the deceased person _____ _____ _____
Name, family name *Signature* *Date*

Doctor / clinician: _____	_____	_____
Name, family name	Signature	Date

Total RNA (including miRNA) extraction from FFPE tissue samples using the QIAGEN miRNeasy FFPE Kit protocol (QIAGEN, ID: 217504)*

Protocol of the procedure:

1) Place two 10 µm sections in a 2 ml Eppendorf tube;
2) add 320 µl of deparaffinisation solution (Deparaffinisation Solution, 16 ml, QIAGEN), vortex (using Vortex V−1 Plus; Biosan) for 10 seconds and centrifuge (using Dynamica 15R centrifuge) for 10 seconds;
3) incubate at 56°C for 3 minutes, then cool to room temperature;
4) add spike-in (exogenous control) UniSp2,4,5;
5) add 240 µl of Buffer PKD solution and mix by vortexing;
6) centrifuge for 1 min at 10 000 rpm;
7) add 10 µl of proteinase K, mix slowly by pipetting;
8) incubate at 56°C for 15 min while ensuring that the solution is mixed (using Thermo-Shaker TS−100; Biosan), then at 80°C for 15 min;
9) transfer the lower, colorless layer of the solution to a new 2 ml Eppendorf tube;
10) incubate on ice for 3 min, then centrifuge for 15 min at 13,500 rpm;
11) transfer the upper, transparent layer to a new 10 ml Eppendorf tube (without disturbing the precipitate that has formed);
12) add 25 µl of DNase Booster Buffer, mix the solution by gently shaking, centrifuge for 10 seconds;
13) incubate at room temperature for 15 minutes;
14) add 500 µl of Buffer RBC and mix by pipetting;
15) add 1750 µl of 96 % ethanol and mix by pipetting;
16) transfer 700 µl of the sample (including the precipitate) to the RNeasy MinElute column placed in a 2 ml collector, close the cap, centrifuge for 15 seconds at a speed of ≥ 10 000rpm, and discard the filtrate;
17) repeat the previous step several times until the entire sample volume has been filtered through the column.
18) add 500 µl of Buffer RPE to the column, close the cap, centrifuge for 15 seconds at ≥ 10 000 rpm, and discard the filtrate;
19) add 500 µl Buffer RPE to the column, close the cap, centrifuge for 2 minutes at ≥ 10 000rpm, discard the collector with all the filtrate;
20) place the RNeasy MinElute column in a new 2 ml collector, open the column cap and centrifuge at maximum speed for 5 min, discard the collector with all the filtrate;
21) place the RNeasy MinElute column in a new 1.5 ml collector, add 30 µl of RNase-free water to the column membrane, close the cap and centrifuge for 1 min at maximum speed;
22) the result of the process is 24 µl of eluate (total RNA), which is placed and stored at −80 °C (24 samples in total).

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* *https://www.qiagen.com/us/products/discovery-and-translational-research/dna-rna-purification/rna-purification/mirna/mirneasy-ffpe-kit*

Measurement of total RNA concentration in samples using the Thermo Fisher Invitrogen Qubit™ RNA BR Assay Kit (ID: Q10210, Q10211) protocol (fluorometric method) and concentration normalisation in all samples.**

Protocol of the procedure:

1) Prepare the Qubit™ working solution by diluting the Qubit™ RNA BR reagent 1:200 with RNA BR Buffer solution (0.95 µl + 189.05 µl =190 µl in one tube);
2) prepare the calibration solution mixture by adding 10 µl of Qubit™ standard solution to the previously prepared working solution (total volume of 200 µl in each sample), thus preparing two calibration solutions;
3) calibrate the Qubit™ 4 Fluorometer;
4) prepare the test sample solutions – each in a separate tube, to which add 198 µl of Qubit™ working solution (prepared as described above) and 2 µl of the test sample (24 tubes in total);
5) vortex each sample for 3-5 seconds, then incubate at room temperature for 2 minutes;
6) perform spectrophotometry with the Qubit™ 4 Fluorometer and read the results (total RNA concentration, ng/ µl);
7) normalise the RNA concentration of each sample by adding the appropriate amount of RNase-free water, calculated in advance.

** *https://assets.thermofisher.com/TFS-Assets/LSG/manuals/Qubit_RNA_BR_Assay_UG.pdf*

Annex 8

Reverse transcription (RT) reaction for cDNA synthesis using the QIAGEN miRCURY LNA RT Kit (QIAGEN, ID: 339340).***

Protocol of the procedure:

1) Prepare the solutions included in the kit – 5x miRCURY RT Reaction Buffer (place on ice), RNase-free water (place at room temperature), 10x miRCURY RT Enzyme (remove from the freezer at -20°C just before use). Gently shake all solutions and centrifuge;
2) prepare UniSp6 RNA spike-in (exogenous control) by adding 80 µl of nuclease-free water, mix by vortexing; leave on ice for 20–30 minutes, then mix by vortexing; divide into aliquots and store the unused portion at −20°C;
3) prepare total RNA samples;
4) prepare reverse transcription MasterMix (keep components on ice) according to the calculation* (Table 8.1):

Table 8.1

Instruction for preparing the MasterMix

MasterMix	Volume per reaction (according to protocol)	Volume adapted to the current RNS concentration (1 reaction)	Volume adapted to the current RNS concentration (24 reactions)
5× miRCURY RT Reaction Buffer	2 µl	8 µl	192 µl
RNase-free water	4.5 µl	18 µl	432 µl
10x miRCURY RT Enzyme Mix	1 µl	4 µl	96 µl
Spike-in	0.5 µl	UniSp6 2 µl + Stem R39 2 µl	48+48 µl
RNS (5 ng/µl)	2 µl	8 µl (20 ng/ µl)	NA
Total volume of the reaction	10 µl	42 µl	NA

*When preparing reaction solutions, possible volume losses during the work process are taken into account, which is why the solution is prepared in an additional 10 % of the total volume (not applicable to biological samples).

1) incubate for 60 min at +42°C using Peqlab PeqSTAR96 X thermocyclers;
2) incubate for 5 min at +95°C using Peqlab PeqSTAR96 X thermocyclers to inactivate the reverse transcriptase enzyme;
3) store at +4°C for further storage (if stored for a long time, store at −20°C).

*** *https://www.qiagen.com/us/products/discovery-and-translational-research/pcr-qpcr-dpcr/qpcr-assays-and-instruments/mirna-qpcr-assay-and-panels/mircury-lna-rt-kit*

Reverse transcription polymerase chain reaction (RT-PCR) was performed to profile miRNA (752 miRNA in total) in selected samples using the miRCURY LNA miRNA miRNome PCR Panels protocol (QIAGEN, ID: 339322).****

Protocol of the procedure:

1) Prepare the necessary elements – pre-prepared cDNA, PCR plates with ready primers (384 reservoir plates containing "dried" primers – each reservoir is intended for one specific reaction; 8 of them – control reservoirs without added primers. The list of MiRNS primers is summarised in Table 9.1), RNase-free water, 2× miRCURY LNA SYBR Green Master Mix (contains QuantiNova® DNA polymerase), ROX reference dye;
2) prepare ROX reference dye at a ratio of 1:200 (low concentration, considering that the subsequent PCR is planned to be performed with the Viia 7 real-time PCR system);
3) dilute each cDNA by adding 1580µl of RNase-free water (diluting the previous concentrate 80x);
4) prepare the reaction solution (at room temperature) according to the calculation* (Table 9.1.):

Table 9.1

Instructions for preparing the reaction solution

Ingredients	Volume per reaction	Volume for 384 reactions
2x miRCURY SYBR Green Master Mix	5µl	1920
ROX reference dye	0.05µl	19.2
cDNS (80x)	4µl	1.536
Rnase-free water	1µl	384
Total volume of the reaction	10.05µl	NA

*When preparing reaction solutions, possible volume losses during the work process are taken into account, which is why the solution is prepared in an additional 10 % of the total volume (not applicable to biological samples).

5) Pipette the reaction solution (repeated with each cDNA) onto PCR plates;
6) wait 5 minutes (to allow the "dried" primers to dissolve), then centrifuge for 10 seconds;
7) place the PCR plate in the Viia 7 real-time PCR system and program it to the appropriate mode (Table 9.2 and Figure 9.1).

Table 9.2

PCR mode in the Viia 7 real-time PCR system

	Time	Temperature	Speed
Step 1 - Initial PCR activation (to activate QuantiNova® DNA polymerase)	2 min	95 °C	Maximum
Step 2 – Denaturation	10 s	95 °C	Maximum
Step 3 – Combined cooling	60s	56 °C	Maximum
Number of cycles	40		
Analysis of the melting curve	60–9 5 °C		

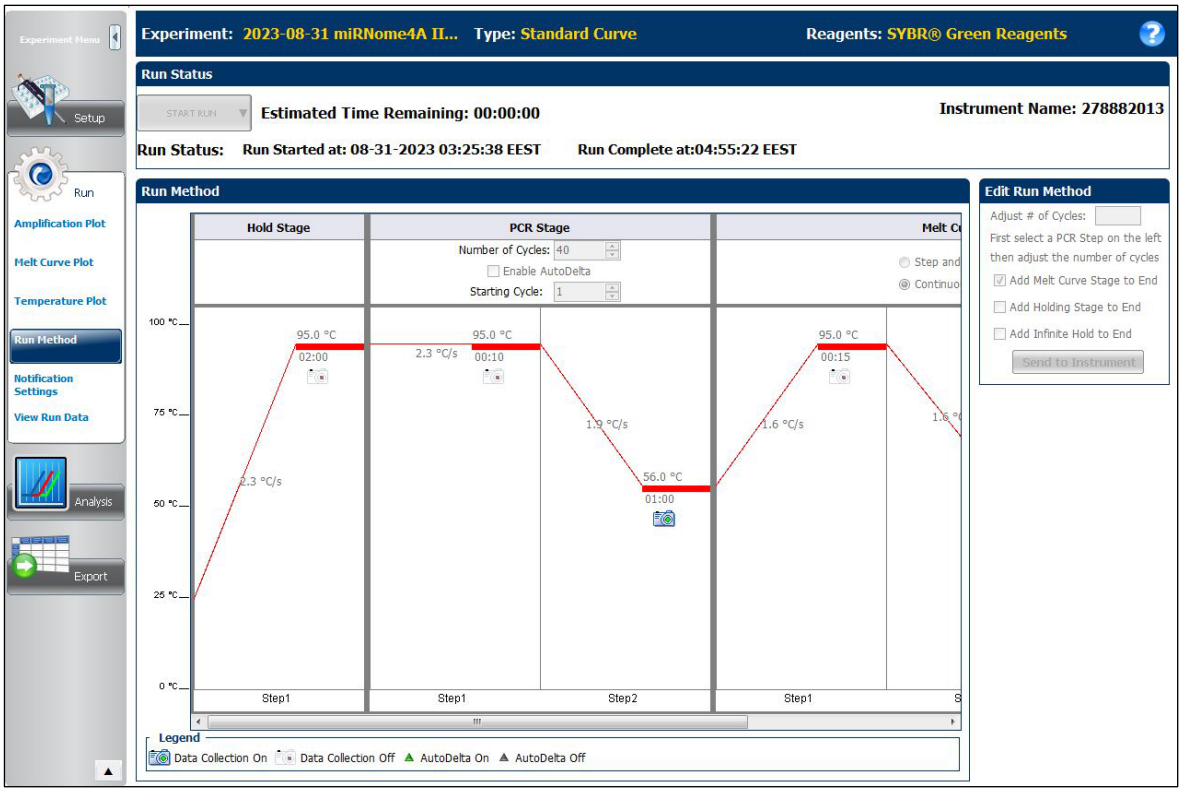


Figure 9.1 **PCR mode in the Viia 7 real-time PCR system**

ViiA 7 automatically generates images with amplification curves (see Figure 9.2) and automatically converts them into Excel file format.

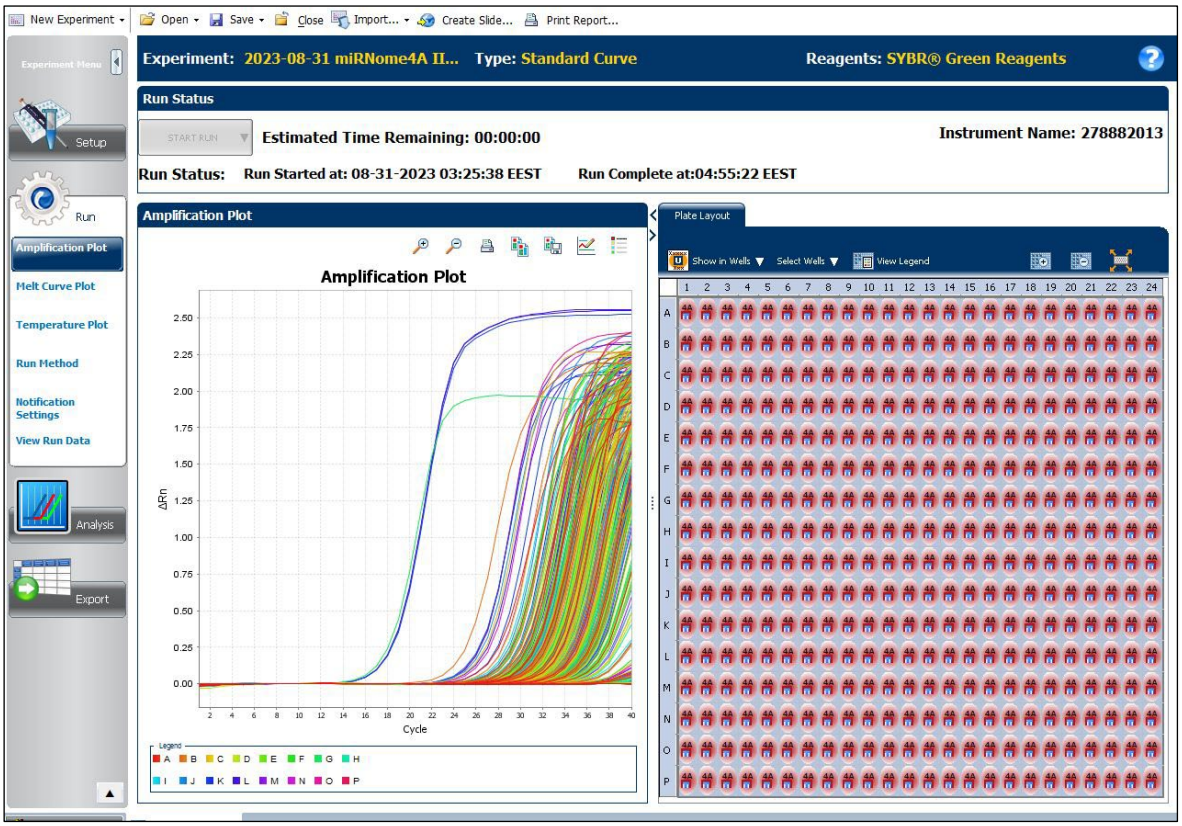


Figure 9.2. **Amplification curves in the Viia 7 real-time PCR system**

Table 9.1

MiRNAs identified during the screening phase

hsa-miR-7-5p	hsa-miR-16-5p	hsa-miR-99b-5p	hsa-miR-654-5p	hsa-miR-631
hsa-miR-217-5p	hsa-miR-98-5p	hsa-miR-431-5p	hsa-miR-545-3p	hsa-miR-34c-5p
hsa-miR-337-5p	hsa-miR-185-5p	hsa-miR-23b-3p	hsa-miR-29b-2-5p	hsa-miR-211-5p
hsa-miR-328-3p	hsa-miR-25-3p	hsa-miR-367-3p	hsa-miR-491-5p	hsa-miR-454-3p
hsa-miR-374b-3p	hsa-miR-765	hsa-miR-505-3p	hsa-miR-92b-3p	hsa-let-7f-5p
hsa-miR-143-3p	hsa-miR-24-3p	hsa-miR-18a-5p	hsa-miR-665	hsa-miR-30e-5p
hsa-miR-623	hsa-miR-369-5p	hsa-miR-92a-3p	hsa-miR-506-3p	hsa-miR-34a-5p
hsa-miR-520c-3p	hsa-miR-425-5p	hsa-miR-500a-5p	hsa-miR-363-3p	hsa-miR-663a
hsa-miR-557	hsa-miR-590-5p	hsa-miR-887-3p	hsa-miR-132-3p	hsa-miR-518e-3p
hsa-miR-218-5p	hsa-miR-760	hsa-miR-491-3p	hsa-miR-651-5p	hsa-miR-29b-3p
hsa-miR-136-5p	hsa-miR-574-3p	hsa-miR-423-3p	hsa-miR-628-3p	hsa-miR-658
hsa-miR-127-5p	hsa-miR-130b-3p	hsa-miR-126-3p	hsa-miR-432-5p	hsa-miR-572
hsa-miR-140-5p	hsa-miR-30c-5p	hsa-miR-421	hsa-miR-154-3p	hsa-miR-802
hsa-miR-31-3p	hsa-miR-133b	hsa-miR-376b-3p	hsa-miR-27a-3p	hsa-miR-521
hsa-miR-20b-3p	hsa-miR-524-5p	hsa-miR-302c-3p	hsa-miR-376c-3p	hsa-miR-433-3p
hsa-miR-325	hsa-miR-23a-3p	hsa-miR-625-3p	hsa-miR-940	hsa-miR-660-5p
hsa-miR-509-3-5p	hsa-miR-193b-3p	hsa-miR-339-5p	hsa-miR-22-5p	hsa-let-7c-5p
hsa-miR-210-3p	hsa-miR-501-5p	hsa-miR-873-5p	hsa-miR-224-5p	hsa-miR-28-5p
hsa-miR-199b-5p	hsa-miR-518c-5p	hsa-miR-323a-3p	hsa-miR-885-5p	hsa-miR-324-5p
hsa-miR-194-5p	hsa-miR-130a-3p	hsa-miR-181d-5p	hsa-miR-320a-3p	hsa-miR-219a-5p
hsa-let-7g-5p	hsa-miR-933	hsa-miR-125a-5p	hsa-miR-18b-5p	hsa-miR-19b-3p
hsa-miR-203a-3p	hsa-miR-379-5p	hsa-miR-129-5p	hsa-miR-187-3p	hsa-miR-526b-5p
hsa-miR-181a-3p	hsa-miR-452-5p	hsa-miR-492	hsa-miR-516b-5p	hsa-miR-215-5p
hsa-miR-137-3p	hsa-miR-589-5p	hsa-miR-20a-5p	hsa-miR-302c-5p	hsa-miR-30b-5p
hsa-miR-551b-3p	hsa-miR-141-3p	hsa-miR-374b-5p	hsa-miR-548b-3p	hsa-miR-184
hsa-miR-524-3p	hsa-miR-342-3p	hsa-miR-302d-3p	hsa-miR-186-5p	hsa-miR-422a
hsa-miR-486-5p	hsa-miR-668-3p	hsa-miR-346	hsa-miR-199a-5p	hsa-miR-199a-3p
hsa-miR-329-3p	hsa-miR-934	hsa-miR-151a-3p	hsa-miR-155-5p	hsa-miR-335-5p
hsa-miR-487b-3p	hsa-miR-101-3p	hsa-miR-493-3p	hsa-miR-107	hsa-miR-519a-3p
hsa-miR-138-5p	hsa-miR-539-5p	hsa-miR-122-5p	hsa-miR-302b-3p	hsa-miR-21-5p
hsa-miR-191-5p	hsa-miR-331-3p	hsa-miR-99a-3p	hsa-miR-662	hsa-miR-129-2-3p
hsa-miR-378a-3p	hsa-miR-499a-5p	hsa-miR-361-5p	hsa-miR-519d-3p	hsa-miR-26b-5p
hsa-miR-103a-3p	hsa-miR-196a-5p	hsa-miR-202-3p	hsa-miR-485-3p	hsa-miR-214-3p
hsa-miR-890	hsa-miR-888-5p	hsa-miR-125b-5p	hsa-miR-200b-3p	hsa-miR-32-5p
hsa-miR-423-5p	hsa-miR-330-3p	hsa-miR-503-5p	hsa-miR-337-3p	hsa-miR-324-3p
hsa-miR-221-3p	hsa-miR-570-3p	hsa-miR-204-5p	hsa-miR-494-3p	hsa-miR-488-3p
hsa-miR-301b-3p	hsa-miR-518c-3p	hsa-miR-30d-5p	hsa-miR-371a-3p	hsa-miR-371a-5p
hsa-miR-550a-5p	hsa-miR-200a-3p	hsa-miR-301a-3p	hsa-miR-637	hsa-miR-455-5p
hsa-miR-532-5p	hsa-miR-188-5p	hsa-miR-362-5p	hsa-miR-144-3p	hsa-miR-891a-5p
hsa-miR-99a-5p	hsa-miR-26a-5p	hsa-miR-30b-3p	hsa-miR-16-1-3p	hsa-miR-549a-3p
hsa-miR-205-5p	hsa-miR-498-5p	hsa-miR-296-5p	hsa-miR-216a-5p	hsa-miR-148a-3p
hsa-miR-518b	hsa-miR-148b-3p	hsa-miR-20b-5p	hsa-miR-424-5p	hsa-miR-146a-5p
hsa-miR-19a-3p	hsa-miR-127-3p	hsa-miR-147a	hsa-miR-921	hsa-miR-139-5p
hsa-miR-150-5p	hsa-miR-598-3p	hsa-miR-198	hsa-miR-513a-5p	hsa-miR-373-5p
hsa-miR-15a-5p	hsa-miR-96-5p	hsa-miR-375-3p	hsa-miR-140-3p	hsa-miR-149-5p

Table 9.1 continued

hsa-let-7d-3p	hsa-let-7d-5p	hsa-miR-517a-3p	hsa-miR-181a-5p	hsa-miR-642a-5p
hsa-miR-608	hsa-miR-135b-5p	hsa-miR-361-3p	hsa-miR-10a-5p	hsa-miR-31-5p
hsa-miR-671-5p	hsa-miR-495-3p	hsa-miR-21-3p	hsa-miR-106a-5p	hsa-miR-451a
hsa-miR-497-5p	hsa-miR-299-5p	hsa-miR-373-3p	hsa-miR-182-5p	hsa-miR-620
hsa-miR-877-5p	hsa-miR-34c-3p	hsa-miR-518f-3p	hsa-miR-370-3p	hsa-miR-27b-3p
hsa-miR-187-5p	hsa-miR-596	hsa-miR-222-3p	hsa-miR-576-5p	hsa-miR-523-3p
hsa-miR-10b-5p	hsa-miR-744-5p	hsa-miR-617	hsa-miR-425-3p	hsa-miR-374a-5p
hsa-let-7i-5p	hsa-miR-145-5p	hsa-miR-154-5p	hsa-miR-450a-5p	hsa-miR-92a-1-5p
hsa-miR-202-5p	hsa-miR-622	hsa-miR-708-5p	hsa-miR-411-5p	hsa-miR-219a-1-3p
hsa-miR-652-3p	hsa-miR-516a-5p	hsa-let-7b-5p	hsa-miR-216b-5p	hsa-miR-1913
hsa-miR-126-5p	hsa-let-7a-5p	hsa-miR-95-3p	hsa-miR-106b-5p	hsa-miR-1245a
hsa-miR-30e-3p	hsa-miR-96-3p	hsa-miR-517c-3p	hsa-miR-22-3p	hsa-miR-522-3p
hsa-miR-181c-5p	hsa-miR-185-3p	hsa-miR-151a-5p	hsa-miR-510-5p	hsa-miR-571
hsa-miR-9-3p	hsa-miR-615-3p	hsa-miR-502-5p	hsa-miR-212-3p	hsa-miR-323a-5p
hsa-miR-548c-3p	hsa-miR-128-3p	hsa-miR-345-5p	hsa-miR-525-5p	hsa-miR-592
hsa-miR-152-3p	hsa-miR-766-3p	hsa-miR-509-3p	hsa-miR-542-5p	hsa-miR-487a-3p
hsa-miR-93-5p	hsa-miR-206	hsa-miR-134-5p	hsa-miR-576-3p	hsa-miR-1249-3p
hsa-miR-365a-3p	hsa-miR-298	hsa-miR-382-5p	hsa-miR-583	hsa-miR-25-5p
hsa-miR-29c-3p	hsa-miR-193a-5p	hsa-miR-490-3p	hsa-miR-483-3p	hsa-miR-922
hsa-miR-372-3p	hsa-miR-449b-5p	hsa-miR-200c-3p	hsa-miR-582-5p	hsa-miR-124-5p
hsa-miR-133a-3p	hsa-miR-520d-5p	hsa-miR-30a-5p	hsa-miR-183-5p	hsa-miR-1264
hsa-miR-124-3p	hsa-miR-192-5p	hsa-miR-181b-5p	hsa-miR-33b-5p	hsa-miR-504-5p
hsa-miR-190a-5p	hsa-miR-29a-3p	hsa-miR-33a-5p	hsa-miR-193a-3p	hsa-miR-138-1-3p
hsa-miR-302a-3p	hsa-miR-18a-3p	hsa-miR-195-5p	hsa-miR-153-3p	hsa-miR-502-3p
hsa-miR-595	hsa-miR-383-5p	hsa-miR-874-3p	hsa-let-7e-5p	hsa-miR-490-5p
hsa-miR-602	hsa-miR-9-5p	hsa-miR-135a-5p	hsa-miR-409-3p	hsa-miR-567
hsa-miR-223-3p	hsa-miR-142-5p	hsa-miR-26a-2-3p	hsa-miR-100-5p	hsa-miR-18b-3p
hsa-miR-627-5p	hsa-miR-363-5p	hsa-miR-146b-5p	hsa-miR-629-5p	hsa-miR-125a-3p
hsa-miR-34b-3p	hsa-miR-147b-3p	hsa-miR-412-3p	hsa-miR-484	hsa-miR-653-5p
hsa-miR-410-3p	hsa-miR-197-3p	hsa-miR-1-3p	hsa-miR-429	hsa-miR-891b
hsa-miR-17-5p	hsa-miR-597-5p	hsa-miR-299-3p	hsa-miR-30c-2-3p	hsa-miR-144-5p
hsa-miR-376a-3p	hsa-miR-326	hsa-miR-142-3p	hsa-miR-518a-3p	hsa-miR-1538
hsa-miR-514a-3p	hsa-miR-15b-5p	hsa-miR-338-3p	hsa-miR-340-5p	hsa-miR-384
hsa-miR-512-5p	hsa-miR-105-5p	hsa-miR-584-5p	hsa-miR-508-3p	hsa-miR-196b-3p
hsa-miR-449a	hsa-miR-196b-5p	hsa-miR-377-3p	hsa-miR-381-3p	hsa-miR-649
hsa-miR-143-5p	hsa-miR-892a	hsa-miR-141-5p	hsa-miR-520h	hsa-miR-518d-3p
hsa-miR-1207-5p	hsa-miR-10b-3p	hsa-miR-1269a	hsa-miR-769-5p	hsa-miR-569
hsa-miR-943	hsa-miR-122-3p	hsa-miR-501-3p	hsa-miR-612	hsa-miR-125b-1-3p
hsa-miR-675-3p	hsa-miR-100-3p	hsa-miR-15b-3p	hsa-miR-1237-3p	hsa-miR-218-2-3p
hsa-miR-200b-5p	hsa-miR-769-3p	hsa-miR-146b-3p	hsa-miR-1908-5p	hsa-miR-519c-3p
hsa-miR-519e-5p	hsa-miR-300	hsa-miR-222-5p	hsa-miR-1260a	hsa-miR-554
hsa-miR-942-5p	hsa-miR-518e-5p	hsa-miR-601	hsa-miR-182-3p	hsa-miR-938
hsa-miR-450b-3p	hsa-miR-489-3p	hsa-miR-924	hsa-miR-365b-5p	hsa-miR-1243
hsa-miR-553	hsa-miR-937-3p	hsa-miR-29a-5p	hsa-miR-508-5p	hsa-miR-708-3p
hsa-miR-605-5p	hsa-miR-381-5p	hsa-let-7a-2-3p	hsa-miR-671-3p	hsa-miR-1185-5p
hsa-miR-24-2-5p	hsa-miR-640	hsa-miR-520f-3p	hsa-miR-941	hsa-miR-512-3p

Table 9.1 continued

hsa-miR-23a-5p	hsa-miR-148b-5p	hsa-miR-101-5p	hsa-miR-23b-5p	hsa-miR-587
hsa-miR-27b-5p	hsa-miR-29c-5p	hsa-miR-520a-3p	hsa-miR-591	hsa-miR-603
hsa-miR-759	hsa-miR-499a-3p	hsa-miR-548m	hsa-miR-26b-3p	hsa-miR-1184
hsa-miR-770-5p	hsa-let-7f-1-3p	hsa-miR-517-5p	hsa-miR-519b-3p	hsa-miR-20a-3p
hsa-miR-585-3p	hsa-miR-382-3p	hsa-miR-448	hsa-miR-30d-3p	hsa-miR-588
hsa-miR-376a-5p	hsa-miR-609	hsa-miR-1296-5p	hsa-miR-518d-5p	hsa-miR-455-3p
hsa-miR-507	hsa-miR-10a-3p	hsa-miR-1537-3p	hsa-miR-212-5p	hsa-miR-582-3p
hsa-miR-520b-3p	hsa-miR-106a-3p	hsa-miR-920	hsa-miR-520e-3p	hsa-miR-409-5p
hsa-miR-302f	hsa-let-7e-3p	hsa-miR-1247-5p	hsa-miR-646	hsa-miR-452-3p
hsa-miR-28-3p	hsa-miR-580-3p	hsa-miR-19b-2-5p	hsa-miR-519e-3p	hsa-miR-19b-1-5p
hsa-miR-875-5p	hsa-miR-761	hsa-miR-558	hsa-miR-626	hsa-miR-610
hsa-miR-219a-2-3p	hsa-miR-643	hsa-miR-106b-3p	hsa-miR-26a-1-3p	hsa-miR-511-5p
hsa-miR-1183	hsa-miR-618	hsa-miR-1258	hsa-miR-190b	hsa-miR-200c-5p
hsa-miR-758-3p	hsa-miR-221-5p	hsa-miR-619-3p	hsa-miR-1471	hsa-let-7a-3p
hsa-miR-1244	hsa-miR-513b-5p	hsa-miR-208a-3p	hsa-miR-548l	hsa-miR-135a-3p
hsa-miR-566	hsa-miR-411-3p	hsa-miR-17-3p	hsa-miR-586	hsa-miR-520a-5p
hsa-miR-1256	hsa-miR-19a-5p	hsa-miR-136-3p	hsa-miR-103b	hsa-miR-1468-5p
hsa-miR-516a-3p	hsa-miR-338-5p	hsa-miR-877-3p	hsa-miR-488-5p	hsa-miR-628-5p
hsa-miR-548c-5p	hsa-miR-1914-3p	hsa-miR-935	hsa-miR-129-1-3p	hsa-miR-552-3p
hsa-miR-496	hsa-miR-323b-5p	hsa-miR-224-3p	hsa-miR-192-3p	hsa-miR-145-3p
hsa-miR-876-3p	hsa-miR-548i	hsa-miR-624-3p	hsa-miR-632	hsa-miR-378a-5p
hsa-miR-532-3p	hsa-miR-541-3p	hsa-miR-767-5p	hsa-miR-181a-2-3p	hsa-miR-7-1-3p
hsa-miR-654-3p	hsa-miR-1272	hsa-miR-559	hsa-miR-1909-3p	hsa-miR-181c-3p
hsa-miR-659-3p	hsa-miR-1205	hsa-miR-449b-3p	hsa-miR-573	hsa-miR-195-3p
hsa-miR-135b-3p	hsa-miR-544a	hsa-miR-205-3p	hsa-miR-302d-5p	hsa-miR-578
hsa-miR-641	hsa-miR-431-3p	hsa-miR-604	hsa-miR-194-3p	hsa-miR-505-5p
hsa-miR-2113	hsa-miR-621	hsa-miR-130b-5p	hsa-miR-302b-5p	hsa-miR-875-3p
hsa-miR-1254	hsa-miR-556-5p	hsa-miR-149-3p	hsa-miR-551b-5p	hsa-miR-450b-5p
hsa-miR-661	hsa-miR-1267	hsa-miR-1271-5p	hsa-miR-635	hsa-miR-876-5p
hsa-miR-362-3p	hsa-miR-379-3p	hsa-miR-92b-5p	hsa-miR-1911-3p	hsa-miR-1224-3p
hsa-miR-624-5p	hsa-miR-556-3p	hsa-miR-551a	hsa-let-7f-2-3p	hsa-miR-1539
hsa-miR-27a-5p	hsa-miR-614	hsa-miR-146a-3p	hsa-miR-155-3p	hsa-miR-663b
hsa-miR-744-3p	hsa-miR-616-5p	hsa-miR-218-1-3p	hsa-miR-105-3p	hsa-miR-1248
hsa-miR-139-3p	hsa-miR-93-3p	hsa-miR-593-5p	hsa-miR-486-3p	hsa-miR-889-3p
hsa-miR-138-2-3p	hsa-miR-1972	hsa-miR-561-3p	hsa-miR-320b	hsa-miR-1227-3p
hsa-miR-655-3p	hsa-miR-616-3p	hsa-miR-767-3p	hsa-miR-296-3p	hsa-miR-548h-5p
hsa-miR-99b-3p	hsa-miR-369-3p	hsa-miR-526b-3p	hsa-miR-7-2-3p	hsa-miR-1255b-5p
hsa-miR-581	hsa-miR-2110	hsa-miR-24-1-5p	hsa-miR-550a-3p	hsa-miR-330-5p
hsa-miR-191-3p	hsa-miR-548a-3p	hsa-let-7b-3p	hsa-miR-380-3p	hsa-miR-1238-3p
hsa-miR-32-3p	hsa-miR-634	hsa-miR-193b-5p	hsa-miR-593-3p	hsa-miR-188-3p
hsa-miR-1204	hsa-miR-320c	hsa-miR-335-3p	hsa-miR-1912-3p	hsa-miR-589-3p
hsa-miR-548j-5p	hsa-miR-636	hsa-miR-541-5p	hsa-miR-493-5p	hsa-miR-125b-2-3p
hsa-miR-555	hsa-miR-606	hsa-miR-30c-1-3p	hsa-miR-432-3p	hsa-miR-16-2-3p
hsa-miR-515-5p	hsa-miR-208b-3p	hsa-miR-629-3p	hsa-miR-454-5p	hsa-miR-650
hsa-miR-340-3p	hsa-miR-367-5p	hsa-miR-377-5p	hsa-miR-936	hsa-miR-1178-3p
hsa-miR-513a-3p	hsa-miR-520d-3p	hsa-miR-630	hsa-miR-30a-3p	hsa-miR-600

Table 9.1 continued

hsa-miR-34a-3p	hsa-miR-1265	hsa-miR-548d-3p	hsa-let-7g-3p	hsa-miR-599
hsa-miR-342-5p	hsa-miR-1203	hsa-miR-885-3p	hsa-miR-214-5p	hsa-miR-520g-3p
hsa-miR-639	hsa-miR-548k	hsa-miR-320d	hsa-miR-183-3p	hsa-miR-564
hsa-let-7i-3p	hsa-miR-548a-5p	hsa-miR-2053	hsa-miR-1179	hsa-miR-132-5p
hsa-miR-543	hsa-miR-1253	hsa-miR-675-5p	hsa-miR-562	hsa-miR-577
hsa-miR-645	hsa-miR-615-5p	hsa-miR-1252-5p	hsa-miR-579-3p	hsa-miR-223-5p
hsa-miR-548d-5p	hsa-miR-607	hsa-miR-548e-3p	hsa-miR-590-3p	hsa-miR-34b-5p
hsa-miR-33a-3p	hsa-miR-1208	hsa-miR-1914-5p	hsa-miR-130a-5p	hsa-miR-888-3p
hsa-miR-664a-3p	hsa-miR-302e	hsa-miR-513c-5p	hsa-miR-563	hsa-miR-424-3p
hsa-miR-1911-5p	hsa-miR-1206	hsa-miR-331-5p	hsa-miR-200a-5p	hsa-miR-339-3p
hsa-miR-33b-3p	hsa-miR-1270	hsa-miR-1182	hsa-miR-483-5p	hsa-miR-380-5p
hsa-miR-638	hsa-miR-525-3p	hsa-miR-611	hsa-miR-15a-3p	hsa-miR-647
hsa-miR-515-3p	hsa-miR-1200	hsa-miR-1181	hsa-miR-944	hsa-miR-518f-5p
hsa-miR-548n	hsa-miR-92a-2-5p			

**** *https://www.qiagen.com/us/products/discovery-and-translational-research/pcr-qpcr-dpcr/qpcr-assays-and-instruments/mirna-qpcr-assay-and-panels/mircury-lna-mirna-mirnome-pcr-panels*

cDNS synthesis and RT reaction using Thermo Fisher Applied Biosystems TaqMan™ MicroRNA Reverse Transcription Kit (ID: 4366596) protocol and primers (Thermo Fisher Applied Biosystems; ID 002681, 002141, 002229, 002248, 002429, 000483, 002642).***

Protocol of the procedure:

1) Prepare the necessary reverse transcription elements by placing them on ice, vortexing them after thawing, and centrifuging them briefly;
2) prepare the reverse transcription reaction mixture (RT Reaction Mix) according to the protocol and calculation* (Table 10.1):

Table 10.1

Instructions for RT Reaction Mix

Component	Volume of a single reaction (according to protocol)	Volume adapted to the current RNS concentration (58 samples, i.e. 464 reactions)
100mM dNTPs	0.15 µl	69.6 µl
MultiScribe reverse transcriptase, 50U/ µl	1.00 µl	464 µl
10x Reverse Transcription Buffer Solution	1.50 µl	696 µl
RNase inhibitor, 20U/ µl	0.19 µl	88.2 µl
Rnase-free water	4.16 µl	1930.2 µl
Total reaction volume	7.00 µl	32485 µl

*When preparing reaction solutions, possible volume losses during the work process are taken into account, which is why the solution is prepared in an additional 10 % of the total volume (not applicable to biological samples).

3) prepare a plate for reverse transcription reaction (Table 10.2.) – one plate can be used for reactions with 12 samples;

Table 10.2

Example of a RT reaction plate

Sample / Primer	3A	3B	5A	5B	13A	13B	14A	14B	20A	20B	21A	21B
hsa-miR-665												
hsa-miR-142-5p												
hsa-miR-548c-5p												
hsa-miR-182-3p*												
hsa-miR-99a-3p*												
hsa-miR-127-5p												
hsa-miR-361-3p												
hsa-miR-151-5p												

4) pipette 7 µl of the prepared reverse transcription mixture into each well of the plate (in all wells, i.e. 96 wells) and 5 µl of total RNA (5 ng/µl) from each sample total RNA dilution into the designated wells (i.e. 8 wells);
5) add 3 µl of reverse transcriptase primer to each designated reservoir (i.e. 12 reservoirs); total reaction volume in one reservoir – 15 µl;
6) place a sealing film on the work plate and centrifuge for 10 seconds;

7) place on ice and proceed to the next step;
8) incubate for 30 min at +16 °C, then 30 min at +42 °C, and then 5 min at +85 °C using Peqlab PeqSTAR96 X thermocyclers;
9) then place the plate at +4 °C (for storage up to 1 week, place at –20 °C);
10) repeat the above steps for all samples.

***** *https://www.thermofisher.com/order/catalog/product/4366596*

Annex 11

Preparation of PCR plates and RT-PCR miRNA quantification using the TaqMan™ Small MicroRNA Assay (Thermo Fisher Applied Biosystems; ID: 4427975) protocol and TaqMan™ Fast Advanced Master Mix (ID: 4444558) and primers (Thermo Fisher Applied Biosystems; ID 002681, 002141, 002229, 002248, 002429, 000483, 002642).******

Protocol of the procedure:

1) Prepare the elements necessary for the reaction – TaqMan Small RNA Assay (20X), PCR MasterMix, RNase-free water;
2) Prepare the PCR mixture according to the protocol* (Table 11.1):

Table 11.1

Instructions for preparation of PCR mixture

	Volume of a single reaction	**Volume required to prepare one specific primer solution (186 reactions)**	**Volume required for all primer reactions (186 x8)**
TaqMan Small RNA Assay (20X)	0.50 µl	93 µl	NA
PCR MasterMix	5.00 µl	930 µl	7440 µl
RNase-free water	3.84 µl	714.24 µl	5713.92 µl

. work process are taken into account, which is why the solution is prepared in an additional 10% of the total volume (not applicable to biological samples).

3) Prepare primer dilutions for all reactions with the specific primer, according to the calculation (186 reactions), and store the unused portion at +4 °C in the dark during this stage;
4) pipette the prepared primer solutions into the appropriate reservoirs on the PCR plate (Table 11.2):

Table 11.2

Example of a PCR work plate

Sample	hsa-miR-665			hsa-miR-142-5p			hsa-miR-548c-5p			hsa-miR-182-3p			hsa-miR-99a-3p			hsa-miR-127-5p			hsa-miR-361-3p			hsa-miR-151-5p		
A																								
B																								
C																								
D																								
E																								
F																								
G																								
H																								
I																								
J																								
K																								
L																								
M																								
N																								
O																								
P																								

5) Add 0.51 µl of each sample cDNA to the corresponding plate wells (one sample is transferred to 3 wells – 0.51 µl each) (Table 11.3);

Table 11.3

Complementary DNA transfer to PCR plates (example)

Sample / Primer	3A	3B
hsa-miR-665		
hsa-miR-142-5p		
hsa-miR-548c-5p		
hsa-miR-182-3p*		
hsa-miR-99a-3p*		
hsa-miR-127-5p		
hsa-miR-361-3p		
hsa-miR-151-5p		

Samples	hsa-miR-665			hsa-miR-142-5p		
A						
B						
C						
D						
E						
F						
G						
H						
I						
J						
K						
L						
M						
N						
O						
P						

6) place an optical adhesive film on the plate, then centrifuge for 10 seconds;
7) create a PCR program in the Viia 7 real-time PCR system according to the protocol (Table 11.4):

Table 11.4

PCR mode in the Viia 7 real-time PCR system

Step	Temperature	Time	Number of cycles
UNG activation*	50 °C	2 min	1
Enzyme activation	95 °C	20 s	1
Denaturation	95 °C	1 s	40
Cooling	60 °C	20 s	

*UNG, or uracil-N-glycosylase (enzyme), is a component of PCR MasterMix whose biological function is to remove uracil from cDNA, creating free uracil and alkali-sensitive apyrimidine sites in DNA. UNG removes uracil incorporated into single-stranded and double-stranded DNA by catalyzing the hydrolysis of the N-glycosyl bond between uracil and sugar. PCR uses it to prevent cross-contamination between samples.

8) Repeat the above steps with the remaining boards.

****** *https://www.thermofisher.com/order/catalog/product/4398987*

Annex 12

Rectal cancer miRNA screening results in patients with LA and SA response to NAT (according to miRCURY LNA miRNA miRNome PCR panel (QIAGEN, ID: 339322)

Table 12.1

Rectal cancer miRNA screening results

MiRNA	GR group		BR group	
	Fold change	p value	Fold change	p value
hsa-miR-7-5p	1.09	0.483537	3.11	0.155110
hsa-miR-217-5p	1.90	0.068450	2.17	0.063208
hsa-miR-212-3p	1.86	0.000011	−1.86	0.001053
hsa-miR-99a-5p	2.13	0.000200	1.74	0.002632
hsa-miR-433-3p	2.00	0.000210	−1.66	0.003340
hsa-miR-34a-5p	1.59	0.000242	2.56	0.004262
hsa-miR-665	2.36	0.000280	−2.33	0.004307
hsa-miR-10b-5p	−1.80	0.000604	1.61	0.004346
hsa-miR-19b-1-5p	−1.82	0.000660	−1.45	0.006249
hsa-miR-342-3p	1.33	0.002123	1.63	0.006325
hsa-miR-100-5p	1.90	0.002513	−2.29	0.006477
hsa-miR-127-5p	2.36	0.002621	−2.64	0.007100
hsa-miR-1260a	−1.37	0.002947	1.50	0.007113
hsa-miR-7-1-3p	−1.51	0.003101	1.82	0.007702
hsa-miR-125b-5p	1.61	0.003375	1.96	0.007869
hsa-miR-141-3p	−1.56	0.003720	9.04	0.007937
hsa-miR-337-3p	1.77	0.003995	2.56	0.007944
hsa-miR-147b-3p	−1.86	0.004063	2.88	0.008054
hsa-miR-141-5p	−2.59	0.004384	1.52	0.008718
hsa-miR-95-3p	−1.57	0.004420	1.26	0.009064
hsa-miR-885-3p	1.69	0.004435	−2.36	0.009481
hsa-miR-577	−1.89	0.004589	1.58	0.010218
hsa-miR-184	−1.96	0.006302	1.63	0.011748
hsa-miR-147a	−1.73	0.006441	−1.64	0.013481
hsa-miR-877-5p	1.33	0.006542	2.32	0.013818
hsa-miR-381-3p	1.96	0.007977	2.82	0.014823
hsa-miR-215-5p	−1.48	0.008166	2.48	0.015213
hsa-miR-543	1.91	0.008240	−1.92	0.015225
hsa-miR-99a-3p	2.52	0.008658	2.12	0.015651
hsa-miR-127-3p	1.79	0.008761	1.50	0.016058
hsa-miR-1244	2.21	0.008839	3.10	0.016115
hsa-miR-192-3p	−1.38	0.008877	−1.60	0.016542
hsa-miR-193b-3p	1.24	0.009186	1.55	0.016622
hsa-miR-192-5p	−1.43	0.009468	2.82	0.017131
hsa-miR-194-5p	−1.34	0.009533	1.82	0.017498
hsa-miR-454-3p	−1.28	0.010086	1.53	0.017723
hsa-miR-410-3p	1.42	0.010723	1.28	0.018518
hsa-let-7a-2-3p	3.67	0.010734	1.29	0.019030
hsa-miR-495-3p	1.79	0.010883	−3.11	0.019105
hsa-miR-34a-3p	1.44	0.011435	1.29	0.019228
hsa-miR-370-3p	2.27	0.012701	1.29	0.019349
hsa-miR-411-3p	2.30	0.013664	1.30	0.019943
hsa-miR-382-3p	1.56	0.013752	1.42	0.019998
hsa-miR-154-5p	1.74	0.013852	2.59	0.020626
hsa-miR-26b-3p	−1.22	0.014187	1.80	0.021591
hsa-miR-485-3p	1.77	0.014586	1.41	0.021752
hsa-miR-30a-3p	−1.30	0.014616	1.41	0.022105
hsa-miR-1185-5p	2.07	0.015143	1.32	0.022577
hsa-miR-190a-5p	−1.72	0.016005	2.27	0.022909
hsa-miR-624-5p	−1.70	0.016067	1.25	0.023196

Table 12.1 continued

MiRNS	GR group		BR group	
	Fold change	p value	Fold change	p value
hsa-miR-203a-3p	−1.56	0.016774	1.32	0.023664
hsa-miR-487b-3p	1.44	0.016966	1.32	0.023789
hsa-miR-132-3p	1.40	0.018624	1.52	0.025154
hsa-miR-103a-3p	−1.08	0.019081	1.56	0.025181
hsa-miR-376a-3p	1.73	0.019127	4.04	0.025515
hsa-miR-504-5p	−1.57	0.019264	1.34	0.026180
hsa-miR-376c-3p	1.65	0.019691	1.34	0.027417
hsa-miR-493-3p	3.43	0.020226	1.65	0.027562
hsa-miR-502-5p	1.31	0.020251	1.60	0.027905
hsa-miR-337-5p	1.72	0.020498	4.00	0.028003
hsa-miR-194-3p	−1.56	0.022224	1.35	0.028032
hsa-miR-520g-3p	−2.37	0.022369	1.42	0.028825
hsa-miR-125b-2-3p	2.21	0.023165	1.41	0.028882
hsa-miR-30d-3p	−1.32	0.023860	3.96	0.028982
hsa-miR-1256	2.02	0.024412	4.00	0.029120
hsa-miR-129-5p	−1.72	0.024751	2.27	0.030003
hsa-miR-200b-5p	−1.48	0.024971	1.46	0.030253
hsa-miR-296-5p	1.19	0.024974	−1.53	0.031037
hsa-miR-200c-5p	−1.82	0.025117	1.75	0.031068
hsa-miR-361-3p	1.13	0.026036	1.40	0.031114
hsa-miR-200c-3p	−1.37	0.026711	1.99	0.031115
hsa-miR-200b-3p	−1.26	0.028358	1.22	0.032104
hsa-miR-20a-5p	−1.30	0.029154	2.29	0.032193
hsa-miR-376b-3p	1.86	0.029312	1.61	0.033827
hsa-miR-24-2-5p	−1.12	0.030495	−2.22	0.034119
hsa-miR-23a-3p	−1.11	0.031680	1.43	0.034403
hsa-miR-132-5p	1.71	0.032215	1.38	0.034695
hsa-miR-340-3p	−1.53	0.032416	1.38	0.034695
hsa-miR-136-5p	1.65	0.032612	1.38	0.034695
hsa-miR-429	−1.21	0.033284	1.38	0.034695
hsa-miR-409-5p	1.94	0.033740	1.38	0.034695
hsa-miR-411-5p	1.55	0.033842	1.38	0.034695
hsa-miR-383-5p	−2.76	0.033981	1.38	0.034695
hsa-miR-1247-5p	2.20	0.034503	1.38	0.034695
hsa-miR-20a-3p	−1.28	0.035418	1.38	0.034695
hsa-miR-379-5p	1.40	0.035757	1.38	0.034695
hsa-miR-125b-1-3p	1.65	0.035813	1.38	0.034695
hsa-miR-199b-5p	1.76	0.035999	1.38	0.034695
hsa-miR-191-5p	−1.12	0.036644	1.38	0.034695
hsa-miR-107	−1.12	0.036805	1.38	0.034695
hsa-miR-152-3p	1.15	0.036867	1.38	0.034695
hsa-miR-1248	1.69	0.037260	1.38	0.034695
hsa-miR-940	1.86	0.037710	1.38	0.034695
hsa-miR-199a-5p	1.56	0.038832	1.38	0.034695
hsa-miR-372-3p	−1.41	0.039636	1.38	0.034695
hsa-miR-214-3p	1.38	0.039638	1.38	0.034695
hsa-miR-423-5p	1.29	0.040093	1.38	0.034695
hsa-miR-566	1.68	0.040930	1.38	0.034695
hsa-miR-151a-5p	−1.13	0.041023	1.38	0.034695
hsa-miR-34b-3p	1.51	0.041297	1.38	0.034695
hsa-miR-136-3p	1.69	0.041733	1.38	0.034695
hsa-miR-494-3p	1.81	0.041890	1.38	0.034695
hsa-miR-377-3p	1.79	0.042696	1.38	0.034695
hsa-miR-139-3p	−1.25	0.043272	1.38	0.034695

Annex 12 continued

Table 12.1 continued

MiRNS	GR group		BR group	
	Fold change	p value	Fold change	p value
hsa-miR-221-3p	1.42	0.044022	1.38	0.034695
hsa-miR-378a-3p	−1.40	0.045188	1.38	0.034695
hsa-miR-1271-5p	−1.14	0.045382	1.38	0.034695
hsa-miR-299-5p	1.61	0.046381	1.38	0.034695
hsa-miR-638	1.69	0.046902	1.38	0.034695
hsa-miR-431-5p	2.56	0.049219	1.38	0.034695
hsa-miR-1913	−1.40	0.049275	1.38	0.034695
hsa-miR-200a-3p	−1.09	0.049451	1.38	0.034695
hsa-miR-431-3p	2.64	0.052336	1.38	0.034695
hsa-miR-654-3p	1.44	0.052838	1.38	0.034695
hsa-miR-539-5p	2.01	0.053585	1.38	0.034695
hsa-miR-92b-5p	1.46	0.055369	1.38	0.034695
hsa-miR-92b-3p	1.42	0.055726	1.38	0.034695
hsa-let-7d-5p	−1.14	0.057293	1.38	0.034695
hsa-miR-300	1.88	0.057539	1.38	0.034695
hsa-miR-374a-5p	−1.25	0.057904	1.38	0.034695
hsa-miR-556-5p	−1.33	0.059204	1.38	0.034695
hsa-miR-590-5p	−1.30	0.061189	1.38	0.034695
hsa-miR-134-5p	1.72	0.061714	1.38	0.034695
hsa-miR-148a-3p	1.33	0.062083	1.38	0.034695
hsa-miR-873-5p	3.33	0.062303	1.38	0.034695
hsa-miR-432-3p	2.32	0.062326	1.38	0.034695
hsa-miR-196a-5p	−2.31	0.064682	1.38	0.034695
hsa-miR-374b-5p	−1.25	0.065574	1.38	0.034695
hsa-miR-181a-2-3p	−1.17	0.066800	1.38	0.034695
hsa-miR-211-5p	2.90	0.068332	1.38	0.034695
hsa-miR-378a-5p	−1.30	0.071049	1.38	0.034695
hsa-miR-34b-5p	1.71	0.071897	1.38	0.034695
hsa-miR-506-3p	2.28	0.072015	1.38	0.034695
hsa-miR-137-3p	−1.75	0.073062	1.38	0.034695
hsa-miR-139-5p	−1.39	0.075009	1.38	0.034695
hsa-miR-744-3p	−1.35	0.075172	1.38	0.034695
hsa-miR-519d-3p	−1.64	0.077841	1.38	0.034695
hsa-miR-26a-2-3p	−1.28	0.078746	1.38	0.034695
hsa-miR-100-3p	1.99	0.078889	1.38	0.034695
hsa-miR-876-5p	2.79	0.079140	1.38	0.034695
hsa-miR-589-5p	1.52	0.080125	1.38	0.034695
hsa-miR-493-5p	2.72	0.080370	1.38	0.034695
hsa-miR-515-3p	1.50	0.082778	1.38	0.034695
hsa-miR-432-5p	1.58	0.082922	1.38	0.034695
hsa-miR-514a-3p	−2.51	0.085900	1.38	0.034695
hsa-miR-196b-3p	−1.19	0.086964	1.38	0.034695
hsa-miR-570-3p	−1.41	0.087735	1.38	0.034695
hsa-miR-663a	2.40	0.088005	1.38	0.034695
hsa-miR-877-3p	−1.18	0.088224	1.38	0.034695
hsa-miR-222-3p	1.31	0.090338	1.38	0.034695
hsa-miR-30c-5p	−1.24	0.091135	1.38	0.034695
hsa-miR-382-5p	1.72	0.094180	1.38	0.034695
hsa-miR-548i	1.84	0.095112	1.38	0.034695
hsa-miR-188-5p	1.39	0.098644	1.38	0.034695
hsa-miR-551a	−1.41	0.099052	1.38	0.034695
hsa-miR-30e-3p	−1.25	0.099471	1.38	0.035150
hsa-let-7a-5p	−1.16	0.099487	1.63	0.035744
hsa-miR-135a-5p	1.28	0.099568	−1.63	0.036602

Table 12.1 continued

MiRNS	GR group		BR group	
	Fold change	p value	Fold change	p value
hsa-miR-2113	1.97	0.101188	1.51	0.036984
hsa-miR-339-3p	−1.07	0.102555	−1.66	0.037443
hsa-miR-552-3p	−1.42	0.103690	1.62	0.037484
hsa-miR-501-3p	1.21	0.105368	1.37	0.037866
hsa-miR-17-5p	−1.22	0.106216	1.22	0.037952
hsa-miR-484	−1.14	0.107821	3.12	0.038175
hsa-miR-518d-5p	−1.26	0.109251	1.36	0.038630
hsa-miR-19b-3p	−1.21	0.109332	1.78	0.039083
hsa-miR-30d-5p	−1.18	0.110030	−1.76	0.039394
hsa-miR-424-5p	1.71	0.111845	1.98	0.039583
hsa-miR-548j-5p	1.25	0.112057	2.01	0.039682
hsa-miR-339-5p	1.12	0.113164	1.34	0.040126
hsa-miR-369-5p	1.38	0.115987	−2.09	0.040908
hsa-miR-326	1.16	0.118636	1.36	0.041206
hsa-miR-22-3p	1.16	0.119021	2.80	0.041530
hsa-miR-409-3p	1.56	0.121077	1.46	0.041622
hsa-miR-106a-5p	−1.19	0.122674	2.16	0.041853
hsa-miR-196b-5p	−1.11	0.122715	−1.62	0.042565
hsa-miR-19a-3p	−1.21	0.123436	−1.46	0.042834
hsa-miR-556-3p	−1.88	0.123537	1.76	0.044661
hsa-miR-424-3p	1.68	0.124076	1.33	0.045056
hsa-miR-199a-3p	1.37	0.124084	1.47	0.045457
hsa-let-7g-5p	−1.20	0.126045	2.23	0.045817
hsa-miR-34c-5p	1.14	0.131162	2.17	0.046501
hsa-miR-154-3p	1.68	0.134508	−1.48	0.047243
hsa-miR-106b-5p	−1.18	0.134922	−1.51	0.047675
hsa-miR-30b-5p	−1.18	0.134953	1.64	0.048050
hsa-miR-515-5p	−1.52	0.135940	2.49	0.048194
hsa-miR-124-3p	−1.23	0.136562	1.41	0.049058
hsa-let-7f-5p	−1.25	0.137697	1.69	0.049065
hsa-miR-1203	1.31	0.138064	1.96	0.049254
hsa-miR-522-3p	−1.27	0.138343	1.43	0.049352
hsa-miR-193b-5p	1.32	0.139381	1.49	0.050276
hsa-miR-661	1.65	0.139503	−1.42	0.050299
hsa-miR-664a-3p	1.21	0.141054	1.57	0.051192
hsa-miR-595	−1.16	0.143440	−1.56	0.051446
hsa-miR-106a-3p	−1.80	0.144418	1.47	0.051755
hsa-miR-34c-3p	1.24	0.145411	1.88	0.052105
hsa-miR-200a-5p	−1.53	0.145577	1.43	0.052758
hsa-miR-15a-3p	1.15	0.146359	3.17	0.053330
hsa-miR-371a-5p	−1.17	0.147943	2.14	0.053451
hsa-miR-92a-1-5p	−1.78	0.148936	1.56	0.053620
hsa-miR-489-3p	1.34	0.151187	−1.36	0.054184
hsa-miR-361-5p	−1.09	0.152678	1.29	0.054321
hsa-miR-425-5p	−1.16	0.155277	−1.48	0.055068
hsa-miR-487a-3p	1.89	0.155364	−5.37	0.055131
hsa-miR-518e-5p	−1.18	0.155390	−1.57	0.056185
hsa-miR-93-5p	−1.19	0.157995	1.72	0.057353
hsa-miR-18b-5p	−1.23	0.158688	1.86	0.059244
hsa-miR-518f-5p	−1.32	0.160173	3.21	0.060612
hsa-let-7b-5p	1.11	0.162161	2.04	0.061626
hsa-miR-503-5p	1.87	0.162304	−1.43	0.061925
hsa-miR-518b	−1.17	0.164295	2.45	0.063617
hsa-miR-186-5p	−1.11	0.168001	3.38	0.063818

Table 12.1 continued

MiRNS	GR group		BR group	
	Fold change	p value	Fold change	p value
hsa-miR-9-3p	−1.35	0.168180	2.51	0.064282
hsa-miR-761	−1.25	0.169064	1.48	0.064563
hsa-miR-658	−1.17	0.171075	1.66	0.065324
hsa-miR-496	1.76	0.175353	1.66	0.067689
hsa-miR-30c−1-3p	−1.27	0.175462	−1.90	0.067983
hsa-miR-512-3p	−1.53	0.176246	−1.61	0.068202
hsa-miR-1-3p	−1.37	0.178263	1.83	0.068828
hsa-miR-624-3p	−1.39	0.180668	−1.86	0.069303
hsa-miR-1181	2.18	0.182966	−2.28	0.070723
hsa-miR-576-5p	−1.22	0.184044	−1.38	0.071343
hsa-miR-92a-2-5p	−1.26	0.184931	2.16	0.071434
hsa-miR-298	−1.26	0.185264	−1.36	0.071882
hsa-miR-146a-5p	1.35	0.185703	−1.71	0.071883
hsa-miR-643	1.58	0.186840	1.66	0.072922
hsa-miR-584-5p	1.33	0.187939	2.04	0.074817
hsa-miR-592	−1.41	0.189114	1.82	0.075468
hsa-miR-380-3p	1.62	0.190991	2.01	0.076153
hsa-miR-423-3p	1.08	0.191345	1.82	0.076216
hsa-miR-542-5p	1.56	0.191509	1.47	0.078741
hsa-miR-605-5p	−1.59	0.193349	−1.48	0.079813
hsa-miR-517-5p	−1.39	0.193525	−1.58	0.079886
hsa-miR-376a-5p	1.34	0.194171	−1.67	0.080278
hsa-miR-9-5p	−1.49	0.195614	1.37	0.080358
hsa-miR-371a-3p	−1.15	0.195889	1.46	0.080897
hsa-miR-138-5p	−1.33	0.196167	−1.66	0.081374
hsa-miR-144-5p	−1.84	0.197148	1.28	0.081821
hsa-miR-579-3p	−1.15	0.197503	1.46	0.082115
hsa-miR-30c-2-3p	−1.33	0.197561	1.30	0.082274
hsa-miR-766-3p	−1.33	0.199671	1.42	0.082446
hsa-miR-340-5p	−1.27	0.200031	2.24	0.082451
hsa-miR-1911-3p	−1.67	0.200144	−1.20	0.082766
hsa-miR-483-3p	1.54	0.200273	1.47	0.084210
hsa-miR-219a-2-3p	−1.35	0.202538	1.86	0.084298
hsa-miR-612	1.71	0.202713	2.25	0.084613
hsa-miR-379-3p	1.58	0.202736	23.63	0.084724
hsa-miR-541-3p	1.30	0.202876	1.55	0.084830
hsa-miR-491-5p	−1.14	0.203061	−1.68	0.085313
hsa-miR-1972	1.30	0.203976	−1.34	0.086844
hsa-miR-758-3p	1.38	0.204434	−1.85	0.087189
hsa-miR-345-5p	1.22	0.206224	1.33	0.088266
hsa-miR-618	1.17	0.206739	1.23	0.089755
hsa-miR-580-3p	−1.51	0.206976	3.49	0.090579
hsa-miR-302c-5p	−1.15	0.207496	1.73	0.090665
hsa-miR-363-5p	1.31	0.208174	3.05	0.091230
hsa-miR-92a-3p	−1.13	0.212928	1.76	0.092069
hsa-miR-554	1.30	0.215840	1.66	0.092365
hsa-miR-647	−1.14	0.215843	3.25	0.092724
hsa-miR-891b	−1.41	0.215926	−1.25	0.093450
hsa-miR-26b-5p	−1.19	0.216834	2.10	0.094350
hsa-let-7f-1-3p	−1.09	0.219843	−1.30	0.094425
hsa-miR-590-3p	−1.46	0.220755	1.23	0.094934
hsa-miR-564	−1.27	0.220796	2.08	0.094995
hsa-miR-802	−1.27	0.220826	−1.34	0.096617
hsa-miR-548h-5p	−1.14	0.220917	1.68	0.097347

Table 12.1 continued

MiRNS	GR group		BR group	
	Fold change	p value	Fold change	p value
hsa-miR-640	−1.34	0.221344	−1.34	0.097958
hsa-miR-212-5p	1.75	0.221613	1.83	0.098277
hsa-miR-499a-5p	2.09	0.225649	1.62	0.098584
hsa-miR-450a-5p	1.33	0.225934	1.88	0.098961
hsa-miR-10b-3p	−1.39	0.228046	2.49	0.100505
hsa-miR-130a-3p	1.11	0.229003	1.57	0.101065
hsa-miR-381-5p	1.60	0.229331	2.96	0.101445
hsa-miR-145-5p	−1.19	0.232376	1.50	0.101547
hsa-miR-1296-5p	−1.18	0.234470	−1.44	0.103092
hsa-miR-541-5p	−1.13	0.234748	1.32	0.103661
hsa-miR-224-5p	1.65	0.236448	−1.61	0.104051
hsa-miR-943	1.35	0.236667	1.65	0.104417
hsa-miR-216a-5p	1.92	0.237060	−1.34	0.104878
hsa-miR-138-1-3p	1.44	0.238120	−1.38	0.105279
hsa-miR-23a-5p	1.67	0.238149	1.96	0.105572
hsa-miR-600	−1.92	0.239523	1.27	0.106194
hsa-miR-129-2-3p	−1.08	0.239629	3.56	0.106639
hsa-let-7e-5p	−1.14	0.239857	1.75	0.107763
hsa-miR-449a	3.50	0.241882	1.73	0.108690
hsa-miR-15b-5p	−1.15	0.242071	1.40	0.109779
SNORD49A (hsa)	1.20	0.242839	2.13	0.110259
hsa-miR-330-5p	1.29	0.243838	2.84	0.112541
hsa-miR-519b-3p	1.40	0.243953	1.62	0.114587
hsa-miR-518f-3p	−1.47	0.244042	1.35	0.115014
hsa-miR-499a-3p	1.65	0.244818	−1.39	0.115570
hsa-miR-144-3p	−1.61	0.244825	1.44	0.115663
hsa-miR-933	−1.13	0.247076	−1.48	0.116516
hsa-miR-616-3p	−1.18	0.247718	1.37	0.116999
hsa-miR-1224-3p	2.12	0.251049	−1.49	0.117179
hsa-miR-129-1-3p	−1.24	0.252307	1.75	0.117282
hsa-miR-942-5p	−1.32	0.253398	1.39	0.118172
hsa-miR-29b-2-5p	−1.13	0.255120	1.83	0.118554
hsa-miR-16-5p	−1.17	0.258190	3.43	0.119061
hsa-miR-105-5p	2.04	0.260558	−1.34	0.119804
hsa-miR-15b-3p	−1.22	0.261916	−1.67	0.121424
hsa-miR-151a-3p	−1.06	0.262845	2.42	0.121476
hsa-miR-550a-3p	−1.17	0.263213	39.20	0.121479
hsa-miR-593-3p	−1.37	0.263820	1.37	0.122651
hsa-miR-505-5p	−1.28	0.264761	1.51	0.123085
hsa-miR-320d	1.14	0.265595	1.31	0.124443
hsa-miR-562	−1.35	0.265834	1.60	0.124921
hsa-miR-567	−1.12	0.266379	−1.42	0.125193
hsa-miR-224-3p	1.49	0.267236	1.56	0.125295
hsa-miR-296-3p	−1.15	0.267405	−2.44	0.127496
hsa-miR-509-3p	2.52	0.267601	1.52	0.128605
hsa-miR-451a	−1.40	0.267876	−1.22	0.128814
hsa-miR-149-5p	−1.05	0.268081	1.90	0.128855
hsa-miR-548k	−1.61	0.269437	3.20	0.130018
hsa-miR-421	1.09	0.270404	1.40	0.130197
hsa-miR-98-5p	1.07	0.274393	2.13	0.131989
hsa-miR-105-3p	1.36	0.277805	−1.86	0.134506
hsa-miR-32-5p	−1.28	0.278829	2.73	0.135138
hsa-miR-30a-5p	−1.12	0.281089	−2.50	0.136081
hsa-miR-18b-3p	−1.25	0.281470	1.24	0.137281

Table 12.1 continued

MiRNS	GR group		BR group	
	Fold change	p value	Fold change	p value
hsa-miR-558	−1.12	0.287641	1.33	0.137668
hsa-miR-377-5p	1.29	0.287643	1.65	0.137905
hsa-miR-653-5p	−1.29	0.290840	1.68	0.137940
hsa-miR-7-2-3p	1.66	0.292057	38.22	0.138214
hsa-miR-589-3p	−1.22	0.292117	2.04	0.139839
hsa-miR-21-3p	1.63	0.293710	1.28	0.140100
hsa-miR-1912-3p	−1.08	0.295052	2.22	0.140731
hsa-miR-145-3p	−1.19	0.295309	2.58	0.141807
hsa-let-7e-3p	−1.19	0.296352	1.31	0.142269
hsa-miR-135b-5p	1.79	0.297367	1.51	0.143187
U6 snRNA	1.36	0.299057	1.23	0.143547
hsa-miR-650	3.87	0.299563	2.40	0.143809
hsa-miR-126-5p	−1.35	0.300824	−2.83	0.144258
hsa-miR-505-3p	1.06	0.302366	1.33	0.145302
hsa-miR-454-5p	1.33	0.303289	1.28	0.147319
hsa-miR-1908-5p	1.47	0.303503	−1.30	0.148362
hsa-miR-133a-3p	−1.07	0.304948	1.32	0.149388
hsa-miR-133b	−1.07	0.306339	1.61	0.149481
hsa-miR-1265	−1.14	0.306463	−1.79	0.149524
hsa-miR-520d-3p	−1.11	0.308003	72.21	0.150234
hsa-miR-1255b-5p	−1.14	0.308103	3.92	0.152125
hsa-miR-187-3p	−1.43	0.308816	−1.57	0.154345
hsa-miR-222-5p	1.43	0.310012	−1.11	0.156024
hsa-miR-33a-3p	−1.29	0.312696	−1.68	0.160552
hsa-miR-130b-5p	−1.19	0.312841	1.29	0.162001
hsa-miR-671-5p	1.39	0.315313	1.61	0.162798
hsa-miR-1207-5p	2.77	0.316437	1.32	0.165084
hsa-miR-143-5p	−1.16	0.316489	4.47	0.165329
hsa-miR-663b	1.62	0.322145	9.57	0.168811
hsa-miR-548c-5p	−1.15	0.326038	2.62	0.170463
hsa-miR-597-5p	1.79	0.328575	2.00	0.171206
hsa-miR-519a-3p	−1.55	0.330486	1.93	0.171957
hsa-miR-122-5p	−2.12	0.330532	1.38	0.173326
hsa-miR-523-3p	−1.15	0.330549	1.41	0.173336
hsa-miR-639	−1.11	0.332381	1.88	0.174679
hsa-miR-130b-3p	−1.12	0.333867	2.11	0.175587
hsa-miR-544a	−1.10	0.335714	−1.46	0.176646
hsa-miR-181c-5p	1.27	0.335813	1.61	0.179380
hsa-miR-193a-3p	1.07	0.337013	1.65	0.179704
hsa-miR-1249-3p	1.13	0.337062	−1.23	0.179965
hsa-miR-208b-3p	−1.12	0.339714	1.75	0.179977
hsa-miR-769-5p	1.04	0.339935	2.29	0.180394
hsa-miR-219a-1-3p	1.37	0.340780	8.59	0.181079
hsa-miR-206	−1.89	0.340960	−1.29	0.183442
hsa-miR-449b-5p	1.68	0.341890	1.84	0.183450
hsa-miR-204-5p	1.26	0.343763	1.24	0.187136
hsa-miR-299-3p	1.32	0.343940	1.69	0.187617
hsa-miR-452-5p	1.50	0.345712	−1.20	0.188232
hsa-miR-18a-5p	−1.17	0.355260	1.17	0.189424
hsa-miR-149-3p	−1.38	0.356469	1.48	0.189519
hsa-miR-532-5p	1.05	0.359464	−1.13	0.190620

Table 12.1 continued

MiRNS	GR group		BR group	
	Fold change	p value	Fold change	p value
hsa-miR-205-3p	−2.52	0.362814	1.64	0.191443
hsa-miR-126-3p	−1.22	0.363066	2.34	0.192858
hsa-miR-623	−1.10	0.363491	1.33	0.193954
hsa-miR-557	−1.10	0.363491	1.49	0.195299
hsa-miR-325	−1.10	0.363491	1.23	0.195542
hsa-miR-181a-3p	−1.10	0.363491	1.55	0.197651
hsa-miR-367-3p	−1.10	0.363491	−1.16	0.197880
hsa-miR-302c-3p	−1.10	0.363491	−1.88	0.198002
hsa-miR-302d-3p	−1.10	0.363491	−1.58	0.198165
hsa-miR-302b-3p	−1.10	0.363491	1.95	0.198489
hsa-miR-662	−1.10	0.363491	1.21	0.201001
hsa-miR-637	−1.10	0.363491	1.36	0.201669
hsa-miR-521	−1.10	0.363491	2.42	0.202769
hsa-miR-422a	−1.10	0.363491	1.28	0.205610
hsa-miR-608	−1.10	0.363491	2.12	0.205982
hsa-miR-548c-3p	−1.10	0.363491	1.61	0.210124
hsa-miR-302a-3p	−1.10	0.363491	1.25	0.212290
hsa-miR-512-5p	−1.10	0.363491	−1.74	0.213165
hsa-miR-498-5p	−1.10	0.363491	−1.56	0.215581
hsa-miR-96-3p	−1.10	0.363491	2.32	0.216206
hsa-miR-520d-5p	−1.10	0.363491	1.55	0.216729
hsa-miR-617	−1.10	0.363491	2.57	0.218908
hsa-miR-412-3p	−1.10	0.363491	2.65	0.219471
hsa-miR-921	−1.10	0.363491	1.60	0.219828
hsa-miR-513a-5p	−1.10	0.363491	1.90	0.220658
hsa-miR-583	−1.10	0.363491	1.32	0.221132
hsa-miR-620	−1.10	0.363491	1.53	0.223143
hsa-miR-922	−1.10	0.363491	−1.22	0.223246
hsa-miR-384	−1.10	0.363491	1.38	0.223461
hsa-miR-553	−1.10	0.363491	1.66	0.226208
hsa-miR-759	−1.10	0.363491	−1.46	0.228174
hsa-miR-520b-3p	−1.10	0.363491	1.60	0.229243
hsa-miR-302f	−1.10	0.363491	1.60	0.230562
hsa-miR-1183	−1.10	0.363491	1.75	0.232364
hsa-miR-516a-3p	−1.10	0.363491	1.61	0.234085
hsa-miR-122-3p	−1.10	0.363491	1.69	0.235007
hsa-miR-323b-5p	−1.10	0.363491	−1.36	0.235886
hsa-miR-1267	−1.10	0.363491	1.41	0.236037
hsa-miR-601	−1.10	0.363491	2.34	0.236181
hsa-miR-924	−1.10	0.363491	1.49	0.237039
hsa-miR-548m	−1.10	0.363491	2.94	0.237353
hsa-miR-448	−1.10	0.363491	2.29	0.237726
hsa-miR-920	−1.10	0.363491	1.58	0.238064
hsa-miR-1258	−1.10	0.363491	−1.30	0.238083
hsa-miR-619-3p	−1.10	0.363491	1.45	0.239820
hsa-miR-208a-3p	−1.10	0.363491	2.07	0.241207
hsa-miR-559	−1.10	0.363491	1.51	0.242077
hsa-miR-591	−1.10	0.363491	1.60	0.242636
hsa-miR-586	−1.10	0.363491	1.25	0.243845
hsa-miR-103b	−1.10	0.363491	1.30	0.244036
hsa-miR-573	−1.10	0.363491	−1.49	0.247110
hsa-miR-302d-5p	−1.10	0.363491	−1.27	0.247404
hsa-miR-302b-5p	−1.10	0.363491	1.35	0.249116
hsa-miR-635	−1.10	0.363491	−1.22	0.252869

Table 12.1 continued

MiRNS	GR group		BR group	
	Fold change	p value	Fold change	p value
hsa-miR-518d-3p	−1.10	0.363491	1.36	0.253283
hsa-miR-519c-3p	−1.10	0.363491	5.53	0.253584
hsa-miR-938	−1.10	0.363491	2.86	0.253708
hsa-miR-587	−1.10	0.363491	−1.22	0.253916
hsa-miR-603	−1.10	0.363491	2.20	0.256117
hsa-miR-1184	−1.10	0.363491	1.22	0.259567
hsa-miR-588	−1.10	0.363491	1.09	0.259638
hsa-miR-452-3p	−1.10	0.363491	1.41	0.260161
hsa-miR-610	−1.10	0.363491	−2.11	0.260992
hsa-miR-578	−1.10	0.363491	1.48	0.261852
hsa-miR-875-3p	−1.10	0.363491	−1.08	0.263860
hsa-miR-581	−1.10	0.363491	−1.25	0.265600
hsa-miR-1204	−1.10	0.363491	1.46	0.266665
hsa-miR-555	−1.10	0.363491	1.44	0.267304
hsa-miR-513a-3p	−1.10	0.363491	−1.11	0.270545
hsa-miR-614	−1.10	0.363491	−1.15	0.274076
hsa-miR-606	−1.10	0.363491	−1.10	0.279234
hsa-miR-367-5p	−1.10	0.363491	1.17	0.279714
hsa-miR-607	−1.10	0.363491	1.20	0.280439
hsa-miR-1208	−1.10	0.363491	−1.38	0.281022
hsa-miR-302e	−1.10	0.363491	1.16	0.281445
hsa-miR-1206	−1.10	0.363491	2.20	0.283923
hsa-miR-888-3p	−1.10	0.363491	1.26	0.284266
hsa-miR-593-5p	−1.10	0.363491	1.34	0.285259
hsa-miR-561-3p	−1.10	0.363491	1.21	0.287167
hsa-miR-630	−1.10	0.363491	1.42	0.290989
hsa-miR-2053	−1.10	0.363491	1.10	0.291293
hsa-miR-1252-5p	−1.10	0.363491	1.14	0.292885
hsa-miR-1182	−1.10	0.363491	1.24	0.296583
hsa-miR-611	−1.10	0.363491	1.63	0.300633
hsa-miR-599	−1.10	0.363491	−1.46	0.301019
hsa-miR-155-3p	−1.10	0.363491	1.18	0.302739
hsa-miR-130a-5p	−1.10	0.363491	1.59	0.303360
hsa-miR-563	−1.10	0.363491	1.51	0.304310
hsa-miR-944	−1.10	0.363491	1.91	0.305213
hsa-miR-548n	−1.10	0.363491	−1.15	0.307760
hsa-miR-205-5p	−3.34	0.364004	−1.25	0.310772
hsa-miR-486-3p	−1.35	0.365224	−1.28	0.310985
hsa-miR-642a-5p	−1.04	0.367061	1.62	0.313016
hsa-miR-29b-3p	−1.06	0.367742	−1.26	0.314025
hsa-miR-548d-3p	−1.09	0.368285	1.50	0.316430
hsa-miR-486-5p	−1.23	0.369272	1.13	0.319075
hsa-miR-138-2-3p	−1.38	0.369497	1.69	0.320173
hsa-miR-29c-5p	−1.12	0.369875	−1.13	0.322624
hsa-miR-548d-5p	−1.33	0.370240	−1.13	0.323677
hsa-miR-1254	1.37	0.372753	1.72	0.324849
hsa-miR-329-3p	1.27	0.373390	−1.23	0.325519
hsa-miR-22-5p	1.13	0.376789	1.59	0.326210
hsa-miR-876-3p	1.36	0.381419	1.30	0.326276
hsa-miR-654-5p	1.44	0.381592	1.36	0.326541
hsa-miR-518e-3p	−1.32	0.382051	1.12	0.327024
hsa-miR-1468-5p	1.30	0.385057	1.23	0.328523
hsa-miR-604	1.13	0.386756	−1.17	0.330457
hsa-miR-93-3p	−1.16	0.388090	1.81	0.330521

Table 12.1 continued

MiRNS	GR group		BR group	
	Fold change	p value	Fold change	p value
hsa-miR-33b-3p	−1.60	0.393527	−1.72	0.330842
hsa-miR-525-3p	−1.14	0.393573	1.37	0.330867
hsa-miR-488-3p	−1.27	0.394365	−1.22	0.332117
hsa-miR-183-3p	1.16	0.395467	1.19	0.332377
hsa-miR-760	1.38	0.396144	1.36	0.332719
hsa-miR-143-3p	−1.13	0.396936	−1.09	0.333784
hsa-miR-301b-3p	1.24	0.399022	−1.18	0.334248
hsa-miR-346	−1.12	0.400411	−1.50	0.334480
hsa-miR-569	−1.09	0.400475	−1.15	0.335737
hsa-miR-320a-3p	1.11	0.400753	2.41	0.338122
hsa-let-7a-3p	−1.19	0.400880	2.51	0.340940
hsa-miR-518a-3p	1.20	0.401302	1.32	0.342266
hsa-miR-622	1.15	0.401666	−1.18	0.343148
hsa-miR-655-3p	1.44	0.402139	1.29	0.343195
hsa-miR-1471	−1.07	0.405179	2.43	0.348091
hsa-miR-1264	−1.09	0.406326	1.30	0.348579
hsa-miR-33a-5p	−1.24	0.408302	1.45	0.349886
hsa-miR-19a-5p	−1.38	0.410265	1.91	0.351086
hsa-miR-548e-3p	−1.21	0.411373	2.72	0.351527
hsa-miR-675-3p	−1.26	0.413487	−1.16	0.352115
hsa-miR-509-3-5p	1.33	0.414987	1.47	0.352299
hsa-miR-218-2-3p	−1.09	0.415657	−1.09	0.356119
hsa-miR-331-3p	1.10	0.419076	4.89	0.360629
hsa-miR-183-5p	1.15	0.419181	1.35	0.363034
hsa-miR-520a-3p	−1.07	0.420949	3.03	0.364710
hsa-miR-188-3p	1.25	0.423571	1.33	0.365219
hsa-miR-146a-3p	−1.31	0.423670	1.17	0.366429
hsa-miR-520e-3p	1.13	0.424289	1.27	0.367629
hsa-miR-602	−1.21	0.424538	1.22	0.367728
hsa-miR-517c-3p	−1.19	0.424710	2.60	0.368802
hsa-miR-10a-5p	1.07	0.428124	1.84	0.369759
hsa-miR-1237-3p	1.26	0.431147	1.75	0.370139
hsa-let-7b-3p	−1.08	0.437720	2.26	0.373098
hsa-miR-1205	1.22	0.439843	−1.62	0.373113
hsa-miR-182-3p	1.58	0.442210	3.87	0.374533
hsa-miR-301a-3p	−1.09	0.447334	1.18	0.376573
hsa-miR-365b-5p	1.37	0.451495	1.12	0.376593
hsa-miR-500a-5p	−1.08	0.452134	1.24	0.380641
hsa-miR-320c	−1.07	0.457863	1.12	0.381329
hsa-miR-551b-3p	1.22	0.458054	1.31	0.383080
hsa-miR-1227-3p	1.10	0.458578	1.12	0.386204
hsa-miR-596	−1.05	0.459222	−1.32	0.386830
hsa-miR-492	1.15	0.459515	1.39	0.389984
hsa-miR-328-3p	1.12	0.459593	1.23	0.395199
hsa-miR-889-3p	1.29	0.460533	−1.08	0.396292
hsa-miR-185-5p	−1.09	0.460919	−1.10	0.402701
hsa-miR-615-5p	1.50	0.463707	1.19	0.403136
hsa-miR-874-3p	1.40	0.464103	1.42	0.404968
hsa-miR-26a-1-3p	1.06	0.466145	1.82	0.405043
hsa-miR-25-3p	−1.10	0.467614	1.36	0.406443
hsa-miR-1911-5p	1.21	0.468341	1.28	0.406511
hsa-miR-511-5p	1.38	0.468726	1.38	0.406832
hsa-miR-26a-5p	−1.07	0.469783	1.36	0.408929
hsa-miR-29a-5p	−1.15	0.472712	1.39	0.409151

Table 12.1 continued

MiRNS	GR group		BR group	
	Fold change	p value	Fold change	p value
hsa-miR-125a-5p	−1.08	0.475652	1.39	0.409196
hsa-miR-202-5p	2.12	0.476287	1.51	0.412138
hsa-miR-520f-3p	−1.19	0.477724	−1.25	0.414746
hsa-miR-1243	−1.06	0.483169	−1.19	0.416237
hsa-miR-769-3p	−1.18	0.489339	1.14	0.416602
hsa-miR-335-3p	−1.30	0.489573	1.13	0.418479
hsa-miR-373-3p	−1.11	0.491171	1.18	0.418859
hsa-miR-510-5p	1.08	0.491409	1.35	0.419955
hsa-miR-221-5p	1.38	0.492649	−1.21	0.426971
hsa-miR-517a-3p	1.47	0.502251	1.27	0.427860
hsa-miR-15a-5p	−1.09	0.502726	1.28	0.428463
hsa-miR-491-3p	−1.18	0.506451	1.15	0.429946
hsa-miR-330-3p	−1.13	0.507324	1.89	0.432257
hsa-miR-338-5p	−1.13	0.508679	1.18	0.432285
hsa-miR-148b-5p	−1.16	0.509337	1.12	0.433534
hsa-miR-490-5p	1.26	0.510190	1.55	0.436824
hsa-miR-210-3p	−1.16	0.510355	1.34	0.437407
hsa-miR-526b-3p	−1.07	0.511102	−1.16	0.438390
hsa-miR-1178-3p	1.16	0.513617	−1.30	0.441649
hsa-miR-17-3p	−1.10	0.519569	−1.21	0.442528
hsa-miR-96-5p	−1.13	0.524567	1.39	0.445487
hsa-miR-483-5p	1.03	0.524902	−1.26	0.445728
hsa-miR-490-3p	1.29	0.529763	1.37	0.449021
hsa-miR-671-3p	1.18	0.531441	1.24	0.449125
hsa-miR-23b-3p	−1.07	0.532715	1.06	0.451183
hsa-miR-1200	1.08	0.533073	−1.01	0.452272
hsa-miR-29a-3p	1.04	0.533662	1.25	0.454282
hsa-miR-628-5p	−1.24	0.534515	1.05	0.455992
hsa-miR-153-3p	−1.34	0.540914	2.13	0.458228
hsa-miR-223-5p	−1.11	0.544626	1.35	0.460244
hsa-miR-628-3p	1.04	0.546057	1.21	0.463101
hsa-miR-935	1.06	0.546404	1.63	0.463828
hsa-miR-30e-5p	−1.11	0.547736	1.50	0.464724
hsa-miR-373-5p	−1.17	0.548375	1.40	0.466106
hsa-miR-520h	−1.14	0.549474	1.28	0.467544
hsa-miR-214-5p	1.18	0.556605	−1.21	0.469188
hsa-miR-508-3p	1.17	0.559842	1.20	0.469202
hsa-miR-323a-5p	1.05	0.563026	1.48	0.469682
hsa-miR-615-3p	2.09	0.568928	−1.10	0.472442
hsa-miR-572	1.11	0.571080	1.15	0.473848
hsa-miR-1914-5p	−1.14	0.571288	1.60	0.475640
hsa-miR-124-5p	−1.06	0.571429	−1.17	0.475784
hsa-miR-1272	−1.06	0.572846	−1.31	0.477120
hsa-miR-323a-3p	1.08	0.575759	−1.09	0.480333
hsa-miR-548b-3p	1.34	0.575976	1.14	0.480566
hsa-miR-571	1.11	0.583272	1.33	0.480655
hsa-miR-21-5p	1.29	0.583962	1.24	0.483546
hsa-miR-106b-3p	−1.04	0.585574	−1.11	0.483857
hsa-miR-10a-3p	1.01	0.585989	−1.58	0.485083
hsa-miR-18a-3p	1.09	0.591963	−1.12	0.486649
hsa-miR-576-3p	−1.34	0.593311	−1.55	0.489058
hsa-miR-155-5p	1.39	0.599144	1.68	0.491837
hsa-miR-770-5p	−1.13	0.603979	−1.42	0.493379
hsa-miR-937-3p	1.12	0.604235	−1.31	0.493830

Table 12.1 continued

MiRNS	GR group		BR group	
	Fold change	p value	Fold change	p value
hsa-miR-20b-5p	−1.11	0.604922	1.52	0.494879
hsa-miR-675-5p	−1.13	0.605664	1.10	0.496304
hsa-miR-649	1.05	0.606704	1.10	0.496995
hsa-miR-190b	−1.24	0.606998	−1.16	0.500278
hsa-miR-33b-5p	1.04	0.609121	1.04	0.502453
hsa-miR-585-3p	1.18	0.620036	1.11	0.502502
hsa-miR-627-5p	−1.13	0.621719	1.20	0.514952
hsa-miR-659-3p	1.09	0.622119	1.54	0.515182
hsa-miR-1245a	−1.28	0.622291	1.03	0.519671
hsa-miR-182-5p	1.01	0.622720	−1.14	0.520268
hsa-miR-652-3p	1.04	0.627185	1.11	0.522552
hsa-miR-181d-5p	−1.05	0.628748	1.06	0.525653
hsa-let-7i-5p	1.22	0.628782	1.25	0.530443
hsa-let-7i-3p	1.47	0.629116	1.17	0.531787
hsa-miR-629-5p	1.05	0.629767	−1.07	0.540363
hsa-miR-128-3p	−1.05	0.631775	1.05	0.542101
hsa-miR-135a-3p	−1.07	0.636541	1.07	0.555151
hsa-miR-27a-5p	1.69	0.639959	1.30	0.556239
hsa-miR-150-5p	1.43	0.640216	−1.06	0.557205
hsa-miR-342-5p	1.33	0.640796	1.58	0.558464
hsa-miR-616-5p	1.23	0.641849	1.43	0.558615
hsa-miR-187-5p	−1.03	0.642323	1.07	0.561725
hsa-miR-142-3p	−1.02	0.645105	1.12	0.564157
hsa-miR-27a-3p	−1.06	0.645938	1.12	0.564304
hsa-miR-28-3p	−1.08	0.646881	−1.01	0.570636
hsa-miR-450b-5p	1.11	0.648633	−1.17	0.571054
hsa-miR-501-5p	1.12	0.650045	1.60	0.578939
hsa-miR-450b-3p	−1.05	0.652389	−1.05	0.581669
hsa-miR-1269a	1.04	0.652779	1.05	0.582296
hsa-miR-532-3p	−1.07	0.654104	−1.16	0.587145
hsa-miR-885-5p	1.19	0.655932	1.10	0.587818
hsa-miR-19b-2-5p	−1.03	0.659140	1.20	0.592136
hsa-miR-449b-3p	1.07	0.660467	1.34	0.594463
hsa-miR-362-3p	−1.08	0.660769	−1.10	0.596100
hsa-miR-2110	1.09	0.661336	1.77	0.602058
hsa-miR-198	−1.02	0.662059	−1.26	0.610900
hsa-miR-28-5p	−1.05	0.664155	1.11	0.615278
hsa-miR-892a	−1.02	0.665888	−1.05	0.616722
hsa-miR-375-3p	2.06	0.665902	1.13	0.616732
hsa-let-7f-2-3p	−1.09	0.666631	−1.11	0.617403
hsa-miR-195-5p	−1.09	0.667728	−1.04	0.629336
hsa-miR-516b-5p	1.06	0.671254	−1.17	0.631204
hsa-miR-331-5p	−1.06	0.671641	−1.26	0.632766
hsa-miR-219a-5p	−1.07	0.679844	1.05	0.634127
hsa-miR-146b-5p	1.37	0.688374	1.09	0.634659
hsa-miR-524-3p	−1.04	0.688555	−1.08	0.639817
hsa-miR-744-5p	1.05	0.688761	−1.40	0.639900
hsa-miR-101-3p	−1.14	0.689738	1.01	0.640711
hsa-miR-335-5p	−1.27	0.693481	−1.55	0.640806
hsa-miR-193a-5p	1.06	0.694743	1.08	0.642998
hsa-miR-875-5p	−1.06	0.695704	1.03	0.643749
hsa-miR-1909-3p	−1.04	0.699004	−1.00	0.648302
hsa-miR-362-5p	−1.03	0.705264	−1.43	0.648332
hsa-miR-29c-3p	−1.04	0.705691	1.08	0.653862

Table 12.1 continued

MiRNS	GR group		BR group	
	Fold change	p value	Fold change	p value
hsa-miR-519e-3p	−1.11	0.708644	1.31	0.654789
hsa-miR-181b-5p	−1.02	0.713704	1.07	0.658153
hsa-miR-641	1.32	0.714565	1.11	0.658199
hsa-miR-548l	1.12	0.720878	−1.09	0.659153
hsa-miR-1539	1.04	0.722273	1.15	0.661756
hsa-miR-455-3p	−1.03	0.723650	1.37	0.664085
hsa-miR-708-5p	1.75	0.726292	−1.79	0.666318
hsa-miR-518c-3p	−1.03	0.728404	−1.27	0.670338
hsa-miR-99b-5p	−1.05	0.728854	1.04	0.670757
hsa-miR-887-3p	1.05	0.729189	1.03	0.672680
hsa-miR-936	1.10	0.729632	1.10	0.674962
hsa-miR-934	−1.05	0.729644	−2.43	0.685215
hsa-miR-668-3p	−1.09	0.729845	1.03	0.686119
hsa-miR-582-3p	−1.18	0.730152	1.07	0.692275
hsa-miR-516a-5p	1.04	0.733758	1.07	0.695091
hsa-miR-621	1.21	0.733864	−1.06	0.698665
hsa-miR-609	−1.04	0.736258	1.18	0.699824
hsa-miR-101-5p	−1.09	0.737502	1.01	0.702613
hsa-miR-135b-3p	1.80	0.738174	−1.05	0.704046
hsa-miR-363-3p	−1.06	0.738485	1.22	0.704841
hsa-miR-651-5p	−1.20	0.739155	1.01	0.706762
hsa-miR-202-3p	−1.04	0.740749	−1.19	0.707259
hsa-miR-30b-3p	1.04	0.741120	1.18	0.716257
hsa-miR-185-3p	1.32	0.742384	1.15	0.720424
hsa-miR-140-5p	−1.06	0.744370	1.05	0.723692
hsa-miR-520c-3p	1.04	0.746786	1.10	0.727629
hsa-miR-625-3p	−1.05	0.752367	−1.02	0.727945
hsa-miR-548a-5p	−1.04	0.753862	−1.35	0.729579
hsa-miR-32-3p	1.01	0.755118	−1.01	0.733003
hsa-miR-324-3p	1.04	0.758354	1.05	0.738387
hsa-miR-24-1-5p	−1.06	0.764561	1.31	0.738390
hsa-let-7g-3p	1.06	0.770378	1.20	0.739330
hsa-miR-524-5p	1.04	0.771436	1.13	0.741270
hsa-miR-765	1.05	0.775273	1.30	0.741438
SNORD38B (hsa)	1.05	0.776131	1.47	0.742740
hsa-miR-24-3p	−1.03	0.776668	1.04	0.749043
hsa-miR-518c-5p	−1.01	0.778935	1.07	0.752677
hsa-miR-195-3p	−1.07	0.779069	1.05	0.754293
hsa-miR-320b	1.04	0.780123	1.04	0.758281
hsa-miR-191-3p	−1.15	0.783185	1.04	0.760946
hsa-miR-1238-3p	−1.07	0.784610	1.08	0.780781
hsa-miR-526b-5p	−1.05	0.785323	−1.09	0.784094
hsa-miR-513b-5p	−1.04	0.792766	1.11	0.788176
hsa-miR-631	−1.12	0.795489	−1.09	0.792817
hsa-miR-488-5p	1.03	0.796065	−1.15	0.793104
hsa-miR-218-1-3p	−1.26	0.796882	1.17	0.793574
hsa-miR-425-3p	1.01	0.802167	1.84	0.799169
hsa-miR-20b-3p	1.27	0.803293	−1.01	0.799599
hsa-miR-27b-3p	−1.05	0.804940	1.17	0.803260
hsa-miR-513c-5p	1.03	0.807762	−1.33	0.810523
hsa-miR-507	−1.02	0.810295	−1.00	0.816547
hsa-let-7c-5p	1.00	0.815440	1.06	0.816837
hsa-miR-629-3p	−1.04	0.819495	1.51	0.817886
hsa-miR-324-5p	−1.01	0.821039	1.35	0.818176

Table 12.1 continued

MiRNS	GR group		BR group	
	Fold change	p value	Fold change	p value
hsa-miR-374b-3p	−1.05	0.829988	1.03	0.821722
hsa-miR-27b-5p	−1.02	0.833154	−1.05	0.827043
hsa-miR-31-5p	9.47	0.833208	−1.06	0.829340
hsa-miR-369-3p	1.09	0.834463	1.02	0.831828
hsa-miR-888-5p	−1.01	0.835456	−1.03	0.832777
hsa-miR-550a-5p	1.20	0.835526	1.24	0.834581
hsa-miR-520a-5p	−1.02	0.837131	−1.12	0.836899
hsa-miR-548a-3p	1.03	0.838626	−1.11	0.837906
hsa-miR-891a-5p	−1.03	0.839367	−1.08	0.838250
hsa-miR-125a-3p	1.09	0.839994	−1.01	0.840412
hsa-miR-941	1.05	0.841229	1.10	0.842156
hsa-miR-632	−1.04	0.844515	−1.15	0.844235
hsa-miR-646	1.03	0.845643	−1.04	0.845681
hsa-miR-598-3p	−1.10	0.848480	1.02	0.851658
hsa-miR-545-3p	1.01	0.850030	1.06	0.860319
hsa-miR-338-3p	−1.08	0.850945	−1.00	0.860673
hsa-miR-380-5p	1.02	0.856338	1.02	0.860870
hsa-miR-365a-3p	1.04	0.862582	−1.07	0.863144
hsa-miR-636	1.01	0.869760	1.01	0.867585
hsa-miR-16-1-3p	−1.02	0.872787	−1.09	0.875163
hsa-miR-890	−1.04	0.875474	−1.53	0.877216
hsa-miR-767-5p	−1.05	0.875894	−1.09	0.878077
hsa-miR-197-3p	−1.01	0.882727	1.10	0.882934
hsa-miR-502-3p	1.02	0.884450	1.10	0.884764
hsa-miR-574-3p	1.01	0.888463	−1.02	0.910288
hsa-miR-142-5p	1.30	0.889447	1.02	0.910619
hsa-miR-551b-5p	1.06	0.891922	1.10	0.915339
hsa-miR-148b-3p	−1.03	0.892464	1.08	0.919231
hsa-miR-519e-5p	−1.03	0.900086	−1.16	0.919267
hsa-miR-1537-3p	1.25	0.901938	1.01	0.927891
hsa-miR-626	−1.00	0.905564	1.03	0.927921
hsa-miR-582-5p	1.07	0.908696	1.11	0.928251
hsa-miR-1179	−1.21	0.913781	−1.06	0.928705
hsa-miR-140-3p	1.01	0.915235	1.01	0.931341
hsa-miR-634	−1.04	0.917552	−1.02	0.932121
hsa-miR-455-5p	−1.04	0.919402	1.18	0.933190
hsa-miR-31-3p	8.82	0.929271	−1.11	0.939987
hsa-miR-16-2-3p	1.00	0.932880	1.14	0.942177
hsa-miR-25-5p	1.02	0.938977	1.25	0.942517
hsa-miR-767-3p	1.03	0.939731	−1.23	0.942839
hsa-miR-645	−1.00	0.941333	−1.54	0.945735
hsa-miR-216b-5p	1.24	0.946140	1.18	0.946932
hsa-miR-1270	−1.13	0.946965	−1.10	0.948963
hsa-miR-525-5p	−1.04	0.952528	1.02	0.952877
hsa-miR-508-5p	−1.01	0.954212	1.00	0.954966
hsa-miR-146b-3p	1.41	0.959618	−1.05	0.958546
hsa-miR-218-5p	−1.06	0.959786	−1.07	0.961454
hsa-miR-1253	1.01	0.963512	1.17	0.963838
hsa-miR-708-3p	1.21	0.966526	1.00	0.965959
hsa-miR-1538	1.04	0.971545	−1.02	0.966907
hsa-miR-549a-3p	−1.02	0.971576	−1.01	0.967273
hsa-miR-99b-3p	−1.00	0.974653	1.00	0.969101
hsa-miR-660-5p	−1.01	0.985794	−1.06	0.978276
hsa-miR-497-5p	−1.03	0.986859	−1.01	0.982992

Table 12.1 continued

MiRNS	GR group		BR group	
	Fold change	p value	Fold change	p value
hsa-miR-181c-3p	−1.04	0.989256	1.14	0.988229
hsa-let-7d-3p	−1.01	0.991244	1.15	0.991796
hsa-miR-181a-5p	1.03	0.995215	−1.05	0.993681
hsa-miR-223-3p	1.19	0.995775	1.13	0.997961
hsa-miR-23b-5p	1.07	0.996984	1.29	0.999012

PSCUH algorithm P-NMS-40/Alg

	P-NMS-40/Alg **Novērošanas taktika pacientiem ar taisnās zarnas adenokarcinomas pilnu klīnisku remisiju pēc neoadjuvantās terapijas**		
	Izstrādāja: Ķirurgi L.Kokaine, A.Gardovskis, M.Pavārs, Onkologi E.Sīviņa, J.Nikolajeva, Radiologs M.Radziņa, Pacientu drošības sistēmas vadītāja A.Jaunzeme	Saskaņoja: galvenā ārsta p.i. J.Vilmanis, Ķirurģijas klīnikas vadītājs J.Gardovskis, Onkoloģijas klīnikas vadītāja A.Geriņa, Diagnostiskās radioloģijas institūta vadītājs K.Kupčs	versija **01** APSTIPRINĀTS ar 07.01.2025 rīkojumu Nr. 1-6/1

Mērķis
Noteikt VSIA "Paula Stradiņa klīniskā universitātes slimnīca" vienotu taktiku, kas ievērojama gadījumā, ja pacientam pēc neoadjuvantās terapijas tiek konstatēta gandrīz pilna vai pilna audzēja klīniska remisija

Procesa turētājs
Onkoloģijas klīnikas vadītājs, Ķirurģijas klīnikas vadītājs

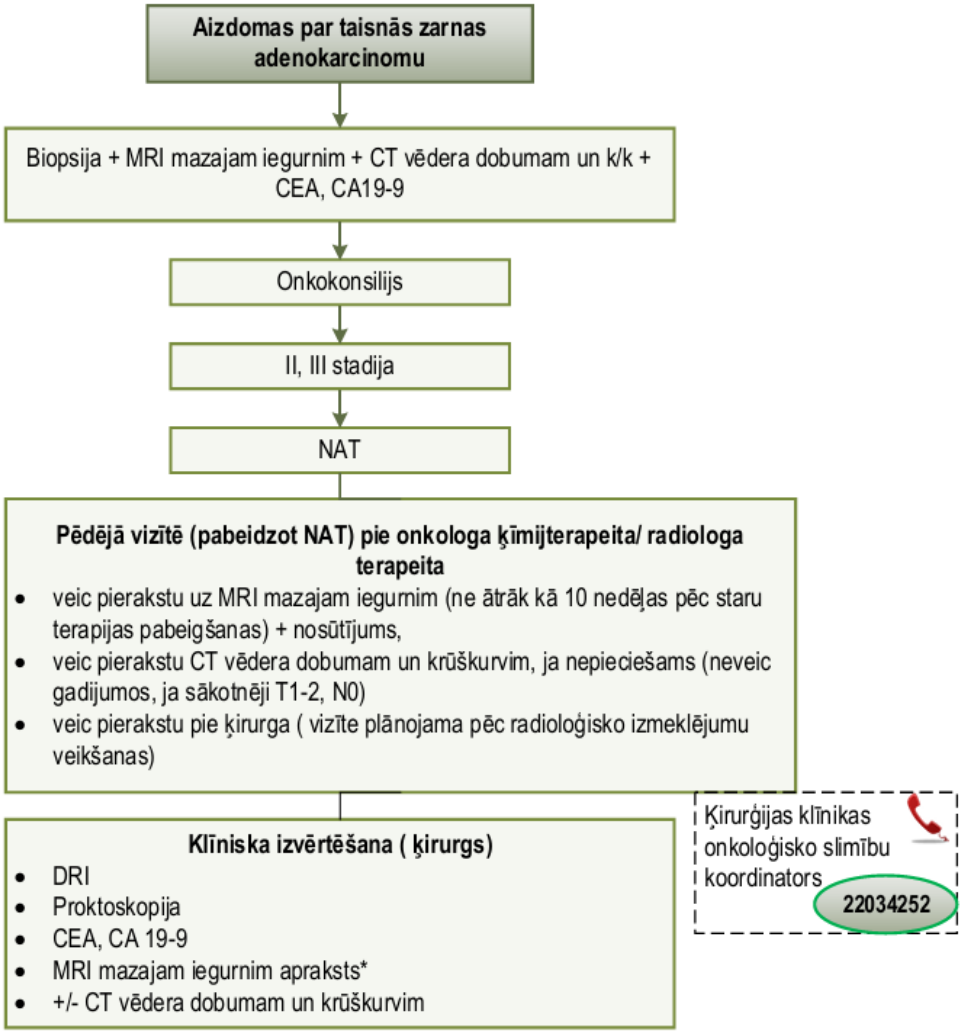
Iesaistītās struktūrvienības
Ķirurģijas klīnika, Onkoloģijas klīnika, Diagnostiskās radioloģijas institūts

Saistošie dokumenti
V-Onko-20 "Pacienta informētā piekrišana W&W"
V-Onko-21 "Pacienta piekrišana savu datu anonīmai ievadei IWWD"

Pielikumi:
1. pielikums - "Watch and Wait" jeb novērošanas taktika pacientiem ar taisnās zarnas vēža pilnu klīnisku remisiju pēc neoadjuvantās terapijas.

Definīcijas un saīsinājumi
CA 19-9 – cancer antigen 19-9 (latv. – audzēja antigēns 19-9)
cCR – complete clinical response (latv. – pilna klīniska remisija)
CD – cukura diabēts
CEA – carcinoembryonic antigen (latv. – karcinoembrionālais antigēns)
CT – computed tomography (latv. – datortomogrāfija)
DRI – digitāli rektāla izmeklēšana
ERUS – endorectal ultrasound (latv. – endorektālā ultrasonogrāfija)
ESMO – European Society for Medical Oncology (latv. – Eiropas Onkologu asociācija)
FKS – fibrokolonoskopija
IWWD – International Watch and Wait Database (latv. – starptautiskā taisnās zarnas vēža pacientu novērošanas taktikas datubāze)
MRI – magnetic resonance imaging (latv. – magnētiskā rezonanse)
mr-TRG – magnetic resonance tumour regression grade (latv. – audzēja regresijas pakāpe magnētiskās rezonanses izmeklējumā)
NAT – neoadjuvantā terapija (staru un ķīmijterapija vai tikai staru terapija)
TRG – tumour regression grade (latv. – audzēja regresijas pakāpe)
W&W – "Watch and Wait" (latv. – novērošanas taktika)

I. Pacienta primārās un pēcterapijas izvērtēšanas vadīšana taisnās zarnas adenokarcinomas gadījumā



* **Radioloģiskajos slēdzienos audzēja atbildes reakcijas aprakstīšanai tiek pielietota mrTRG klasifikācija (mrTRG1-mrTRG5)**

TRG1	Pilna radioloģiska atbildes reakcija	nav norādījumu par ārstēto tumoru
TRG2	Laba atbildes reakcija	blīva fibroze (>75%); nav redzams reziduāls tumors
TRG3	Vidēja atbildes reakcija	>50% fibrozes vai mucīna un redzama vidēja T2 signāla intensitāte
TRG4	Vāja atbildes reakcija	nelieli fibrozes vai mucīna apvidi, bet prevalē tumorozi audi
TRG5	Nav atbildes reakcijas	vidēja T2 signāla intensitāte; atrade atbilst sākotnējam tumorozam procesam

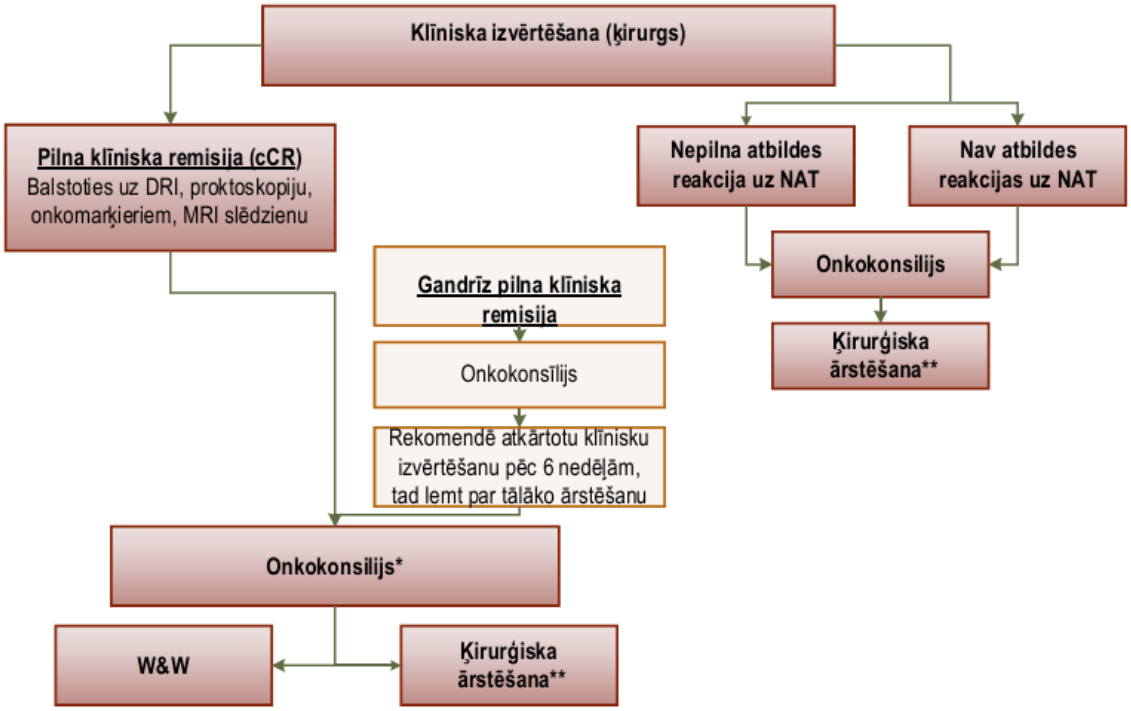
Pilna klīniska remisija

Definīcija	Par pilnu klīnisku remisiju pēc NAT ir uzskatāma atrade, ja audzējs nav nosakāms endoskopiski un klīniskās izmeklēšanas laikā, vismaz 4 nedēļas pēc NAT pabeigšanas
ESMO (2017.g.) **kritēriji** pilnai klīniskai remisijai	Minimālie kritēriji: ✓ DRI laikā nav konstatējams veidojums vai zarnas sieniņas neregularitāte ✓ endoskopiskas izmeklēšanas laikā nav vizualizējams veidojums, bet var būt plakana rēta, teleangiektāzijas vai gļotādas bālāks apvidus Papildus kritēriji: ✓ nav norādījumu par reziduālu audzēju tā primārajā lokalizācijā un reģionālajos limfmezglos MRI vai ERUS ✓ biopsijā no rētas – nav malignitātes ✓ CEA ir normas robežās (5 ng/mL), ja pirms NAT tas bijis paaugstināts
Slimnīcas noteiktie **kritēriji** pilnas klīniskas remisijas apstiprināšanai	1. DRI laikā nav konstatējams veidojums vai zarnas sieniņas neregularitāte 2. endoskopiskas izmeklēšanas laikā nav vizualizējams veidojums, bet var būt plakana rēta, teleangiektāzijas vai gļotādas bālāks apvidus 3. nav norādījumu par reziduālu audzēju tā primārajā lokalizācijā un reģionālajos limfmezglos MRI 4. CEA un CA 19-9 ir normas robežās, ja pirms NAT marķieri bijuši paaugstināti (CEA norma 0-5 ng/mL; CA 19-9 norma (0-37 U/ml)

Gandrīz pilna klīniska remisija

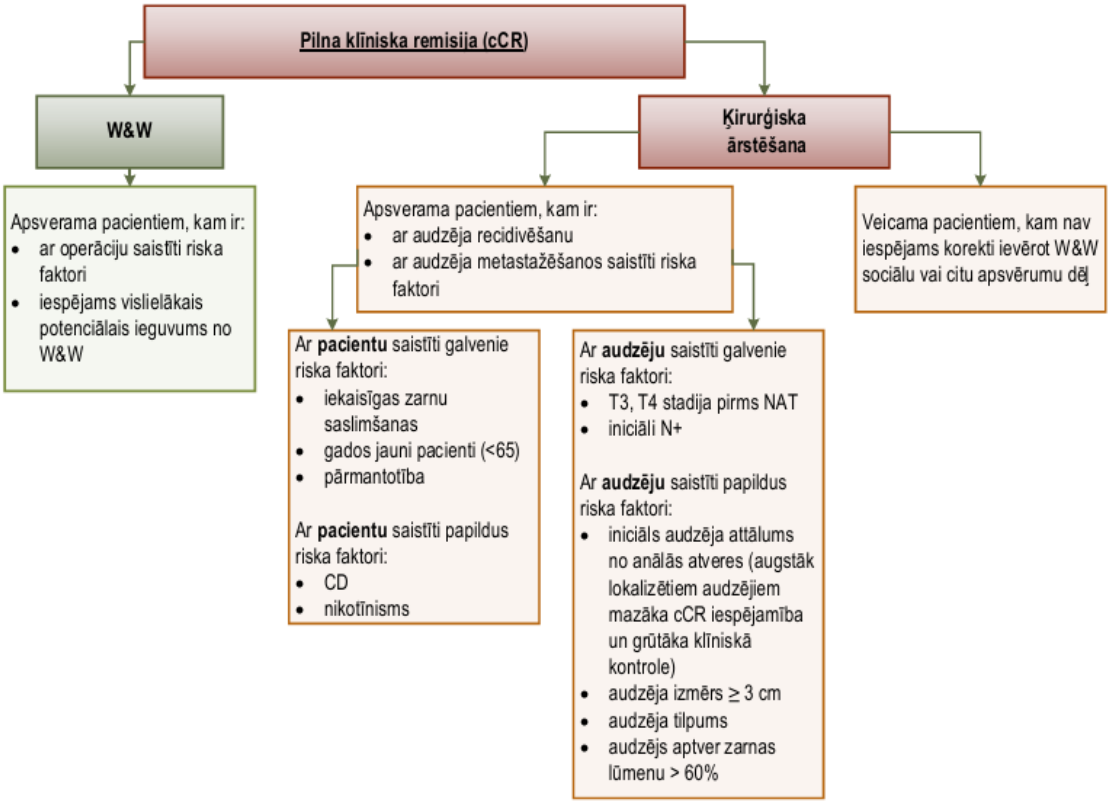
Definīcija	Par gandrīz pilnu klīnisku remisiju ir uzskatāma atrade, ja DRI un endoskopiskas izmeklēšanas laikā konstatē indurāciju vai čūlu, kas ir mazāka par 1 cm, un MRI nav norādījumu par izplatību reģionālajos limfmezglos, un atrade atbilst mr-TRG1 vai mr-TRG2

II. Taktika gadījumā, ja tiek konstatēta taisnās zarnas adenokarcinomas pilna klīniska remisija vai gandrīz pilna klīniska remisija pēc NAT



*Pacienta gadījums izskatāms onkokonsīlijā ne vēlāk kā 12 nedēļas pēc NAT pabeigšanas.
**Par ķirurģiskās ārstēšanas termiņu visos gadījumos (neatkarīgi no audzēja atbildes reakcijas uz NAT) tiek lemts onkokonsīlija ietvaros.

III. Taktika riska grupas pacientiem (ar pacientu vai audzēju saistīti riski) gadījumā, ja tiek konstatēta taisnās zarnas adenokarcinomas pilna klīniska remisija pēc NAT



IV. Novērošanas taktikas principi gadījumā, ja tiek konstatēta taisnās zarnas adenokarcinomas pilna klīniska remisija pēc NAT

- Kopējais novērošanas laiks: 5 gadi
- Intensīvās novērošanas laiks: 2 gadi
- Intensīvās novērošanas perioda kontroles vizīšu biežums: ik 3 mēnešus

W&W

Laika periods	Izmeklējums	Laiks
1., 2.gads	DRI	Ik 3 mēneši
	Proktoskopija	Ik 3 mēneši
	CEA, CA 19-9	Ik 3 mēneši
	MRI mazajam iegurnim	Ik 3 mēneši (4x/gadā)
	CT vēdera dobumam un krūškurvim	Ik 6 mēneši (2x/gadā)
	FKS	Ik 12 mēneši

Vizīte pie onkologa – 2x/gadā
Vizīte pie ķirurga – 4x/gadā

Laika periods	Izmeklējums	Laiks
3.-5.gads	DRI	Ik 6 mēneši
	Proktoskopija	Ik 6 mēneši
	CEA, CA 19-9	Ik 6 mēneši
	MRI mazajam iegurnim	Ik 12 mēneši (1x/gadā)
	CT vēdera dobumam un krūškurvim	Ik 12 mēneši (1x/gadā)
	FKS	Ik 12 mēneši (1x/gadā)

Vizīte pie onkologa – 1x/gadā
Vizīte pie ķirurga – 2x/gadā

V. Vizītes un to laikā veicamie nozīmējumi novērošanas taktikas protokola ietvaros

0. vizīte	Vizīte pie ķirurga pēc neoadjuvantās terapijas, kuras laikā, ja tiek konstatēta audzēja pilna klīniska remisija, pacientam tiek sniegta informācija par novērošanas taktiku, turpmākajiem izmeklējumiem un vizītēm, tiek iedoti nepieciešamie dokumenti. Tiek iedots nosūtījums MRI veikšanai mazajam iegurnim, nozīmējums onkomarķieru veikšanai. Pēc vizītes pacienta gadījums tiek izskatīts onkokonsīlijā, un novērošanas taktikas izvēle tiek dokumentēta onkokonsīlija slēdzienā.	
	1. un 2.gads	
1.vizīte – pēc 3 mēnešiem	**Ķirurgs**	✓ izvērtē lokālo stāvokli – veic taisnās zarnas digitālu izmeklēšanu, proktoskopiju; ✓ izvērtē MRI mazajam iegurnim atbildi; ✓ izvērtē onkomarķieru rādītājus; ✓ iedod nosūtījumu, lai veiktu CT vēdera dobumam un krūškurvim; ✓ iedod nosūtījumu, lai veiktu MRI mazajam iegurnim; ✓ iedod nosūtījumu onkomarķieru noteikšanai; ✓ pacientam izskaidro nākamās vizītes plānu.
2.vizīte – pēc 6 mēnešiem	**Ķirurgs**	✓ izvērtē lokālo stāvokli – veic taisnās zarnas digitālu izmeklēšanu, proktoskopiju; ✓ izvērtē MRI mazajam iegurnim atbildi; ✓ izvērtē CT vēdera dobumam un krūškurvim atbildi; ✓ izvērtē onkomarķieru rādītājus; ✓ iedod nosūtījumu, lai veiktu MRI mazajam iegurnim; ✓ iedod nosūtījumu onkomarķieru noteikšanai; ✓ pacientam izskaidro nākamās vizītes plānu.
	Onkologs	✓ izvērtē MRI mazajam iegurnim atbildi; ✓ izvērtē CT vēdera dobumam un krūškurvim atbildi; ✓ izvērtē onkomarķieru rādītājus; ✓ sniedz informāciju par nākamo vizīti
3.vizīte – pēc 9 mēnešiem	**Ķirurgs**	✓ izvērtē lokālo stāvokli – veic taisnās zarnas digitālu izmeklēšanu, proktoskopiju; ✓ izvērtē MRI atbildi mazajam iegurnim; ✓ izvērtē onkomarķieru rādītājus; ✓ iedod nosūtījumu, lai veiktu CT vēdera dobumam un krūškurvim; ✓ iedod nosūtījumu, lai veiktu MRI mazajam iegurnim; ✓ iedod nosūtījumu onkomarķieru noteikšanai; ✓ iedod nosūtījumu FKS veikšanai; ✓ pacientam izskaidro nākamās vizītes plānu.
4.vizīte – pēc 12 mēnešiem	**Ķirurgs**	✓ izvērtē lokālo stāvokli – veic taisnās zarnas digitālu izmeklēšanu, proktoskopiju; ✓ izvērtē MRI mazajam iegurnim atbildi; ✓ izvērtē CT vēdera dobumam un krūškurvim atbildi; ✓ izvērtē FKS atbildi; ✓ izvērtē onkomarķieru rādītājus; ✓ iedod nosūtījumu, lai veiktu CT vēdera dobumam un krūškurvim (tikai 1.gadā);

	Ķirurgs	✓ iedod nosūtījumu, lai veiktu MRI mazajam iegurnim (tikai 1.gadā); ✓ iedod nosūtījumu onkomarķieru noteikšanai; ✓ pacientam izskaidro nākamās vizītes plānu.
	Onkologs	✓ izvērtē MRI mazajam iegurnim atbildi; ✓ izvērtē CT vēdera dobumam un krūškurvim atbildi; ✓ izvērtē FKS atbildi; ✓ izvērtē onkomarķieru rādītājus; ✓ sniedz informāciju par nākamo vizīti.
5., 6., 7. un 8. vizīte otrajā novērošanas gadā notiek pēc 1.-4.vizītes plāna		
3.-5.gads		
9.vizīte – pēc 6 mēnešiem	**Ķirurgs**	✓ izvērtē lokālo stāvokli – veic taisnās zarnas digitālu izmeklēšanu, proktoskopiju; ✓ izvērtē onkomarķieru rādītājus; ✓ iedod nosūtījumu, lai veiktu CT vēdera dobumam un krūškurvim; ✓ iedod nosūtījumu, lai veiktu MRI mazajam iegurnim; ✓ iedod nosūtījumu onkomarķieru noteikšanai; ✓ iedod nosūtījumu, lai veiktu FKS; ✓ pacientam izskaidro nākamās vizītes plānu.
10.vizīte – pēc 12 mēnešiem	**Ķirurgs**	✓ izvērtē lokālo stāvokli – veic taisnās zarnas digitālu izmeklēšanu, proktoskopiju; ✓ izvērtē MRI mazajam iegurnim atbildi; ✓ izvērtē CT vēdera dobumam un krūškurvim atbildi; ✓ izvērtē FKS atbildi; ✓ izvērtē onkomarķieru rādītājus; ✓ iedod nosūtījumu onkomarķieru noteikšanai; ✓ pacientam izskaidro nākamās vizītes plānu.
	Onkologs	✓ izvērtē MRI mazajam iegurnim atbildi; ✓ izvērtē CT vēdera dobumam un krūškurvim atbildi; ✓ izvērtē FKS atbildi; ✓ izvērtē onkomarķieru rādītājus; ✓ pacientam izskaidro nākamās vizītes plānu.
11., 12., 13. un 14. vizīte ceturtajā un piektajā novērošanas gadā notiek pēc 9.un 10. vizītes plāna		

VI. Atbildības sadalījums starp speciālistiem attiecībā uz nosūtījumu sagatavošanu pacientam novērošanas taktikas ietvaros

1. Nosūtījumus visiem nepieciešamajiem izmeklējumiem (onkomarķieri, MRI mazajam iegurnim, CT vēdera dobumam un plaušām, FKS) sagatavo un pacientam izsniedz ķirurgs.
2. Nosūtījumos obligāti norādāmā informācija:
   2.1. primārā diagnoze un cTNM stadija;
   2.2. saņemtā ārstēšana un ārstēšanas periods;
   2.3. norāde par pilnu klīnisku remisiju un tās noteikšanas datums;
   2.4. atzīme par to, ka tiek veikta «novērošanas taktika», nosūtījumā ietverot apzīmējumu «W&W».
3. Ikvienā no vizītēm, ja pastāv klīniski norādījumi par lokālu vai sistēmisku recidīvu:
   3.1. novērošanas taktika tiek pārtraukta, pacients par to tiek informēts;
   3.2. tiek paņemta biopsija no taisnās zarnas iepriekšējās tumora ložas vai aizdomīgajiem audiem;
   3.3. pacients tiek nosūtīts uz radioloģiskajiem izmeklējumiem (izņemot tos, kuri ir jau veikti pēdējā mēneša laikā) – MRI mazajam iegurnim, CT vēdera dobumam un krūškurvim – vai uz citiem izmeklējumiem pēc indikācijām;
   3.4. pacienta gadījums tiek apspriests onkokonsīlijā, lai lemtu par tālāko ārstēšanas taktiku.
4. Informācijas dokumentēšana:
   4.1. pacientam tiek iedota informētas piekrišanas forma (V-Onko-20), kurā izskaidrots par novērošanas taktikas būtību. Piekrišanas forma tiek parakstīta divos eksemplāros – viens eksemplārs pacientam, otrs ārstam;
   4.2. pacientam tiek sniegta rakstiska informācija, kurā atspoguļots veicamo izmeklējumu un kontroles vizīšu plāns (1. pielikums);
   4.3. informācija par pacienta slimības gaitu un turpmāko novērošanas plānu anonīmā veidā tiek ievadīta starptautiskā datubāzē – IWWD (International Watch and Wait Database), https://cast-cancer.eu/. Ja pacients piekrīt savu datu anonīmai ievadei IWWD, tad paraksta atsevišķu piekrišanas formu (V-Onko-21). Piekrišanas forma tiek parakstīta divos eksemplāros – viens eksemplārs pacientam, otrs ārstam;
   4.4. katras vizītes izvērtēšanas rezultāts tiek fiksēts pacienta elektroniskajā ambulatorajā kartiņā "Ārsta Birojā", norādot, kura izvērtēšanas vizīte tā ir, atbilstoši protokolam;
   4.5. pacienta ģimenes ārstam jābūt informētam, kādēļ pacients netiek operēts (tas jāatspoguļo onkokonsīlija slēdzienā).

P-NMS-40/Alg **Novērošanas taktika pacientiem ar taisnās zarnas adenokarcinomas pilnu klīnisku remisiju pēc neoadjuvantās terapijas** versija **01**	1. pielikums

"*Watch and Wait*" jeb novērošanas taktika pacientiem ar taisnās zarnas vēža pilnu klīnisku remisiju pēc neoadjuvantās terapijas.

Informācija pacientam

1.GADS

		1.vizīte		**2.vizīte**		**3.vizīte**		**4.vizīte**
Vizīte, kurā tiek konstatēta audzēja pilna klīniska remisija	**3 mēneši**	• MRI mazajam iegurnim • onkomarķieri • **vizīte pie ķirurga** - taisnās zarnas izmeklēšana ar pirkstu - proktoskopija	**6 mēneši**	• MRI mazajam iegurnim • CT vēdera dobumam un krūškurvim • onkomarķieri • **vizīte pie ķirurga** - taisnās zarnas izmeklēšana ar pirkstu - proktoskopija • **vizīte pie onkologa**	**9 mēneši**	• MRI mazajam iegurnim • onkomarķieri • **vizīte pie ķirurga** - taisnās zarnas izmeklēšana ar pirkstu - proktoskopija	**12 mēneši**	• MRI mazajam iegurnim • CT vēdera dobumam un krūškurvim • onkomarķieri • kolonoskopija • **vizīte pie ķirurga** - taisnās zarnas izmeklēšana ar pirkstu - proktoskopija • **vizīte pie onkologa**

2.GADS

	5.vizīte		**6.vizīte**		**7.vizīte**		**8.vizīte**
1 gads un 3 mēneši	• MRI mazajam iegurnim • onkomarķieri • **vizīte pie ķirurga** - taisnās zarnas izmeklēšana ar pirkstu - proktoskopija	**1 gads un 6 mēneši**	• MRI mazajam iegurnim • CT vēdera dobumam un krūškurvim • onkomarķieri • **vizīte pie ķirurga** - taisnās zarnas izmeklēšana ar pirkstu - proktoskopija • **vizīte pie onkologa**	**1 gads un 9 mēneši**	• MRI mazajam iegurnim • onkomarķieri • vizīte pie ķirurga - taisnās zarnas izmeklēšana ar pirkstu - proktoskopija	**2 gadi**	• MRI mazajam iegurnim • CT vēdera dobumam un krūškurvim • Onkomarķieri • kolonoskopija • **vizīte pie ķirurga** - taisnās zarnas izmeklēšana ar pirkstu - proktoskopija • **vizīte pie onkologa**

P-NMS-40/Alg **Novērošanas taktika pacientiem ar taisnās zarnas adenokarcinomas pilnu klīnisku remisiju pēc neoadjuvantās terapijas** versija **01**	1. pielikums

3.GADS

	9.vizīte		**10.vizīte**
2 gadi un 6 mēneši	• onkomarķieri • **vizīte pie ķirurga** - taisnās zarnas izmeklēšana ar pirkstu - proktoskopija	**3 gadi**	• MRI mazajam iegurnim • CT vēdera dobumam un krūškurvim • onkomarķieri • kolonoskopija • **vizīte pie ķirurga** - taisnās zarnas izmeklēšana ar pirkstu - proktoskopija • **vizīte pie onkologa**

4.GADS

	11.vizīte		**12.vizīte**
3 gadi un 6 mēneši	• onkomarķieri • **vizīte pie ķirurga** - taisnās zarnas izmeklēšana ar pirkstu - proktoskopija	**4 gadi**	• MRI mazajam iegurnim • CT vēdera dobumam un krūškurvim • onkomarķieri • kolonoskopija • **vizīte pie ķirurga** - taisnās zarnas izmeklēšana ar pirkstu - proktoskopija • **vizīte pie onkologa**

5.GADS

	13.vizīte		**14.vizīte**
4 gadi un 6 mēneši	• onkomarķieri • **vizīte pie ķirurga** - taisnās zarnas izmeklēšana ar pirkstu - proktoskopija	**5 gadi**	MRI mazajam iegurnim • CT vēdera dobumam un krūškurvim • onkomarķieri • kolonoskopija • **vizīte pie ķirurga** - taisnās zarnas izmeklēšana ar pirkstu - proktoskopija • **vizīte pie onkologa**