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Response to neoadjuvant treatment in rectal cancer surgery

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"Writing a book is an adventure. To begin with it is a toy and an amusement. Then it becomes a mistress, then it becomes a master, then it becomes a tyrant. The last phase is that just as you are about to be reconciled to your servitude, you kill the monster and fling him to the public."

Sir Winston Churchill.

To my family

Contents

1. Abstract.....	9
2. List of publications.....	11
3. Abbreviations.....	13
4. Summary in Swedish.....	15
5. Introduction and background	
Introduction.....	19
Molecular background to colorectal cancer.....	21
Radiation therapy.....	25
Neoadjuvant chemotherapy.....	27
Rectal cancer flow chart.....	28
Surgical treatment.....	29
Magnetic resonance imaging in rectal cancer.....	31
6. Aims of the thesis.....	33
7. Patients and methods	
Study I.....	35
Study II.....	37
Study III.....	39
Study IV.....	41
8. Results and discussion	
Study I.....	43
Study II.....	45
Study III.....	47
Study IV.....	51

9. General discussion and future perspectives.....	53
10. Acknowledgements.....	57
11. References.....	61
12. Studies I-IV	67

Abstract

Rectal cancer is one of the three most common malignancies in Sweden with an annual incidence of about 2000 cases. Current treatment consists of surgical resection of the rectum including the loco-regional lymph nodes in the mesorectum. In advanced cases, neoadjuvant chemo-radiotherapy (CRT) prior to the operative treatment reduces local recurrences and enables surgery. The neoadjuvant treatment can also eradicate the tumour completely, i.e. complete response. This research project was designed to investigate the effects of preoperative radiotherapy/ CRT and analyze methods to predict response to CRT.

Study I investigated the expression of the FXYD-3 protein with immunohistochemistry in rectal cancer, with or without preoperative radiotherapy. The results from the total cohort showed that, strong FXYD-3 expression was correlated to infiltrative tumour growth ($p = 0.02$). In the radiotherapy group, strong FXYD-3 expression was related to an unfavourable prognosis ($p = 0.02$). Tumours with strong FXYD-3 expression had less tumour necrosis ($p = 0.02$) after radiotherapy. FXYD-3 expression in the primary tumour was increased compared to normal mucosa ($p=0.008$). We concluded that FXYD-3 expression was a prognostic factor in patients receiving preoperative radiotherapy for rectal cancer.

Study II investigated FXYD-3 expression in tumours that developed local recurrences following surgery and compared this with expression in tumours that did not develop local recurrences. There was no difference in the expression of FXYD-3 between the group that developed local recurrences and the group that did not develop local recurrences. There was no difference in survival between those with strong or weak FXYD-3 expression. We concluded that this study could not confirm the findings from study 1 i.e. that FXYD-3 expression has prognostic significance in rectal cancer.

Study III was a register-based study on the incidence and effects of complete response to neoadjuvant treatment. Eight per cent of the patients with adequate CRT to achieve complete response also had a complete histological response of the luminal tumor in the resected bowel. Sixteen per cent of that group had remaining lymph node metastases in the operative specimen. Chemotherapy together with radiotherapy doubled the chance of complete response in the luminal tumour. Patients with remaining lymph node metastases had a lower survival rate compared to those without. We concluded that residual nodal involvement after neoadjuvant treatment was an important factor for reduced survival after complete response in the luminal tumour.

Study IV followed up the results from the previous study by re-evaluating magnetic resonance imaging (MRI)- images in patients with complete tumour response. Two experienced MRI radiologists performed blinded re-staging of post CRT MR- images from patients with complete response in the luminal tumour. One group with lymph node metastases and another one without were studied and the results compared with the pathology reports. The sensitivity, specificity, and positive and negative predicted values for correct staging of positive lymph nodes was 37%, 84%, 70% and 57%. The size of the largest lymph node (4.5 mm, $p=0.04$) seemed to indicate presence of a tumour positive lymph node. We concluded that MRI couldn't correctly stage patients for lymph node metastases in patients with complete response to CRT in the luminal tumour.

List of publications

1. Loftås P, Önneshö S, Widegren E, Adell G, Kayed H, Kleeff J, Zentgraf H, Sun X-F. **Expression of FXYD-3 is an Independent Prognostic Factor in Rectal Cancer Patients With Preoperative Radiotherapy.** *International Journal of Radiation OncologyBiologyPhysics* 2009;75(1): 137-142.
2. Loftas P, Arbman G, Sun XF, Edler D, Syk E, Hallbook O. **FXYD-3 expression in relation to local recurrence of rectal cancer.** *Radiation oncology journal* 2016;34(1): 52-58.
3. Loftas P, Arbman G, Fomichov V, Hallbook O. **Nodal involvement in luminal complete response after neoadjuvant treatment for rectal cancer.** *European journal of surgical oncology : the journal of the European Society of Surgical Oncology and the British Association of Surgical Oncology* 2016;42(6): 801-807.
4. Loftås P, Sturludóttir, Hallböök O, Almlöv K, Arbman G, Blomqvist L. **Assessment of remaining tumour positive lymph nodes with MRI in patients with complete luminal response after neoadjuvant treatment of rectal cancer** *In manuscript.*

Abbreviations

• 3-D	Three Dimensional
• 5-FU	5-Fluorouracil
• AJCC	American Joint Committee on Cancer
• AR	Anterior Resection
• APR	Abdomino Perineal Resection.
• ATP	AdenosinTriPhosphate
• C.I	Confidence Interval
• cCR	Clinical Complete Response
• CRM	Circumferential Resection Margin
• CRT	Chemo-Radio Therapy
• DAB	DiAmineBenzidine tetrahydrochloride
• DCE	Dynamic Contrast Enhanced
• EMVI	ExtraMural Venous Invasion
• FXYD-3	Probably eponym for “fixed ID”
• Gy	Gray
• IQR	InterQuartile Range
• kD/kDa	kiloDalton
• LCR	Luminal Complete Response
• LR	Local Recurrence
• LRT	Long term Radiotherapy Treatment
• MAT-8	Mammary Tumour Protein
• MCF-7	Michigan Cancer Foundation
• MRI	Magnetic Resonance Imaging
• mRNA	Messenger RiboNuklein Acid
• MDT	Multi Disciplinary Team
• MV	Mega Volt
• NCR	National Cancer Registry
• NPV	Negative Predictive Value
• p21	Protein 21
• p53	Protein 53
• P73	Protein 73
• PBS	Phosphate Buffered Saline
• pCR	pathologic Complete Response
• PPV	Positive Predictive Value
• PRL	Phosphatase of Regenerating Liver
• R0	Pathologically confirmed Radical Resection
• RT	Radiotherapy
• SCRCR	Swedish ColoRectal Cancer Register
• SPSS	Statistical Package for the Social Science
• SRT	Short-term Radiotherapy Treatment
• T	Tesla/Tumour
• T1-T2-weighted	Tissue-weighted
• TME	Total Mesorectal Excision
• TNM	Tumour, Node, Metastasis (classification)

Sammanfattning på svenska

Ändtarms- och tjocktarmscancer är två av de vanligaste cancerformerna i Sverige och i västvärlden. Ändtarmscancer drabbar vanligtvis individer i åldern 70-75 år, men sjukdomen kan även drabba betydligt yngre. Den är något vanligare hos män än hos kvinnor. Precis som för de flesta andra cancersjukdomar finns ingen enskild orsak till att man drabbas av sjukdomen, men västerländsk kost, vissa kroniska tarmsjukdomar samt ärftlighet kan öka risken. Som alla andra cancersjukdomar kan ändtarmscancer sprida sig i kroppen med dottertumörer, vanligtvis i lever och lungor. I undantagsfall återkommer tumören även i bäckenet efter botande behandling, så kallat lokalrecidiv. Behandlingen för ändtarmscancer har länge varit kirurgi då hela eller delar av ändtarmen opereras bort. Ibland tas också ändtarmsöppningen bort och patienten får en permanent tarmöppning på magen, en stomi. Om patienten strålbehandlas före operationen minskar risken att få återfall i bäckenet. Strålningen minskar också tumörens storlek vilket ger bättre förutsättningar för operation. Behandling med både cellgifter och strålning är effektivare och kan även påverka tumörceller som tagit sig vidare i kroppen.

Kombinationen av strålning och cellgifter är ibland så lyckad att hela tumören och även tumör som spridit sig till de regionala lymfkörtlarna försvinner helt innan den planerade operationen. Hittills har patienten ändå blivit opererad eftersom det anses vara den säkraste metoden.

Både strålning och cellgifter har svåra och många gånger livslånga biverkningar och dessa måste vägas mot vinsterna med behandlingen. Det innebär att bara patienter med mer avancerad sjukdom får förbehandling med strålning och/eller cellgifter eftersom nyttan av behandlingen i de fallen väger tyngre än biverkningarna.

Den första studien fokuserade på ett protein, FXYD-3. Alla tumörer innehåller inte detta protein, medan en del har höga halter av proteinet, andra avsevärt lägre. Syftet med studien var att undersöka om tumörer som har höga halter av detta protein har en sämre prognos och därmed ett större behov av strålbehandling. Studien visade att FXYD-3 var vanligare i tumörvävnad än i normal vävnad. Tumörer som hade mycket FXYD-3 växte också aggressivare än tumörer som inte hade så höga halter av proteinet. Hos de patienter som strålades innan operationen kunde man se att mycket FXYD-3 i tumören medförde sämre överlevnad och sämre svar på strålbehandlingen. Dessa skillnader kunde man inte se i den patientgrupp som inte fick någon strålbehandling.

Tolkningen av dessa resultat är att FXYD-3 verkar kunna tydliggöra vilka patienter som har en mer aggressiv sjukdom och som därför inte svarar lika bra på strålbehandling och därmed också får en sämre prognos.

Den andra studien är en fortsättning på den första där vi ville bekräfta att de patienter som har FXYD-3 i tumören kan förutsägas ha sämre resultat av strålbehandling. Då det tydligaste resultatet av strålbehandling innan operation visat sig vara en minskning av antalet återfall i bäckenet, lokalrecidiv, valde vi att analysera tumörer som utvecklat lokalrecidiv. I den här studien samarbetade vi med Karolinska Institutet i Stockholm som tidigare studerat samma patientgrupp.

Vi studerade mängden FXYD-3 hos patienter med lokalrecidiv, och jämförde dem med en grupp patienter som inte utvecklat lokalrecidiv. Vi kunde inte bekräfta att tumörer med mycket FXYD-3 i högre grad riskerar återfall till bäckenet. Tolkningen försvåras dels av att det var få patienter med mycket FXYD-3 i tumören som fick strålning och även utvecklade lokalrecidiv. I den här studien kunde vi inte bekräfta resultatet i den första studien.

I den tredje studien valde vi att studera den patientgrupp som fick ett så bra resultat av strål- och cellgiftsbehandlingen innan operation att hela tumören försvann, så kallad *complete response*. Vi ville genom registerforskning ta reda på förekomst och prognos för dessa patienter som opererades utan att man fann någon kvarvarande tumör vid mikroskopisk undersökning av den bortopererade tarmen,

Knappt 30 procent får en så pass avancerad behandling med cellgifter och/eller strålning att det finns en teoretisk möjlighet att tumören kan försvinna. Av dessa hade åtta procent ingen kvarvarande tumör i den bortopererade ändtarmen vid den efterföljande mikroskopiska undersökningen. Av de patienter där tumören försvunnit helt hade 16 procent trots det en spridning av sjukdomen till regionala lymfkörtlar. Spridning till dessa lymfkörtlar innebär en ökad risk för spridning av sjukdomen till andra organ. Det visade sig också att dessa patienter hade sämre överlevnad än de patienter som inte hade någon spridning till lymfkörtlar, trots att både ändtarm med lymfkörtlar var bortopererade.

Den fjärde studien följer upp resultatet av den tredje. I och med att antalet patienter med *complete response* blir fler så har man i andra länder börjat avstå från att operera dessa patienter. Detta för att patienten ska slippa en stor operation med stomi som följd eftersom tumören redan tycks vara borta. Det är relativt enkelt att fastställa om tumören i tarmen försvunnit helt, men det är svårare att med magnetröntgen skilja friska lymfkörtlar från de som fått spridning av tumörer. Då vi vet från den föregående studien att det inte är helt ovanligt med spridning till lymfkörtlar även efter att tumören försvunnit, och att det dessutom medför sämre överlevnad, är det viktigt att identifiera de patienter som fått en sådan spridning till körtlarna innan det fattas beslut om att inte operera. Studien genomfördes i samarbete med Karolinska Institutet i Stockholm. Två erfarna röntgenläkare med stor kunskap i att bedöma magnetröntgen av den här typen av patienter fick bedöma tidigare magnetröntgenundersökningar från två grupper av patienter. Båda grupperna hade ändtarmscancer och samtliga hade fått strålbehandling, med eller utan cellgiftsbehandling så att tumören försvunnit och därefter opererats så att operationspreparaten kunde undersökas. Alla patienter hade också genomgått magnetundersökning av bäckenet före och efter strål-/cellgiftsbehandlingen, men innan operationen. Hälften av patienterna hade tumör kvar i lymfkörtlarna medan den andra

hälften inte hade det. Röntgenläkarna som gjorde bedömningarna visste inte vilka patienter som hade friska lymfkörtlar och vilka som inte hade det. När vi sammanställde resultatet visade det sig att man inte med tillräcklig stor säkerhet vare sig kunde bekräfta eller utesluta tumör i lymfkörtlarna med magnetröntgenundersökning. Bedömning av den största lymfkörteln storlek hos varje patient visade sig kunna vara ett sätt att identifiera de patienter som hade en spridning av tumörer till lymfkörtlar

Slutsatsen blev att magnetröntgenundersökning inte ensamt kan användas för att bedöma om körteln är frisk eller sjuk på dessa patienter, men att storleken på den största körteln skulle kunna användas för att påvisa misstanke om att patienten har fått en spridning av sjukdomen till lymfkörteln

Introduction.

Colorectal cancer is an age-dependent malignancy that is the second most common cause of cancer-related death in the Western world and the second most common cancer in women and men in Sweden. The incidence is almost equal in men and women in and is fairly stable though there is a slight increase due to the increase in average age in Sweden. Rectal cancer accounts for approximately 30% of all colorectal malignancies (1, 2).

The introduction of preoperative radiotherapy (RT) in the treatment of rectal cancer has reduced the frequency of local recurrence, and RT is now a part of the standard treatment regime for advanced rectal cancer. Radiotherapy is often combined with chemotherapy, chemoradiotherapy (CRT). CRT induces downsizing and downstaging of the tumour and regional lymph nodes thereby enabling surgery of an otherwise inoperable tumour(1, 3, 4). There are well-known short-term and long-term side-effects of pelvic radiation and chemotherapy. Furthermore studies have established that the response to RT is highly individual and that this may partly be explained at the molecular level(5-8). In order to avoid unnecessary side effects it is important to discover biological factors that could influence recurrence and survival, and to identify patients that will probably not benefit from RT/CRT. A number of clinically available biomarkers have been investigated as to their ability to predict radio sensitivity, but so far none has come into clinical use. Some are regarded as promising. Examples of such biomarkers are epidermal growth factor, (EGFR) thymidylate synthase, p21 and carcinoembryonic antigen (CEA) (8-10).

A dependable predictor for neoadjuvant treatment response would spare probable non-responders unnecessary side effects and reduce undue time waiting for surgery. Identification of potential responders would enable more tailored neoadjuvant treatment and individual follow up programmes.

In patients with an outstanding response to neoadjuvant treatment the entire luminal tumour may have disappeared prior to surgery, a so-called complete response, (ypT0N0M0). Extended times between chemoradiotherapy and surgery, novel chemotherapeutic agents and change in indications for neoadjuvant treatment has led to an increase in the frequency of complete response(11-15).

Complete response can occur as a result of standard treatment following national guidelines for preoperative down staging. It could also be viewed as a preferred outcome in a goal directed treatment aiming to avoid surgery. Hence the indications for neoadjuvant treatment can be extended to include less advanced tumours as well(11). The rate of complete response varies with the indication, as there is a better chance of complete response in the less advanced tumours (11, 16). Some single institution centers have presented high complete response rates in small tumours and in a highly selected patient population(12). There is a need to know the frequency of complete response in a large unselected cohort following national guidelines where factors influencing incidence and outcome may be analyzed.

When considering a conservative wait-and-watch strategy in patients with complete response both the surgeon and the patient need to know the individual risk factors for recurrent disease and the limitations of the imaging techniques used for staging and follow up(17). Staging and restaging is of great importance when deciding on neoadjuvant treatment and non-surgical management. As yet there are no implemented guidelines for follow-up after non-surgical management. Highly frequent follow-ups for

many years including physical examination, endoscopy, CT-scans and MRI are necessary(17, 18)

Figure 1.

MRI and endoscopic images of rectal tumours before and after CRT, with luminal complete response in the same patient.

- A. Sagittal MR T2 image of rectal cancer (marked T).
- B. Endoscopic view of the tumour in same patient
- C. Post CRT MR T2 image from the same patient with no visible tumour, (arrow).
- D. Post CRT endoscopic view showing only telangiectasia at the former tumour site (arrow).

The molecular background to colorectal cancer.

We do not know any single etiology of colorectal cancer or any endogenous or exogenous causative carcinogens responsible for the development of colorectal cancer. Every human is built of approximately 60 billion cells that are constantly dividing. With every cell division there is a risk for error in the DNA replication that may be inherited and multiplied. The longer we live the greater the number of mitoses that have occurred, as well as the greater the exposure to environmental carcinogens such as radiation, chemicals and drugs. Very effective cellular system exists in the body that repair or terminate mutations in a cell that could otherwise be replicated and passed on to daughter cells. This system of repair is however not foolproof, and some mutations are not corrected. These mutations together with accumulated oncogenic events eventually lead to the development of cancer(19).

In 1990 Fearon and Vogelstein proposed the adenoma to carcinoma sequence as a model with successive genetic mutations leading to colorectal cancer. This model describes the stepwise transition from normal epithelium to cancer through multiple mutations in oncogenic and tumour suppressor genes(20) (Figure 2 and 3).

Figure 2.
The adenoma-carcinoma sequence.
(Modified from Fearon-Vogelstein (20))

Despite almost 25 years of research into the development of cancer, the Vogelstein model, with some important modifications still stands as the accepted model for colorectal cancer development, though there are other models, such as the theory of cell fusion(21, 22).

We know that many mutations take place in the various steps towards invasive cancer, and the greater the number of mutations occurring the greater the risk for cancer. Furthermore, the cumulative number of mutations is more important than the sequential order (20) (Figure 2)

Figure 3.
Common oncogenes in colorectal cancer.

Oncogenes in colorectal cancer
Proto oncogenes
K-ras (Kirsten-rat sarcoma)
Supressor genes
APC (Adenomatous Polyposis Coli)
p53 (Protein 53 kDa DCC (Deleted in Colorectal Carcinoma)
DNA mismatch repair genes. (Responsible in hereditary non polyposis colon cancer (HNPCC))
hMSH2 hMLH1 hPMS1 hPMS 2

The colon and rectum are formed of 10⁷ glandular crypts each with thousands of differentiated cells of polyclonal origin and a small number of stem cells (less than 10 per crypt). These stem cells are protected from the hostile intestinal environment at the bottom of the crypts. The differentiated cells divide rapidly and migrate to the top of the crypt where 10¹⁰ cells are shed each day(19). Proliferation is limited to the lower two thirds of the crypts and the mature cells are shed at the surface.

The first step in the monoclonal malignant transformation is caused by individual mutations to both alleles of the tumour suppressor Adenomatous Polyposis Coli (APC) gene. This initial mutation shifts cell proliferation to the upper portion of the crypt where the dividing cells are more exposed to the hazardous contents of the bowel. The APC gene is a restrainer of proliferation and the lack of this function leads to uncontrolled cell growth and the formation of a polyp(20). Over expression of COX1 and COX2 cyclooxygenases, catalyzes the arachidonic acid derivate malondialdehyde which itself is mutagenic and intensifies the malignant process by promoting cell migration and production of angiogenic stimulatory factors(23). This is thought to occur after the mutation of the second allele of the gene.

These initial steps of malignant transformation are reversible as seen after administration of NSAID, where there is a reduction in both size and number of polyps through COX2 inhibition, this led to the concept of COX2 inhibitors in prophylaxis or treatment of colon adenomas(24).

Further mutations are needed to transform the adenoma to invasive cancer and a mutation of the Ki-ras proto-oncogene increases cell division and leads to increased dysplasia in the adenoma. Mutated Ki-ras is not expressed in early benign adenomas but increases in expression in dysplastic polyps and cancers and is thus important for malignant transformation in many, but not all colorectal cancers(25). Mutations in the Deleted in Colorectal Carcinoma (DCC) gene and SMAD 4 gene, both of which play a role in cell adhesion and stromal proliferation (20, 26) are also important steps. One of the final steps on the path to malignancy is the mutation of both alleles (double hit) of the tumour suppressor gene p53. The p53 is known as the “guardian of the genome” by recognizing DNA damage and inducing G1 cell cycle arrest during DNA reparation or, in the case of irreparable damages, initiating apoptosis(27). Mutations of the p53 gene are found in 75% of all colorectal cancer cells but are rare in adenomas(28). Mutation of p53 results in reduced p21 transcription, cumulative DNA damage and uncontrolled premature replication of p53 seems to have a moderate effect in normal cells but has a greater effect in cells stressed by radiation or cytotoxic drugs(29).

DNA Mismatch Repair genes (MMR) are responsible for the repair of mismatches in base pairs. Cells with abnormally functioning MMR are unable to correct errors that occur during DNA replication and consequently DNA errors accumulate. This leads to the creation of novel microsatellite fragments. Microsatellites are composed of repeated DNA sequences. These sequences can be made up of repeating units of one to six base pairs in length. Mutation of the gene will result in defective repair, microsatellite instability and an increase in dysplasia, both of which are seen in 13% of sporadic colorectal carcinomas and in all HNPCC (30).

The ability to metastasize is the least understood stage in the stepwise progression theory. For cells to be able to metastasize the cell-cell adhesions must be disrupted. Reduction in cell adhesions is partly due reduced expression of E cadherin (31). Various metalloproteases take part in a proteolytic cascade that degrades ECM components and disrupts extracellular matrix (ECM) cell interaction (32). Intravasation of malignant cells in to the blood stream and resistance to the major histocompatibility complex system, which removes circulating tumour cells, is poorly understood. Animal studies have demonstrated that only a small portion of the malignant cells entering the bloodstream have the ability to establish metastases by avoiding being killed by circulating immune cells or by establishing a colony at a distant site. Carcinoembryonic antigen that is used as a tumour marker in colorectal cancer is believed to play a role in this process as well as endothelial growth factor that is a target for novel cytotoxic drugs (33, 34).

Radiation therapy.

The effects of radiation therapy may be divided into three separate phases.

The physical phase

The physical phase is short and based on the interactions of charged particles with the tissue DNA. It takes an electron 10^{-18} seconds to pass through the DNA molecule and the journey through the entire cell takes around 10^{-14} s. During this short time the electron or photon will interact and eject electrons from atoms (ionization). These ejected electrons may ionize nearby atoms giving rise to an ionization cascade. For every Gray of absorbed radiation 10^5 ionizations take place for every $10\mu\text{m}$ of tissue penetrated.

The chemical phase

The chemical phase is the time period when damaged atoms react with cellular components, breaking chemical bonds and forming split molecules, i.e. free radicals. During this time span of 1ms, protective scavengers reactions including sulphhydryl compounds, chemically inactivate these free radicals.

The biological phase

This phase includes all other processes that follow.

Most damaged DNA is successfully repaired. However, cancer cells with mutations in genes, such as p53 are rapidly dividing and have a defect DNA repair system causing increased cell death compared to normal tissue after radiation. When the mutations lead to cell death, a process that takes time, a cell may undergo many divisions before it dies. The early effects of radiation on skin, mucosa or blood components are due to the killing of stem cells. The late effects of radiation therapy such as secondary tumours are also due to the initial DNA damage(35).

Radiation therapy is used as neoadjuvant treatment for rectal cancer either alone or in combination with chemotherapy. Two different regimens exist, Short-term Radiotherapy (SRT e.g. 5×5 Gy) is delivered over five days followed by surgery within five days. The indication is usually T3b tumours in the middle and lower rectum as well as mesorectal N+ tumours provided the CRM is clear. SRT has no down staging effect as the time between radiation and surgery is too short. It is used to sterilize the pelvic lymph nodes in order to reduce local recurrence i.e. within the pelvis(1). However surgery may be postponed for several reasons thereby increasing the chance that SRT also has a down-staging effect. This is termed "SRT with delay" which is usually defined as a delay of more than four weeks between radiotherapy and surgery. Long Term Radiotherapy (LRT (e.g. $28 \times 1.8-2$ Gy), has an interval of 6-8 weeks before surgery and can lead to sterilization and down staging of both the tumour and adjacent lymph nodes and is indicated for advanced T3 and T4 tumours. There are several short- and long-term side effects of pelvic radiation such as urinary and fecal incontinence, sexual dysfunction, peripheral nerve damage and pathological fractures. Small bowel toxicity with an increased risk for bowel obstruction is probably the most frequent side effect (5, 36, 37). The incidence of severe radio toxicity with leukopenia, abdominal pain and diarrhea is around 5% (38, 39). The late effects of RT may add to the complications of pelvic surgery. Both irradiation and the surgical trauma in the vicinity of pelvic nerves can harm the nerves controlling sexual function and continence of both the bowel and bladder.

The increased risk for nerve damage after RT, independent of surgical trauma, has been described in several studies (5, 36). The risk for secondary cancer is increased after irradiation (36)

Several studies have demonstrated that LRT and SRT with delay have comparable down staging effect and reduction in the rate of local recurrence(38-40). There is a clear dose-response between the RT-dose and the tumour down staging when the same fractionations are compared(35, 41). With tailored extended delays between CRT and surgery, an increase in the frequency of complete pathological response, (ypT0N0M0) is evident (11-15).

Neoadjuvant chemotherapy

The fluoropyrimidines (5-fluorouracil and fluorodeoxyuridine), when given in combination with radiation increase the effectiveness of neoadjuvant treatment. Increased radiation sensitivity occurs in cells that progress inappropriately into the S phase due to DNA damage inflicted by radiation. This could provide the molecular basis for the selective killing of tumour cells rather than normal tissue cells(42).

Fluoropyrimidine based chemotherapy can be used in conjunction with both LRT and SRT, in the most advanced forms of rectal cancer (chemoradiotherapy, CRT), acting as a radio-sensitizer to increase the effect of radiotherapy (43). CRT increases the rate of complete response compared to radiotherapy alone (40).

Cisplatin, a platinum based chemotherapy, has multiple effects on cells, one of them being DNA damage repair inhibition but the exact mechanism is not known(42).

The addition of oxaliplatin, another platinum-based chemotherapy agent, to 5-FU for adjuvant treatment(44), reduces the risk for recurrence by 25% in rectal cancer. In the neoadjuvant setting, oxaliplatin has not been shown to be a better radio sensitizer. Other chemotherapeutic agents such as irinotecan or monoclonal antibodies such as bevacizumab have not been shown to have this effect either(45).

The side-effects of 5-FU based chemotherapy as single therapy or in combination with other therapies in the neoadjuvant setting are usually well tolerated with less than 5% having severe toxic reactions because of the short-term exposure(46).

Current treatment schedules include nearly 6 weeks of neoadjuvant chemo radiation, 6-8 weeks of recovery prior to surgery, and another 4 weeks of recovery prior to consideration for adjuvant therapy. In this way there is a minimum 4 months delay before initiation of full dose systemic therapy, (see flow chart following page). Starting full dose chemotherapy at an earlier stage has the advantage of treating micro-metastatic disease earlier, thereby reducing the incidence of distant recurrence. Furthermore in most studies radiotherapy has not improved survival but primarily reduced local recurrence (1). Thus an alternative could be neoadjuvant chemotherapy alone. There is limited experience of chemotherapy as single treatment modality but initial reports are encouraging in terms of both down-staging and outcome but further research with long-term follow-up is needed and initiated(43, 46, 47).

Figure 4. Example of a theoretical flow chart with treatment options for a Swedish patient with rectal cancer 2016.

Surgical treatment.

Jacque Lisfranc performed the first formal perineal resection for rectal cancer in 1826. Gausseubauer and Hartmann 1879 addressed the problem with limited exposure by performing resections via an abdominal approach(48). Ernest Miles studied the lymphatic spread and demonstrated an upward direction of lymph flow. Between 1899 and 1906 he performed 57 perineal resections of which 95% developed early recurrence. In the post mortem he found recurrence in the peritoneum and in lymph nodes around the left iliac artery. He stated, "I have failed in one important aspect-namely the complete eradication of the zone of upward spread"(48).

In 1907 he performed the first abdominoperineal resection enabling resection of the upward spread to lymph nodes, the Miles' Operation(49).

In 1923 he had a recurrence rate of 29.5 %, which was outstanding compared to previous results(50).

The Miles' procedure became the gold standard and was improved by Lloyd-Davis as a one-stage operation in contrast to Miles' two-stage procedure. Moynihan advocated for the high tie of the inferior mesenteric artery so as to include lymph nodes that could be a future locus for metastatic growth, a controversy that is not yet solved(51).

In 1948 Claude Dixon presented the results of 400 patients operated with anterior resection for middle and upper third rectal cancer maintaining bowel continuity instead of the permanent stoma used in the Miles' operation(52).

In 1982 Heald described the lymphatic spread within the mesorectal sleeve and formulated the concept of Total Mesorectal Excision (TME) with *en bloc* resection of the rectum and mesorectum and sharp dissection in well defined planes as opposed to blunt manual extraction used previously. In an article from 1986 he reported on 115 patients where only 3 local recurrences had been seen after a four-year follow up (53, 54). The TME concept revolutionized rectal cancer surgery and is now the gold standard worldwide.

Anterior resection.

Anterior resection (AR) is the most common surgical procedure for rectal cancer being 40% of the surgical procedures for rectal cancer in Sweden 2015(2). The procedure is decreasing in popularity probably due to a greater awareness amongst surgeons and patients of poor postoperative bowel function with a very low anastomosis and the fear of complications. The operation includes resection of the rectum, mobilization of the left colon, and division of the inferior mesenteric artery. The left colic flexure is usually taken down to allow a tension-free anastomosis. The rectum is mobilized circumferentially to the pelvic floor, or 5 cm distal to the tumour according to the TME technique. The descending colon and the distal end of rectum is divided and a colorectal anastomosis is created using a circular stapling device. Diverting ileostomy is indicated with a low rectal anastomosis(2). The procedure carries a 10-15% risk for anastomotic leakage leading to pelvic abscess and septicemia.

There is a risk for damaging the nerves controlling bladder, anal sphincter and sexual function, a risk that is also increased by preoperative radiation.

The operation is a sphincter-sparing procedure if the diverting stoma is reversed.

Recently the Low Anterior Resection Syndrome (LARS) with incontinence, urgency, and frequent bowel movements has been highlighted and has been shown to cause considerable functional long-term bowel function problems after surgery (55).

Abdominoperineal Resection

An abdominoperineal resection (APR) is indicated for the most distal tumours. The abdominal part of the operation is performed as in an AR. The second part of the operation is performed with a perineal approach. The anus, anal canal and parts of the pelvic floor are included in the resection. The patient is left with a permanent colostomy. In 2015, 25% of the rectal cancer operations in Sweden, were APR(2).

Hartmann's Resection.

In a Hartmann's resection the same abdominal procedure as in AR is performed, but as the indications for this procedure are concerns about anastomotic complications in old age, poor health or expected postoperative functional problems, no anastomosis is constructed. The patient is left with a colostomy and the closed rectal stump is left in situ. Hartmann's resections is performed in less than 10% of the abdominal resections (2).

Local Excision and Transanal Endoscopic Microsurgery (TEM).

These operations are indicated for T1 cancers or as a palliative procedure in patients unfit for abdominal resection. TEM uses a large endoscope encompassing instruments for dissection and gas insufflation. Full-thickness resections may be obtained and the bowel defect is closed or left open for secondary healing.

The major disadvantage of this technique is that even if the tumour is radically resected, the regional lymph nodes are left in situ and cannot be examined pathologically.

Minimally invasive techniques such as laparoscopic and robotic surgery have improved visualization and further reduced the perioperative trauma still without improved oncological outcome (56, 57).

Magnetic resonance imaging in rectal cancer.

High-resolution T2-weighted imaging is the key sequence in the magnetic resonance (MR) imaging evaluation of rectal cancer both for the primary staging and in restaging after chemoradiotherapy. The technique can, with high accuracy differentiate between rectal tumours confined to the rectal wall (Stage T2) from those that extend beyond the muscularis propria (Stage T3)(58, 59).

Several studies have shown that high-resolution MR imaging is a reliable and reproducible technique with high specificity for assessing the distance of the tumour from the CRM (60).

However, assessment of nodal involvement remains problem. Prior to starting neoadjuvant treatment, the size and morphology of nodes may to a certain degree be used to discriminate between benign and malignant lymph nodes. However, after CRT treatment morphology is no longer a reliable criterion and size alone has significant limitations. Assessment of nodal stage seems to be highly dependent upon the experience of the radiologist(61-63).

Diffusion weighted imaging (DWI) is an MRI technique that quantifies the movement of water molecules at the cellular level. Since the properties of water vary in areas of necrosis, inflammation and fibrosis, the DWI technique improves the staging of cancer and facilitates lymph node detection. There is no consensus on the use of DWI in rectal cancer staging, and it is not reliable for differentiating between benign and malignant lymph nodes(64, 65).

MR imaging of the pelvis with rectal cancer.

- A. Sagittal T2 view of a large rectal cancer.
- B. Transverse T2 view of circumferential rectal cancer.
- C. Transverse T2 view with a lateral lymph node indicated with arrow.

A

B

C

Aims of the thesis

The aims of the thesis were;

- to investigate the expression of FXYD-3 in rectal cancer and the relationship of FXYD-3 expression to survival in patients with or without preoperative radiotherapy:

- to investigate the potential role of FXYD-3 as a biomarker for decreased radio-sensitivity, using local recurrence of rectal cancer as a proxy:

- to investigate in a prospective national register-based study the impact of neoadjuvant therapy on the incidence and outcome of pathologically confirmed complete response:

- to assess the accuracy of MRI in predicting the presence of remaining mesorectal lymph node metastases (ypT0N+) in patients with complete pathologically confirmed luminal response (ypT0) after neoadjuvant radiotherapy or chemo- radiotherapy.

Patients and Methods

Study I

In study I, 140 specimens from patients that participated in a Swedish randomized trial on preoperative radiotherapy (RT) between 1987 and 1990 were analyzed. All patients had primary rectal carcinoma and 65 of the 140 received 5x5Gy RT over a five-day period, followed by surgery within a week. None of the patients had adjuvant chemotherapy and the mean follow-up time was 84 months (66) (Table 1). Patients were analyzed for expression of FXYD-3 in the primary tumour, normal mucosa and regional lymph node metastases. Immunohistochemical analysis was performed on paraffin-fixed sections using a monoclonal anti-FXYD-3 primary antibody (Fig. 1) Degree of staining was graded as negative, weak, moderate or strong based on the intensity of the staining. The stained sections were microscopically examined and scored independently by two investigators blinded to the clinical, pathological and biologic data.

Statistics.

McNamara's method and Chi-square were used to test the significance of differences in FXYD-3 expression between distant normal mucosa, adjacent normal mucosa, the primary tumour and lymph node metastases, and the association of FXYD-3 expression with clinical, pathologic and biologic variables. The relationship between FXYD-3 expression and survival was tested using Cox's proportional hazards model. Survival curves were calculated using the Kaplan-Meier method. All tests were two-sided and a p-value of less than 0.05 was considered as statistically significant.

Figure 1. Immunohistochemical staining for FXYD-3 expression from the same patient. A: weak expression in distant normal mucosa. B: Strong expression in primary tumour, C: weak expression in lymph node metastases.

Table 1. Characteristics of patients and rectal cancers

Table 1. Characteristics of patients and rectal cancers				
Characteristics	Non-Radiotherapy		Radiotherapy	
	No.	%	No.	%
Gender				
Male	44	59	41	63
Female	31	41	24	37
Age (years)				
≤ 67	31	41	28	43
> 67	44	59	37	57
TNM stage				
I	20	27	21	32
II	19	25	20	31
III	54	41	17	26
IV	5	7	7	11
Differentiation				
Well	5	7	5	8
Moderately	53	71	40	62
Poorly	17	23	20	31
Numbers of tumors				
Single	63	84	48	74
Multiple*	10	13	16	25
Unknown	2	3	1	2
Surgical type				
Rectal amputation	37	49	25	38
Anterior resection	38	51	40	62
Resection margin				
Tumour free	73	97	60	92
Tumour involved margin	2	3	5	8
Distance to anal verge (cm)				
Mean	7.6		8.6	
* Other colorectal cancer or other type of tumour besides the present rectal cancer.				

Study II

Data on patients with rectal cancer from three healthcare regions during the time period 1985-2000 were extracted from a database. All patients had been operated for rectal cancer. The majority of patients receiving RT received 5x5 Gy followed by surgery within a week. Patients with locally advanced disease received 25x1.8 Gy and surgery after 6-8 weeks. 1180 patients were included and operated on with radical resection (R0) using the TME technique (67, 68).

Forty-eight patients were diagnosed with a local recurrence (LR) of the rectal cancer within the pelvis. Patients with liver or lung metastases were also included.

A nested case-control study was designed with two control patients for every LR case. Controls and cases were matched for gender and preoperative RT (Table 1). The median time for follow-up for the case group was 79 weeks and for the control group 316 weeks. We were able to retrieve formalin- fixed blocks from the primary tumour of 48 cases and 81 controls. FXYD-3 expression was analyzed using immunohistochemistry on tumor tissue from the index rectal cancer operation.

The intensity of staining was graded as negative, weak, moderate or strong based on the intensity of the staining. The cases with negative, weak and moderate intensity were grouped together as the weak FXYD-3 expression group and the cases with strong intensity formed the strong FXYD-3 expression group.

The staining and examination processes were performed at the Karolinska Institute, Solna by two histo-pathologists blinded for clinic-pathologic and biologic data.

Statistics.

The Chi-square method was used to test the significance of the differences in FXYD-3 expression between the strong FXYD-3 expression group and the weak FXYD-3 expression group. The figure depicting time to local recurrence was calculated using the Kaplan-Meier method. All tests were two-sided, and a value of $p < 0.05$ was considered statistically significant. Logistic regression and Cox regression were used to analyze the effect of differences between the case group and the control group.

Figure 1

Immunohistochemistry showing FXYD-3 expression in the primary tumour from different patients. A Weak FXYD-3 expression. B Strong FXYD-3 expression

Table 1

Patient characteristics at the index operation and FXYD-3 expression

Characteristics		Local recurrence Group	Control group value (Adjusted) *	P-value (Unadjusted)	P-
Patients n, (%)		48	81		
Time to recurrence (mo)	median	18	-	-	-
	Range	3-60			
Age (y)	Median	71	72	0.36	-
	Range	34-85	34-95	-	-
Gender (n)	Male	24 (50)	45 (56)	0.54	0.96
	Female	24 (50)	36 (44)	-	-
Type of surgery (n)	Anterior resection	32 (67)	65 (80)	0.08	0.88
	Abdominoperineal resection	16 (33)	16 (20)	-	-
Distance of primary tumor from anal verge (n)	11-15 cm	15 (31)	28 (35)	0.71	0.48
	6-10 cm	16 (33)	31 (38)	-	-
	0-5 cm	17 (35)	22 (27)	-	-
Radiotherapy (n)	Yes	18 (38)	25 (31)	0.44	0.97
	No	30 (62)	56 (69)	-	-
TNM stage (n)	I	4 (8)	26 (32)	0.01	0.02
	II	19 (40)	36 (44)	-	-
	III	25 (52)	19 (23)	-	-
Tumour differentiation	High	3 (6)	6 (8)	-	0.03
	Moderate	30 (64)	61 (81)	-	-
	Poor	14 (30)	8 (11)	-	-
FXYD-3 Expression	Weak (Grade 0+1+2)	39 (81%)	67 (83%)		
	Strong (Grade 3)	9 (19%)	14 (17%)	p>0.05	

*Adjusted for all other variables in the table

Study III.

Study III is a register-based study on the incidence and outcome of complete pathological response of rectal cancer to neoadjuvant treatment in Sweden between 2007 and 2012. The Swedish Colo-Rectal Cancer Register (SCRCR) is a prospective population-based register collecting data from all colorectal cancer cases in Sweden (69). This is a validated register with 99.5% immediate coverage and five-year follow-up data from 98% of patients. The study cohort consisted of 11226 patients with a median age of 71 years 59% were males. An abdominal procedure was performed on 7885 of the patients. Twenty-six per cent (2063 patients) were classified as having potential for pCR after receiving long-term radiotherapy treatment (28x 1.8 Gy, LRT) or short-term radiotherapy treatment (5x5 Gy, SRT) with a delay of more than four weeks, with or without chemotherapy before abdominal surgery. This group was labeled “potential complete responders” and formed the basis of this study (Table 1). A majority of the 2063 potential responders (84%) were cT3 or cT4 cancers, and 56% had received neoadjuvant chemotherapy. Patients with ypT0 were identified from that group. The group of 161 patients (8%) with complete response was further analyzed for lymph node status and during that process errors and misclassifications in the register were discovered and corrected.

Statistics. Differences between the proportions of groups were tested with a two-sided Z-test of proportion. The non-parametric Mann-Whitney u-test was applied for differences between median times. Overall survival was presented as a Kaplan-Meier curve, and differences in survival were tested with Gehan’s generalized Wilcoxon test. Five-year survival rates were calculated using life tables. Relative risks for mortality were compared using hazard ratios calculated with COX regression. Binomial logistic regression was implemented to get odds ratios.

Table 1. Patient characteristics

			Female	M1*
Rectal cancer (2007-2012)	<i>n - %</i>	11226	41%	20%
	<i>age</i>	71	71	
	<i>(median)</i>			
Abdominally operated **	<i>n - %</i>	7885	40%	8%
	<i>age</i>	69	69	
	<i>(median)</i>			
Anterior resection	<i>n - %</i>	3940	42%	12%
	<i>age</i>	67	66	
	<i>(median)</i>			
Abdominoperineal resection	<i>n - %</i>	2837	38%	11%
	<i>age</i>	70	70	
	<i>(median)</i>			
Hartmann’s procedure	<i>n - %</i>	1108	42%	8%
	<i>age</i>	78	77	
	<i>(median)</i>			
Potential complete responders	<i>n - %</i>	2063	40%	13%
(Long-term RT or short-term RT with delay, +/- chemotherapy.)	<i>age</i>	66	67	
	<i>(median)</i>			
Long-term radiation.	<i>n - %</i>	1179	41%	10%
	<i>age</i>	64	64	
	<i>(median)</i>			
Short-term radiation with delay.	<i>n - %</i>	884	38%	16%
	<i>age</i>	71	73	
	<i>(median)</i>			

* 10 of the ypT0 patients had distant metastases (M1). * 368 patients with rectal cancer had a local excision of the tumour and were not included in this cohort

Study IV.

Study IV was a follow-up of the findings of study III on remaining loco-regional lymph node metastases in luminal complete response and the following reduced survival. The SCRCR was scrutinized and from 11226 patients with rectal cancer diagnosed between 2007 and 2012 a group of 161 patients with luminal complete response (ypT0Nall) were identified. From that group 26 patients with ypT0N+ were selected in order to study the accuracy of MRI in detecting remaining tumour positive lymph nodes. We were able to retrieve medical records on oncological treatment and complete MRI imaging from the staging and restaging MRI investigations from 19 patients. Another 19 patients with ypT0N0 were matched for treating hospital and comprised the control group (Table1). All images were assessed by two radiologists blinded to surgical and pathology findings first independently and then in consensus. Images before and after neoadjuvant treatment were assessed together.

Extramural depth of tumour invasion and involvement of the mesorectal fascia were noted according to previously established criteria(60). Nodal number, size and position were identified in each patient for both benign and tumour positive lymph nodes. The lymph nodes identified on MRI were judged to be malignant or benign using morphologic criteria (62, 70).

On the pre-treatment MRI a lymph node was considered to be malignant if it showed an irregular outer border or internal signal heterogeneity. On the post CRT MRI, size was used as the only criterion, where lymph nodes exceeding 5 mm were regarded as malignant.

Statistics

- **Sensitivity** measures the proportion of positives that are correctly identified as such (e.g. the percentage of sick people who are correctly identified as having the condition).
- **Specificity** measures the proportion of negatives that are correctly identified as such (e.g., the percentage of healthy people who are correctly identified as not having the condition).
- **Positive predictive value** = probability of disease among patients with a positive test
- **Negative predictive value** = probability of no disease among patients with a negative test

The Student's T-test was used to test the significance of the differences between the cases and the control group.

All tests were two-sided, and a value of $p < 0.05$ was considered statistically significant. Logistic regression was used to analyze the effect of differences between the case group and the control group.

Table 1.
Description of the study population.

Gender	All	N+	N0	p
Female	17	11	6	0.10
Male	21	8	13	
Average age at surgery (range). years	57(37-74)	54 (37-69)	61(37-74)	0.05
T-stage				0.18
T1-T2	9	5	4	
T3	14	8	6	
T4	14	5	9	
Tx	1	1		
Radiation				0.73
28x2 Gy	27	13	14	
5x5 Gy with delay	11	6	5	
Chemotherapy				0.18
None	7	3	4	
5-FU/ Capecitabine	18	7	11	
5-FU/ Capecitabine + Oxaliplatiine	13	9	4	
Sum	38	19	19	

Results and discussion

Study I

The expression of FXYD-3 was significantly increased in the primary tumour compared to normal mucosa ($p=0.008$) (Fig 1). There was no difference in expression between the primary tumour and tumour positive lymph nodes. There was a higher FXYD-3 expression in men than in women ($p=0.04$) and in tumours with infiltrative growth compared to tumours with expansive growth ($p=0.02$).

FXYD-3 expression was positively related to both phosphatase of regenerating liver (PRL) ($p=0.001$) and p73 ($p=0.03$).

In patients receiving RT, FXYD-3 expression was stronger in tumours with less necrosis than in tumours with extensive necrosis ($p=0.02$). In both uni- and multivariate analysis patients with strong FXYD-3 expression alone ($P=0.02$) or in combination with PRL ($p=0.02$) had reduced survival in the RT group, regardless of tumour stage or differentiation (Fig 2).

Fig. 1. Strong FXYD-3 expression in the radiotherapy (RT) group and non-RT group in distant normal mucosa, adjacent normal mucosa, primary tumour and tumour positive lymph nodes.

Figure 2. Univariate analysis of survival vs. FXYD-3 and PRL expression in the RT group

FXYD-3 is a trans-membrane protein normally present in body tissues but overexpressed in several cancers. Increased expression in early tumour differentiation and tumour proliferation has indicated that it could possibly be used as a tumour marker(19, 71-75). There are also indications that FXYD-3 may be involved in tumour resistance to 5-FU chemotherapy (76). At the time of the study no previous research had analyzed the clinical significance of FXYD-3 in colorectal cancer. Our aim was to determine whether there was an increased expression of FXYD-3 in rectal cancer compared to normal mucosa, and to identify differences in outcome between patients with or without RT and relate these to FXYD-3 expression. Our results showed that there was increased FXYD-3 expression in the primary tumour compared to normal mucosa, and in tumours with infiltrative growth. The association of FXYD-3 expression with p73 and PRL is of interest as in previous studies both PRL and p73 have been shown to be related to radio-sensitivity. Furthermore, overexpression of p73 is also related to reduced survival in colorectal cancer (6, 77-79). Less tumour necrosis and poorer survival were seen in patients with increased FXYD-3 expression in the RT group but not in the group without RT.

A lower degree of tumour necrosis can be seen as an indicator of reduced radio-sensitivity. There was also a non-significant increase in distant metastases ($p=0.08$) in this group. These results taken together suggested to us that expression of FXYD-3 could be a prognostic factor and an indicator of reduced radio sensitivity. Since this was the first study on FXYD-3 and colorectal outcome, our findings required confirmation. A study on reduced radio-sensitivity using local recurrence as a proxy was considered to be more accurate since few studies have been able to show a correlation between RT and survival (1). We therefore went on to attempt to confirm the significance of FXYD-3 in rectal cancer by studying the frequency of local recurrence after RT (study II).

Study II

There was no difference in FXYD-3 expression in relation to RT neither in the control group nor in the local recurrence (LR) group ($p>0.05$) (Table 2). There was no significant difference in time to diagnosis of a local recurrence, between tumours with strong FXYD-3 expression and those with weak FXYD-3 expression (Figure 1). When all patients were analyzed together, i.e. both those with and without LR, there was no difference in survival in the radiated group between patients with strong or weak FXYD-3 expression ($p=0.43$). There was no difference in survival in the non-radiated group between the patients with strong or weak FXYD-3 expression ($p=0.30$). There was a tendency towards a lower incidence of strong FXYD-3 expression in patients with LR who had been treated with preoperative RT (Table 3).

Figure 1. Time to local recurrence in all 129 patients.

Table 1.
FXYD-3 Expression in the control group in relation to preoperative radiotherapy (RT) (n= 81)

FXYD-3 expression	No RT	RT
Weak (0,1,2)	46 (84%)	21 (81%)
Strong (3)	9 (16%)	5 (19%)

$p>0.05$

Table 2.
FXYD-3 expression in the local recurrence group in relation to preoperative radiotherapy (RT) (n=48)

FXYD-3 expression	No RT	RT
Weak (0,1,2)	23 (77%)	16 (89%)
Strong (3)	7 (23%)	2 (11%)

$p>0.05$

The results from study I indicated that FXYD-3 could possibly serve as a prognostic indicator for survival and also an indicator of reduced radio-sensitivity. In many large studies radiotherapy has reduced the incidence of LR but its effect on survival is not so clear (1, 80-83). It was thus our ambition to analyse FXYD-3 expression in tumours from rectal cancer patients that developed a local recurrence and to see if these tumours have a strong FXYD-3 expression, or if strong FXYD-3 expression is related to time to LR. Researchers at the Karolinska Institute in Stockholm had previously performed a study on a cohort of patients regarding the relationship of LR to RT. In cooperation with the Karolinska Institute we designed this study using their former study cohort, which also made it possible to confirm our results from study I at another laboratory.

We were not able to confirm our findings of FXYD-3 as a prognostic factor for reduced survival or an indicator of radio-resistance in this study. Several factors could have contributed to this;

Firstly there were few of the patients in both the LR group (36%) and the control group (31%) that received RT (Figure 1). This in combination with the fact that only 19% in the LR group and 17% in the control group had strong FXYD-3 expressions made it hard to reach statistically significant results. Furthermore, patients and controls were only matched for gender and RT while both tumour stage and differentiation differed between the groups. Immunohistochemistry is a semi-quantitative method and analysis of old formalin fixed specimen could well be an issue too. Blinded examinations of immunohistochemistry slides were performed at both hospitals to ensure objective grading.

Even though several findings, together and independently from the study I indicated that FXYD-3 could be a prognostic factor and an indicator of reduced radio-sensitivity, the results of study III could not support this. The collection of a larger cohort of patients with local recurrence in order to gain greater power would be a large long-term project. Still, a large prospective cohort with samples from preoperative biopsies analysed with RNA techniques would be of interest in the search for predictive markers of reduced response to neoadjuvant treatment.

Study III.

Complete eradication of the luminal tumour, ypT0 was found in 161 patients (8%). The ypT0 rate increased with decreasing cT-stage, the highest rate being in cT1-2 cancers (Tables 2-3). The addition of chemotherapy resulted in 10% ypT0 compared to 5.1% in the group without chemotherapy ($p < 0.00004$)

The length of delay between CRT and surgery did not affect the ypT0 rate regardless of whether LRT or SRT with delay was used.

Of the ypT0 patients 83 % had tumour-negative regional lymph nodes (ypT0N0), 12% had 1-3 tumour positive lymph nodes (ypT0N1) and 4% had more than three tumour positive lymph nodes (ypT0N2). The distribution of pN status depended significantly on the cN-status ($p = 0.000$).

There was a significantly reduced risk for tumour-positive lymph nodes in younger patients and in cT1-2 tumours compared to cT3-4 ($p = 0.01$).

There was significantly greater survival in ypT0 compared to ypT+ patients (hazard ratio 0.38 (C.I 0.25-0.58)) and survival was significantly greater in patients with ypT0N0 compared to ypT0N1-2 (hazard ratio 0.36 (C.I 0.15-0. 86)). (Figure 1)

Table 1.

Odds ratios of complete response (ypT0) in clinical tumour stages

cT-stage	Complete response (ypT0) (n, %)		Odds ratio (CI) of having CR*
	161	8%	
cT1-2	21	14%	1
cT3	77	9%	0.401 (0.230-0.698)
cT4	58	6%	0.227 (0.125-0.413)
cTX	5	2%	0.179 (0.058-0.555)

*Adjusted for: age (under/over 66 years $p = 0.019$), gender (0.081), tumour level (under/over 8 cm $p = 0.020$), preoperative chemotherapy (yes/no $p = 0.001$) and preoperative radiotherapy (Long-term radiotherapy vs. short-term radiotherapy with delay ($p = 0.666$))

Table 2

Luminal complete response (ypT0) in relation to different neoadjuvant treatments.

Luminal complete response (ypT0) in relation to different neoadjuvant treatments.		Preoperative treatment									
		Long radiotherapy (25x1.8/2Gy) and preop. chemotherapy		Long radiotherapy (25x1.8/2Gy)		Short radiotherapy (5x5Gy) and wait >4 weeks and preop. chemotherapy		Short radiotherapy (5x5Gy) and delay >4 weeks		Total	
		Luminal complete response		Luminal complete response		Luminal complete response		Luminal complete response		Luminal complete response	
		No	Yes	No	Yes	No	Yes	No	Yes	No	Yes
Clinical T-stages.	cT1-	14	6	8	0	3	3	108	12	133	21
		70 %	30 %	100 %	0 %	50 %	50 %	90 %	10 %	86 %	14 %
	cT3	304	45	79	6	70	8	368	18	821	77
		87 %	13 %	93 %	7 %	90 %	10 %	95 %	5 %	91 %	9 %
	cT4	533	43	74	4	69	7	174	4	850	58
		92 %	8 %	95 %	5 %	91 %	9 %	98 %	2 %	94 %	6 %
	cTX	41	2	14	1	2	0	32	1	89	4
		95 %	5 %	93 %	7 %	100 %	0 %	97 %	3 %	96 %	4 %
	Total	892	96	175	11	144	18	682	35	1893	160*
		90 %	10 %	94 %	6 %	89 %	11 %	95 %	5 %	92 %	8 %

- Information on clinical T-stages is missing for nine patients; one of them had luminal complete response.

Figure 1 cumulative survival in relation to luminal complete response and nodal pathology status

Adjusted for adjuvant chemotherapy p=0000.
 HR (No adjuvant chemotherapy given): 1.48 (1.24-1.77)

In this register based study we found a low ypT0 rate, (8%) compared to other studies that have shown rates of 13-45% (84-86). Our results may reflect the broad inclusion of rectal cancer patients in Swedish normal clinical practice with a high proportion of T4 tumours and the inclusion of patients with LRT, and SRT with delay but without chemotherapy.

As both local and systemic recurrences are reduced after pCR (86-89) the significance of tumour positive nodes in the resected specimen is not clear, but the results of this study indicate a reduction in survival in patients with ypT0N1-2 compared to ypT0N0. Our results show that the survival benefit from complete response is dependent upon nodal involvement, with a survival curve for ypT0N+ similar to the curve for patients with ypT+.

We could not confirm previous findings of improved ypT0 rates with increased delay between RT and surgery(15). There was, however a narrow time span between LRT and surgery for the group as a whole in this study due to adherence to Swedish guidelines. The rationale behind non-surgical management is based on the clinical ability to rule out remaining tumour in the pelvis, including the regional lymph nodes. A prerequisite is that eventual regrowth can be detected and treated with results that are not inferior to

surgery. For advanced tumours the risk for lymph node metastases despite luminal complete response is high and this study indicates a less favorable prognosis even with radical surgery. The prognosis for patients with pT0N+ is significantly worse than for pT0N0 even with radical surgery. We also found that chemotherapy seems to have little effect on lymph node metastases. When considering a “ watchful waiting” policy these findings should be taken into account.

Study IV.

Thirty-eight patients were evaluated, 19 with ypT0N0 and 19 with ypT0N+. The mean time between post-CRT MRI staging and surgery was 21 days (6-68) with no statistical difference between the N0 and N+ group (21 and 25 days $p=0.27$).

At the pre-CRT MRI staging a total number of 314 lymph nodes were detected, and 122 (39%) of these were suspected to be malignant. The average number of lymph nodes in the ypN0 group was 9 and in the ypN+ group 7 ($p=0.5$). (Figure 1). The average size of the lymph nodes in the ypN0 group was 4.0 mm and in the ypN+ group it was 3.8 mm ($p=0.65$). The total sum of sizes was 645 and 612 mm respectively ($p=0.81$).

At post-CRT MRI staging, 107 lymph nodes were detected and 20 (19%) of these were suspected to be malignant. Twenty-nine out of 38 patients had no suspected malignant node. On average there were 2.7 nodes detected in the ypT0 group compared to 2.9 nodes in the ypT+ group ($p=0.75$) (Figure 1). The average lymph node size in the ypT0N0 group was 1.9 mm and 3.0 mm in the ypT0N+ group ($p=0.12$). The total sum of size was 144 and 225 mm respectively ($p=0.21$).

When comparing the average size of the largest node for all patients there was a significant difference between the ypN0 and ypN+ groups (2.6 mm vs. 4.5mm) ($p=0.04$) (Figure 2). Nodes larger than 8 mm were only found in N+ patients ($n=3$). Two patients with no lymph node detected on MRI staging were staged ypN+ at the pathology report. Of the 19 patients who were ypN+ 12 were MRI staged as N0, and 3 of the 19 ypN0 were staged as N+. Sensitivity, specificity, PPV and NPV for the expert radiologists to correctly detect lymph node metastases in ypT0 patients were 37%, 84%, 70% and 55% respectively.

Size and morphology together are the two most promising lymph node features used to discriminate between malignant and benign nodes. We still need to find a threshold value that can rule out malignant lymph nodes by size alone. The cut-off size is probably related to population factors such as initial tumour stage, CRT regimen and the time between CRT treatment and reevaluation MRI.

The results of this study on ypT0 and lymph node staging shows that CRT has a substantial effect on the size and number of lymph nodes detected. The small numbers of patients, causing a Type 2 error made it hard to find significant changes between the two groups. With regard to suspected malignant lymph nodes, we could see a trend towards there being more suspect lymph nodes both before and after CRT in the ypT0N+ group (Fig 1). Furthermore there was no significant difference in size of nodes between the two groups and which is in contrast to other studies (61, 90). We excluded all but complete responders in our study population in contrast to other studies, which comprised a greater variety in post CRT T-stage. When we analyzed the average size of the largest lymph node we found that the ypT0N+ group had a significantly larger paramount gland than the ypT0N0 group. This may indicate N+stage in LCR. This is one of the first studies on luminal complete response and lymph node restaging by MRI. We showed a large reduction in both suspected malignant and benign-looking lymph nodes after CRT. The size of the largest lymph node seemed to predict node malignancy. The results of this study indicate the need for a better method to adequately predict lymph node status in patients with luminal complete response after CRT in rectal cancer.

Figure 1. Detected lymph nodes in relation to N-stage

Figure 2. Average size of the largest lymph node in relation to N-stage.

General discussion and future perspectives

After Claude Dixon's development of the anterior resection in 1948 (52) little happened in rectal cancer treatment for almost forty years until the mid 1980's with both the development of TME surgery and perioperative radiotherapy(53, 54). During the years following there was a rapid evolution in rectal cancer treatment with neoadjuvant and adjuvant chemotherapy, laparoscopy, enhanced recovery programmes, multidisciplinary team conferences, non-operative management and robotic surgery(43, 45, 57). All these measures aimed at improving patient outcome and minimizing morbidity. In our efforts to maximize the effects of anti tumour treatments there is always the risk that those patients not responding, gain nothing and end up paying the price of side-effects and a longer time waiting for curative treatment. It is therefore important to find indicators that predict or indicate response to CRT treatment in perioperative oncological management.

In study I we investigated the protein FXYD-3 that theoretically could serve as an indicator of malignant progression. Our results showed that tumours with increased FXYD-3 expression showed less necrosis after RT and more infiltrative growth coupled with reduced survival. This suggested that FXYD-3 could be a predictor of reduced survival as a result of lower radio-sensitivity.

To confirm these findings we designed study II on FXYD-3 expression related to local recurrence where we could directly pinpoint the actual issue on radiosensitivity. We also attempted to confirm our findings by comparison of immunohistochemistry results, a semi-quantitative method at another renowned center, (Karolinska Institutue). In study II none of the findings from study I on the specific issues of radio-sensitivity and local recurrence due to over-expression of FXYD-3 could be confirmed. This may be due to the fact that there is no relationship between the expression of FXYD-3 and local recurrence, it could also be that we did not have the statistical power to prove it. In conclusion the significance of FXYD-3 expression in rectal cancer is still not clear.

The paradigm of non-operative management of rectal cancer was pioneered in Brazil(91). Cultural differences may trigger and propel surgical development in one part of the world that would probably not have evolved in other places. One example is the general view regarding stomas, where both surgeons and patients in northern Europe seem to have a more permissive view than those from southern Europe or South America. This is probably reflected in the rate of coloanal anastomoses and inversely in the rate of permanent stomas(92).

Cultural differences like this could have been a contributing factor why the concept of non-operative management was first explored in Brazil(12, 13, 91). In the case of complete response with absence of cancer cells in the rectum, it is even harder for a patient to accept a permanent stoma. When new treatment options and therapies develop in centers outside the national welfare system, conclusions regarding the effects of a new paradigm at the national level are difficult to draw. Large national registers can be used to validate data from small centers by identifying low frequency side-effects, as well as cultural differences in outcome and indications(69).

In study III we aimed to investigate the rate of complete pathological response using data from a national register. We found a low rate of complete response compared to other single-center studies(11, 13, 91), but our results reflect an older population with

more advanced tumour stages and treatment with no intent to achieve complete response per se.

The findings of a relatively high rate of remaining tumour positive lymph nodes in the surgical specimen reflected by a lower survival rate is probably a consequence of the high proportion of advanced tumours in our study. This stresses the importance of careful restaging and patient selection when considering a non-operative management. In order to make well-founded decisions on non-operative management there is a need to validate methods used for restaging after neoadjuvant treatment.

In study IV we came to the conclusion that despite assessment by expert radiologists, present MRI technique seem not to be adequate enough to discriminate between benign and malignant lymph nodes. This is a finding that is in line with previous research(93). Unfortunately MRI is the best we have in clinical practice at the moment.

The search for new predictors of response to neoadjuvant or adjuvant treatment will go on. So far there has been much research but with dismal results, which leads us to believe that it will take time before a predictor of response to neoadjuvant treatment comes into clinical use (9).

Non-operative management of rectal cancer will probably have a major impact on future management. The concept of neoadjuvant treatment using only chemotherapy will probably continue to develop. With early full dose chemotherapy we can be able to treat micro-metastatic disease and at the same time omitting radiotherapy with its long-term side effects. Although data from large prospective studies is still lacking.

As the rate of both local and distant recurrence is reduced with complete response to CRT, outcome will be improved(11, 13, 87). If that holds true in non-operative management, rectal cancer treatment will undergo major changes in the near future.

Furthermore long-term follow-up and its impact on the health care system and its resources, and patient concern about having to live with a life-long diagnosis of cancer are issues that must be discussed.

With our present goal of developing diagnostic tools that are not dependent on ionizing ray techniques, we will certainly see rapid improvements in MRI technology in the near future.

I am interested to continue the research on complete response in rectal cancer in the future. I believe it would be safer to select patients for a non-operative management if we could better identify patients at risk of having a regrowth of the luminal tumour or developing metastatic disease. Presence of tumour positive lymph nodes after CRT seems to be a risk factor for reduced survival. It is unfortunately difficult to identify these tumour positive lymph nodes after CRT by using MRI only. I would like to further analyze the findings from study IV where the size of the largest lymph node (4,5mm) seemed to indicate N+ stage. Of interest is that the size of 4.5 mm also has been suggested as cut-off size for identifying malignant nodes after CRT in another study(61). We have initiated a plan for a study where we would identify and follow-up patients who developed distant metastases after CRT in operated rectal cancer. Initially we plan to retrospectively analyze the restaging MRI images and measure the size of the largest lymph node. We would then analyze if there is a correlation between the size of the largest lymph node and the development of distant metastases. A control group with the same treatment but no metastases would be used for comparison. If we could confirm that the size of the largest lymph node correlates to reduced survival it could indicate a sentinel node function in rectal cancer. Identification of a single large index lymph node

as an indicator for N+ stage would be more reproducible than morphologic criteria of individual lymph nodes.

We could use this pilot-study for a future larger prospective study. In that study we could include other factors such as extra-mural vascular invasion (EMVI) and tumour regression. These factors could then be analyzed and compared to outcome in order to safer select patients for a non-operative management of rectal cancer.

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References

1. Glimelius B, Gronberg H, Jarhult J, Wallgren A, Cavallin-Stahl E. A systematic overview of radiation therapy effects in rectal cancer. *Acta Oncol.* 2003;42(5-6):476-92.
2. Swedish Rectal Cancer Registry. Årsrapport 2016 [Internet]. 2016.
3. Bosset JF, Collette L, Calais G, Mineur L, Maingon P, Radosevic-Jelic L, et al. Chemotherapy with preoperative radiotherapy in rectal cancer. *N Engl J Med.* 2006;355(11):1114-23.
4. Ceelen WP, Van Nieuwenhove Y, Fierens K. Preoperative chemoradiation versus radiation alone for stage II and III resectable rectal cancer. The Cochrane database of systematic reviews. 2009(1):CD006041.
5. Birgisson H, Pahlman L, Gunnarsson U, Glimelius B. Late adverse effects of radiation therapy for rectal cancer - a systematic overview. *Acta Oncol.* 2007;46(4):504-16.
6. Pfeifer D, Gao J, Adell G, Sun X. Expression of the p73 protein in rectal cancers with or without preoperative radiotherapy. *International Journal of Radiation OncologyBiologyPhysics.* 2006;65(4):1143-8.
7. Pfeifer D, Wallin Å, Holmlund B, Sun X-F. Protein expression following γ -irradiation relevant to growth arrest and apoptosis in colon cancer cells. *Journal of Cancer Research and Clinical Oncology.* 2009;135(11):1583-92.
8. Molinari C, Matteucci F, Caroli P, Passardi A. Biomarkers and Molecular Imaging as Predictors of Response to Neoadjuvant Chemoradiotherapy in Patients With Locally Advanced Rectal Cancer. *Clin Colorectal Cancer.* 2015;14(4):227-38.
9. Kuremsky JG, Tepper JE, McLeod HL. Biomarkers for Response to Neoadjuvant Chemoradiation for Rectal Cancer. *International Journal of Radiation OncologyBiologyPhysics.* 2009;74(3):673-88.
10. Agostini M, Crotti S, Bedin C, Cecchin E, Maretto I, D'Angelo E, et al. Predictive response biomarkers in rectal cancer neoadjuvant treatment. *Front Biosci (Schol Ed).* 2014;6:110-9.
11. Glynne-Jones R, Hughes R. Critical appraisal of the 'wait and see' approach in rectal cancer for clinical complete responders after chemoradiation. *Br J Surg.* 2012;99(7):897-909.
12. Habr-Gama A, Perez RO. Non-operative management of rectal cancer after neoadjuvant chemoradiation. *Br J Surg.* 2009;96(2):125-7.
13. Habr-Gama A, Perez RO, São Julião GP, Proscurshim I, Gama-Rodrigues J. Nonoperative Approaches to Rectal Cancer: A Critical Evaluation. *Seminars in Radiation Oncology.* 2011;21(3):234-9.
14. Maas M, Beets-Tan RG, Lambregts DM, Lammering G, Nelemans PJ, Engelen SM, et al. Wait-and-see policy for clinical complete responders after chemoradiation for rectal cancer. *J Clin Oncol.* 2011;29(35):4633-40.
15. Sloothaak DA, Geijsen DE, van Leersum NJ, Punt CJ, Buskens CJ, Bemelman WA, et al. Optimal time interval between neoadjuvant chemoradiotherapy and surgery for rectal cancer. *Br J Surg.* 2013;100(7):933-9.
16. Pucciarelli S, Bacigalupo L, Maretto I. Predicting response to neoadjuvant therapy for rectal cancer. *Techniques in coloproctology.* 2014;18(8):683-4.
17. Dedemadi G, Wexner SD. Complete response after neoadjuvant therapy in rectal cancer: to operate or not to operate? *Dig Dis.* 2012;30 Suppl 2:109-17.

18. Lambregts DM, Maas M, Bakers FC, Cappendijk VC, Lammering G, Beets GL, et al. Long-term follow-up features on rectal MRI during a wait-and-see approach after a clinical complete response in patients with rectal cancer treated with chemoradiotherapy. *Dis Colon Rectum*. 2011;54(12):1521-8.
19. Rajagopalan H, Nowak MA, Vogelstein B, Lengauer C. The significance of unstable chromosomes in colorectal cancer. *Nat Rev Cancer*. 2003;3(9):695-701.
20. Fearon ER, Vogelstein B. A genetic model for colorectal tumorigenesis. *Cell*. 1990;61(5):759-67.
21. Arends JW. Molecular interactions in the Vogelstein model of colorectal carcinoma. *J Pathol*. 2000;190(4):412-6.
22. Parris GE. Historical perspective of cell-cell fusion in cancer initiation and progression. *Crit Rev Oncog*. 2013;18(1-2):1-18.
23. Prescott SM, White RL. Self-promotion? Intimate connections between APC and prostaglandin H synthase-2. *Cell*. 1996;87(5):783-6.
24. Giardiello FM, Hamilton SR, Krush AJ, Piantadosi S, Hyland LM, Celano P, et al. Treatment of colonic and rectal adenomas with sulindac in familial adenomatous polyposis. *N Engl J Med*. 1993;328(18):1313-6.
25. Ando M, Maruyama M, Oto M, Takemura K, Endo M, Yuasa Y. Higher frequency of point mutations in the c-K-ras 2 gene in human colorectal adenomas with severe atypia than in carcinomas. *Japanese journal of cancer research : Gann*. 1991;82(3):245-9.
26. Tannapfel A, Nusslein S, Fietkau R, Katalinic A, Kockerling F, Wittekind C. Apoptosis, proliferation, bax, bcl-2 and p53 status prior to and after preoperative radiochemotherapy for locally advanced rectal cancer. *Int J Radiat Oncol Biol Phys*. 1998;41(3):585-91.
27. Reed JC. Bcl-2 and the regulation of programmed cell death. *J Cell Biol*. 1994;124(1-2):1-6.
28. Baker SJ, Preisinger AC, Jessup JM, Paraskeva C, Markowitz S, Willson JK, et al. p53 gene mutations occur in combination with 17p allelic deletions as late events in colorectal tumorigenesis. *Cancer research*. 1990;50(23):7717-22.
29. Levine AJ. p53, the cellular gatekeeper for growth and division. *Cell*. 1997;88(3):323-31.
30. Thomas G. Advances in the genetics and molecular biology of colorectal tumors. *Current opinion in oncology*. 1994;6(4):406-12.
31. Ilyas M, Tomlinson IP. The interactions of APC, E-cadherin and beta-catenin in tumour development and progression. *J Pathol*. 1997;182(2):128-37.
32. Boland CR. The biology of colorectal cancer. Implications for pretreatment and follow-up management. *Cancer*. 1993;71(12 Suppl):4180-6.
33. Jessup JM, Giavazzi R, Campbell D, Cleary K, Morikawa K, Fidler IJ. Growth potential of human colorectal carcinomas in nude mice: association with the preoperative serum concentration of carcinoembryonic antigen in patients. *Cancer research*. 1988;48(6):1689-92.
34. Folkman J. Tumor angiogenesis and tissue factor. *Nature medicine*. 1996;2(2):167-8.
35. Joiner M. Basic clinical radiobiology: CRC Press; 2009.
36. Birgisson H, Pahlman L, Gunnarsson U, Glimelius B. Adverse effects of preoperative radiation therapy for rectal cancer: long-term follow-up of the Swedish Rectal Cancer Trial. *J Clin Oncol*. 2005;23(34):8697-705.

37. Birgisson H, Pahlman L, Gunnarsson U, Glimelius B. Occurrence of second cancers in patients treated with radiotherapy for rectal cancer. *J Clin Oncol.* 2005;23(25):6126-31.
38. Pettersson D, Cedermark B, Holm T, Radu C, Pålman L, Glimelius B, et al. Interim analysis of the Stockholm III trial of preoperative radiotherapy regimens for rectal cancer. *British Journal of Surgery.* 2010;97(4):580-7.
39. Pettersson D, Holm T, Iversen H, Blomqvist L, Glimelius B, Martling A. Preoperative short-course radiotherapy with delayed surgery in primary rectal cancer. *Br J Surg.* 2012;99(4):577-83.
40. Loftas P, Arbman G, Fomichov V, Hallbook O. Nodal involvement in luminal complete response after neoadjuvant treatment for rectal cancer. *European journal of surgical oncology : the journal of the European Society of Surgical Oncology and the British Association of Surgical Oncology.* 2016;42(6):801-7.
41. Appelt AL, Ploen J, Vogelius IR, Bentzen SM, Jakobsen A. Radiation dose-response model for locally advanced rectal cancer after preoperative chemoradiation therapy. *Int J Radiat Oncol Biol Phys.* 2013;85(1):74-80.
42. Lawrence TS1 BA, McGinn C. The mechanism of action of radiosensitization of conventional chemotherapeutic agents. *Semin Radiat Oncol.* Jan;13(1):13-21.
43. Boland PM, Fakih M. The emerging role of neoadjuvant chemotherapy for rectal cancer. *J Gastrointest Oncol.* 2014;5(5):362-73.
44. Petersen SH, Harling H, Kirkeby LT, Wille-Jørgensen P, Mocellin S. Postoperative adjuvant chemotherapy in rectal cancer operated for cure. *The Cochrane database of systematic reviews.* 2012(3):CD004078.
45. Aschele C, Cionini L, Lonardi S, Pinto C, Cordio S, Rosati G, et al. Primary tumor response to preoperative chemoradiation with or without oxaliplatin in locally advanced rectal cancer: pathologic results of the STAR-01 randomized phase III trial. *J Clin Oncol.* 2011;29(20):2773-80.
46. AlGizawy SM, Essa HH, Ahmed BM. Chemotherapy Alone for Patients With Stage II/III Rectal Cancer Undergoing Radical Surgery. *The oncologist.* 2015;20(7):752-7.
47. Jalil O, Claydon L, Arulampalam T. Review of Neoadjuvant Chemotherapy Alone in Locally Advanced Rectal Cancer. *Journal of gastrointestinal cancer.* 2015;46(3):219-36.
48. Lange MM, Rutten HJ, van de Velde CJ. One hundred years of curative surgery for rectal cancer: 1908-2008. *European journal of surgical oncology : the journal of the European Society of Surgical Oncology and the British Association of Surgical Oncology.* 2009;35(5):456-63.
49. Miles W. A method of performing abdomino-perineal excision for carcinoma of the rectum and of the terminal portion of the pelvic colon (1908). *The Lancet.* 1908(172(4451)):1812-3.
50. Nestorovic M. ONE HUNDRED YEARS OF MILES' OPERATION- WHAT HAS CHANGED? *Acta Medica Medianae.* 2008;47((4)):43-6.
51. Cirocchi R, Farinella E, Trastulli S, Desiderio J, Di Rocco G, Covarelli P, et al. High tie versus low tie of the inferior mesenteric artery: a protocol for a systematic review. *World J Surg Oncol.* 2011;9:147.
52. Dixon CF. Anterior Resection for Malignant Lesions of the Upper Part of the Rectum and Lower Part of the Sigmoid. *Annals of surgery.* 1948;128(3):425-42.
53. Heald RJ, Husband EM, Ryall RD. The mesorectum in rectal cancer surgery--the clue to pelvic recurrence? *Br J Surg.* 1982;69(10):613-6.

54. Heald RJ, Ryall RD. Recurrence and survival after total mesorectal excision for rectal cancer. *Lancet*. 1986;1(8496):1479-82.
55. Emmertsen KJ, Laurberg S. Low anterior resection syndrome score: development and validation of a symptom-based scoring system for bowel dysfunction after low anterior resection for rectal cancer. *Annals of surgery*. 2012;255(5):922-8.
56. Moghadamyeghaneh Z, Phelan M, Smith BR, Stamos MJ. Outcomes of Open, Laparoscopic, and Robotic Abdominoperineal Resections in Patients With Rectal Cancer. *Dis Colon Rectum*. 2015;58(12):1123-9.
57. Biffi R, Luca F, Bianchi PP, Cenciarelli S, Petz W, Monsellato I, et al. Dealing with robot-assisted surgery for rectal cancer: Current status and perspectives. *World journal of gastroenterology*. 2016;22(2):546-56.
58. Koh DM, Brown G, Husband JE. Nodal staging in rectal cancer. *Abdominal imaging*. 2006;31(6):652-9.
59. Koh DM, Brown G, Temple L, Blake H, Raja A, Toomey P, et al. Distribution of mesorectal lymph nodes in rectal cancer: in vivo MR imaging compared with histopathological examination. Initial observations. *European radiology*. 2005;15(8):1650-7.
60. Group MS. Extramural depth of tumor invasion at thin-section MR in patients with rectal cancer: results of the MERCURY study. *Radiology*. 2007;243(1):132-9.
61. Heijnen LA, Maas M, Beets-Tan RG, Berkhof M, Lambregts DM, Nelemans PJ, et al. Nodal staging in rectal cancer: why is restaging after chemoradiation more accurate than primary nodal staging? *Int J Colorectal Dis*. 2016;31(6):1157-62.
62. Brown G, Richards CJ, Bourne MW, Newcombe RG, Radcliffe AG, Dallimore NS, et al. Morphologic predictors of lymph node status in rectal cancer with use of high-spatial-resolution MR imaging with histopathologic comparison. *Radiology*. 2003;227(2):371-7.
63. De Nardi P, Carvello M. How reliable is current imaging in restaging rectal cancer after neoadjuvant therapy? *World journal of gastroenterology*. 2013;19(36):5964-72.
64. Heijnen LA, Lambregts DM, Mondal D, Martens MH, Riedl RG, Beets GL, et al. Diffusion-weighted MR imaging in primary rectal cancer staging demonstrates but does not characterise lymph nodes. *European radiology*. 2013;23(12):3354-60.
65. Boone D, Taylor SA, Halligan S. Diffusion weighted MRI: overview and implications for rectal cancer management. *Colorectal Dis*. 2013;15(6):655-61.
66. Improved survival with preoperative radiotherapy in resectable rectal cancer. Swedish Rectal Cancer Trial. *N Engl J Med*. 1997;336(14):980-7.
67. Syk E, Glimelius B, Nilsson PJ. Factors influencing local failure in rectal cancer: analysis of 2315 patients from a population-based series. *Dis Colon Rectum*. 2010;53(5):744-52.
68. Syk E, Lenander C, Nilsson PJ, Rubio CA, Glimelius B. Tumour budding correlates with local recurrence of rectal cancer. *Colorectal Dis*. 2011;13(3):255-62.
69. Pahlman L, Bohe M, Cedermark B, Dahlberg M, Lindmark G, Sjodahl R, et al. The Swedish rectal cancer registry. *Br J Surg*. 2007;94(10):1285-92.
70. Kim JH, Beets GL, Kim MJ, Kessels AG, Beets-Tan RG. High-resolution MR imaging for nodal staging in rectal cancer: are there any criteria in addition to the size? *European journal of radiology*. 2004;52(1):78-83.
71. Anderle P, Rakhmanova V, Woodford K, Zerangue N, Sadee W. Messenger RNA expression of transporter and ion channel genes in undifferentiated and

- differentiated Caco-2 cells compared to human intestines. *Pharmaceutical research*. 2003;20(1):3-15.
72. Bibert S, Roy S, Schaer D, Felley-Bosco E, Geering K. Structural and Functional Properties of Two Human FXYD3 (Mat-8) Isoforms. *Journal of Biological Chemistry*. 2006;281(51):39142-51.
73. Kayed H, Kleeff J, Kolb A, Ketterer K, Keleg S, Felix K, et al. FXYD3 is overexpressed in pancreatic ductal adenocarcinoma and influences pancreatic cancer cell growth. *International journal of cancer*. 2006;118(1):43-54.
74. Morrison BW, Moorman JR, Kowdley GC, Kobayashi YM, Jones LR, Leder P. Mat-8, a novel phospholemman-like protein expressed in human breast tumors, induces a chloride conductance in *Xenopus* oocytes. *The Journal of biological chemistry*. 1995;270(5):2176-82.
75. Vaarala MH, Porvari K, Kyllonen A, Vihko P. Differentially expressed genes in two LNCaP prostate cancer cell lines reflecting changes during prostate cancer progression. *Laboratory investigation; a journal of technical methods and pathology*. 2000;80(8):1259-68.
76. Maxwell PJ, Longley DB, Latif T, Boyer J, Allen W, Lynch M, et al. Identification of 5-fluorouracil-inducible target genes using cDNA microarray profiling. *Cancer research*. 2003;63(15):4602-6.
77. Bardelli A, Saha S, Sager JA, Romans KE, Xin B, Markowitz SD, et al. PRL-3 expression in metastatic cancers. *Clin Cancer Res*. 2003;9(15):5607-15.
78. Liu SS, Leung RC, Chan KY, Chiu PM, Cheung AN, Tam KF, et al. p73 expression is associated with the cellular radiosensitivity in cervical cancer after radiotherapy. *Clin Cancer Res*. 2004;10(10):3309-16.
79. Wallin AR, Svanvik J, Adell G, Sun XF. Expression of PRL proteins at invasive margin of rectal cancers in relation to preoperative radiotherapy. *Int J Radiat Oncol Biol Phys*. 2006;65(2):452-8.
80. Bujko K, Kolodziejczyk M, Nasierowska-Guttmejer A, Michalski W, Kepka L, Chmielik E, et al. Tumour regression grading in patients with residual rectal cancer after preoperative chemoradiation. *Radiother Oncol*. 2010;95(3):298-302.
81. Glimelius B, Isacson U, Jung B, Pahlman L. Radiotherapy in addition to radical surgery in rectal cancer: evidence for a dose-response effect favoring preoperative treatment. *Int J Radiat Oncol Biol Phys*. 1997;37(2):281-7.
82. Graf W, Dahlberg M, Osman MM, Holmberg L, Pahlman L, Glimelius B. Short-term preoperative radiotherapy results in down-staging of rectal cancer: a study of 1316 patients. *Radiother Oncol*. 1997;43(2):133-7.
83. Holm T, Rutqvist LE, Johansson H, Cedermark B. Postoperative mortality in rectal cancer treated with or without preoperative radiotherapy: causes and risk factors. *Br J Surg*. 1996;83(7):964-8.
84. Bujko K, Nowacki MP, Nasierowska-Guttmejer A, Michalski W, Bebenek M, Kryj M. Long-term results of a randomized trial comparing preoperative short-course radiotherapy with preoperative conventionally fractionated chemoradiation for rectal cancer. *Br J Surg*. 2006;93(10):1215-23.
85. Hartley A, Ho KF, McConkey C, Geh JI. Pathological complete response following pre-operative chemoradiotherapy in rectal cancer: analysis of phase II/III trials. *The British journal of radiology*. 2005;78(934):934-8.
86. Maas M, Nelemans PJ, Valentini V, Das P, Rodel C, Kuo LJ, et al. Long-term outcome in patients with a pathological complete response after chemoradiation for

rectal cancer: a pooled analysis of individual patient data. *The Lancet Oncology*. 2010;11(9):835-44.

87. Capirci C, Valentini V, Cionini L, De Paoli A, Rodel C, Glynne-Jones R, et al. Prognostic value of pathologic complete response after neoadjuvant therapy in locally advanced rectal cancer: long-term analysis of 566 ypCR patients. *Int J Radiat Oncol Biol Phys*. 2008;72(1):99-107.

88. Vecchio FM, Valentini V, Minsky BD, Padula GD, Venkatraman ES, Balducci M, et al. The relationship of pathologic tumor regression grade (TRG) and outcomes after preoperative therapy in rectal cancer. *Int J Radiat Oncol Biol Phys*. 2005;62(3):752-60.

89. Martin ST, Heneghan HM, Winter DC. Systematic review and meta-analysis of outcomes following pathological complete response to neoadjuvant chemoradiotherapy for rectal cancer. *Br J Surg*. 2012;99(7):918-28.

90. Kim IK, Kang J, Lim BJ, Sohn SK, Lee KY. The impact of lymph node size to predict nodal metastasis in patients with rectal cancer after preoperative chemoradiotherapy. *Int J Colorectal Dis*. 2015;30(4):459-64.

91. Habr-Gama A, Perez RO, Nadalin W, Sabbaga J, Ribeiro U, Silva e Sousa AH, et al. Operative Versus Nonoperative Treatment for Stage 0 Distal Rectal Cancer Following Chemoradiation Therapy. *Transactions of the Meeting of the American Surgical Association*. 2004;CXXII(&NA;):309-16.

92. Holzer B, Matzel K, Schiedeck T, Christiansen J, Christensen P, Rius J, et al. Do geographic and educational factors influence the quality of life in rectal cancer patients with a permanent colostomy? *Dis Colon Rectum*. 2005;48(12):2209-16.

93. Memon S, Lynch AC, Bressel M, Wise AG, Heriot AG. Systematic review and meta-analysis of the accuracy of MRI and endorectal ultrasound in the restaging and response assessment of rectal cancer following neoadjuvant therapy. *Colorectal Dis*. 2015;17(9):748-61.

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