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DOCTORAL DISSERTATION

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Abstract

Background: Squamous cell cancer of the anus (SCCA) is a rare malignancy, but the incidence is increasing. It is associated with humman papilloma virus infection. The standard treatment is radiotherapy (RT) combined with chemotherapy, usually 5-fluorouracil (5FU)/Mitomycin C (MMC). This treatment is relatively effective, but recurrence still represents a problem especially in locally advanced SCCA.

The overall aim of this thesis was to improve the treatment of SCCA by analysing a large Nordic population-based cohort and to explore a new treatment strategy in a prospective phase I study, NOAC 8.

Methods: Studies I-III were based on a retrospective cohort comprising 1266 patients with SCCA treated according to Nordic guidelines between 2000 and 2007 (cohort 1), with definitive RT, alone or combined with chemotherapy (CRT), stratified by tumor stage.The second cohort included 13 patients with locally advanced SCCA enrolled in the NOAC 8 trial, investigating RT combined with cetuximab and 5FU/MMC, a combination that had not been tested before. The primary aim was to determine the maximum tolerated dose (MTD) of chemotherapy using a pre-defined dose escalation scheme.

Results: High age, male gender, large primary tumor, lymph node metastases, distant metastases, poor performance status and non-inclusion into a protocol were all independent factors associated with worse outcome.The treatment results were good, well in accordance with published randomized trials. A high incidence (11%) of inguinal lymph nodes recurrence was observed in patients with small tumors where adjuvant lymph irradiation was omitted. Surgery alone of early SCCA was associated with a high locoregional recurrence rate and poor survival, which were significantly improved with postoperative RT/CRT.The outcome in patients with metastatic SCCA was poor, but it was significantly better in patients receiving active treatment. Male gender, metachronous disease and multiple metastatic sites were identified as prognostic factors associated with worse prognosis.

The MTD of 5FU/MMC in combination with cetuximab and RT was determined.Dose limiting toxicity were diarrhoea, febrile neutropenia and thrombocytopenia.

Conclusions: Good treatment results were obtained with widely implemented Nordic guidelines. We recommend prophylactic inguinal lymph node irradiation also for small tumors. Postoperative RT/CRT is effective after primary surgery for early SCCA. The addition of cetuximab to 5FU/MMC in combination with RT was a rather toxic regimen but the side-effects were manageable.

Key words: anal cancer, radiotherapy, chemotherapy, surgery, prognosis, phase I study, cetuximab

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To my patients

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Thesis at a glance

Paper	Material	No of patients	TNM	Method	Aim
I	NOAC database Cohort 1	1266	All stages	Retrospective	To evaluate prognostic factors and treatment outcome in a large population-based cohort of patients with anal cancer .
II	NOAC database Cohort 1	93	TxT1-T2N0	Retrospective	To study the recurrence patterns and survival outcome in patients treated with surgery alone compared with patients treated with surgery followed by postoperative (chemo)radiotherapy.
III	NOAC database Cohort 1	185	M1	Retrospective	To analyse the overall survival and prognostic factors in patients with synchronous and metachronous metastatic anal cancer.
IV	NOAC 8 study Cohort 2	13	T2≥4 cm – T4N0-3M0	Prospective, phase I study	To evaluate the feasibility and the safety of the addition of cetuximab to standard chemoradiotherapy. To determine the maximum tolerated dose of chemotherapy in this combination.

List of papers

I. **Leon O**, Guren M, Hagberg O, Glimelius B, Dahl O, Havsteen H, Naucler G, Svensson C, Tveit KM, Jakobsen A, Pfeiffer P, Wanderås E, Ekman T, Lindh B, Balteskard L, Frykholm G, Johnsson A. Anal carcinoma - Survival and recurrence in a large cohort of patients treated according to Nordic guidelines. *Radiother Oncol* 2014;113: 352-358.

II. **Leon O**, Hagberg O, Johnsson A. Primary surgery with or without postoperative radiotherapy in early stage squamous cell carcinoma in the anal canal and anal margin. *Acta Oncol* 2018;57:1209-1215.

III. **Leon O**, Hagberg O, Johnsson A. Prognostic factors and survival outcome in metastatic anal cancer: a population-based study (manuscript)

IV. **Leon O,** Guren MG, Radu C, Gunnlaugsson A, Johnsson A. Phase I study of cetuximab in combination with 5-fluorouracil, mitomycin C and radiotherapy in patients with locally advanced anal cancer. *Eur J Cancer* 2015;51:2740-2746

Abbreviations

AJCC	American Joint Committee on Cancer
AIN	Anal intraepithelial neoplasia
APR	Abdominoperineal resection
CFS	Colostomy-free survival
CR	Complete Response
CRT	Chemoradiotherapy
CT	Computed tomography
CTV	Clinical tumor volume
CSS	Cancer specific survival
DFS	Disease-free survival
DSS	Disease specific survival
ESMO	European Society for Medical Oncology
EGFR	Epidermal growth factor receptor
FDG-PET	Fluorodeoxyglucose-positron emission tomography
5-FU	5-Flourouracil
GTV	Gross tumor volume
HIV	Human immunodeficiency virus
HNSCC	Head and neck squamous cell carcinoma
HPV	Human papilloma virus
HSIL	High-grade Squamous Intraepithelial Lesions
IHC	Immunohistochemistry
ISH	In situ Hybridization
IMRT	Intensity Modulated Radiotherapy
ITT	Intention to treat
KRAS	Kirsten-ras proto-oncogene
LRR	Locoregional recurrence
LSIL	Low-grade Squamous Intraepithelial Lesions

MDT	Multidisciplinary Team conferences
MMC	Mitomycin C
MRI	Magnetic resonance imaging
MSM	Men who have sex with men
MTD	Maximum Tolerated Dose
NCCN	National Cancer Comprehensive Network
NOAC	Nordic Anal Cancer group
OS	Overall survival
ORR	Objective Response Rate
P16	Protein 16
PCR	Polymerase Chain Reaction
PFS	Progression-free survival
PTV	Planning target volume
PP	Per protocol
QoL	Quality of Life
RECIST	Response Evaluation Criteria in Solid Tumors
RFS	Recurrence-free survival
RT	Radiotherapy
SCCA	Squamous cell carcinoma of the anus
SEER	Surveillance, Epidemiology, and End Results Program
SIB	Simultaneous Integrated Boost
RTK	Tyrosine Kinase Receptor
UICC	International Union Against Cancer
VMAT	Volumetric Arc Therapy

Background

Epidemiology

Anal cancer is an uncommon malignancy and accounts for 2-2.5% of all gastrointestinal malignancies (1). The incidence of anal cancer has continuously increased worldwide during the last years. In Sweden, the Cancer Register reported in 2017 about 150 new cases. The median age at diagnosis is 65 years and the incidence is higher in women than in men.

The overall 5-year survival is 60-80% in patients with localized anal cancer and below 20% for patients with metastatic disease (1).

Etiology

Risk factors

Several studies have found associations between the incidence of anal cancer and female gender, infection with human papilloma virus (HPV), immunosuppression in transplant recipients, infection with human immunodeficiency virus (HIV), cigarette smoking, receptive anal intercourse, lifetime number of sexual partners and genital warts (2-6). The incidence of anal cancer is higher among men who have sex with other men (MSM) especially among HIV positive MSM (7).Women with anal cancer have a higher prevalence of prior vulvar, cervical or vaginal cancers supporting the common HPV etiology (8, 9).

Human Papilloma virus

HPV infection is the most common sexually transmitted disease and can be found in many premalignant and malignant lesions of the anogenital tract (10, 11). There are more than 150 different strains of HPV and they are classified in low-and high-risk groups according to their potential for oncogenesis. Malignancies induced by HPV infection include cervical, anal, vaginal, vulvar, oropharyngeal and penis cancer.

The vast majority of anal cancer, nearly 90%, are associated with HPV infection. The most frequently isolated HPV strains in anal malignancies are HPV 16 and 18, similar to that found in genital malignancies (11, 12). There are different diagnostic tests to detect HPV: 1) HPV polymerase chain reaction (PCR), 2) HPV in situ hybridization (ISH) analysis and 3) immunohistochemical (IHC) expression for p16. P16 is a cell cycle inhibitor and is used as a surrogate marker for the HPV status. Most anal cancers are p16 positive (13, 14).

Serup-Hansen et al evaluated both HPV 16 and p16 status as prognostic factors for patients with anal cancer in a Danish population-based cohort. In the univariable analysis HPV positivity was significantly correlated with improved overall survival (OS) and p16 positivity was significantly correlated with improved OS and disease-specific survival (DSS). In multivariable analysis, p16 positivity remained an independent prognostic factor for OS. There was also a significantly higher proportion of males among p16 negative patients (13). Women are more likely to have HPV associated anal cancer than men. HPV infection has higher prevalence in MSM (15, 16).

Some HPV types are associated with anal intraepithelial neoplasia (AIN), also known as squamous intraepithelial lesions (ASIL). Squamous intraepithelial lesions can be low-grade (LSIL) or high- grade (HSIL). HSIL is considered to be the precursor of anal cancer. Several studies have reported a transformation rate from HSIL to invasive anal cancer of approximately 11% (17).

Vaccines against the HPV infection have been developed, and there are three different vaccines depending on the type of HPV they protect against: 1) bivalent vaccine, targets HPV 16 and 18, 2) quadrivalent, targets HPV 16, 18, 6 and 11, 3) 9-valent vaccine, targets HPV 16, 18, 6, 11, 31, 33, 45, 52 and 58. These are prophylactic vaccines that are used mainly for prevention of cervical neoplasia in women (18). The bivalent HPV vaccination efficacy regarding prevention of AIN has been studied in 4210 women who received bivalent vaccine against cervical HPV 16/18 infections. They found that the bivalent HPV vaccination also reduced the incidence of anal infection with HPV 16/18 (19).

The efficacy of quadrivalent vaccine has been investigated also in males. The results from a randomized trial in 4065 males reported that the quadrivalent HPV vaccine was effective in preventing infection with HPV 6, 11, 16 and 18. They showed a decreased incidence of AIN associated with HPV 6, 11, 16 and 18 among men who received the vaccine compared with placebo (20).

Anatomy and histology

The anal canal is the terminal part of the large intestine and it is 3-4 cm long. It begins at the upper border of the anorectal ring and ends at the anal verge where the squamous cells histologically blend with the perianal skin (Figure1).

Histologically, the mucosa of the anal canal can be divided into three zones. The proximal zone is covered by a glandular mucosa. The middle part is an epithelial transitional zone and it extends 0.5-1.0 cm above the dentate line. It consists of columnar, transitional-cuboidal, and squamous epithelium. In this part endocrine cells and melanocytes can also be present. The inferior part, below the dentate line is characterized by squamous cell epithelium. The anal margin or perianal skin is defined as the pigmented hair-bearing skin within a radius of 5 cm surrounding the anal orifice. In the past several authors have used a different definition for anal margin, including all tumors located below the dentate line (21, 22).

Squamous cell carcinoma (SCC) is the predominant type. Other histopathologies are rare, e.g. malignant melanoma, sarcoma, Paget disease, verrucous carcinoma (Buschke-Lowenstein tumor). In this thesis, we analysed only patients with SCC of the anus (SCCA), all other histological variants were excluded.

The lymphatic drainage of the anal canal depends on the anatomic site of the primary tumor. Tumors originating above the dentate line drain primarily to the mesorectal and internal iliac lymph nodes. Tumors arising below the dentate line drain to the superficial inguinal and external iliac lymph nodes. The anal margin drains mainly to the inguinal lymph nodes.

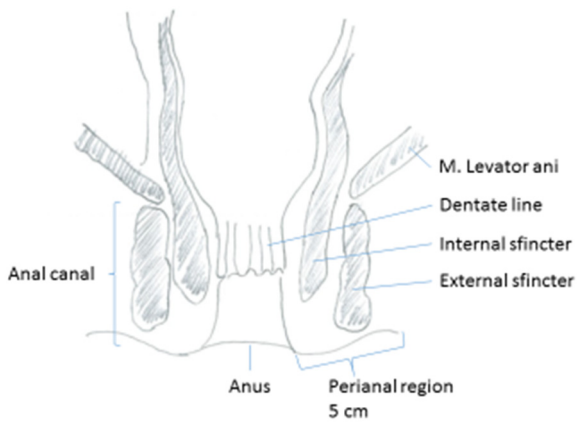

Figure. 1
Anatomy of the anus.

Clinical presentation and diagnostics

The most frequent symptoms are pain, bleeding and sensation of an anal mass. Other clinical symptoms may include anal discomfort and pruritus, change in bowel habits, fistula, fissures or faecal incontinence. Patient´s and physician´s delay are common and many patients have symptoms for several months or years before the SCCA is diagnosed.

SCCA is mainly a locoregional disease. At the initial diagnosis most patients have a T2 tumor and approximately 20% have regional lymph node metastases (23, 24). The probability of nodal involvement is related to tumor size and location. Distant metastases at the initial diagnosis are rare, about 5% and the most common sites are lung and liver (25, 26).

Pretreatment clinical staging includes physical examination, digital rectal examination and palpation of inguinal lymph nodes, proctoscopy and biopsy of the primary tumor. In addition, magnetic resonance imaging of the pelvis (MRI) is performed for local staging of primary tumor. Endoanal ultrasound (EUS) can be used if MRI is contraindicated. A computed tomography (CT) of the thorax and abdomen with intravenous contrast is performed for detection of regional and distant metastases.

Several studies have shown superiority of fluorodeoxyglucose-positron emission tomography (FDG-PET) /CT scan compared to CT alone. Trautmann et al showed that FDG-PET/CT had high sensitivity for detection of regional nodal involvement and distant metastases (27). Changes in tumor stage were observed in 24% of cases, compared to CT alone. Cotter et al reported abnormal nodes in 20% of patients with normal CT and in 23% of patients with normal physical examination (28). Therefore, FDG-PET/CT is a useful tool for radiotherapy dose planning (29). FDG-PET/CT can be also used to detect para-aortic lymph node metastases.

National Comprehensive Cancer Network (NCCN) guidelines recommend FDG-PET /CT scan as a part of the pretreatment staging for anal canal cancers, but not perianal cancers (30). European Society for Medical Oncology (ESMO) guidelines recommend PET/CT for staging of both locations (31).

A gynecological examination should be recommended for women, including screening for cervical cancer. Overall, blood samples for HIV testing of patients are recommended (31).

TNM staging

Anal cancer staging is performed according to Tumor Node Metastasis (TNM) classification by the American Joint Committee on Cancer (AJCC) and International Union Against Cancer (UICC). It is based on tumor size, invasion of adjacent structures, involvement of regional lymph nodes and the presence or not of distant metastases.

In this thesis, the TNM classification used is according to UICC 4th edition 1997 (Table 1). The newest version (eight edition, 2017) is outlined in Table 2.

Table 1: TNM classification of anal cancer according to the AJCC and UICC 1997

Primary tumor (T)

T category	T criteria
Tx	Primary tumor not assessed
T0	No evidence of primary tumor
Tis	Carcinoma in situ
T1	Tumor ≤2 cm
T2	Tumor >2 cm but ≤5 cm
T3	Tumor >5 cm
T4	Tumor of any size invading adjacent organs: vagina, urethra, bladder

Regional lymph node (N)

N category	N criteria
Nx	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis
N1	Metastasis in perirectal lymph node(s)
N2	Metastasis in unilateral internal iliac and/or unilateral inguinal lymph node(s)
N3	Metastasis in perirectal and inguinal lymph nodes and/or bilateral internal iliac and/or bilateral inguinal lymph nodes

Distant metastasis (M)

M category	M criteria
M0	No distant metastasis
M1	Distant metastasis

Table 2. TNM classification of anal cancer according to the AJCC and UICC 2017

Primary tumor (T)

T category	T criteria
TX	Primary tumor not assessed
T0	No evidence of primary tumor
Tis	High grade squamous intraepithelial lesion (previously termed carcinoma in situ, Bowen disease, AIN II-III, high-grade anal intraepithelial neoplasia)
T1	Tumor ≤2 cm
T2	Tumor >2 cm but ≤5 cm
T3	Tumor >5 cm
T4	Tumor of any size invading adjacent organ(s): vagina, urethra, or bladder

Regional lymph nodes (N)

N category	N criteria
Nx	Regional lymph nodes cannot be assessed
N0	No regional lymph nodes metastasis
N1	Metastasis in inguinal, mesorectal, internal iliac, or external iliac nodes
N1a	Metastasis in inguinal, mesorectal, or internal iliac lymph nodes
N1b	Metastasis in external iliac lymph nodes
N1c	Metastasis in external iliac with any N1a nodes

Distant metastasis (M)

M category	M criteria
M0	No distant metastasis
M1	Distant metastasis

TNM: tumor, node, metastasis ; AJCC: American Joint Committee on Cancer; UICC: Union for International Cancer Control eight edition

NCCN guidelines recommend the same TNM staging system for both anal canal and anal margin cancer.

Treatment of localized anal cancer

Treatment of anal cancer is a multidisciplinary teamwork involving surgeons, radiologists, pathologists and oncologists. All new cases should be discussed at multidisciplinary team (MDT) conferences in order to determine which approach is best for the individual patient.

Surgery

In the past, surgery and especially abdominoperineal resection (APR) with a permanent colostomy was the standard treatment of SCCA with 5-year survival rates ranging from 40% to 70% (32-34).

Surgery as primary treatment in SCCA had been examined in small retrospective studies in patients treated in the 1950s and 1970s (32, 34-37). The vast majority of them concluded that APR was the treatment of choice in patients with SCCA, except for small perianal lesions where local excision could be used (38).

Several studies reported that local excision should be recommended only in very carefully selected cases, especially in patients with small carcinoma in situ perianal with a choice of APR in case of invasive perianal tumors and anal canal tumors in order to reduce the rate of local recurrence and to improve survival. Sawyers et al showed that local excision should be chosen only for patients with in situ perianal tumors (34). Beahrs et al reported a 5 years OS of 74.2% for patients with perianal lesions treated with local excision. They found good results mainly in patients with small superficially invasive or carcinoma in situ lesions <3 cm, but not in the patients with invasive lesions. For those and for patients with anal canal tumors the recommended treatment was APR (32). Schraut et al showed better overall prognosis after local excison of carcinoma in situ or microinvasive tumors ≤2 cm and less favourable prognosis for larger microinvasive tumors, invasive tumors and for anal canal tumors. They concluded that perianal and anal canal cancers are two separate clinical entities and that the depth of invasion, the location and the size of tumor are very important for the selection of surgical procedure (38). The histological differentiation had no impact on the outcome. Greenall et al reported a cause-specific 5-year survival of 88% after local excision of anal margin tumors, but a high locoregional recurrence (LRR) rate of 46% (36). We have to take into consideration that in this study they defined the perianal cancer as tumors located within 5 cm radius of the dentate line (22). That differs from other studies which define the perianal cancers as tumors arising within 5 cm of the anal verge (32). Thus, some of the patients included in the study by Greenall et al had tumors which were rather localized in the anal canal according to current definitions. Boman et al reported successful outcome in 12 out of 13 patients treated by local excision for small superficially invasive tumors of the anal canal (≤2 cm) (35). For larger tumors they recommended APR. They showed a local and distant recurrence of 40% after APR with a 5-year OS of 71% and a preoperative mortality of 2.5-4.5%. On the other hand, Longo et al reported high local recurrence and a worse outcome after local excision of invasive tumors in the anal canal with local failure rates of 44% and 100% after local excison of a T1N0 tumor and T2-T3N0 tumors, respectively. The median survival after local excision of a T1N0 tumor was 33 months (37).

These studies report good results with surgery alone mainly in patients with superficially lesions, which today would be classified as carcinoma in situ or HSIL for which local excision is still a treatment of choice.

In 1974 Nigro et al reported high response rates in three patients treated with preoperative combined chemoradiotherapy (CRT) (39). These patients received radiotherapy (RT) to a total dose of 30 Gray (Gy) in combination with one cycle of 5-fluorouracil (FU) with Mitomycin C (MMC). APR was performed 6 weeks after the end of CRT and in 2 of 3 patients no residual tumor was found in the pathological specimens. The third patient was only followed up with no evidence of tumor after one year.

Subsequently, several non-randomized studies using RT with or without chemotherapy confirmed the results of Nigro et al and gradually RT/CRT replaced APR as standard treatment of localized anal cancer (23, 40-44). This strategy has significantly improved locoregional control without the need of a colostomy.

Randomized studies comparing surgery with RT/CRT as primary treatment in anal cancer are lacking. However, several retrospective studies showed that patients treated with RT/CRT had a more favourable outcome than patients treated with primary surgery alone (local excision, APR). Goldman et al evaluated the treatment results between two population-based groups of patients with SCC of the anal canal, treated with different modalities (local excision, APR alone or followed by RT, primary RT/CRT followed by surgery or definitive RT/CRT) in a non-randomised study. They reported a LRR of 78% in patients treated with local excision and 57% in patients treated with APR for T1-T2 tumors, with a better survival after non-surgical approach and concluded that the initial treatment for SCC of the anal canal should be RT/CRT (45).

According to ESMO guidelines local excision is recommended only for small, <2 cm perianal cancer, not poorly differentiated and not invading the sphincter (31). In case of involved margins, re-excision or postoperative RT/CRT is recommended. However, data supporting these strategies are very limited (46, 47).

Currently, APR is recommended as salvage treatment in patients with residual or recurrent tumor and in patients who had previously been irradiated to pelvis due to other malignancies. Five-year OS after salvage surgery is 50-60% according to results from a Swedish and a Danish study, respectively (48).

Recently, Chai et al reported in a study based on the National Cancer Database on patients with T1N0 SCCA of the anal canal that the proportion of patients treated with local excision has increased with time in the US, especially for tumors ≤1 cm. They presented similar OS rates in patients treated with local excision compared with those treated by curative CRT. However, data regarding LRR and the outcome of patients that had underwent local excision prior to CRT are not presented (49).

Combined chemoradiotherapy

The standard treatment for localized SCCA is definitive CRT. The most widely used chemotherapy regimen is a combination of 5FU and MMC concomitant with RT to a total radiation dose of 50-60 Gy, with salvage surgery in case of local failure. The efficacy of this strategy has been confirmed by several randomized trials performed during the last decades.

The UKCCCR trial (ACT I) included 585 patients with T1-T4 or N+ who were randomized between RT alone or RT with concomitant 5FU and MMC (50). The RT dose to the primary tumor was 45 Gy. After six weeks break the patients with a tumor response ≥50% (good responders) received a boost with external beam RT of 15 Gy or with 25 Gy brachytherapy, whereas poor responders underwent salvage surgery. The local failure rate was significantly lower in the CRT arm compared to RT alone arm (36% vs 59%), but no significant difference in 3-year OS (65% vs 58%). However, the cancer specific survival (CSS) was significantly improved in the CRT arm (72% vs 61%). Long-term follow-up showed a lower LRR and improvement in recurrence-free survival (RFS) even 12 years after starting treatment (51).

The European Organization for Research and Treatment of Cancer Radiotherapy and Gastrointestinal Cooperative Groups (EORTC 22861) performed a similar study on 110 patients with locally advanced tumors (T3-T4N0-3 or T1-2N1-3) (52). They were randomized between RT alone and CRT. RT consisted of an initial course of 45 Gy combined with 5FU plus MMC. After sex weeks break the patients with partial or complete response received a RT boost of 15 or 20 Gy. In case of poor response salvage surgery was performed. They found a significant increase of complete remission rate from 54% in RT alone group to 80% for CRT group. This led to a significant improvement of locoregional control and colostomy free interval for CRT group. The OS rate remained similar in both groups. Skin ulceration, nodal involvement and gender were the most important prognostic factors for both local control and survival.

To evaluate the role of MMC the Radiation Therapy Oncology Group (RTOG)/Eastern Cooperative Oncology Group (ECOG) 87-04 performed a study on 310 patients with any T any N, which randomized to RT with 5FU or RT with 5FU and MMC (53). The RT consisted in 45 Gy. After the end of RT the response was assessed by biopsies and patients with positive biopsies received boost RT of 9 Gy and additional chemotherapy. Patients with persistent tumor following boost RT underwent APR. Colostomy-free survival (CFS) and disease free survival (DFS) were significantly higher in the MMC arm (71% vs 59% and 73% vs 51%). OS was not significantly different. The CFS difference was noted especially in patients with T3-T4 tumors, while there was no difference for patients with smaller tumors. Early toxicity was significantly higher in the MMC arm.

Cisplatin is a chemotherapeutic compound that has been widely used as a radiosensitizer in other SCC malignancies such as SCC of head and neck (HNSCC) and esophageal cancer. In the early 1990s several phase II studies, in which MMC was replaced by cisplatin was performed, yielding encouraging results (54, 55). In a study by Peiffert et al 80 patients with locally advanced SCCA were treated with 2 cycles of neoadjuvant 5FU/cisplatin followed by RT 45 Gy concomitant with 2 cycles of 5FU and cisplatin. After 4-8 weeks the good responders received a boost RT of 15-20 Gy. After neoadjuvant chemotherapy, most of patients were objective responders. All patients but 5 achieved complete response (CR). The 3-year OS and RFS was 86 % and 70%, respectively (55).

The role of induction (neoadjuvant) chemotherapy and of cisplatin compared to MMC was studied in a randomized trial conducted by the United States Intergroup (RTOG 98-11) (56). The patients were randomized to induction chemotherapy consisting of 5FU and cisplatin, followed by concomitant CRT using the same chemotherapy or the standard regimen with 5FU and MMC. 644 patients with SCCA were enrolled, 27% with tumor >5 cm, 35% T3-T4 and 26% with positive nodes. The 5-year LRR and distant metastases rates were 25% and 15%, respectively in the MMC arm and 33% and 19% in the cisplatin arm. The cumulative rate of colostomy was significantly better in the MMC arm. The 3-year DFS and OS were not statistically significant. However, a recent update found that the 5-year DFS and OS were significantly better in the MMC arm than in the cisplatin arm 67.8% vs 57.8% and 78.3% vs 70.7%, respectively. In multivariate analysis male gender, positive nodes and tumor size >5 cm were independent prognosticators for worse DFS. Hematologic toxicity was worse in the MMC arm, but late RT toxicities were similar. The study showed no benefit for the induction chemotherapy. The overall treatment time (OTT) was longer in the cisplatin arm, 101 days vs 49 days in the MMC arm. They speculate that this delay in CRT start might have had a negative impact on the outcome in the cisplatin arm.

Another randomized trial using cisplatin was ACCORD 03, designed to determine if induction chemotherapy or RT dose escalation improves CFS (57). 307 patients with tumors ≥4 cm or <4 cm and N+M0 were randomized to one of following arms: 1) 2 courses of neoadjuvant 5FU-cisplatin followed by concomitant CRT with 45 Gy and boost 15 Gy. 2) similar treatment as 1) with a high- dose boost of 20-25 Gy. 3) concomitant CRT with a standard dose boost of 15 Gy 4) concomitant CRT with a high-dose boost of 20-25 Gy. Chemotherapy in CRT consisted of 5FU-cisplatin. The boost was given to responders three weeks after CRT was completed. No benefit was observed in CFS, local control or OS with neither neoadjuvant chemotherapy nor high-dose RT boost.

The role of maintenance chemotherapy was studied in the ACT II trial (58). 940 patients with SCCA, 43% T3-T4 and 30% positive nodes received CRT with 5FU

plus cisplatin or 5FU plus MMC. The RT dose was 50.4 Gy/28 fractions without gap. The maintenance chemotherapy started four weeks after the end of CRT and consisted of 2 courses of 5FU and cisplatin. No difference in the CR rate (90%), progression-free survival (PFS), CFS and OS were observed between the 5FU/cisplatin and the 5FU/MMC arms. The toxicity was similar in all groups. No benefit was seen with the maintenance chemotherapy. Local control was observed in 75% of patients. Grade 3 hematological toxic effects were more in the MMC group than in the cisplatin group. Despite this, they concluded that 5FU/MMC should remain the standard of care because of similar efficacy, but also because less resources needed to administer MMC compared to cisplatin.

In conclusion combined CRT is the standard of care in treatment of localized SCCA by which 60-80% of patients are cured. The studies have shown a benefit for 5FU plus MMC over 5FU and cisplatin for local control, DFS, CFS and OS. The chemotherapy doses vary between the studies. In ACT trials the MMC dose was 12 mg/m^2 day 1 only (58) while in the RTOG trials MMC dose was 10 mg/m^2 on days 1 and 29 (56). The 5FU doses are usually the same across studies, 1000 mg/m^2/ day on days 1-4 and 29-32.

In the late of 1990s in Sweden, Norway and Denmark the SCCA was treated according to the NOAC treatment protocols issued by Nordic Anal cancer group (NOAC) which included 7 different treatment schedules consisting of definitive RT with/without chemotherapy stratified by tumor stage (NOAC 1-7) .The chemotherapy consisted mainly of 5FU/MMC or 5FU/cisplatin. The NOAC treatment schedules are presented in detail in the chapter “Material and methods”.

Currently, in Sweden the treatment of localized SCCA consists of RT to a total dose of 54 Gy to the primary tumor and 40 Gy to the elective lymph nodes, respectively combined with one cycle of 5FU/MMC in the early tumors, while in tumors T2 (≥4cm) – T4 /N+ the recommended RT dose to the primary tumor is 57.5 Gy and 41.6 Gy mot elective lymph nodes, combined with two cycles of 5FU/MMC in the first and fifth week of RT.

However, in some cases that 5FU is contraindicated a combination of MMC and cisplatin may be used together with RT, according to a study by Matzinger et al. This was a randomized phase II trial to assess the feasibility of CRT with MMC/cisplatin with reference to RT combined with FU/MMC which showed that the combination had acceptable toxicity and a good objective response rate (ORR >75%) (59).

Recent phase II studies suggested that daily oral Capecitabine in combination with MMC and RT had acceptable toxicity and that the Capecitabine could be an alternative to infusional 5FU (60-63).

Radiotherapy

The optimal dose of external beam RT to primary tumor has been examined in several retrospective studies which suggest that an RT dose of at least 45 Gy is required to achieve a better local control, DFS and OS (64). The total dose is a significant prognostic factor for both local control and survival (65). In the majority of the randomized trials the patients received an initial RT dose of 45 Gy followed by a boost of 10-20 Gy with concomitant chemotherapy, usually 5FU plus MMC.

Other small retrospective series suggest that 30 Gy with combined chemotherapy could be an adequate dose after an excisional biopsy for patients with an early SCCA (46).

NCCN guidelines recommend a minimum RT dose of 45 Gy to the primary tumor and an additional boost of 10-14 Gy to a total dose of 55-59 Gy for patients with T3-T4 and nodal involvement or T2 with residual disease after 45 Gy (30).

In patients with high comorbidity, RT in combination with lower chemotherapy doses is recommended. In cases when chemotherapy is contraindicated a higher RT dose of 60-64 Gy to primary tumor should be recommended.

The most used technique is external beam RT using fields that encompass the primary anal tumor, the pathological lymph nodes and the elective regional lymph nodes (presacral, perirectal, internal iliacal, inguinal).

The target of elective lymph nodes depends on the tumor localization and lymph drainage. The role of prophylactic inguinal lymph nodes irradiation is controversial. Some retrospective studies reported that the prophylactic RT of inguinal nodes can be safely omitted in T1N0M0 (66, 67) or T2N0 (68) , while others showed higher inguinal recurrences about 10% when elective inguinal nodes irradiation was omitted (69, 70).

RT techniques have improved considerably during the last decades, from simple opposed fields or “four-field boxes” or three dimensional conformal RT (3D-CRT) to more advanced methods such as intensity-modulated RT (IMRT) and volumetric-modulated arc therapy (VMAT) or proton beam irradiation. Imaging, treatment planning and modalities of fixation have also been developed.

The primary tumor volume is defined as Gross Tumor Volume (GTV) including anal tumor and metastatic lymph nodes. The clinical tumor volume (CTV) consists of the GTV and a margin around it for assumed possible microscopic tumor spread. The PTV (planning target volumes) including the CTV and a margin around it for organ movement and for set-up uncertainties. The surrounding normal tissues in the area are called organs at risk (OARs). OARs for radiation of the SCCA are the small bowel, urinary bladder, ureters, femoral heads, nerves, genitalia and anal sphincter.

Several retrospective and prospective studies evaluated the safety of IMRT and chemotherapy (5FU/MMC) for SCCA. A phase II study was conducted by RTOG (RTOG 0529) and they reported a lower dermatological, gastrointestinal and hematological toxicity compared with those from the RTOG 98-11 trial (56, 71). Pepek et al found a clear reduction of hematologic toxicity using IMRT compared to 3D-CRT used in the RTOG 98-11 trial, whereas Salama et al demonstrated no difference (72).

Some centers use brachytherapy for the boost instead of external beam RT, but there are no randomized trials comparing the two methods regarding efficacy and toxicity. There are retrospective trials which reported similar or superior tumor control with brachytherapy boost compared to external beam RT boost, however with no difference in OS (47, 73-75).

Radiotherapy could be also an option in the palliative setting to relieve pain, stop bleeding and delay local progression.

Overall treatment time

The OTT seems to be significantly associated with locoregional control and an inferior tumor local control has been seen with increasing total treatment time. Graf et al (76) evaluated the clinical outcome in 111 patients with T1-T4NxM0 SCCA treated with 45 Gy (given as a split course or continuously) with concomitant 5FU/MMC. They concluded that advanced tumor stage, size, nodal status and an OTT >41 days significantly decreased the 5-year local control rate. The 5-year local control rate was 58% for OTT >41 days and 79% for OTT ≤41 days; p=0.04). The predominant determinant of local control was OTT and not the administration schedule, split or continuous RT.

Chemoradiotherapy in elderly patients

In elderly and frail patients it may be necessary to modify the chemotherapy/RT schedule. Charnley N. et al (77) reported high rates of local control (73%) in 16 patients with SCCA with median age of 81 years treated with a low dose of RT 30 Gy with concomitant reduced 5FU. They concluded that this is a well-tolerated regimen for elderly patients or patients with poor performance status. A Finnish study reported the results of CRT in elderly patients treated at Helsinki University Central Hospital and concluded that the treatment should not be determine by age (78). Dale et al. evaluated the outcome after CRT for patients above 80 years compared with younger patients in 35 patients with SCCA treated in Norway. Half of the patients could tolerate the CRT despite high age. Anyway, in fragile patients not suitable for CRT, RT alone or surgery could be an alternative to control local

disease. CSS was significantly lower in the patients above 80 years 50% vs 60% (79).

HIV and chemoradiotherapy

The treatment of anal cancer in HIV positive patients is similar to those without HIV infection and the guidelines recommended the same CRT doses if the CD4 count is >200 cells/microL (80). Blazy et al reported no association between CRT-related toxicity and CD4+ cell count in patients with HIV associated SCCA treated with highly active antiretroviral therapy (HAART) (81). It is uncertain whether the treatment with HAART has an impact on the incidence of anal cancer. Bower et al reported in 2004 no significant difference in clinical features, incidence and OS in patients with HIV associated anal cancer since the introduction of HAART (80).

Follow-up and surveillance

According to the guidelines a close follow-up is mandatory and includes clinical examination and a PET/CT and/or MRI at 3 months after the completion of the curative CRT in order to evaluate the treatment response. The clinical examination includes digital rectal examination and palpation of inguinal lymph nodes. The guidelines recommend also gynaecological examination in women. Patients should be follow-up every 3 months in the first 2 years and after that, every six months until 5 years after the end of the CRT. The most recurrences occur locoregionally rather than distantly. For patients with locally advanced anal cancer, T3-T4N0 or any TN+ who have 15% risk to develop distant metastasis within 3 years it is recommended to perform a CT of the thorax and abdomen at 1, 2, respectively 3 years after the completion of CRT. According to the results of ACT II trial only 1% of patients recur after 3 years so the guidelines do not recommend any further imaging after this period of time (30, 31).

It is known that some tumors may require 6 months or more before complete response has been achieved. In case of persistent or recurrent tumor, a biopsy should be performed in order to confirm the presence of cancer. If the biopsy is positive, PET/CT and MRI are recommended for staging before planning of salvage surgery (APR). All patients should be discussed at MDT with surgeons, radiologists, pathologists and oncologists.

Prognosis

The prognosis is good for patients with localized SCCA treated with curative CRT, with a 5-years OS of 60-80% (1). Unselected cohorts tend to have lower rates

compared to randomized trials because they also include elderly patients and patients with severe comorbidities.

Prognostic factors for RFS and OS in patients treated for SCCA have been investigated in several randomized and non-randomized trials and include male sex, primary tumor size >5 cm, positive lymph nodes, particularly positive inguinal lymph nodes (82, 83). Results from the ACT II trial showed that T stage, gender and haemoglobin were prognostic factors for local regional failure and CFS (84).

A number of other factors such as lower haemoglobin levels and skin ulceration have also been evaluated as prognostic factors for worse local control and survival (52).

Late toxicity

Thus, CRT for localized SCCA is an effective treatment, but many anal cancer survivors suffer from late effects from the treatment with impact on their health-related quality of life (HRQOL). Bentzen et al (85, 86) reported the late effects and the faecal incontinence in a Norwegian cohort of 128 anal cancer survivors treated according to NOAC treatments schedule. To evaluate the late effects the EORTC QLQ-C30 and EORTC QLQ-CR 29 questionnaires were used. The study showed a significant impairment of function, especially role and social function in anal cancer survivors compared to a reference group of volunteers from the normal population. The majority of patients reported symptom as flatulence, buttock pain, pollakiuria, nocturia, diarrhoea, flatulence, faeces incontinence, skeletal pain and sexual dysfunction.

The faecal incontinence was evaluated by two instruments, the St. Mark's score and the EORTC-QLQ-CR 29. The St. Mark's score is a validated instrument to score the frequency and degree of faecal incontinence during the last four weeks. According to this score urgency and faecal incontinence occur in 64% and 43%, respectively in anal cancer survivors after the completion of CRT. These symptoms have significant consequences in alteration in lifestyle in 54% of the survivors compared to 3% in the reference group of volunteers.

Treatment in metastatic anal cancer

Distant metastases in SCCA are rare, 5-8% of patients have synchronous distant metastases at the time of initial diagnosis and another 10-20% of patients develop metachronous distant metastases after the end of curative CRT (31). The most common metastatic sites are liver, lung and extrapelvic lymph nodes.

The guidelines recommend a combination of 5FU and cisplatin as first line treatment of metastatic SCCA, based on small retrospective studies (87, 88). Other active chemotherapeutic agents include carboplatin, paclitaxel and docetaxel (89-91). Recently preliminary results were presented from the first prospective randomized trial on patients with recurrent or metastatic SCCA, the InterAACT trial (NCT 02051868), showing a significantly better OS and less toxicity in patients treated with carboplatin/paclitaxel compared to those treated by 5FU plus cisplatin and this regimen could be an alternative for first line treatment in metastatic SCCA.

Due to the rarity of the SCCA there are no uniformly accepted treatment algorithms for metastatic SCCA. Therefore, multidisciplinary management including discussion of all new diagnosed patients with surgeons, radiologists, pathologists and oncologists on the MDT is recommended.

Some retrospective studies showed a significantly improved median OS in patients after multimodality treatment including surgical resections, radiofrequency ablation or definitive CRT of distant metastases (25, 92). A retrospective multicentre study with 27 patients who underwent liver surgery for liver metastases reported a median OS after hepatic resection of 22.3 months and a 5-year OS rate of 20.5%. Synchronous metastases, liver metastasis size >5 cm and a positive surgical resection margin were independent factors associated with higher recurrence after metastatic surgery and worse OS (93). They concluded that long-term survival could be achieved in patients with SCC after surgery for liver metastases, especially in patients with limited metachronous disease amenable to radical resection. These results support an aggressive approach in selected patients with limited distant metastases from SCCA.

There are a few case reports which showed good response with treatment included EGFR inhibitors (e.g. cetuximab) as monotherapy or in combination with chemotherapy, e.g. irinotecan (94) or cisplatin and 5FU (95, 96).

Recently early studies have shown promising results on PD-1 inhibitors (e.g. pembrolizumab and nivolumumab) (97-99).

The prognosis of metastatic SCCA is generally poor, with a median survival of 8-34 months. Results from a US study based on the SEER database for the 1973-2000 period showed a 5-year survival of 10% for men and 20% for women (1). The literature describing clinical outcome and prognostic factors influencing OS in patients with metastatic anal cancer is limited.

EGFR-inhibitors in chemoradiation

Thus, even though the cure rate with standard CRT is relatively high, approximately 25% of patients, especially with large tumors develop locoregional recurrence and 10-20% distant metastases (31) and therefore more effective treatments are warranted.

Several studies have shown that the epidermal growth factor receptor (EGFR) is often overexpressed in SCC, including SCC of head and neck (HNSCC), non-small cell lung cancer (NSCLC) and SCCA (100).

The epidermal growth factor receptor (EGFR, erbB1) is a member of the tyrosine kinase receptor (RTK) family, which includes also erbB2 (HER2/neu), erbB3, (HER 3) and ErbB4 (HER4). The erbB receptor is present at the cell surface and has a common structure composed of an extracellular ligand-binding domain, transmembrane segment and an intracellular tyrosine kinase domain. The EGFR is involved in cell proliferation, metastasis and angiogenesis and is expressed in the majority of epithelial tumors. The two main classes of EGFR inhibitors are the RTK inhibitors and the monoclonal antibody.

Cetuximab (Erbitux) is a chimeric IgG1 mouse antibody directed against the extracellular domain of EGFR. The binding of cetuximab to EGFR prevents dimerization that is required for the activation of the receptor and its signalling pathways, including key proteins like KRAS (Kirsten rat sarcoma viral oncogene), BRAF, etc. This may lead to inhibition of e.g. cycle progression, proliferation and angiogenesis (Figure 2).

Cetuximab is approved for treatment of metastatic colorectal cancer, either as monotherapy or in combination with chemotherapy. Translational studies have shown that cetuximab is effective only against tumors that are KRAS- and NRAS-wild type. In anal cancer the frequencies of KRAS mutations are very low in comparison with mCRC (101-103).

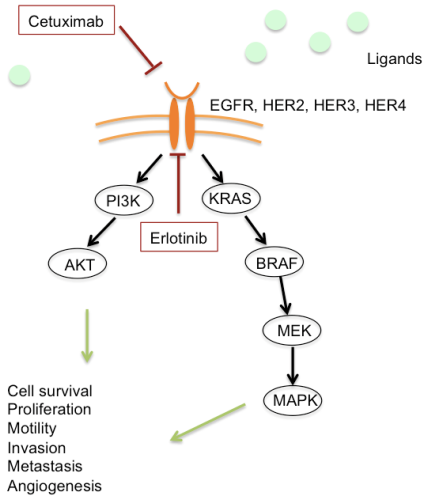

Figure 2
Ilustration of the EGFR signalling pathway. Reprinted with courtesy of dr. Margareta Heby

In addition, cetuximab has been found to potentiate the effects of radiotherapy and it was the first targeted therapy approved for use in combination with RT for treatment of patients with locally advanced HNSCC (104, 105).

Side effects include allergic reactions, acneiform rash and hypomagnesemia. Allergic reactions are more commonly associated with cetuximab compared to the human monoclonal antibody panitumumab.

In SCCA cetuximab had been studied in patients with localized anal cancer in a few phase I-II trials. In the study by Olivatto et al (106) the cetuximab was added to CRT based on 5FU and cisplatin and their conclusion was that the combination had unacceptable high toxicity, but the locoregional response rate was encouraging. The ACCORD 16 (107) trial evaluated the objective response rate in locally advanced SCCA treated with the same chemotherapy and RT doses up to 65 Gy. The study was stopped prematurely due to serious adverse events. In the VITAL study Feliu et al (108) examined the combination of panitumumab with RT and 5FU/MMC in patients with stage >T2N0 SCCA and concluded that the combination was tolerable with a good compliance and an acceptable toxicity, but it didn't reach the primary endpoint concerning DFS and OS. Phase II trials of cetuximab plus cisplatin, 5FU and RT in immunocompetent (ECOG 3205) and HIV positive (AMC 045) patients with stage I-III SCCA showed that the addition of cetuximab to CRT might reduce LRR, but the combination was rather toxic (109).

The combination of cetuximab with RT and 5FU/MMC has not been studied before.

Aims of the thesis

The overall aim of this thesis was to improve the treatment of SCCA by analysing a large Nordic population-based cohort and to explore a new treatment strategy in a prospective phase I study.

Specific aims:

- To analyse prognostic factors and treatment outcome in terms of local control, recurrence patterns and survival in a large cohort of patients with SCCA treated according to Nordic guidelines
- To evaluate the results of surgery alone and postoperative radio(chemo)therapy after local excision of early anal cancer
- To evaluate survival outcomes and prognostic factors in patients with synchronous and metachronous metastatic anal cancer
- To explore the role of cetuximab in combination with standard chemoradiation for treatment of locally advanced anal cancer in a phase I study, the NOAC 8 trial

Material and methods

Patients

Cohort 1

This cohort was based on a large population of 1266 patients with SCC of the anal canal and anal margin diagnosed from 2000-07-01 to 2007-06-30 in 16 oncological departments in Sweden, Norway and Denmark. All patients' data were retrieved retrospectively and were entered into a Nordic database (Figure 3).

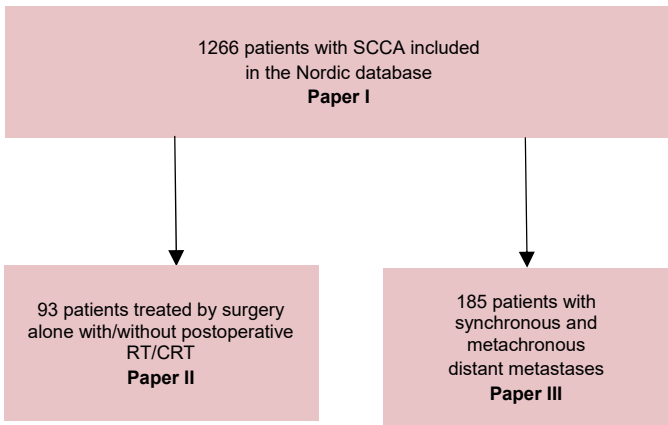

Figure 3
Consort diagram NOAC database

The NOAC treatment protocols were issued by the Nordic Anal Cancer Group (NOAC) in the late of 1990s. There were 7 different treatment schedules consisting of definitive RT with or without chemotherapy stratified by tumor stage (NOAC 1-7) except in patients with well or moderately differentiated anal margin tumors <1cm (without muscular invasion) who could be treated by local surgery. An overview of NOAC protocols is presented in Table 3.

Table 3
NOAC treatment schedules (1-7)

Treatment schedule	Stage	RTdose primary tumor (Gy)	RTdose adjuvant nodes (Gy)	Induction chemotherapy	Chemotherpay during RT
NOAC 1	T1N0 well/mod diff	64			
NOAC 2	T1poorly-diff-T2N0	64	46		MMC/5FU x 1
NOAC 3	T1poorly-diff-T2N0	54	42		
NOAC 4	T3-4/N+	64	46	CisPt/5FU x 3	
NOAC 5	T3-T4/N+	60	46	CisPt/5FU x 2	CisPt/5FU x 1
NOAC 6	T3-4/N+M	64	46	CisPt/5FU/Ifo x 3	
NOAC 7	T3-T4/N+	60	46		MMC/5FU x 2

The Nordic (NOAC) database contained information of patient, tumor and treatment characteristics, treatment results and follow-up data, previous malignancy etc. No assessment of the HPV status was done. The database included all patients diagnosed with SCCA during that time period, regardless of tumor stage and treatment.

Tumor staging was performed according to institutional standards, with digital rectal examination and examination of inguinal lymph nodes, anorectoscopy, biopsy, CT of the abdomen and thorax as a minimum. EUS was frequently used initially, but it was gradually replaced by MRI during the study period. The 4th edition of the UICC TNM staging system was used (Table 1). The study was approved by the Ethics Committees in all participating countries.

After treatment the patients were followed up according to institutional routines, usually with clinical examination every 3 months the first 2 years and then every 6 months to 5 years. CT scans and biopsy were performed when clinically indicated.

In paper I we evaluate the outcome for all 1266 patients with SCCA included in NOAC database. 886 patients were treated according to one of the predefined NOAC protocols (NOAC 1-7) whereas 380 patients were not (Figure 4). This database included >90% of all patients with anal cancer in Sweden and Norway and approximately 25% of those diagnosed in Denmark.

We determined the recurrence patterns, RFS, CFS and OS in patients with early SCCA and advanced SCCA, respectively. For patients with early cancers, T1 and T2N0 there were three protocols NOAC 1, NOAC 2 and NOAC 3. For patients with stages T3-4N0 or any TN+, four protocols were available, NOAC 4 –NOAC 7. Patients with large T2 tumors (>4 cm) N0 could also be treated according to these protocols. The most widely used protocols were NOAC 1-5 (Table 3).

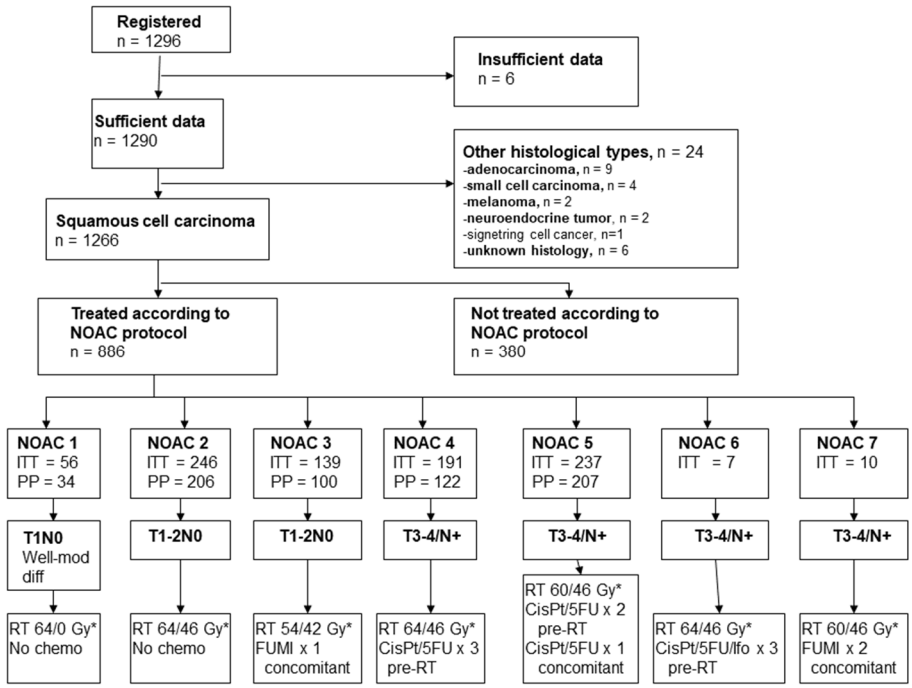

Figure 4

Flow-chart NOAC database study I. RT xx/yy Gy= xx Gy to primary tumor, yy Gy to adjuvant lymph nodes. 5FU= 5-fluorouracil, FUMI = 5FU+ Mitomycin C, cisPt = cisplatin, Ifo = ifosfamide, ITT = intention-to-treat population, PP = per protocol population

Variables including gender, age, primary tumor size, TNM stage, localization of tumor and given treatment were recorded in order to determine the risk factors for recurrence. Surgery, usually APR was performed as salvage if there was residual tumor or local recurrence after completion of RT/CRT. In some cases with very large tumors, APR was performed as an integrated part of the therapy, after a preoperative RT dose of 41-48 Gy.

From the Nordic database we identified 93 patients with stage TxT1-T2N0M0 treated with surgery alone, group S (n=59) or surgery followed by postoperative RT/CRT (n=34), group S+RT/CRT (Figure 3) within 6 months after surgery. Surgery consisted of local excision in 86 patients and APR in 7 patients (due to previous RT, high age and comorbidities), all of them in the surgery alone group. There was no information regarding exact type of excision or whether the cancer diagnosis had been established preoperatively or not. For resection radicality (R) the following classification was used: R0= microscopically radical with >1mm margin, R1= macroscopically but not microscopically radical and R2= macroscopically not

radical. The R assessment was based on the original medical reports from the surgeon and pathologist. No pathological re-evaluation was performed.

The primary tumor was localized merely in the anal margin in 41% of patients. There were no pre-specified dose recommendations in the NOAC protocol for the postoperative setting. The postoperative median RT dose used to the tumor bed was 54 Gy (range 46-66) and RT of elective lymph nodes (perirectal, presacral, iliacal, inguinal) was given in 75% of the patients to a median dose of 46 Gy (range 26-46). Half of the patients received concomitant chemotherapy and the most used chemotherapy was a combination of 5FU and MMC. Outcomes after surgery with or without postoperative RT/CRT, with regard to locoregional recurrence and survival were analysed and summarized in paper II.

From 1266 patients included in the NOAC database we identified 185 (15%) patients with metastatic disease. Sixty-nine of them (37%) were diagnosed with synchronous distant metastases and 116 (63%) with metachronous distant metastases. Outcome and prognostic factors influencing OS were analysed in this patient cohort and presented in paper III (manuscript).

Cohort 2

In 2012, we initiated a phase I/II trial, NOAC 8, on cetuximab in combination with RT and 5FU/MMC as primary treatment for patients with locally advanced anal cancer, T2(≥4cm)-T4 N0-3M0 or any TN2-3M0. Our purpose was to explore the role of cetuximab in combination with RT and 5FU/MMC, a combination that had not been tested before. There was a prospective non-randomized, multicenter phase I study, including patients from the oncological departments of Lund, Uppsala and Oslo. A total of 13 patients were included between 2012 and 2014.

The regimen consisted of weekly standard doses of cetuximab starting one week before start of CRT. IMRT or VMAT with simultaneous integrated boost (SIB) was given to 57.5/54.0/48.6 Gy in 27 fractions to primary tumor/lymph node metastases/elective lymph nodes. 5FU/MMC was given on week 1 and 5 of RT (Figure 5).

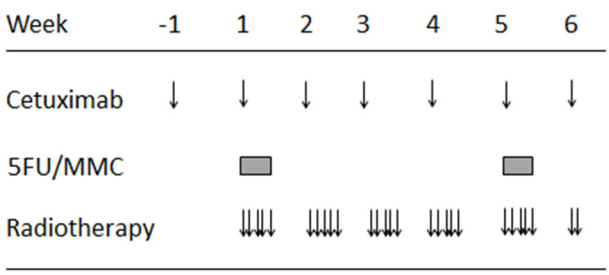

Figure 5
Summary of NOAC 8 study. 5FU, 5-fluorouracil; MMC, mitomycin C

The primary aim was to establish the maximum tolerated dose (MTD) of chemotherapy (5FU and MMC) in combination with standard CRT using a pre-specified dose escalating scheme (Table 4). According to this scheme the first patients received a reduced chemotherapy dose, which was then adjusted for subsequent patients based on the side effects. This schedule followed a 3+3 design, one of the most used methods for defining optimal treatment dose in phase I clinical trials.

RT and cetuximab doses were the same in all patients. Secondary endpoints included acute toxicity, response rate, RFS and OS. HPV status was analysed by p16 staining. The study was approved by the Ethics Committee in all participating countries (paper IV).

Table 4
Dose escalation schedule

Dose level	No of patients accrued	Cetuximab first dose (mg/m2)	Cetuximab Weekly (mg/m2)	5-fluorouracil* (mg/m2)	Mitomycin C (mg/m2)
-1	0	400	250	800	6
0	6	400	250	800	8
1	7	400	250	1000	8
2	0	400	250	1000	10

*The dose per day, given for 4d(96h) continuously

Chemotherapy regimens used in NOAC protocols

5FU/MMC: 5-Fluorouracil 1000 mg/m^2/24 h continuous infusion days 1-4 and Mitomycin C 10 mg/m^2 bolus day 1.

Cis/5FU: Cisplatin 75 (60-100) mg/m^2 day 1 and 5-Fluorouracil 750-1000 mg/m^2/24h continuous infusion days 1-4 or days 1-5. Concomitantly the cisplatin

dose was reduced to 60 (50-75) mg/m^2. If contraindication cisplatin could be replaced by carboplatin (AUC 4-7).

Cis/Ifo/5FU: Cisplatin 37.5 mg/m^2, 5-Fluorouracil 500 mg/m^2 and Ifosfamide 2g/m^2, days 1-2.

In the NOAC 8 trial a combination of 5FU and MMC on days 1-4 was administered intravenously as a continuous infusion according to a pre-specified dose-escalating schedule. Firstly, 3 patients will receive chemotherapy at dose level 0, combined with cetuximab and RT. If none of 3 patients had dose limiting toxicity (DLT), escalation to the next step was to be performed in the following 3 patients. If 1 of 3 patients had DLT, an additional 3 patients were treated at that dose level. If no further patients had DLT, dose escalation would proceed to the next level. If ≥2 of these 6 patients or ≥2 of the first patients exhibited DLT, one dose level below would be investigated. At least 6, but not more than 9 patients should be treated at the MTD. MTD was defined as dose level below the one with DLT in ≥2 patients and from which a de-escalation step was made.

Dose Limiting Toxicity (DLT) was defined as:

- Neutrofila <0.5 x 10^9/L for >5 days
- Febrile neutropenia (neutrofila <1.0 x 10^9/L and fever >38.5^{0}C)
- Platelets <25 x 10^9/L
- Diarrhoea grade ≥ grade 3 for >5 days despite optimal loperamide use
- In-field radiation dermatitis grade 4
- Cumulative dose intensity <70% of any delivered treatment components due to intolerance
- Other treatment induce adverse events >grade 3, except cetuximab related skin toxicity outside the RT field

Radiotherapy planning and treatment technique

RT techniques varied between institutions and changed during the study period from 2-field AP-PA to conformal methods with multiple fields.

Target volume definitions were based on CT scans. RT was delivered by linear accelerators with photon energy of 6 to 18 MV, 5 fractions per week, without break. The GTV included macroscopic primary tumor and lymph node metastases. Two CTV were delineated. CTV-t was created by adding a margin of 1.5-2 cm (3 cm in NOAC1) to the GTV. CTV-n included elective lymph nodes, depending on tumor

stage and localization, usually presacral, perirectal, internal iliacal, inguinal and sometimes external iliacal.

In the NOAC 8 protocol RT was delivered five days/week, without any planned gap. The total radiation treatment time should not excide 39 days. Patients were treated in a supine position using IMRT (2 patients) or VMAT (9 patients) with SIB. CT was used for dose-planning. The target volumes (GTV, CTV and PTV) were similar with those described above.

Evaluation of treatment

The tumor response was based on clinical evaluation consisted in digital rectal examination and palpation of inguinal lymph nodes. In the Nordic guidelines from 2000 CT scans were not mandatory. However, in the NOAC 8 trial a PET-CT was mandatory for evaluation of tumor response 3 months after the completion of CRT and the response was determined according to Response Evaluation Criteria in Solid Tumors (RECIST, version 3.0). In both cohorts the first clinical control was at 4-6 weeks after the end of the RT/CRT for evaluation of the acute side effects due to radiotherapy. The treatment outcome was first registered 3 months after the end of CRT. Patients without sign of tumor were followed-up every 3 months the first 2 years, then every 6 months up to 5 years. Patients in whom a local failure was diagnosed within 6 months after the completion of CRT/RT were classified as having persistent disease (residual) and were considered for salvage surgery. Patients who presented with a locoregional failure later than 6 months were classified as having recurrent tumor. Locoregional recurrence was defined as tumor recurrence in the pelvic or regional lymph nodes. Distant failure was defined as any distant metastases outside the pelvic or regional nodes, independent of locoregional status.

In addition, in the NOAC 8 trial patients were evaluated weekly and toxicity was assessed according to the Common Terminology Criteria for Adverse Events (CTCAE) version 3.0.

Statistical analysis

Descriptive statistics were used in all papers. Comparison between the treatment groups were performed using Student t-tests or Mann-Whitney U test for continuous variables and Chi-square or Fischer's test for categorical variables.

Recurrence was defined as the first event of any tumor relapse, locoregional or distant. LRR was defined as any tumor recurrence in the anorectal area or pelvic or regional lymph nodes. Distant failure was defined as any distant metastasis outside the pelvic and regional lymph nodes. Colostomy failure was defined as colostomy for progression, relapse or complication at the time of analysis.

OS was calculated from the date of diagnosis to death from any cause or last follow-up. RFS was defined as the period from the date of diagnosis to LRR, distant metastasis or death. Patients alive or lost to follow-up were censored. Survival analyses were made by Kaplan-Meier estimates and comparisons between different groups were made by log-rank test.

In paper I the RFS and OS analyses were performed in an "intention-to-treat" population (ITT) population, comprising all patients treated according to one of the predefined NOAC protocols, regardless of actual tumor stage and delivered treatment and in a "per protocol population" (PP), comprising only patients with correct tumor stage and adequate treatment given. For details see the paper I.

Uni- och multivariable analysis were performed with Cox regression proportional hazards models using variables including gender, age, primary tumor size, nodal involvement, distant metastasis, localization of tumor and given treatment in order to determine prognostic factors for recurrence and survival.

Cumulative time of relapse in paper III was defined as the time from primary diagnosis of anal cancer to occurrence of metachronous distant metastases.

In all papers, statistical significance was accepted at p-value ≤ 0.05.

The NOAC 8 trial was a non-randomized, multicentre phase I study with the main purpose of determining the MTD of 5FU and MMC in combination with cetuximab and RT by applying a pre-specified dose escalating schedule (design 3+3). The number of patients could not be determined beforehand. A minimum of 6 and a maximum of 21 would be included.

Statistics analyses were performed in cooperation with statistician Oskar Hagberg using SPSS version 22.0 (SPSS Inc Chicago, IL, USA) and R package, version 2.15.2 and 3.2.2.

Results and Discussion

Prognostic factors and treatment outcome in patients with SCCA (paper I)

The median follow-up was 4.2 years (range 0.1-9.1). The median age of patients treated within NOAC protocols was 63 years, with a female predominance (73%), 87% had WHO performance status 0-1, 51% had T1-T2 tumors and 32% had nodal involvement. There were 196 (15.5%) patients with a history of previous malignancies. A significantly higher than expected frequency of cancers of the cervix uteri (4x), vulva (12x) and lung (4x) was found, based on the prevalence of these cancers in the Nordic countries, matched by age and gender (Table 5). These results indirectly confirm the role of HPV infection and smoking as risk factors for SCCA, in accordance with previous published studies (2, 4, 11).

Table 5
Prevalence of previous malignancies in all 1266 patients with SCCA

Site of previous malignancy	Observed, n	Expected, n	RR*, (95% CI)
Lung	13	3.12	4.17 (2.42-7.18)
Vulva	10	0.82	12.20 (6.56-22.67)
Cervix uteri	16	3.92	4.08 (2.50-6.66)

*RR: Risk Ratio

The LRR rate was 17% and the distant metastases occurred in 11% of patients. In 4% of patients both LRR and distant recurrence were registered. Most LRR occurred in the primary tumor site and a high rate of inguinal recurrences (11%) was noted in the NOAC 1 cohort, most likely because that the prophylactic radiation of the inguinal lymph nodes had been omitted in the NOAC 1, while it was generally performed in NOAC 2-3. The role of prophylactic inguinal irradiation is controversial. Some authors suggest that elective lymph node irradiation can be safely omitted in T1N0 tumors (66, 67) while others reported higher inguinal recurrences if the irradiation of inguinal lymph node was not performed (69). Our results support the inclusion of inguinal lymph nodes in the prophylactic areas of lymph nodes irradiation in T1 tumors regardless of differentiation (70).

The rate of distant metastases was higher in the NOAC 4-5 groups consisting of patients with more advanced tumors than in the NOAC 1-3.

The 3-year RFS for patients with early SCCA treated according to NOAC 1, 2 and 3 was 70%, 67% and 76% respectively, with no significant differences between protocols. The 3-year OS was very similar between the three protocols, approximately 80% and the CFS was significantly better in NOAC 3 than in NOAC 2 (p=0.03) suggesting that 54 Gy RT with one cycle of 5FU/MMC might be better treatment for early tumors than 64 Gy alone.

The 3-year RFS for patients with locally advanced anal cancer, treated according to NOAC4 and NOAC 5 protocol was 63% and 64% respectively, with a tendency for better OS (p=0.065) in NOAC 5 suggesting that better results could be obtained with concomitant CRT. The CFS was significantly better in NOAC 5 compared to NOAC 4 (p=0.011) probably because of a higher proportion of patients who underwent pre-planned APR after CRT in NOAC 4.

According to multivariable analysis high age, male gender, large T, lymph node involvement, distant metastases, poor performance status and non-inclusion in a NOAC protocol were all independent factors associated with worse outcome (Table 6-7). This confirms previous findings published in several reports (82, 83, 110) .

Our results are similar with those reported in the randomized trials (56, 58) and indicate that concomitant CRT is superior to induction chemotherapy followed by RT alone for locally advanced SCCA. There are also evidence that combined CRT should be preferred to RT alone for early SCCA. The question regarding concomitant chemotherapy used, cisplatin or MMC could not be elucidated in our study, since very few patients with advanced cancers received MMC-containing chemotherapy. However, our results support the use of cisplatin/5FU with RT as a treatment alternative for locally advanced SCCA.

Generally, the survival rates of patients with SCCA are good, but locoregional recurrence is a major problem particularly in patients with locally advanced disease. New treatment approaches are needed to improve outcomes in these patients.

Table 6
Cox-proportional hazard regression for RFS according to patient, tumor and treatment characteristics

			Univariate		Multivariate	
	n. pats	**n.events**	**Hazard ratio (95% CI)**	**p**	**Hazard ratio (95% CI)**	**p**
Sex						
Male	349	182	1.46	<0.001	1.56	<0.001
Female	888	358	(1.33-1.60)		(1.28-1.90)	
Age						
≥ 65	582	321	1.91	<0.001	1.59	<0.001
< 65	654	218	(1.75-2.09)		(1.31-1.93)	
WHO						
1-4	393	256	2.82	<0.001	2.15	<0.001
0	696	217	(2.57-3.09)		(1.77-2.61)	
T stage						
T3-4	543	299	1.93	<0.001	1.26	0.0028
T0-2	668	224	(1.76-2.11)		(1.03-1.55)	
N stage						
N 1-3	388	211	1.68	<0.001	1.38	0.002
N0	849	329	(1.54-1.84)		(1.12-1.70)	
M stage						
M1	68	57	4.86	<0.001	3.15	<0.001
M0	1169	483	(4.22-5.61)		(2.27-4.36)	
NOAC protocol*						
No	362	227	2.21	<0.001	1.78	<0.001
Yes	874	313	(2.03-2.42)		(1.47-2.16)	

*treated according to any of the NOAC protocols

Table 7
Cox-proportional hazard regression for OS accordning to patient, tumor and treatment characteristics

			Univaraiate		Multivariate	
	n. pats	**n.events**	**Hazard ratio (95% (CI)**	**p**	**Hazard ratio (95% CI)**	**P**
Sex						
Male	349	142	1.49	<0.001	1.63	<0.001
Female	888	269	(1.35-1.66)		(1.31-2.05)	
Age						
≥65	582	261	2.31	<0.001	1.94	<0.001
<65	654	149	(2.09-2.56)		(1.54-2.43)	
WHO						
1-4	393	220	3.83	<0.001	2.77	<0.001
0	696	138	(3.44-4.28)		(2.21-3.47)	
T stage						
T3-4	543	246	2.32	<0.001	1.40	0.006
T0-2	668	152	(2.09-2.58)		(1.10-1.78)	
N stage						
N1-3	388	178	1.95	<0.001	1.58	<0.001
N0	849	233	(1.76-2.15)		(1.25-2.01)	
M stage						
M1	68	54	5.49	<0.001	3.49	<0.001
M0	1169	357	(4.74-6.37)		(2.48-4.90)	
NOAC prot*						
No	362	188	2.56	<0.001	1.89	<0.001
Yes	874	223	(2.32-2.82)		(1.51-2.36)	

*treated according to any of the NOAC protocols

Primary surgery of early SCCA (paper II)

From the NOAC database we identified 59 patients who underwent surgery alone (group S) and 34 patients who received postoperative RT/CRT after primary local excision (group S+RT/CRT). Our purpose was to study the locoregional recurrence rate and the survival outcome in these patients groups.

The majority of patients in the group S (88%) and all patients in the group S+RT/CRT underwent local excision. There was a significantly higher percentage of R1 and R2 resections (83%) in the S+ RT/CRT group compared to the S group, probably because the patients in the S+RT/CRT group had larger tumors at diagnosis. That could also explain the physicians' choice to treat these patients with postoperative RT/CRT. Despite small tumors and higher percentage of R0 resection in the S group the LRR rate was significantly higher after surgery alone compared to surgery followed by postoperative RT/CRT, 36% vs 9% (p=0.006). Most locoregional recurrences occurred in the anal region and all were seen in the S group whereas in the S+RT/CRT group the only recurrence occurred in the inguinal lymph nodes, where the majority did not receive prophylactic RT.

When analysing the S group by localization we found a LRR rate of 43% for tumors localized in the anal canal, but also a high LRR of 30% for tumors localized in the anal margin (Table 8).

Tabel 8
Locoregional recurrence in the surgery alone group

	Number of patients, n=59	Number of recurrences, n=22	%
T stage			
Tx	5	3	60
T1	38	12	32
T2	16	7	44
Localization			
Margin only	23	7	30
Canal	35	15	43
Unknown	1	0	0
Type of surgery			
Local excision	52	19	37
APR	7	3	43
Radicality			
R0	37	14	38
R1-2	17	5	30
Unknown	5	3	60
Histological differentiation	41	14	34
Well-moderately	5	2	40
Poorly-undifferentiated	13	6	46
Unknown			

When the analysis was restricted to patients who underwent local excision for small well differentiated perianal tumors ≤2 cm in size (T1N0) with a R0 resection, there were 11 patients and 4 of them recurred locally (36%). These data suggest that only local excision may be an inadequate treatment for a small ≤2 cm anal margin tumors and that local control can be improved with postoperative RT/CRT.

Our results are in line with other published studies which reported a high locoregional failure after local excision of small T1 deeply invasive anal margin tumors or T1-T2N0 anal canal cancers (36-38). Previous studies showed good results with local excision mainly in patients treated for superficially perianal lesions or carcinoma in situ for which local excision is still the treatment of choice. Moreover, the local control and survival were not better for patients treated with APR than those treated with local excision, results that had also been observed previously in a population-based study by Goldman et al (45).

The multivariable analysis showed that the addition of RT/CRT was the only factor with significant influence on RFS.

The RFS and OS were significantly better in patients treated with postoperative RT/CRT than in patients who did not (3-year RFS 84.2% vs 52.7% log-rank $p < 0.001$ and 3-year OS 87.2% vs 70%, log-rank $p=0.026$). Similar results have been reported in previous publications which showed good local control and outcome in patients who received postoperative RT/CRT after local excision for a small T1-T2N0 anal margin or anal canal cancer (46, 111, 112). The optimal RT dose in this setting is not known. However, based on the literature it seems that lower doses of 30-40 Gy could be sufficient after R0/R1 resection in order to prevent late toxicity. After R2 resection, indicating macroscopically residual tumor tissue, a higher RT dose of 50-60Gy could be necessary (112).

Our results indicate that the addition of RT/CRT improves the locoregional control and the survival outcome after surgery alone in early SCCA. In addition our results provides evidence for avoiding surgical treatment in patients with early SCC of the anal canal which is in line with international guidelines. Regarding surgery in early perianal cancer, our study showed a high LRR despite small tumors ≤2 cm (T1N0), well differentiated and R0 resection, which suggest that this issue merits further investigation.

Survival in metastatic SCCA (paper III)

The treatment outcome and the prognostic factors influencing OS in patients with metastatic SCCA was studied in 185 patients retrieved from the NOAC database, out of them 69 (37%) with synchronous and 116 (63%) with metachronous distant metastases.

The outcome in our cohort was poor with a median OS of 6.9 months and a 3-year OS rate of 14%. The median OS for untreated patients was only 3.2 months, whereas for those who received treatment against metastatic disease (chemotherapy, RT or surgery) was 11.4 months. The latter fits into the wide OS range between 8 and 34 months reported in previous small retrospective studies of patients treated with different chemotherapy regimens (25, 26, 113). The poorer OS in our study could be due to the fact that 42% of the patients did not receive any treatment for their metastatic disease.

There was a significantly better OS among patients in the synchronous group compared to patients in the metachronous group (8.3 vs 5.8 months, p=0.0048).

Regarding the patterns of metastasization the most frequent metastatic site was liver (43%), followed by lung (32%), extrapelvic lymph nodes (25%), bone (11%) and brain (2%) with a significant difference between the synchronous group compared to the metachronous group (p=0.007). There is a significantly higher proportion of liver metastases in the patients with synchronous disease, whereas in the patients with metachronous disease a higher incidence of bone metastases and unusual sites, e.g. peritoneum, abdominal wall, genitalia was observed. The reason for this difference is unclear and to the best of our knowledge, no previous studies have examined this issue.

In the metachronous group the median interval between diagnosis of the primary tumor and recurrence with distant metastases was 14.2 months and 89% of distant metastases occurred within the first three years. Our results showed that the rate of relapse is very low 3 years after the end of curative CRT. This is in line with the surveillance recommendations issued by ESMO guidelines and support the follow-up with CT scans for no longer that 3 years (31).

In our study male gender, metachronous disease, multiple metastatic sites and no treatment for metastatic disease are all independent prognostic factors for poor prognosis (Table 9). No significant association was found between initial T or N stage, histological differentiation or metastatic site and OS. We showed in the paper I that male gender was an independent prognostic factor for poorer OS and RFS in localized SCCA which in accordance with previously published results (82, 83). Male gender remains an independent prognostic factor for poorer OS also in metastatic disease. An explanation could be that a larger proportion of male patients

had HPV/p16 negative tumors, which is a negative prognostic factors in both localized and metastatic SCCA (13, 114). However, in our study HPV/p16 status is unknown because this test was not routinely performed in the study period.

Recent studies showed an impressive median OS of 53 months in patients with metastatic SCCA treated with multimodality treatment including surgical resection, radiofrequency ablation or definitive CRT of distant metastases which indicate that there is room for improvement of treatment for metastatic disease (25). Several case reports and phase II study showed promising results with both EGFR-inhibitors (e.g. cetuximab) and PD1-inhibitors (e.g pembrolizumab and nivolumumab) (94, 97-99, 115).

Table 9
Factors influencing overall survival, uni- and multivariable analyses

		Univariable		Multivariable	
	No. of patients	**RR**	**p value***	**RR**	**p value***
Gender					
Male	65	1	0.084	1	0.012
Female	120	0.752		0.649	
Age					
≤ 65 years	91	1	0.051	1	0.845
> 65 years	94	1.366		1.033	
Distant metastases					
Synchronous	69	1	0.005	1	<0.001
Metachronous	116	1.605		2.103	
Number of metastatic sites			0.006		0.009
One site	138	1		1	
Multiple sites	47	1.638		1.609	
Chemotherapy of DM					
No	96	1	<0.001	1	<0.001
Yes	89	0.379		0.327	
Radiotherapy of DM					
No	162	1	0.120	1	0.008
Yes	23	0.677		0.494	
Surgery of DM					
No	168	1	0.066	1	0.009
Yes	17	0.575		0.442	

*Cox proportional regression analysis; RR, risk ratio; DM, distant metastases

Cetuximab in combination with standard CRT in SCCA (paper IV)

Our purpose was to study if the addition of cetuximab to RT and 5FU/MMC is a tolerable combination and if so, to initiate subsequent studies to test whether this regimen would lead to improved local control and prolonged OS.

A total of 13 patients were included. Two patients discontinued cetuximab due to hypersensitivity reaction and were withdrawn from the study. The median follow-up was 22 months (range 12-27) and 85% of the patients had stadium IIIB.

The MTDs of 5FU/MMC in combination with cetuximab were determined as 5FU continuous infusion 800 mg/m2 on days 1-4 and 29-32 and MMC 8 mg/m2 on days 1 and 29 when combined with RT 57.5 Gy/27 fractions using SIB and weekly cetuximab. Dose-limiting toxicity (DLT) events occurred in 3 of 11 patients: febrile neutropenia, diarrhoea and thrombocytopenia (Table 10).

Table 10
Common Terminology Criteria for adverse events grade 3 and 4 toxicity in patients during treatment

	All patients, number of patients(%) n=11	
	Grade	Grade
	3	4
Radiation dermatitis	7 (63%)	0
Diarrhoea	4 (36%)	0
Genito/urinary	0	0
Rash	0	0
Thrombosis/embolism	1 (9%)	0
Anaemia	0	0
Neutropeni	3 (27%)	3 (27%)
Febrile neutropenia	1 (9%)	0
Thrombocytopenia	2 (18%)	1 (9%)

Three other phase I/II trials have investigated the combination of EGFR inhibitors (cetuximab or panitumumab) with chemotherapy, using cisplatin/5FU (106, 107) or 5FU/MMC (108) and they also reported a high frequency of grade 3-4 toxicities. In ACCORD 16 (107) trial the most common grade 3-4 toxicities were “general” (81%) and digestive (56%), whereas in the Olivatto et al study (106) the most common grade 3-4 were diarrhoea (44%) and neutropenia (17%). Both concluded that this combination is not feasible due to the high toxicity rate. The VITAL study by Feliu et al (108) enrolled 58 patients with T2-T4N0-3M0 and the most common grade 3-4 toxicities were radiation dermatitis (19%), diarrhoea (10%) and neutropenia (9%). They concluded that the addition of panitumumab to CRT was not efficient enough, with a 3-year OS rate of 78.4%.

Analysis of toxicity profile of our 11 evaluable patients showed that the most common grade 3-4 toxicity was radiation dermatitis (63%), hematologic toxicity (54%) and diarrhoea (36%). No treatment related deaths occurred. Concerning the hematologic toxicity our results were in line with previous published data from RTOG 98-11 trial (56) which also reported a high incidence of grade 3-4 myelotoxicity (61%). One explanation might arise from the administration of the MMC twice at day 1 and day 29 in our study, similar to RTOG 98-11, while MMC in the ACT II trial (58) was given only on day 1, yielding grade 3-4 haematological toxicity of only 26%. Another explanation could be the use of IMRT/VMAT with SIB in contrast with the conventional RT in the other studies. Some studies have reported a reduced myelotoxicity using IMRT (116), while others showed no difference in myelotoxicity with IMRT (72).

Radiation dermatitis is a common toxicity in patients with SCCA treated with CRT. To what extent the addition of cetuximab may have added to the radiation toxicity cannot be determined. Skin rash due to cetuximab was generally mild.

Despite the DLT observed in our study, the tumor control rates were encouraging. At three months control ten patients (91%) had local CR, but two patients had developed liver metastases, yielding a total complete rate of 73%. One possible explanation could be that the patients enrolled in our study had very advanced tumors with a median tumor size of 6 cm and 92% had lymph nodes involvement, which are negative prognostic factors (82).

Our conclusion was that the combination of cetuximab and standard CRT using IMRT/VMAT with SIB was a rather toxic regimen, but the acute side-effects were manageable.

Strengths and limitations

SCCA is a rare malignancy and the number of randomised trials are limited. Therefore results of studies from population-based series can add useful information to those obtained from randomized controlled trials. Our NOAC database is a large population-based cohort which gave us the opportunity to evaluate the treatment results and outcome in patients with SCCA in routine practice.

The main strength of this thesis is that the first three studies are based on a large unselected cohort of patients with SCCA, covering the vast majority of patients with this disease in the Nordic countries between 2000 and 2007. The size of the cohort allowed us to perform multiple comparisons between defined subgroups of patients, with regards to tumor stage and treatment strategies, that have not been previously described in the literature.

A limitation of the NOAC database is that data were collected retrospectively, which is always a possible source of error. Data were initially monitored by a study nurse and queries were sent out to sites if necessary, but we did not have the resources to perform a complete independent monitoring of all the data entered into the database. In study I, patients were mainly analysed according to the choice of treatment protocol. In some cases, deviations were noted regarding choice of schedule, tumor stage and actual treatment delivered. To "adjust" for this, data were analysed in two different ways, in an "intention-to treat" (ITT) population containing all patients treated according to one of the predefined NOAC protocols and in a "per protocol population" (PP), comprising only patients with correct tumor stage that had received "adequate treatment", according to predefined criteria. The results were only marginally different between those populations, indicating that the findings in the ITT population may be applied to the intended target population of each protocol.

Patients included in the NOAC database were treated between 2000 and 2007, before MRI and FDG-PET/CT had become widely used for staging of anal cancer. The only recommended radiological examination for tumor staging was a CT scan of the thorax and abdomen, which would be regarded as suboptimal by modern standards. Thus there is a risk that some of our patients were understaged, which should be recognized when interpreting our results.

Another limitation is that we did not collect information on tumor response after oncological treatments. Therefore we could neither determine the objective response rates after CRT in localized disease nor analyse PFS after chemotherapy of metastatic disease.

Regarding the comparison between outcomes in different treatments protocol, our findings must be interpreted with caution, since this was not a randomized study. Several factors, besides the given treatment, such as differences in age, gender distribution, comorbidity and treatment site may also have influenced the results.

Study II included patients who had undergone primary surgery, where evaluation of the resection margin was crucial. The radicality assessment was based on the original medical reports from the surgeon and pathologist and no pathological re-evaluation was performed, which is a potential weakness.

As opposed to the first three studies in the thesis, study IV was a prospective clinical trial, where data were collected rigorously during and after the treatment, which gives a much higher level of validity than can be achieved in retrospective studies. The main objective of the NOAC 8 trial was to determine MTD of 5FU/MMC when combined with RT and cetuximab. For this purpose we used a standard pre-defined dose escalation schedule, by which the MTDs were defined after treatment of 11 patients. From a pure methodological view this was sufficient, but with so few patients, conclusions must be drawn with great caution. We observed that 3 out of 11 patients developed early distant metastases, which is higher than expected. The reason for this is unclear, but it could be a coincidental finding due to small sample size.

Conclusions and clinical importance

The conclusions derived from our studies are as follows:

- A higher than expected prevalence of previous cervical and vulva cancer was observed emphasizing the common etiological factor of HPV infection
- A higher than expected prevalence of lung cancer was found indicating the role of smoking as risk factor for SCCA
- High age, male gender, large primary tumor, lymph node metastases, distant metastases, poor performance status and non-inclusion into a protocol were all independent factors associated with worse outcome
- The treatment results with these widely implemented guidelines were good, well in accordance with recently published randomized trials
- In early SCCA combined CRT with 54 Gy and one cycle of 5FU and MMC was at least as good as 64 Gy RT alone, indicating that one cycle of 5FU/MMC corresponds to approximately 10 Gy radiation
- A high incidence of inguinal lymph nodes recurrence (11%) was observed in patients with T1N0 well and moderately differentiated tumors treated without inguinal irradiation. Therefore, prophylactic inguinal irradiation should be recommended also for patients with T1N0 tumors
- Locoregional recurrence is significantly higher in patients with early SCCA treated with surgery alone, compared with patients treated with surgery followed by postoperative RT/CRT within 6 months
- The addition of postoperative RT/CRT was the only factor with significant influence on the RFS
- The median OS in patients with untreated metastatic SCCA was poor but was significantly improved with systemic chemotherapy
- Male gender, multiple metastatic sites, metachronous metastatic disease and no treatment for metastatic disease were independent prognostic factors associated with poor OS
- Our results support the surveillance with CT scans for no longer than 3 years after curative treatment of localized SCCA

- The combination of cetuximab with standard CRT using IMRT/VMAT with SIB was a rather toxic regimen, but the acute side-effects were manageable.
- The MTDs were determined as 5FU 800 mg/m^2 on RT days 1-4 and 29-32 and MMC 8 mg/m^2 on days 1 and 29 when combined with IMRT/VMAT with SIB and cetuximab in locally advanced SCCA

Future perspectives

SCCA is a rare malignancy but the incidence is steadily increasing. Treatment advancements during the last decades have led to improved survival, particularly in patients with early SCCA. However, new challenges with respect to prevention, diagnosis, accurate staging, therapy and survivorship need to be overcome. It is known that >85-90% of SCCA is associated with HPV infection. Therefore, vaccination to prevent HPV infection should be an effective way to reduce the incidence of SCCA (18). Vaccination against HPV, with the main purpose of preventing cervical cancer was implemented in Sweden around a decade ago, for girls in the school age. Besides prevention of cervical cancer, the HPV vaccination will probably lead to a decreased incidence of other HPV-related cancers, such as SCCA, but since the median age at diagnosis of SCCA is 65 years, it will take four to five decades before we see this effect.

Since anal cancer is associated with HPV infection and develops from a precursor lesion there may be a role for anal cancer screening in high-risk populations (e.g. HIV positive patients, MSM or transplant recipients), using anal Pap smears or high-resolution anoscopy in order to detect precancerous lesions (7).

A recently published registry study conducted in the US on patients with SCC of the anal canal ≤ 1cm reported that the use of local excision has increased over time, with good survival. They suggest that local excision is a valid treatment option for these patients (49, 117). Their conclusions are contradictory to our findings in study II where we noted a high local recurrence rate after surgery alone, leading to a recommendation that the majority of patients should be offered postoperative RT/CRT. However this treatment is associated with a substantial risk or late side-effects and some patients do not need the treatment. Future studies should aim at identifying subgroups of patients with early SCCA where local excision alone might be sufficient.

Standard treatment for localized SCCA consisting of definitive CRT is an effective treatment with a 5-year overall survival of 60-80%. However, many survivors suffer from late effects caused by RT, e.g. faecal incontinence, buttock pain, vaginal stenosis and impotence (85, 86). Therefore, it is of great clinical importance to optimize the RT with the purpose of reducing the late sequele, without compromising locoregional control, by e.g. using new and more conformal RT techniques such as IMRT/VMAT.

Our results showed a high rate of inguinal recurrences if elective RT of the inguinal lymph nodes was omitted, which is in line with previous studies. However, these studies were conducted before the modern staging era with MRI and PET/CT. Thus, the true proportion of positive lymph nodes in our study may have been higher. It may be that elective lymph node irradiation could be avoided in some patients with T1 tumors that are clearly N0 according to MRI and PET/CT. However this remains to be proven in future investigations.

Factors associated with poor prognosis include male gender, advanced tumor stage, p16 negative tumors and current smoking (6, 13, 82, 83). These patients may require intensified treatment. Even though RT dose escalation has not generally proven beneficial, it may be an attractive approach to investigate in patients with poor prognosis. This is supported by an extended dose-effect analysis from the NOAC database that was recently published (118).

Regarding treatment of metastatic SCCA universally accepted guidelines are largely lacking due to the rarity of the condition. In study III we found a poor prognosis with a median OS of 6.9 months in patients with metastatic SCCA treated between 2000 and 2007, partly reflecting a low treatment intensity. Forty-two % did not receive any treatment against their metastatic disease and only 9% were subjected to surgical metastasectomy. A recent study has shown a high median OS of 53 months in patients with metastatic SCCA treated with curative intention, including surgical resection and other local ablative methods of oligometastases (25). These results support an aggressive approach in some patients with limited metastatic disease, but a number of issues remain to be investigated, regarding e.g. treatment sequence and choice of chemotherapy: 1) Should induction chemotherapy be used or should one start with CRT upfront in patients with synchronous metastatic SCCA? 2) What is the optimal chemotherapy in this situation, 5FU/MMC or cisplatin/5FU? 3) When should the metastases be resected? Directly after completion of CRT? After complete regression of the primary tumor? 4) Is there a role for postoperative chemotherapy in this setting? 5) Which patients benefit from aggressive treatment of metastatic disease? All these issues need to be addressed in future studies.

Another track of development is to explore new drugs. Since EGFR is usually overexpressed in SCCA, incorporation of EGFR inhibitors seems logical. The addition of anti-EGFR agents to standard CRT has been tested recently, both by us (study IV) and other investigators. The general conclusion from these is that the addition of EGFR inhibitors in this setting seems to add toxicity without any obvious improvement of antitumoral effect, indicating that the future of these drugs as radiosensitizer in SCCA is questionable. However EGFR inhibitors may still have a role in the treatment of metastatic SCCA, as suggested by early reports, but further studies are needed.

SCCA is associated with HPV infection and is believed to often be immunogenic and thereby susceptible to immunotherapy. Lately several agents targeting immune checkpoints, such as PD-1, have been proven highly effective in subsets of patients with malignant melanoma, lung cancer and renal cell cancer. In metastatic SCCA, phase II studies on pembrolizumab and nivolumab have shown promising efficacy (98, 99). Several studies are ongoing to further investigate PD-1 inhibitors against SCCA, both in metastatic disease and integrated with CRT for localized disease.

Populärvetenskaplig sammanfattning (Summary in Swedish)

Optimering av analcancerbehandling

Analcancer är en ovanlig cancersjukdom och utgör 2-2,5 % av alla gastrointestinala tumörer. I Sverige diagnostiseras ca 150 fall/år. Sedan 1970-talet har incidensen ökat stadigt sannolikt pga en ökad förekomst av HPV-(humant papilloma virus) infektion, framförallt HPV 16 och 18 vilka har en central etiologisk roll för utveckling av analcancer. Histologin är huvudsakligen skivepitelcancer. Medianålder vid insjuknande är 65 år och incidensen är högre hos kvinnor.

Historiskt har behandlingen varit kirurgisk, vilket i de flesta fall innebär rektumamputation med permanent stomi. Behandlingsstrategin har ändrats under de senaste decennierna och kirurgisk behandling har ersatts av radioterapi (RT) kombinerad med kemoterapi (radiokemoterapi, CRT), vilket idag utgör standardbehandling av analcancer. Kirurgi sparas till ”salvage-situationer”, dvs. om tumören inte går i komplett remission på CRT eller vid lokalt återfall.

Randomiserade fas III-studier har visat att CRT är bättre än enbart RT, att Mitomycin C (MMC) är bättre än cisplatin, att neoadjuvant eller adjuvant cytostatikabehandling, underhållsbehandling med cytostatika eller ökning av strålbehandlingsdosen inte förbättrar resultaten. Standardbehandling av analcancer är därför RT kombinerad med cytostatika i form av 5fluorouracil (5FU) och MMC. Behandlingen är relativt effektiv med en 5-årsöverlevnad på 60-80%, men ca 25-30% av patienterna recidiverar locoregionalt och 10-20% med fjärrspridning.

Analcancer är således en tumörform som i stor utsträckning kan botas med icke-kirurgisk behandling. Dock är det efter RT mot bäckenet vanligt med sequele, som analinkontinens, blödningar, sexual dysfunktion och smärttillstånd i bäckenet. Därför finns behov att förbättra behandlingen av analcancer och om möjligt minska förekomst av sena biverkningar.

Epidermal tillväxtfaktorreceptorn (EGFR) är ofta överuttryckt i analcancer och därför förefaller det logiskt att undersöka effekten av EGFR-hämmare vid denna malignitet. Ett sådant läkemedel är cetuximab, vilket är en antikropp riktad mot EGFR. cetuximab i kombination med RT har visat sig förbättra överlevnaden hos

patienter med skivepitelcancer i huvud-hals området, jämfört med enbart RT. Erfarenheten av cetuximab vid behandling av analcancer är begränsad, men resultaten från fas I studier och case reports har visat lovande behandlingsresultat.

CRT utgör standardbehandling av analcancer för majoriteten av patienterna, förutom för dem med små högt differentierade tumörer < 2cm lokaliserade perianalt, där internationella riktlinjer rekommenderar lokal excision. Vid icke-radikal resektion föreslås re-excision eller postoperativ radiokemoterapi. Den vetenskapliga grunden för dessa rekommendationer är dock mycket begränsad.

Behandling av generaliserad analcancer är otillräckligt studerat och pga den låga frekvensen av tillståndet saknas randomiserade studier. Befintliga riktlinjer är baserade på små retrospektiva studier.

Det övergripande målet med detta avhandlingsarbete är att förbättra behandlingen av analcancer, genom att analysera en stor nordisk populationsbaserad kohort och utforska en ny behandlingsstrategi i en prospektiv fas I studie.

De särskilda målen är:

1. Att analysera prognostiska faktorer och behandlingsresultat i en stor patientkohort med analcancer, behandlad enligt nordiska riktlinjer
2. Att studera lokoregionalt recidiv efter lokal excision av en tidig analcancer med eller utan tillägg av postoperativ RT eller CRT
3. Att analysera prognostiska faktorer och överlevnad hos patienter med synkron resp. metakron generaliserad analcancer.
4. Att studera biverkningar och tolerans med tillägg av cetuximab som tillägg till standard CRT i en prospektiv fas I-studie (NOAC 8), en behandlingsprincip som inte hade testats tidigare

Avhandlingsarbetet bestod av fyra projekt enligt ovan.

De första tre arbetena baserades på en retrospektiv kohort bestående av 1266 patienter med skivepitelcancer i anus, behandlade enligt nordiska riktlinjer mellan 2000 och 2007. Dessa riktlinjer utfärdades av Nordic Anal Cancer Group (NOAC) i slutet av 1990-talet. Sexton onkologiska kliniker från Norge, Danmark och Sverige deltog. Det fanns sju olika behandlingsprotokoll för olika tumörstadier. Huvudprincipen i dessa protokoll var att ge enbart RT till små tumörer och CRT till stora tumörer. Uppgifter om tidigare maligniteter, tumörstadium, behandling, recidivmönster och överlevnad insamlades retrospektiv från patienternas journal.

Totalt 1266 patienter inkluderas och 886 av dem behandlades inom något av NOAC protokollen. Täckningsgraden var god, i Norge och Sverige låg den på >90%, varför kohorten väl avspeglar patienter med analcancer som hanterats i rutinsjukvården.

De viktigaste resultaten from de första tre arbetena var:

1. Bland analcancerpatienterna sågs en klart ökad risk för tidigare förekomst av cervical- (4x) och vulvacancer (12x), vilket stämmer väl med en gemensam etiologisk faktor i form av HPV-infektion
2. För de patienter som behandlades enligt de föreslagna protokollen, var 3-årsöverlevnaden för dem med små tumörer T1-T2 ca 80 % och för dem med mer avancerade tumörer (T3-T4 eller med lymfkörtelmetastaser) ca 70 %, väl i linje med nyligen publicerade randomiserade studier. Detta visar att man kan uppnå goda behandlingsresultat i rutinsjukvården med hjälp av nordiska terapiriktlinjer
3. Recidivrisk och överlevnad i patientgruppen med små tumörer behandlade med CRT med 54 Gy och en cykel 5FU/MMC var minst lika bra som i patientgruppen behandlade med enbart RT 64 Gy vilket indikerar att 1 cykel 5FU/MMC motsvarar ungefär 10 Gy
4. En hög förekomst av inguinalt lymfkörtelrecidiv (11 %) observerades hos patienter med små tumörer (<2cm) där elektiv inguinal lymfkörtelbestrålning inte gavs. Därför rekommenderas numera inguinal profylaktisk lymfkörtelbestrålning även för patienter med små tumörer
5. Hög ålder, manligt kön, stor primärtumör, lymfkörtelmetastaser, fjärrspridning, nedsatt allmäntillstånd och behandling utanför protokoll var alla oberoende faktorer för sämre prognos
6. Lokoregionalt recidiv är signifikant högre hos patienter med tidig analcancer behandlad med enbart lokal excision jämfört med patienter som behandlades med kirurgi följt av postoperativ RT eller CRT
7. Medianöverlevnaden hos patienter med obehandlad generaliserad analcancer är dålig, men kan förbättras med systemisk cytostatikabehandling
8. Manligt kön, multipla fjärrmetastaslokaler, metakron sjukdom och ingen behandling för generaliserad sjukdom var oberoende prognostiska faktorer för dålig prognos vid generaliserad analcancer
9. Vid metakron metastaserad analcancer diagnostiserades fjärrspridningen i 89 % av fallen inom de första 3 åren efter avslutat kurativ CRT, vilket stödjer att röntgenkontroller inte behöver fortgå längre än 3 år efter avslutat kurativ behandling för lokaliserad sjukdom

Det fjärde arbetet, NOAC 8 studien, inkluderade patienter med lokal avancerad analcancer från tre onkologkliniker (Lund, Uppsala och Oslo) i en multicenter prospektiv fas I-studie för att testa tillägg av cetuximab till standard CRT. Det

primära syftet var ett bestämma den maximalt tolererade dosen (MTD) av cytostatikabehandling 5FU/MMC, med hjälp av ett standardiserat doseskaleringsschema, där de första patienterna erhöll reducerad dos, som sedan justerades för efterföljande patienter baserat på biverkningarna. Totalt 13 patienter inkluderades mellan 2012 och 2014. De vanligaste grad 3-4 biverkningarna var stråldermatit (63 %), benmärgspåverkan (54 %) och diarré (36 %). MTD för 5FU/MMC i kombination med RT och cetuximab fastställdes till 5FU 800mg/m^2 dag 1-4 och dag 29-32 och MMC 8mg/m^2 dag 1 och dag 29. Tio patienter hade komplett lokalt respons (91 %), men två av dem fick tidig levermetastasering, vilket gav en total komplett responsrat på 75 %. Vår slutsats var att denna kombination var en ganska toxisk behandling, men de akuta biverkningarna var hanterbara.

Sammanfattningsvis har våra analyser av en stor populationsbaserad kohort av patienter med analcancer, lett fram till flera kliniskt betydelsefulla resultat, som påverkat handläggningen av dessa patienter i klinisk praxis.

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Improved oncological treatment of anal cancer

Otilia Leon is a medical oncologist working at Skåne University Hospital in Lund, Sweden.

Her clinical area is treatment of patients with gastrointestinal cancers. The overall aim of this thesis was to improve the treatment outcome of patients with anal cancer by analysing a Nordic population-based cohort and to explore a new treatment strategy in a prospective phase I study.

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