DEPARTMENT OF ONCOLOGY AND PATHOLOGY
Karolinska Institutet, Stockholm, Sweden

OUTCOME OF PREOPERATIVE RADIOTHERAPY IN THE TREATMENT OF CERVICAL CANCER WITH FOCUS ON PROTEIN EXPRESSION AND DOSIMETRY

Catharina Beskow

Karolinska Institutet

Stockholm 2011

Published by Karolinska Institutet.

ISBN 978-91-7457-213-1

Printed by Reproprint AB
Gårdsvägen4, 169 70 Solna
www.reproprint.se

Carpe diem

ABSTRACT

Treatment with preoperative intracavitary radiotherapy (ICRT) is used in cervical cancer with the intention to reduce the tumour burden and sterilize microscopic disease in the paracervical tissues. Preoperative ICRT is, however, a controversial treatment regime since it is unclear if addition of ICRT to surgery improves treatment outcome compared to surgery alone. This question constitutes the basis of this thesis. In relation to this issue we have studied clinical, dosimetric and molecular key factors with focus on DNA damage repair signalling molecules of possible importance for radiotherapy sensitivity.

One possible way to investigate the potential benefit of preoperative ICRT is to analyze whether complete tumour remission in the surgical specimen after preoperative ICRT is correlated to treatment outcome or not. In paper I we analyzed treatment results after preoperative ICRT in patients with cervical cancer stage IB-IIA. We found a strong correlation between pathologic complete remission (pCR) and survival with a 5-year survival of 95% in patients with pCR compared to 46% in patients with residual tumour (non-pCR) ($p<0.0001$). These results indicate that the use of preoperative ICRT may contribute to treatment outcome compared to surgery alone.

DNA double strand breaks (DNA DSBs) and their repair has in tumour cell lines been linked to radiosensitivity. In paper II we therefore analyzed the expression of proteins related to the DNA-PK repair pathway; DNA-PKcs, Ku70, Ku86, p53, p21 and Mdm-2 in pre-treatment tumour tissue with the aim to find predictive markers for radiotherapy response. Our hypothesis was that high DNA repair capacity in the primary tumour, reflected by a high frequency of cells positive for DNA-PK proteins, would correlate with non-pCR cases. However, we did not find that any of the analyzed proteins were predictive for radiotherapy response. Our hypothesis in paper III was that residual tumours that survived radiotherapy would display a higher frequency of DNA-PK positive cells reflecting a higher capacity to DNA DSB repair compared to their corresponding primary tumours. The expression of DNA-PKcs, Ku70, Ku86, p53, p21 and Mdm-2 proteins was compared in pre- and post-treatment tumour tissue. We found that residual tumours showed an increased frequency of tumour cells positive for DNA-PK complex proteins compared to the frequency in the primary tumour. This result may be interpreted as that radiation causes a selection pressure allowing tumour cells with high DNA-PK expression to survive RT.

Biological effective dose (BED) can be used to predict the influence on outcome of different treatment schedules for individual patients. In paper IV we evaluated BED with respect to survival, local control and late toxicity in patients treated either with radiotherapy and surgery or with radiotherapy alone. We found a correlation between BED and treatment outcome for patients treated with radiotherapy alone but not for patients treated with radiotherapy and surgery. No correlations were found between BED and late toxicity from bladder and rectum.

In conclusion this thesis illustrates that treatment with preoperative intracavitary radiotherapy may be beneficial for patients with cervical cancer stage IB-IIA. We found that the expression of the DNA-PK complex proteins in primary tumour cannot predict RT response. We did, however, find that the frequency of the DNA-PK complex proteins is higher in residual tumour after ICRT than in corresponding primary tumour. BED cannot be used as a predictive factor for the outcome in patients treated with pre-and postoperative radiotherapy or for the late side effects but does correlate with local control of the tumour and survival in patients treated with RT alone.

LIST OF PUBLICATIONS

I. **Catharina Beskow**, Anna-Karin Ågren-Cronqvist, Fredrik Granath, Bo Frankendal and Rolf Lewensohn. Pathologic complete remission after preoperative intracavitary radiotherapy of cervical cancer stage Ib and IIa is a strong prognostic factor for long-term survival: analysis of the Radiumhemmet data 1989-1991 *International Journal of Gynecologic Cancer 2002;12:158-170*

II. **Catharina Beskow**, Lena Kanter, Åsa Holgersson, Bo Nilsson, Bo Frankendal, Elisabeth Åvall-Lundqvist and Rolf Lewensohn. Expression of DNA damage response proteins and complete remission after radiotherapy of stage IB-IIA of cervical cancer *British Journal of Cancer 2006;94:1683-1689*

III. **Catharina Beskow**, Jurate Skikuniene, Åsa Holgersson, Bo Nilsson, Rolf Lewensohn, Lena Kanter and Kristina Viktorsson. Radioresistant cervival cancer shows upregulation of the NHEJ proteins DNA-PKcs, Ku70 and Ku86 *British Journal of Cancer 2009;101:816-821*

IV. **Catharina Beskow**, Anna-Karin Ågren-Cronqvist, Rolf Lewensohn and Iuliana Toma-Dasu. Biological effective dose evaluation and assessment of rectal and bladder complications for cervical cancer treated with radiotherapy and surgery *Manuscript*

LIST OF ABBREVIATIONS

3D	3-dimensional
AKT	v-akt murine thymoma viral oncogene homolog
ATM	ataxia-telangiectasia-mutated
BED	biological effective dose
BT	brachytherapy
CDK	cyclin dependent protein kinase
DNA DSB	DNA double strand break
DNA-PK	DNA dependent protein kinase complex
DNA-PKcs	catalytic subunit of DNA-PK
EGFR	epidermal growth factor receptor
EBRT	external beam radiotherapy
FEN-1	Flap endonucleases 1
FIGO	Federation Internationale de Gynecologie et d´Obstetrique
Gy	Gray (SI-unit for absorbed energy per mass-unit)
HDR	high dose rate
HPV	human papilloma virus
HR	homologous recombination
ICRT	intracavitary radiotherapy
IGFR	insulin-like growth factor receptor
IHC	immunohistochemistry
IMRT	intensity-modulated radiotherapy
LQ	linear quadratic
LDR	low dose rate
Mdm-2	Mouse double minute 2 protein
MDR	medium dose rate
Mghr	milligram-hours of radium
MRI	magnetic resonance imaging
NHEJ	non-homologous end joining
Non-pCR	non-pathologic complete remission
pCR	pathologic complete remission
PCR-SSCP	PCR-single-strand conformation polymorphism
PDR	pulsed dose rate
Rb	retinoblastoma protein
RT	radiotherapy
XLF	XRCC4-like factor
XRCC4	X-ray complementing Chinese hamster gene 4

CONTENTS

1 INTRODUCTION

Gynaecologic cancer has been treated by irradiation for more than a century since the discovery of radioactivity by Becquerel 1896 and the extraction of radium 1898 by Marie Curie. Radiotherapy (RT) can be delivered as external beam irradiation or as brachytherapy (BT) where the radioactive source is placed in near vicinity to the tumour. In cervical cancer BT is most often given as intracavitary radiotherapy (ICRT) with the radioactive source placed in the uterus and in the vagina. For the treatment of cervical cancer RT can be used as a single modality or in combination with surgery either as preoperative or postoperative treatment. Treatment with preoperative ICRT for early stage cervical cancer is questioned since it is unclear if BT in the preoperative setting contributes to treatment outcome. One possible way to study this question is to investigate whether complete tumour remission in the surgical specimen after preoperative ICRT is correlated to treatment outcome or not. In this thesis the role of pathologic complete remission (pCR) for survival and local control after treatment preoperative ICRT has been studied.

Although RT is a well established method in the treatment of cervical cancer not all cases respond properly to such treatment. The underlying molecular processes that are causative for the lack of proper RT response in the individual patient are not fully elucidated. On the cellular level, the primary action of ionising radiation is the formation of DNA-damage of which DNA double-strand breaks (DNA DSBs) are considered the most severe. Within cells, DNA DSBs are predominantly repaired by the non-homologous end joining (NHEJ) repair system in which the DNA dependent protein kinase complex (DNA-PK) has a central function. With the aim to find predictive markers for RT response we have in the current thesis analyzed the expression of proteins related to DNA-PK repair pathway; DNA-PKcs, Ku70, Ku86, p53, p21 and Mdm-2 in pre- and post-treatment tumour tissue.

Radiotherapy can be delivered with treatment schedules that differs in fraction size, dose rate and treatment time. When planning radiation treatment for the individual patient it is important to consider these parameters. Moreover, the effect of irradiation differs between tumour and normal tissue. The linear quadratic model is a mathematic model that can adjust for those above mentioned dosimetric parameters as well as for variations in the radiobiological effect on different tissues. In this thesis we have studied the prognostic value of the biological effective dose (BED), calculated according to the linear quadratic model, with respect to survival, local control and late toxicity in patients treated either with radiotherapy and surgery or with radiotherapy alone.

The present thesis is based on a retrospective analysis of patients with cervical cancer stage IB-IIB treated during 1989-1991 at the Department of Gynaecologic Oncology at Radiumhemmet. We have focused on the role of pathologic complete remission (pCR), molecular markers and radiobiological mathematical modelling as prognostic factors for the outcome of preoperative ICRT with the purpose to better tailor the treatment strategy for patients with cervical cancer.

1.1 BACKGROUND CERVICAL CANCER

1.1.1 Epidemiology

Cervical cancer is the third most common cancer globally among women with an estimated 529 000 new cases 2008[1]. Approximately 85% of the worldwide burden of this tumour disease occurs in the developing countries. In Sweden the incidence of cervical cancer has decreased throughout the last fifty years most likely attributed to the organised cytological screening programmes[2]. However, during the last decade the incidence has been rather stable with about 460 new cases yearly[3].

1.1.2 Etiology

Persistent genital infection with oncogenic types of human papilloma virus (HPV) is the main risk factor for developing cervical cancer[4]. HPV is found in almost all invasive cervical cancers and the most frequent oncogenic types are HPV 16 and 18[5 6 7]. HPV infection is a common, sexually transmitted disease and the majority of HPV infections heal spontaneously[8]. Only very few women with HPV infection develop cervical cancer, implying the involvement of other etiologic cofactors in cervical carcinogenesis. Factors such as smoking, use of oral contraceptive, early age at sexual debut and immunosuppressive medication has all been associated with increased risk of cervical carcinoma[9 10 11]. The introduction of HPV vaccines has shown to be effective in prevention cervical intraepithelial neoplasia and will hopefully lead to reduced incidence of cervical cancer in the future[12].

1.1.3 Staging

Staging of cervical cancer is based on a clinical examination performed during anaesthesia according to definitions from “Federation Internationale de Gynecologie et d´Obstetrique (FIGO). These guidelines have continuously been revised and the current definitions are presented in Table 1[13]. It may be noted that according to the FIGO guidelines, the staging is not governed by the lymph node status although it is an important prognostic factor (see 1.1.5). Patients with stage IB-IIB are studied in this thesis illustrated in Figure 1.

Table 1.Clinical stages of cervical cancer according to FIGO *

Stage I	The carcinoma is strictly confined to the cervix
IA1,IA2	Invasive carcinoma is identified microscopically, with deepest invasion ≤ 5.0 mm and largest extension ≤ 7.0 mm
IB	Clinically visible lesions limited to the cervix uteri or pre-clinical cancers greater than stage IA
IB1	Clinically visible lesion ≤ 4.0 cm in greatest dimension.
IB2	Clinically visible lesion > 4.0 cm in greatest dimension
Stage II	The tumour extends the cervix, but not to the pelvic wall or to the lower third of the vagina
IIA	Without parametrial invasion
IIA1	Clinically visible lesion ≤ 4.0 cm in greatest dimension
IIA2	Clinically visible lesion > 4.0 cm in greatest dimension
IIB	With obvious parametrial invasion
Stage III	The tumour extends to the pelvic wall and/or involves lower third of the vagina and/or causes hydronephrosis or non-functioning kidney
IIIA	Tumour involves lower third of the vagina, with no extension to the pelvic wall
IIIB	Extension to the pelvic wall and/or hydronephrosis or non-functioning kidney
Stage IV	The carcinoma has extended beyond the true pelvis or has involved the mucosa of the bladder or rectum.
IVA	Spread of the growth to adjacent organs
IVB	Spread to distant organs

*FIGO Federation Internationale de Gynecologie et d´Obstetrique[13].

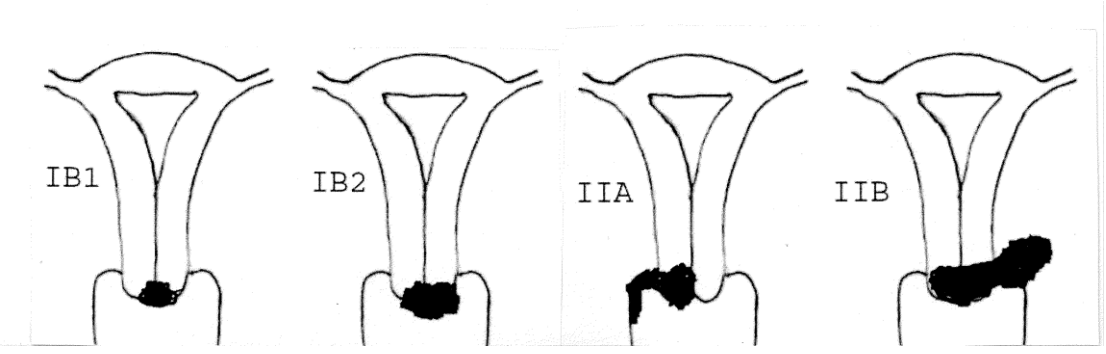

Figure 1*. Schematic picture of cervical cancer stage IB-IIB studied in this thesis.*

1.1.4 Histology

Squamous cell carcinoma is the most frequent type of cervical carcinoma and accounts for approximately 75-90 % of the cases. Over the past 50 years there have been an increase in the relative proportion of cervical adenocarcinoma in Sweden and other western countries[14 15]. The cause of this increase is indefinite but one explanation suggested is that the current screening methods are less effective in detecting adenocarcinomas relative to squamous cell cancer[14 16].

1.1.5 Survival and prognostic factors

The outcome of patients with cervical cancer is closely related to tumour stage. Thus, the 5-year survival in stage IA is almost 100% compared to approximately only 20% in stage IVA[17]. For patients in stage IB and IIA 5-year survival rates of 60-89% and 60- 85% respectively, has been demonstrated[18 19 17]. Other important prognostic factors are tumour size and nodal status[18 20 21 22 23 17]. Furthermore, histopathologic factors revealed at surgery such as deep invasion of the cervical stroma and tumour invasion of the capillary-lymphatic space have shown to correlate with poor survival[24 25 26 27].

1.1.6 Treatment

Stage IB and IIA cervical cancer can be cured by surgery or by definitive radiotherapy[19 28 17]. The therapeutic strategy is governed by operability of the individual patient and by presence of bad prognostic factors. Primary radical surgery is the standard treatment for early stages of the disease. Postoperative adjuvant RT has shown to prolong progression-free survival and significantly reduce the risk of recurrences for patients with unfavourable prognostic factors found at surgery (see 1.1.5)[29 30]. Recent reports have suggested concomitant chemotherapy with radiotherapy (chemoradiotherapy) in this situation[31].

Chemoradiotherapy represents the current standard of care in patients diagnosed with locally advanced disease (tumours > 4cm) stage IB and IIA[32 33 34 35]. Chemoradiotherapy is also the standard treatment for patients with more advanced stages of cervical cancer (IIB-IVA)[36].

Preoperative intracavitary radiotherapy (ICRT) has been reported as an efficient therapeutic option for early stage cervical cancer but remains controversial[37 38 39 40 41 42]. Thus, it is still unclear if ICRT can improve the treatment results as compared to primary surgery. A randomised study between primary surgery and preoperative ICRT would possibly give answer to that question.

1.1.6.1 Surgery

Standard radical surgery for early stage cervical carcinoma comprise radical hysterectomy with complete resection of the parametrium and the paracervical tissue, pelvic lymphadenectomy and resection of the proximal portion of the vagina[43]. Radical surgery may cause long-term side effect such as lymphoedema and bladder dysfunction[44]. Development of nerve sparing surgical techniques has shown to be beneficial in reducing postoperative morbidity[45].

1.1.6.2 Radiotherapy

Definitive RT for cervical cancer includes external beam radiotherapy (EBRT) and intracavitary radiotherapy (ICRT). The use of ICRT is reported to be one of the most important treatment factors with respect to local control and survival in patients treated with definitive RT[46]. The pelvic field for EBRT in cervical cancer usually includes the internal and external iliac nodes and the lower common iliac nodes up to the level of the space between lumbar vertebra L4 and L5. The treatment is ordinarily delivered with a daily fraction of 1.8-2.0 Gy (except for week ends) over a period of 5-6 weeks. The total prescribed radiation dose for definitive EBRT in combination with ICRT differs depending on tumour extension and individual clinical factors. A total central dose of ≥ 80 Gy is often considered to be needed for local control of the tumour[47]. On the other hand, the incidence of serious side-effects from the rectum and bladder is reported to increase for doses ≥ 70-80Gy[48 46 49]. New techniques for delivering EBRT such as intensity-modulated radiation therapy (IMRT) have made it possible to reduce the dose to the normal tissue in the pelvis, especially the small intestine, and thereby opens for an increase of the total prescribed dose[50 51 52].

1.1.6.3 Brachytherapy

The first brachytherapy (BT) treatment in Sweden of carcinoma of the cervix was performed in 1910 by Dr Gösta Forsell and the technique was described 1914[53]. During the following decades different treatment schedules for intracavitary radiotherapy of cervical cancer developed such as the Paris, Manchester and Stockholm systems which differed in applicator design, in the geometry of the insertion and packing technique as well as in the treatment time[54]. After the discovery of artificial radioactivity by Irene Curie and Frederick Joliot in 1934 new highly radioactive sources were developed such as iridium (^{192}Ir) and cesium (^{137}Cs). Treatment with wire sources lead to the development of flexible afterloading applicators which made it possible to modify the shape of the isodose surfaces thus improving the quality of treatment. The remote after-loading technique was first described by Walstam 1962[55]. With this technique the radioactive sources are operated from a control panel distant from the patient thus eliminating the exposure of radioactivity to the staff.

During the "radium era" the dosage was most often quoted in milligram-hours of radium (mghr) which was defined as the product of the total radium content in the applicator and the treatment time. A new dosimetric system for cervical cancer was introduced by the Manchester system[56]. In this system, the dose was prescribed and reported at a selected reference point "Point A" located 2 cm lateral of the central axis of the uterus and 2 cm above the vaginal surface (Figure 2). Point A, as a reference point for dose prescription is still widely used.

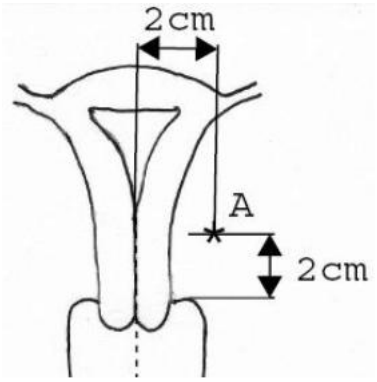

Figure 2*. The dose reference "Point A" is located 2 cm lateral of the central axis of the uterus and 2 cm above the vaginal surface.*

When using BT it is of great importance to cover the complete tumour volume in order to achieve local tumour control. The introduction of magnetic resonance imaging (MRI) has made it possible to more properly assess the tumour size and configuration. The development of three-dimensional (3D) treatment planning systems based on MRI has allowed an individual adaptation of dose distribution to the tumour volume for cervical cancer ICRT[57 58]. 3D treatment planning has proven to increase local tumour control parallel to low treatment related morbidity for patients with locally advanced cervical cancer[59].

BT can be delivered with different dose rates which are determined by the radioactivity in the source used. Dose rate definitions according to ICRU report no 38[60] are;

Low dose rate (LDR):	0.4-2Gy/hr
Medium dose rate (MDR):	2-12Gy/hr
High dose rate (HDR):	>12Gy/hr

Pulsed dose rate (PDR) is a technique that has been developed during recent years, which delivers the HDR in a large number of small fractions aiming at a radiobiological effect similar to LDR (see below 1.3).

Changes in dose rate will affect various tissues in different ways (see below 1.3). Historically, LDR treatment has been considered as the dose delivery with the most optimal therapeutic window for cervical carcinoma. However, several reports on long-term results of HDR in cervical cancer points towards comparable results as with LDR with respect to survival and late complications[61 62 63].

1.1.6.4 Treatment related side-effects after radiotherapy

RT induced normal tissue toxicity is characterized by acute and late reactions. Acute reactions, with symptoms usually appearing within two to three weeks after initiating RT, originate from tissues with rapidly proliferating cells such as intestinal mucosa and bone marrow. Late reactions which occur months or years after completion of RT represent damage to slowly reacting tissue, resulting in fibrosis, vascular damage and necrosis. Symptoms of serious late toxicity after pelvic and intracavitary irradiation for cervical cancer may be bleeding from the bowel, bowel stricture, urethral stenosis and fistulas between vagina and bladder or rectum[64 65]. RT related factors such as the total dose, the fractionation schedule and the irradiated tissue volume are of importance for the risk of developing side effects[66 49]. Also patient related factors such as previous surgery[64 66], inflammatory bowel disease [67], smoking [68]and co-morbidity such as hypertension and diabetes have been reported to increase the risk for late complications[69].

1.2 RADIOTHERAPY ON CELLULAR AND MOLECULAR LEVEL

1.2.1 DNA repair and non-homologous end joining (NHEJ)

When cells are irradiated by X-rays various DNA damages occur. DNA double strand breaks (DNA DSBs) are considered to be the most difficult ones to repair among them. Two distinct pathways for DNA DSB repair are found in eukaryotes, homologous recombination (HR) and non-homologous end joining (NHEJ)[70]. Since HR makes use of the sister chromatid as a template it is most active under late S and G2 phases of the cell cycle when such a template is available. Moreover, as HR utilizes the non-damaged DNA copy as a template for repair it is considered less error prone than NHEJ[71]. A large number of proteins are involved in the different steps of HR such as RAD51, RAD54 and BRCA1 and BRCA2[70].

In mammalian cells DNA DSBs formed in cells in G0 and G1 phases of the cell cycle are repaired by NHEJ[72]. It is therefore not surprising that mutations affecting the NHEJ pathway severely impair the repair of DNA DSBs and results in increased cell death upon irradiation[73 74]. By utilizing the NHEJ repair pathway, the cell may repair the DNA lesions efficiently but in an error prone way since no homologues DNA strand is available that can be used as a template[75]. The main proteins required to repair DNA DSB by NHEJ are DNA-PK, XRCC4 (X-ray complementing Chinese hamster gene 4), DNA ligase IV, Artemis and XLF (XRCC4-like factor)[76] (Figure 3).

DNA-PK comprises the Ku heterodimer Ku86/Ku70 and the DNA-PKcs (catalytic subunit)[77]. DNA-PKcs is a serine/threonine protein kinase belonging to the phosphatidylinositol-3-OH kinase protein family[78 79].The initial step in NHEJ is the detection and binding of the Ku heterodimer Ku86/Ku70 to the DNA DSB that will recruit the DNA-PKcs to the site of the damage[80]. Two DNA-PKcs bound to Ku-DNA complex forms a synaptic complex and tether the DNA-DSB[81]. The synaptic complex is believed to protect the DNA ends and recruit additional NHEJ proteins such as XRCC4, ligase-IV to the DNA break[82].

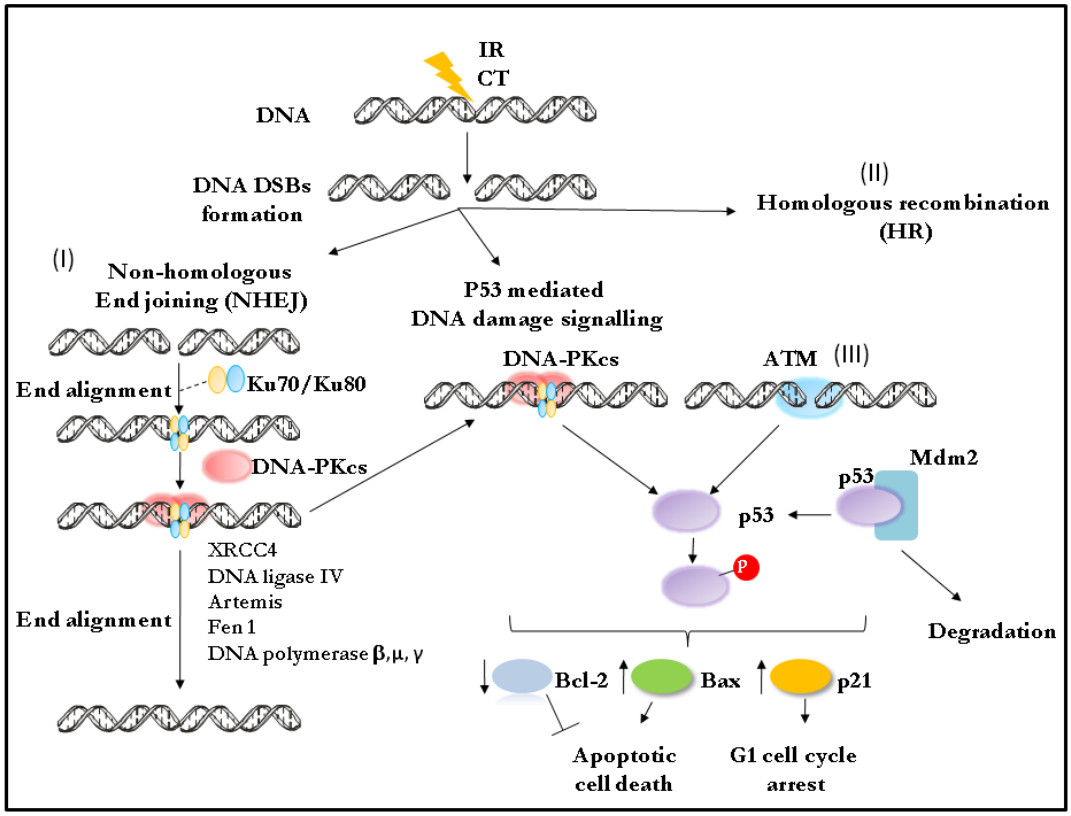

Figure 3*.Radiation induced DNA DSBs are detected and repaired by different molecular pathways among them NHEJ (I), HR (II), and ATM (III). In NHEJ mediated repair the Ku-proteins are recruited to the DNA DSBs followed by binding of DNA-PKcs. The DNA within the DNA DSB is processed and re-ligated in a process involving Fen1, Artemis, DNA polymerases and DNA ligase 4. p53 is stabilized in response to DNA DSB as a consequence of DNA-PK and ATM-mediated phosphorylation which inhibits Mdm-2 mediated degradation. In turn the accumulated p53 protein activates both cell cycle checkpoint control by transcriptional regulation of p21 as well as induces apoptotic signalling through transcriptional activation of Bax and Bcl-2, respectively.*

Some of the X-ray induced DNA breaks are highly complex and in order for NHEJ to accurately perform repair of these their DNA ends needs to be trimmed. Candidate proteins for this processing are among others the exo- and endonuclease Artemis and Flap endonucleases 1 (FEN-1) as well as DNA-polymerases β, γ and μ which trims the DNA ends and add single DNA bases respectively[83 76]. Following the processing the DNA ends are ligated in a process catalyzed by the XRCC4 homodimer and DNA-ligase IV[84].

The critical role of the different DNA-PK proteins in cellular radiosensitivity is illustrated by the DNA-PKcs defective rodent scid cells and the Ku86 defective xrs6-cells both which show a dramatic increase in radiosensitivity compared to their parental cell lines[85 86]. Also human tumour cell lines with defective DNA-PKcs e.g. glioblastoma cells (MO59J) has been shown to be highly radiosensitive relative to their DNA-PKcs proficient counterpart (MO59K)[87 88].

In response to DNA DSBs cells not only activate DNA repair pathways but also cell cycle checkpoints are triggered enabling the DNA repair processes to take place. The cell cycle checkpoints in response to DNA DSBs are regulated mainly by the ATM (ataxia-telangiectasia-mutated) kinases. However, DNA-PK is after detecting DNA DSBs believed also to transmit DNA DSBs signals to cell cycle regulating proteins such as p53 (see below)[76].

1.2.2 Cell cycle control and DNA damage response

Dividing cells continuously go through the cell cycle which is divided into four different stages, G1, S, G2 and M. During S phase the DNA is replicated and during M phase mitosis is taking place. At the heart of the cell cycle is a system of protein kinases, cyclin dependent protein kinases (CDKs) and their regulatory proteins the cyclins (Figure 4).

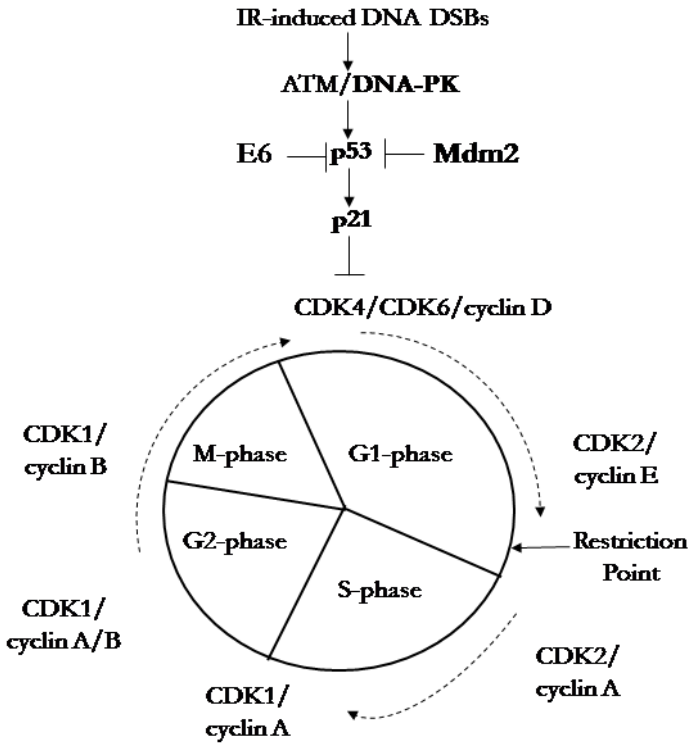

Figure 4. *Normal cell cycle progression is regulated by the levels of different cyclins and by the activity of their binding partner CKDs (for details see text). Upon radiation both ATM and DNA-PK gets activated as a consequence of IR-induced DNA DSBs. ATM and DNA-PK in turn activates a number of proteins controlling different checkpoints of the cell cycle. One important control mechanism is the activation and stabilization of p53 which inhibit the Mdm-2 mediated degradation. The increased p53 expression causes increased transcription of the CDK inhibitor p21. In G1, increased expression of p21 inhibits CDK4/6 and thereby impedes Rb phosphorylation and the release of E2F transcription factor hence causing arrest in G1. In HVP-infected cells, the HPV E6 protein target p53 for degradation.*

Different cell cycle check points ensure that progression within the cell cycle is not triggered if the DNA contains damages. Thus replication is not carried out on damaged DNA and a cell does not go through mitosis with lesions in the chromatin.

Passage through G1 is catalyzed by CDK 4/6 and cyclin D. This protein kinase complex phosphorylates and inactivates the retinoblastoma protein (Rb). Upon inactivation Rb releases transcription factor E2F allowing transcription of gene products necessary for G1-S transition, S phase and DNA replication. In G1 CDK2 and cyclin E is required for transition into S phase[89]. In late G1 there is a restriction check point[90]. During the replication of DNA in S phase CDK2 is complex binding with cyclin A. The G2 and M cell cycle phase is regulated by CDK 1 and cyclin A/B.

Following exposure to ionizing radiation the inflicted DNA DSBs arrests the cells in late G1 (G1/S check point) and at the G2/M border (G2/M check point). During these transient cell cycle arrests DNA DSBs are detected and repaired (Figure 3).

One of the most prominent regulators of DNA DSB cell cycle effects is the tumour suppressor protein p53[91]. In undamaged cells the p53 is unstable and present in minute amounts. The concentration of p53 in cells without DNA damages are regulated by the ubiquitin ligase Mdm-2 which targets p53 for degradation by proteasomes[92].

When cells encounter DNA damage such as DNA DSBs the cell cycle progression is inhibited[93].Thus the activated ATM and DNA-PK causes phosphorylation of several cell cycle regulating proteins i.e. checkpoint regulators among them p53. As a consequence the association between p53 and Mdm-2 is blocked and p53 is stabilized (Figure 3). Activated p53 subsequently induce the transcription of p21 and in G1 increased p21 levels thus blocks CDK4/6 activity and thereby phosphorylation of Rb and hence the release of E2F transcription factor preventing S phase entry (Figure 4).

Depending on the extent of DNA damage p53 is able to direct the cell into cell cycle arrest or cell death e.g. apoptosis[94]. Thus it is believed that severe DNA damage not only results in p53-mediated activation of p21 transcription but causes increased expression of the pro-apoptotic protein Bax as well as repression of anti-apoptotic Bcl-2 expression resulting in increased apoptosis.

Somatic p53 mutations, which impede its transcriptional function, are frequently found in many cancers[95] but this is not the case for cervical cancer where the frequency of mutated p53 is low[96 97 98 99 100]. In cervical cancer the persistent infection with oncogenic types of HPV is considered to be the most important factor for inactivating p53 signalling. Thus the E6 and E7 products of HPV interfere with p53 and Rb functions and deregulate the cell cycle. HPV E6 inactivates and degrades p53 via the ubiquitination pathway resulting in at least a partial loss of DNA-damage induced p53 cell cycle arrest and apoptosis control[101 102 103].

1.3 CLINICAL RADIOBIOLOGY

The biological effects of RT depend on radiation type and energy, total dose, fractionation schedule, irradiated volume and the duration of treatment as well as the dose rate and dose distribution within the tumour. The importance of these various factors for determining the outcome differs between EBRT and BT. In conventional EBRT the treatment is delivered with a high dose rate to a large volume with a homogenous dose distribution and with a treatment time that proceeds over several weeks as EBRT is given as small daily fractions. In BT on the other hand, the dose distribution is heterogeneous within a small target volume and the dose is delivered in one or a few fractions with a dose rate depending on the activity of the source used. BT is characterized by steep dose gradients in the vicinity of the sources resulting in a rapid dose fall to the surrounding tissue. However, since the prescribed dose is delivered as the minimum target dose, the tissue close to the source will receive twice or three times the prescribed dose which is positive for the local control of the tumour but may cause serious complications on normal tissue.

The biological effect of radiotherapy does not only depend on the physical factors characterising the treatment but also on the biological characteristics of the cells, tissues or organs that are irradiated. In vitro studies have shown differences in cell survival after irradiation between cells originating from various tumour types[104]. Moreover, cells from diverse normal tissue vary in their response to irradiation[105].

Comparisons of the effectiveness between different treatment schedules that varies in fractionations, dose rates and overall treatment time is possible through the use of bioeffect dose models that take into account the variation of response with the above mentioned dosimetric parameters. The linear quadratic (LQ) model is currently the most used bioeffect dose model that take into account dosimetric parameters as well as the radiobiological differences between various tissues[106 105 107]. The LQ model is described by the following equation:

$$S = \exp (- \alpha D - \beta D^2)$$

where S is the surviving fraction of cells and D is the radiation dose.

A large α/β value relates to small repair capacity and a small α/β to a large repair capacity. The α/β values varies with tumour type, for cervical cancer the value is high[108].

It is known from results obtained by irradiating cells in vitro that the fraction of cells killed by a given dose increases with dose rate and also that the effect differs between cell of different origin[109 110]. Fast proliferating tissues are more sensitive to radiation than slow proliferating tissues and respond differently to changes in dose rate. Increasing the dose rate will increase the effects in normal tissue more than it will increase the effect on the tumour i.e. normal tissue is more sensitive to changes in dose rate than tumour tissue[111 112 105]. This relation is described as "The terapeutic ratio" (Figure 5).

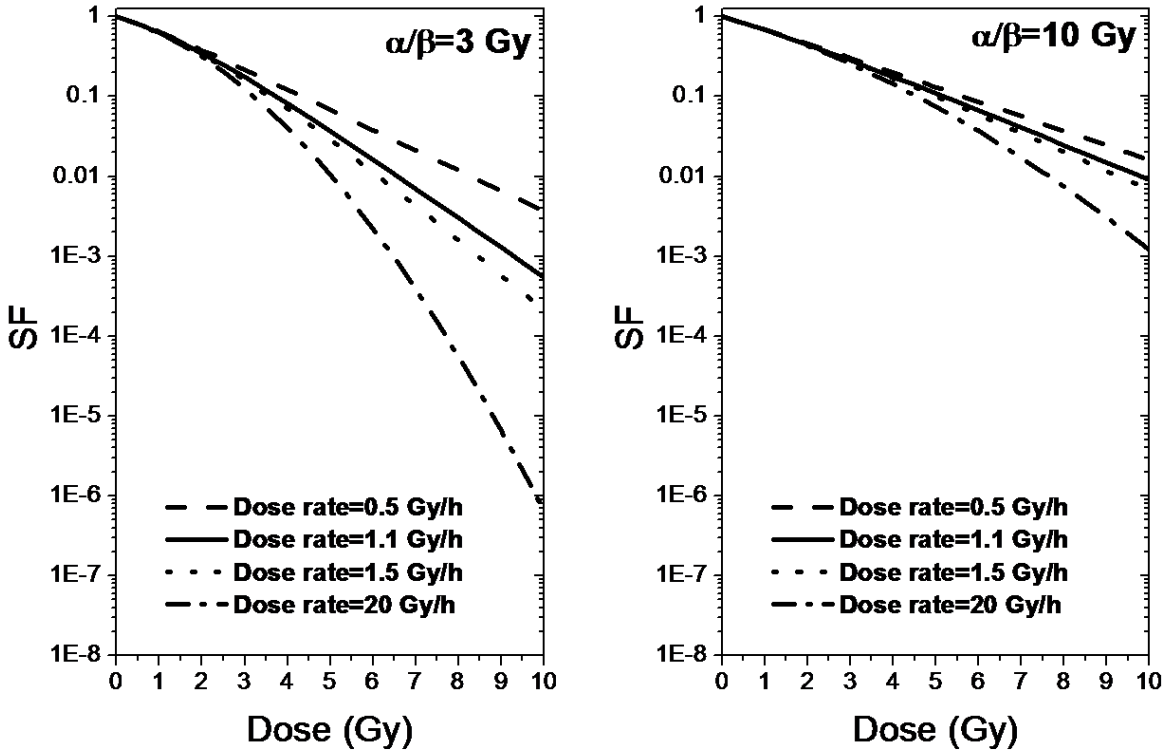

Figure 5*. Illustration of the effect of late (α/β = 3 Gy) and early (α/β = 10 Gy) reacting tissue calculated with the LQ formula adapted to account for the dose rate and the rate of repair of sublethal lesions. Courtesy of I. Toma-Dasu.*

A number of biological processes are taking place during irradiation that will influence the survival curve. Some of them are described as the "4 Rs" of radiobiology: repair of sublethal DNA-damage, repopulation, reoxygenation and redistribution[113]. The relative influence of these factors on the cellular response to a given radiation quality depends of the cell type, duration of irradiation and the dose rate as well as the overall treatment time[104, 114].

2 AIMS OF THE THESIS

The overall aim of this thesis was to study the response to preoperative radiotherapy in patients with cervical cancer with respect to clinical, dosimetric and molecular key factors focusing on DNA-DSB signalling molecules.

Specific aims

To analyse the outcome of treatment with preoperative intracavitary radiotherapy in patients with cervical cancer stage IB and IIA *(Paper I).*

To evaluate the impact of tumour remission after preoperative RT on survival *(Paper I).*

To investigate if DNA-DSB signalling proteins DNA-PKcs, Ku70, Ku86, p53, p21and Mdm-2 can be used as predictive markers for radiotherapy response in cervical cancer *(Paper II).*

To investigate whether resistant parts of cervical tumours remaining after fulfilled radiotherapy have an altered expression of DNA-PK protein complex compared with corresponding pre-treatment cervical cancer biopsies *(Paper III).*

To correlate dosimetric parameters calculated according to the linear quadratic model to survival, local control and late toxicity in patients treated with radiotherapy and surgery or radiotherapy alone for cervical cancer stage IB-IIB *(Paper IV).*

3 MATERIAL AND METHODS

3.1 STUDY POPULATION

These studies were performed on a study group that included 199 patients with cervical cancer stage IB, IIA and IIB treated at Radiumhemmet, Karolinska University Hospital during January 1989 to December 1991. Diagnosis was based on multiple cervical biopsies and fractionated curettage. Clinical staging procedure was performed according to the FIGO guidelines used at that time including staging investigations such as a chest X-ray, cystoscopy and intravenous pyelogram[115]. Small tumours were defined as <2 cm, medium size tumours between 2 - >4 cm and large tumours ≥4 cm. Assessment of tumour remission in patients treated with preoperative radiotherapy was carried out on the surgical specimen retrieved at surgery performed 4 weeks after second fraction of ICRT. Tumour remission was classified as pathologic complete remission (pCR) if no microscopic tumour was found or as incomplete pathologic remission (non-pCR) if microscopic residual tumour was evident. Follow-up was carried out at our clinic during five years and then by the referring gynaecologist. Moderate (grade 2) and major (grade 3) late side effects were classified retrospectively according to the glossary of Chassagne *et al*[116] and were defined as complications persistent or occurring more than 3 months after end of RT.

In paper I the outcome of 185 consecutive patients with stage IB and IIA cervical cancer was studied with focus on 121 patients primarily treated with preoperative ICRT and radical surgery. Out of 121 patients, stage IB and IIA were diagnosed in 97 and 24 patients, respectively. Median age at diagnosis was 44 years (range 22-75). Squamous cell carcinoma was diagnosed in 84 cases and adenocarcinoma in 29 cases. Small tumours were found in 31 patients, medium and large tumours in 42 and 48 patients, respectively. The manual technique for BT was used in 45 cases and the remote afterloading technique in 76 cases.

In the second study pretreatment tumour biopsies were obtained from 109 patients. Stage IB and IIA were found in 85 and 24 patients, respectively. Squamous cell carcinoma was diagnosed in 77 cases and adenocarcinoma in 26 cases and small, medium and large tumours were detected in 25, 37 and 47 patients, respectively. Preoperative ICRT was performed with the manual technique in 40 patients and 69 patients were treated with the remote afterloading technique. The frequency of DNA-PKcs, Ku86, Ku70, p53, p21 and Mdm-2 positive cells were analyzed in formalin-fixed tumour biopsies using immunohistochemistry (IHC). The frequency of stained cells for each case was correlated to tumour remission assessed as pCR and non-pCR in the surgical specimen. Comparison between pCR or non-pCR cases with respect to expression of DNA-PKcs, Ku70 and Ku86 were based on samples with low percentage stained cells (< median value) or high percentage stained cells (> median value) within each tumour specimen.

The third study included 22 patients in which residual cervical tumour in the surgical specimen after preoperative radiotherapy were detected. Stage IB and IIA were diagnosed in 13 and 9 patients, respectively. Tumour size differed between 1.0 and 8.0 cm with a median of 5 cm. Squamous cell carcinoma was found in 13 patients and

adenocarcinoma in 9 cases. The grade of differentiation was high and moderate in 5 and 8 cases respectively, low grade of differentiation was found in 9 cases. Eleven patients were treated with the after-loading technique while the manual technique was used in 10 patients. Three patients were treated with a combination of EBRT and BT preoperatively and one patient received only EBRT. The frequency of DNA-PKcs, Ku86, Ku70, p53, p21 and Mdm-2 positive tumour cells were assessed by IHC in residual cervical tumours after preoperative ICRT and in corresponding primary tumour material.

In paper IV biological effective dose (BED) calculations were performed on 171 patients with cervical cancer stage IB-IIB. Stage IB was diagnosed in 113 patients, stage IIA and IIB were found in 44 and 14 patients, respectively. Treatment included preoperative ICRT and radical surgery for 128 patients of which 31 cases received additional postoperative EBRT. Definitive radiotherapy consisting of ICRT and EBRT were used in 43 patients. Median age at diagnosis was 43 years for patients treated with radio-surgery and 72 years for patients treated with RT alone. The manual technique and the remote after-loading technique for BT were used in 91 and 80 patients, respectively.

3.2 TREATMENT

Throughout the time period for this study the standard treatment at the Department of Gynecologic Oncology, Radiumhemmet, for operable patients with cervical cancer stage IB-IIA was preoperative ICRT followed by surgery. Surgery included radical hysterectomy according to the Wertheim-Meig procedure[43] but usually only a small portion, 1-2 cm, of the vaginal cuff was resected. Postoperative external beam radiotherapy (EBRT) over a pelvic and para-aortic field was added to patients with lymph node metastases diagnosed at surgery while cases with non-radical resection margins received EBRT over the pelvis only. It has to be mentioned that residual tumour in the cervical specimen (non-pCR) was not an indication for postoperative adjuvant treatment.

Patients not suitable for surgery due to poor medical status and patients diagnosed with stage IIB disease (paper IV) were treated with two fractions of BT followed by EBRT over a pelvic field. EBRT was delivered by 6-21MV linear accelerators over anterior-posterior fields with a daily fraction of 1.6 Gy. The prescribed dose to the pelvic and para-aortic field was 45 and 40 Gy, respectively. All patients receiving EBRT after BT were treated with a central shield placed in accordance to the intracavitary applicators with an individualized shielding to the bladder and rectum depending on the dose already given by BT. A guideline for the total clinical dose, i.e the sum of the doses from ICRT and EBRT, was 60 Gy to the bladder and 55 Gy to the rectum, respectively.

3.2.1 Brachytherapy

All patients received low dose rate BT but during the studied period our technique for BT gradually changed from a manual technique with radium applicators to a remote after-loading technique using cesium-137. Using either technique patients were treated with two fractions of ICRT with a 3-week interval followed by either surgery or EBRT 4 weeks after the second insertion.

In the manual technique a combination of an intrauterine tube (43-70mg Ra) and a vaginal applicator (50-70 mg Ra) that were not fixed to each other was used. The dose rate in the bladder and rectum, measured by a gammameter, served as the basis for determining the treatment time and thus the total given dose. The dosage was quoted in milligram-hours of radium (mghRa) with two fractions giving a total uterovaginal mean dose of 6500 mghRa (4800-7900 mghRa). The estimated dose rate at point A varied between 1.0 and 1.23 Gy/hr (mean 1.1 Gy/hr).

In the remote after-loading technique a circular-shaped vaginal applicator fixed to the intrauterin applicator was used (Selektron®). With this technique the dose rate at point A varied between 1.20 and 1.45 Gy/hr (mean 1.35 Gy/hr). The prescribed dose for each fraction to point A varied between 20 and 22.5 Gy, giving a total dose of 40-45 Gy. The rectal point dose was calculated on a lateral radiograph and located at the anterior rectal wall in the plane of a line through the upper and lower periphery of the ring-applicator. The dose to the bladder was estimated at a point in the posterior surface of a catheter balloon according to the recommendations from ICRU[60] (Figure 6).

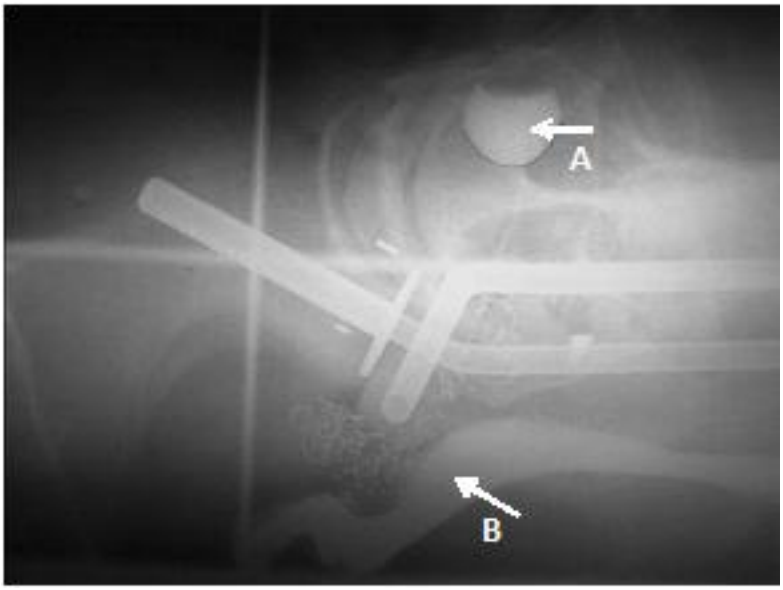

Figure 6. *Lateral x-ray showing intracavitary brachytherapy with the remote after-loading technique. The circular-shaped vaginal applicator is fixed to the intrauterine applicator. Two silver markers are placed in the cervical tumour. The bladder and the rectum are visualised by contrast medium in the catheter balloon (A) and the rectum (B), respectively*

3.3 BIOEFFECT DOSE CALCULATIONS

Biologically effective dose (BED) is a convenient way of converting any fractionation or protraction scheme to an equivalent isoeffective dose independent of fractionation pattern or delivery time. This allows comparison and addition of the effects of various complete or partial treatment regimens irrespective of pattern of dose delivery. In paper IV we used the below described calculations.

For EBRT, the BED was calculated according to equation 1.

$$BED_{EBRT} = nd\left(1+\frac{d}{\alpha/\beta}\right) \qquad (1)$$

where n is the number of fractions, d is the dose per fraction and α/β is the ratio of the LQ parameters of the tissue investigated. It has to be pointed out that we performed separate calculations for each tissue, according to their fractionation sensitivity quantified as α/β. For low dose rate BT where significant repair of damage takes place during the treatment duration, the BED for each session was given by equation 2.

$$BED_{BT} = D\left\{1+\frac{2D}{\alpha/\beta\,\mu T}\left[1-\frac{1}{\mu T}\left(1-e^{-\mu T}\right)\right]\right\} \qquad (2)$$

where D is the radiation dose, T is the duration of the BT session and μ is a parameter characterizing the repair of sublethal damage in the irradiated tissues; $\mu=\ln(2)/T_{1/2}$, where $T_{1/2}$ is half-life of sublethal damage repair.

For combined treatment schedules, the total BED was calculated as:

$$BED_{tot} = \sum_i BED_i \qquad (3)$$

where BED are the individual biologically effective doses from each session of RT. Equation 3 gives the general BED expression that does not take into account the proliferation during the treatment. The effects of this latter aspect could be expressed through a supplementary term subtracted from the expression in equation 3 to describe the loss of effect due to proliferation of tissues during the treatment (equation 4).

$$BED_{prolif} = \sum_i BED_i - \frac{\ln(2)}{\alpha}\frac{T_{treat}-Tk}{Tp} \qquad (4)$$

where T_{treat} is the overall treatment time, T_k is the lag time for the onset of proliferation [117], T_p is the effective doubling time during proliferation and α is the linear parameter of the LQ model. It should be noted that no correction for proliferation is needed if the overall treatment time is shorter than T_k.

The expressions in equations 1-4 were used to calculate the relevant BED for tumors and normal tissues in paper IV. The parameters used for calculations have been chosen in agreement with existing literature[108 118 119]. Thus, generic values of 10 Gy

and 3 Gy have been assumed for the fractionation sensitivities of tumors and normal tissues respectively. The repair half-life for sublethal damage was 1.5 h resulting in a repair parameter μ=0.46 h^{-1}, the lag time for the onset of proliferation T_k=21 days, the effective proliferation doubling time T_p=5 days and α=0.3 Gy^{-1}.

3.4 IMMUNOHISTOCHEMISTRY

Immunostaining of the DNA DSB signalling proteins was performed on archival, formalin fixed tumour material obtained from the patients described in section 3.1. For antigen retrieval the paraffin sections (4 µm) were boiled in citrate buffer. After blocking with 1% BSA staining were performed with the following antibodies; DNA-PKcs, Ku86, Ku70 (Neo Markers, Union City, Ca, USA) , p53 (clone DO-7, DAKO, Kyoto, Japan), p21 (clone EA10, Oncogene research products, Cambridge, MA, USA) and Mdm-2 (SMP14, Santa Cruz Biotechnology, Santa Cruz, CA, USA). The ABC-technique (Vector Laboratories, Elite Standard Kit, Burlingame, CA, USA) was used for visualize the immunostaining. Staining evaluation was performed in a blinded fashion, without prior knowledge of the clinical data or outcome for the individual patient. Counting of 500 tumour cells was carried out on four representative areas in a light microscope and the percentage of stained cells was determined. Intensity of immunostaining was judged as low, intermediate or strong. Morphologically normal squamous epithelial and cervical glandular cells in each specimen served as internal negative control. The intraobserver reproducibility was tested and showed significant correlation between the two series of evaluations ($r=0.95$, $p<0.001$).

4 RESULTS AND DISCUSSION

4.1 PAPER I

The purpose of this study was to evaluate the treatment results of preoperative ICRT in stage IB and IIA cervical cancer with focus on the prognostic value of complete tumour remission for recurrence rate and survival. In patients treated with preoperative ICRT we found a 5-year disease specific survival rate of 87% and 75% for stages IB and IIA, respectively, which corresponds well with other reports on survival for patients in these stages of cervical carcinoma (see 1.1.5). The importance of complete tumour remission for survival after preoperative ICRT has been addressed in earlier studies but the results are inconclusive with some studies reporting a correlation between tumour sterilisation and patient survival[120 39 41 121] while others do not find such a correlation[37 122 40].

In this study we found that complete tumour remission after preoperative BT is of strong prognostic value when it comes to patient survival. Patients with pathologic complete remission (pCR) had a 5-year disease-specific survival of 95% compared to 46% in patients with non-pCR ($p < 0.0001$) (Figure 7). To further investigate the prognostic value of pCR we analyzed the importance of pCR for survival in relation to node status. Among node negative patients we found a 5-year survival rate of 98% in cases with pCR compared to 64% in cases with non-pCR ($p < 0.0001$). Node positive patients with pCR had a 5-year survival rate of 67% compared to 25% in patients with non-pCR, however, this difference was not statistically significant ($p=0.11$). Moreover, patients with large tumours and pCR had a 5-year survival rate of 96% compared to 40% in patients with large tumours and non-pCR ($p < 0.0001$).

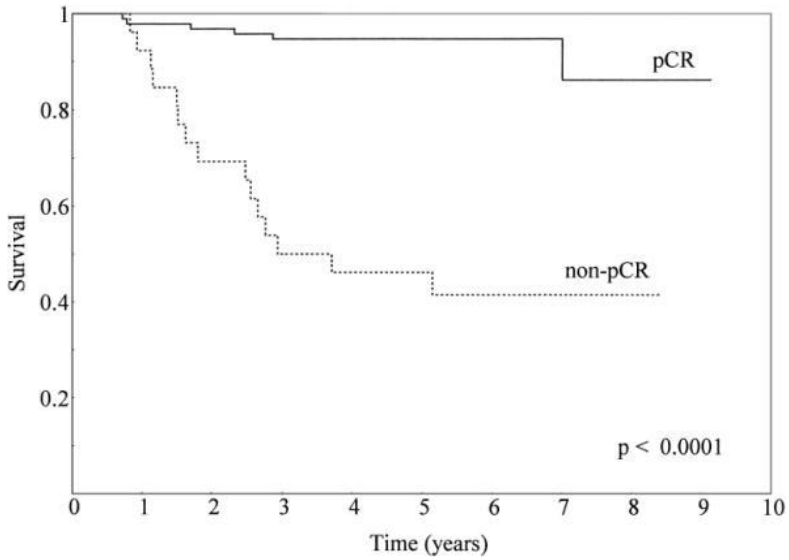

***Figure 7**. Five-year disease-specific survival in patients with cervical cancer stage IB-IIA in relation to pathologic complete remission (pCR) or residual tumour (non-pCR) after treatment with preoperative brachytherapy.*

Several factors may be of importance for this result. The frequency of pCR is an issue of interest. We found pCR in 79% of the patients (Table 2). As expected, pCR was more common in patients with small tumours compared to patients with large tumours and node negative patients more frequently reached pCR than node positive patients. A small tumour has obviously a higher possibility to be fully targeted with ICRT than a large tumour which is a probable explanation to our finding that small tumours more often reached pCR than large tumours. Consequently, the higher frequency of pCR among node negative cases compared to node positive cases may well be explained by the known correlation between large tumour size and increased frequency of pelvic lymph node metastases[123 24]. Still, the possibility of node positive tumours representing a more radioresistant type of tumour also must be considered.

Table 2. Tumour characteristics in relation to tumour remission.

	Pathologic complete remission	Non-pathologic complete remission	Total no. of patients
No. of patients	95 (79%)	26 (21%)	121
Tumour characteristics			
Stage IB	79 (81%)	18 (19%)	97
Stage IIA	16 (67%)	8 (33%)	24
Node negativ	86 (86%)	14 (14%)	100
Node positive	9 (43%)	12 (57%)	21
Histologic type			
Squamous	68 (81%)	16 (19%)	84
Adenocarcinoma	22 (76%)	7 (24%)	29
Others	5 (63%)	1 (37%)	8
Grade of differentiation			
High-moderate	60 (81%)	14 (19%)	74
Low	29 (73%)	11 (27%)	40
Unknown	6 (86%)	1 (14%)	7
Tumour size			
Small	30 (97%)	1 (3%)	31
Medium size	37 (88%)	5 (12%)	42
Large	28 (58%)	20 (42%)	48

Our result of pCR in 79% of the patients is of the same order as found by others[124 125 126 37 39 121]. However, there are reports on lower frequencies of tumour sterilization[127 128 40]. Factors such as differences in studied patients regarding tumour stage and size as well as differences in the total radiation dose and dose rate should be considered when comparing results obtained in different studies. In a randomised trial examining two low dose rates (0,38 and 0.73Gy/hr) on cervical cancer patients stage I

and II treated with preoperative ICRT a higher grade of tumour sterilizations was reported in the lower dose rate group for a subgroup of patients in stage II disease[128]. That result may lead to the assumption that a higher dose rate will result in less degree of pCR. However, this assumption is not likely since the mean dose rate in our study was almost twice as high (1.2-1.35Gy/hr) as in the randomised trial, yet in spite of that we found a higher frequency of complete remissions pCR, 79% in our study compared to 47% in the randomised study. Likewise, in our study there was some variation in the dose rate between the two BT techniques, however, we did not find that the method of administrating BT had any impact on the frequency of pCR.

Furthermore, the time interval between RT and surgery is of importance when comparing results from different studies on pCR after preoperative ICRT. It has been shown that the rate of eradicated cancer increases with a longer time interval between end of RT and surgery[128 129]. The relevance of the time factor between end of RT and surgery is illustrated by the findings of Jacinto *et al* on cervical cancer patients with stage IIB treated with both EBRT and BT preoperatively[129]. Thus, they showed that patients who underwent surgery earlier than 80 days after end of RT showed a significantly lower rate of pCR (28%) than patients with surgery performed later than 80 days after end of RT (57%). In our study the time interval between BT and surgery was shorter (range 28-35 days) thus this factor is unlikely to explain our relatively high frequency of pCR.

The definition of tumour remission is also of importance for the frequency of pCR. In our study we classified tumour remission as pathologic complete remission (pCR) if no microscopic tumour was found or as incomplete pathologic remission (non-pCR) if microscopic residual tumour was found in the surgical specimen. This is in agreement with most other studies[37 41 121 42]. One objection to this definition could be that the response to RT may be more gradual and that it would be more accurate to measure a partial response. It is however, difficult to get that type of pathological judgement performed with a high reproducibility.

The importance of pathologic complete remission (pCR) on survival in our study is further demonstrated by the result of the multivariate analysis where only non-pCR and pelvic lymph node metastases were found to be of independent significance for decreased survival while large tumour size was not. This is in agreement with the report from Grigsby *el al* who found no effect of tumour size on the prognosis for patients with cervical cancer stage IB and IIA treated with preoperative RT[130]. One hypothetical explanation for this lack of impact of tumour size on prognosis after preoperative RT is that pretreatment microscopic tumour spread in the paracervical tissue is correlated to tumour size. When using preoperative ICRT the surrounding paracervical tissue will be reached by a low but sufficient radiation dose for targeting potential microscopic disease. This theory is supported by the findings that postoperative additional RT has shown to reduce local recurrence rate and prolong disease-free survival for patients with bad prognostic factors such as large tumour size, capillary-space tumour invasion and deep stromal invasion of the cervix revealed at surgery (see 1.1.6). Furthermore, this hypothesis is compatible with our finding of a significant lower frequency of locoregional recurrences in patients with pCR compared to non-pCR patients.

In some of the studies which analyze the outcome after preoperative ICRT, survival with respect to pCR is not reported[38 42]. However, there is one study of 192 patients with stage IA2-IIA cervical cancer treated with preoperative ICRT that reports a correlation between distant recurrences and non-pCR[41]. Another study of 264 patients

with stage IB1-IIB reports significantly decreased 5-year survival for patients with residual tumour in the surgical specimen[121]. In a study of 115 patients with stage IB and stage II no significant difference in survival between cases with and without residual carcinoma after preoperative ICRT was reported[37]. The explanation of these diverging results is unclear but may be attributed to the difference in technique for BT, dose rate as well as in the clinical material that all are factors that may have an impact on both frequency of pCR and survival of the patients.

When discussing different treatment options it is important to take into account treatment related side effects. We registered no grade 3 complications such as vaginal fistulas among the 121 patients analyzed in this study. Grade 3 late complications after preoperative ICRT is reported in a frequency varying between 0.5% and 6% [37 38 40 41]. One factor of importance for our result of no vaginal fistulas might be that the "Stockholm technique" is based on individualization which resulted in a reduction of the prescribed dose if the estimated dose to bladder and rectum was too high. Another factor of importance may be our strict time interval between the insertions of BT as well as between BT and surgery which may provide time for normal tissue to repair. It is, however, important to point out that a retrospective analysis of patient records as ours in general will give a lower frequency of complications than the result of a prospective study or a personal questionnaire. However, when it comes to serious, grade 3 complications we consider our result reliable since symptoms leading to investigation procedures or surgery are well reported.

4.2 PAPER II AND III

Given our finding in paper I that complete tumour remission after preoperative BT did strongly correlate with long-term survival we were interested to find potential molecular markers in the primary tumour that could predict response to BT. Others have performed similar studies on pre-treatment cervical cancer tumour specimens aiming at finding biological markers for RT response but have focused on other markers. Thus, increased survival signalling trough Her-2[131] and epidermal growth factor receptor (EGFR)[132 133] as well as insulin-like growth factor receptor (IGFR)[134] have all been shown to correlate to decreased responsiveness to RT.

In paper II we have investigated the expression of proteins known to be involved in the NHEJ DNA repair process with the aim to find possible markers for radiosensitivity. Using IHC we analyzed the DNA-PK proteins DNA-PKcs, Ku70, Ku86 and their downstream signalling proteins p53, p21 and Mdm-2.

Importantly, we found that non-neoplastic squamous epithelium and tumour-free cervical glands showed low expression of all of the DNA-PK subunits (Figure 8).

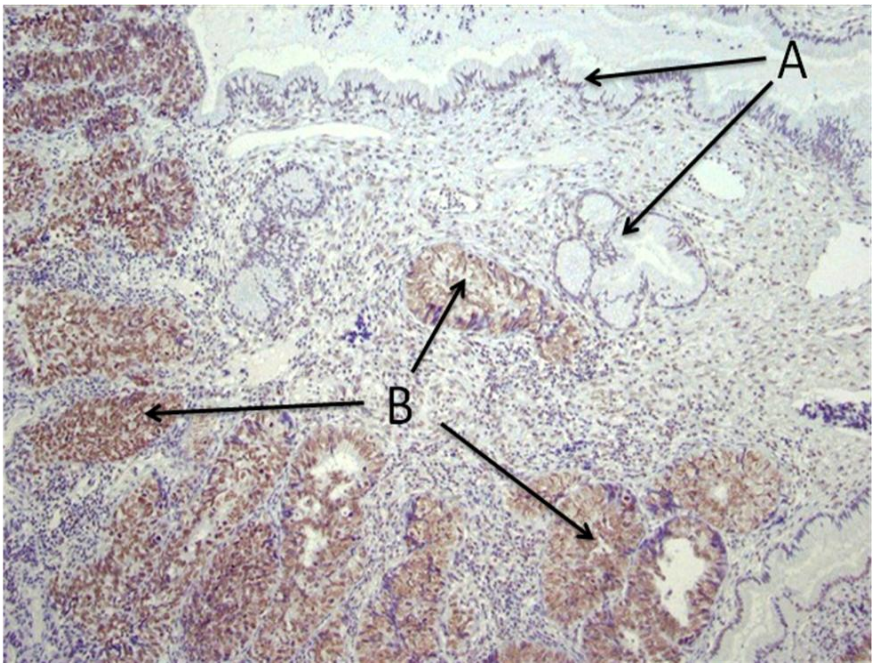

Figure 8*. DNA-PKcs protein expression in non-neoplastic tissue (A) and partly poor differentiated adenocarcinoma (B). A clear difference in immunostaining of DNA-PKcs is observed between the non-neoplastic tissue (A) and the tumour tissue (B).*

Our finding of almost complete lack of immunostaining in non-neoplastic tissues versus the high staining frequency of DNA-PK complex proteins within cervical tumour tissue is in agreement with reports from other studies on other tumour types i.e. bladder- and colorectal carcinoma in which DNA-PKcs, Ku86 and Ku70 all were found to have an increased expression relative to the corresponding non-neoplastic tissue[135] [136]. This result is compatible with the report by Bartkova *et al* showing that other DNA DSBs response proteins such as ATM, Chk2, p53 and γ-H2AX are constitutively activated and expressed at higher levels early in the tumour genesis as compared to normal tissue[137].

We found a positive nuclear staining of DNA-PKcs in all of the 109 tumours analysed, with a median of 66% positive cells (range 20-100%). Staining for Ku 86 was detected in 107 cases and the median percentage of positive cells was 74% (range 24-100%). Expression of Ku70 was found in 108 tumours with a median of 78% positive cells (range 15-99%). The staining levels of Ku70 and Ku86 detected in our study are comparable with values reported by others [138]. When comparing small/intermediate and large tumours we could not find any significant difference in expression of neither DNA-PKcs nor Ku70 or Ku86. This result may be interpreted as that DNA-PK is not upregulated during tumour progression which could be supported by findings in other tumour types where other DNA damage response proteins are constitutively activated early in tumour genesis[137, 139 140].

With an attempt to identify predictive markers for radiosensitivity we analyzed if the expression of the above described markers in the primary tumour were correlated to treatment outcome measured as pathologic complete remission (pCR) after

preoperative ICRT. We could not detect any significant difference in expression of DNA-PKcs primary tumour between pCR and non-pCR patients. Similar to DNA-PKcs, we did not detect any significant difference in the expression of the subunits Ku70 and Ku86 between pCR and non-pCR patients (Figure 9).

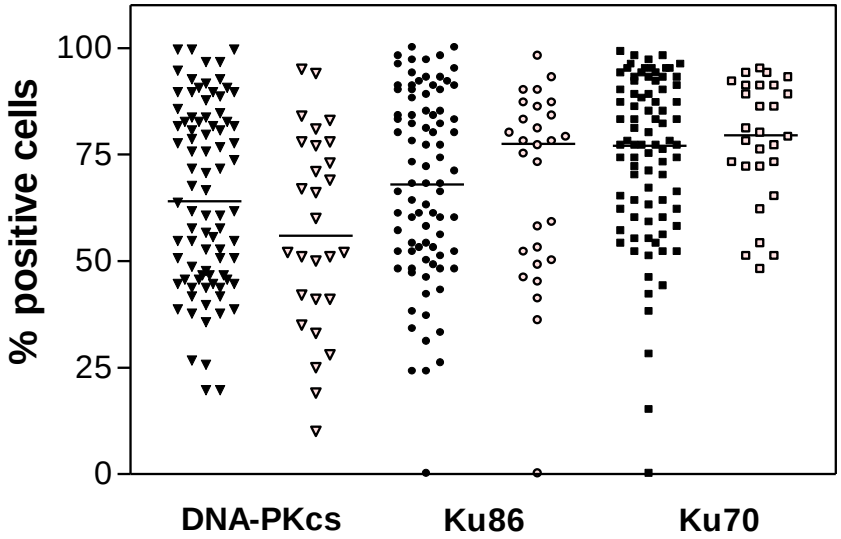

Figure 9*. Expression of DNA-PKcs (triangles), Ku86 (circles) and Ku70 (squares) in primary biopsies from patients with cervical carcinoma and comparison between pCR (filled symbols) and non-pCR cases (unfilled symbols). Median values are indicated.*

Studies on cells lines have shown that deficiency in any of the DNA-PK subunits leads to impaired DNA DSB repair and increased sensitivity to ionising radiation[141 142 143]. Moreover, it has been shown in our group that high expression of DNA-PK correlates with resistance to irradiation in lung cancer cells lines[144]. However, the significance of DNA-PK expression levels in biopsy specimens for the clinical response to RT in a patient is clearly not as obvious. This discrepancy may be explained by multiple factors. First, the level of DNA-PK in cervical tumours with low DNA-PK expression may still be sufficient to repair RT-inflicted DNA damage and consequently no correlation between non-pCR and a high protein expression will be found. Moreover, tumours with low DNA-PK may induce an upregulation and thereby handle the RT-induced DNA damage leading to non-pCR. One possible pathway involved in such an upregulated function of DNA-PK might be the EGFR signalling cascade as it has been shown that irradiation may induce accumulation of EGFR within the cell nucleus which subsequently causes an increased function of the DNA-PK complex[145].

Furthermore, in a tumour, the surrounding tissue may influence the tumour response to RT as compared to cell lines. Thus, one may speculate that tumour associated fibroblasts would produce factors that influence the response to RT. The heterogeneity of a tumour, in contrast to cell lines, with a various degree of hypoxia may also influence the response to treatment.

The lack of a correlation between expression of DNA-PK and tumour response in our study differs from other reports indicating a positive role for DNA-PK as a predictive marker for radioresistance. Clinical studies on DNA-PK and outcome after RT on various tumour sites show diverging results. In head and neck cancer patients it is reported that high levels of Ku86 correlate with better locoregional control measured one month after end of treatment[146]. Komuro *et al* reports on patients with rectal carcinoma treated with preoperative RT that low levels of Ku70 correlate with radiosensitivity[147]. However, in that study radiosensitivity was defined in the surgical specimen as shrinkage and fibrosis of more than two-thirds of the tumour which is in marked contrast to our study where radiosensitivity was defined as complete tumour remission. This variation in endpoint for radiosensitivity may explain why different results were obtained. Moreover, Komuro *et al* RT used EBRT for treatment whereas we used low dose rate BT. It is known that irradiation with high dose rate such as EBRT result in different cellular radiobiological effects than low dose rate irradiation[114] which also may explain the diverging result regarding DNA-PK expression levels and correlation to tumour remission between the two studies.

Also in cervical cancer, there are reports showing a relation between expression of DNA-PK and outcome after RT. In a study on patients with cervical cancer stage I-III treated by RT a higher survival rate was found for patients with low expression of Ku70 in the primary tumour while the expression of Ku80 did not correlate with survival [138]. Similarly, a report on cervical cancer patients stage IA-IVB reports better survival for patients after RT if the tumour was Ku80 negative[148]. The reasons for the diversity of results in these clinical studies for the predictive role of DNA-PK expression for RT response are not obvious. One important issue, however, is the definition of RT response. Survival is often used as an endpoint in studies searching for potential predictive biomarkers for radiosensitivity. This is problematic since survival is closely related to the clinical stage of the disease as well as to other factors such as age and co-morbidity. This is especially apparent when the analyzed patients are heterogeneous with respect to stage. In our study we had a more homogenous patient material as we only included patients with stage IB-IIA cervical cancer. Furthermore, we defined RT response as complete tumour remission in the surgical specimen thereby avoiding confounding factors related to survival. In addition, the total dose and treatment type as well as time interval between treatment and evaluation of DNA-PK expression differ between studies. In the above referred clinical reports on DNA-PK expression and cervical cancer, treatment have included EBRT with or without BT. In our study patients were treated only with low dose rate BT which has a different radiobiological effect than EBRT as discussed above[114]. This might result in a different cellular respond with respect to DNA-PK and DNA repair.

We also examined if the expression of p53, p21 and Mdm-2 within the primary tumour specimen from 109 cervical cancer patients could predict their RT treatment response. We found no correlation between either Mdm-2 or p21 to pCR. We found p53 staining in 35% of the analyzed tumours, however, with a low frequency of stained cells within each positive tumour (range 5 - 33%). We found that p53 positivity in the primary tumour correlated with cases showing residual tumour after RT i.e. to non-pCR. This is in agreement with Mukherjee *et al* that report a correlation between p53 staining in primary tumour biopsies and residual tumours after preoperative EBRT in patients with cervical cancer stage IIA-IIB[149]. In contrast, Ebara *et al* found no correlation between p53 staining in primary tumour and treatment outcome defined as local control and survival on stage III cervical cancer[150]. In the study by Ebara *et al* the same antibody was used as we applied which is an antibody that detects p53 regardless of mutation. Thus we do not know if the p53 staining we observed within the cervical cancer specimens represents mutated or wild type p53. However, mutations of p53 are rarely detected in cervical carcinoma and the issue of a correlation between mutated p53 and immunopositivity for p53 have been investigated in several studies on cervical cancer patients reporting no such correlation[97 99 151 98]. Moreover, in a study analyzing the mutational status of p53 by PCR-single-strand conformation polymorphism (PCR-SSCP) in relation to response to RT in patients with cervical cancer stage IIIB it was reported that p53 mutation was significantly correlated with local recurrence[152]. It is shown that the expression of p53 increases under hypoxic conditions in cell cultures on cells from HPV-infected cervical carcinomas[153]. One explanation to our finding of a correlation between the frequency of p53 positivity and non-pCR may be that in radioresistant tumours i.e. non-pCR cases, p53 positivity represents tumours with a higher degree of hypoxic cells that may be more resistant to RT. We also found that p53 positive tumours showed a significantly higher expression of DNA-PKcs than p53 negative tumours. This points to that the DNA-repair capacity might be increased in p53 positive tumours.

Few clinical studies have so far compared the expression of DNA-PK proteins in primary and residual tumour tissue after RT. One may hypothesize that tumours surviving irradiation would display increased function of a DNA-PK signalling network. In paper III we therefore compared the expression of DNA-PKcs, Ku70 and Ku86 as well as p53, p21 and Mdm-2 in primary tumours and corresponding residual cervical tumours after RT.

We found that the residual tumour showed higher percentages of DNA-PKcs immunopositive cells compared with the corresponding primary tumour biopsy. Similarly, the frequency of tumour cells staining positive for Ku70 and Ku86 were higher in residual than in primary tumours.

To our knowledge this is the first study of clinical cervical carcinoma in which the DNA-PK expression has been examined in both primary and residual tumour tissue after RT. However, there is one study by Shintani *et al* on oral squamous carcinoma which analyzed DNA-PK proteins in tumour biopsies pre- and post RT delivered as EBRT to a median total dose of 32.9 Gy[154]. It was demonstrated that these residuals of oral squamous carcinoma obtained by surgery two or three weeks after fulfilled RT indeed had an increased expression of DNA-PKcs and Ku70 whereas the expression of

Ku80-positive cells was found to be similar in tumour tissue pre- and post RT. The results by Shintani *et al* are partly in agreement with our findings while there is a discrepancy with respect to Ku80. It may be related to the effect of the chemotherapy that was given to half of the patients in the study by Shintani *et al* which might have induced a different selection pressure on the primary tumour than RT alone. Moreover differences in treatment type, ICRT *vs* EBRT, as well as in the time span between end of RT and surgery may contribute.

One interpretation of our finding that residual tumour tissue displayed increased frequency of cells staining positive for DNA-PK components compared to primary biopsies is that radiation causes a selection pressure allowing DNA-PK high expressing tumour cells to survive RT and conquer the tumour. Provided there are both DNA-PK low expressing radiosensitive and DNA-PK high expressing radioresistant tumour cells in the primary tumour, radiation will lead to death of the DNA-PK low expressing cells as they will not have enough capacity to repair the radiation inflicted DNA DSBs. Therefore the DNA-PK high expressing tumour cells will constitute a greater part of the total amount of residual tumour cells. Yet, another possibility might be that radiation to a tumour may induce an upregulation of the DNA-PK proteins and that individual tumour cells will have different susceptibility for triggering such an upregulation as a result of different signalling aberrations in different parts of a tumour.

We also analysed the expression of p53, p21 and Mdm-2 in primary and residual tumours after RT. We observed a decrease in the frequency of p21 positive cells in residual tumours after RT compared to corresponding primary tumour. In contrast, no difference in the frequency of p53 and Mdm-2 cells was found.

This result is contradictory to studies on cell lines showing a radiation induced increase in p21 expression[155]. Also in the clinical situation, an increase of p21 positive cells in tumour tissue analyzed after RT as compared to the frequency in pre-treatment tumour biopsies has been reported in patients with cervical cancer[156]. However, in that study by Niibe *et al* the biopsies were taken in an early phase of the radiation course, at a total dose of 10 Gy delivered by EBRT, which is in contrast to our study using ICRT with a total dose of at least 30 Gy. Moreover, in that study p21 expression was evaluated at 6 hours post radiation while our analysis was performed four weeks after end of BT. Hence we did not measure the acute radiation-induced activation of p21 expression as was the case in the study by Niibe *et al* which may explain the different results.

4.3 PAPER IV

The purpose in paper IV was to evaluate the biological effective dose (BED) defined by the LQ model with respect to local tumour control, survival and late toxicity of the bladder and rectum in patients treated with either radiotherapy (RT) in combination with surgery or RT alone in patients with cervical cancer stage IB-IIB.

We found a disease specific 5-year survival rate of 87% for stage IB, 75% for stage IIA and 54% for stage IIB.

Our analysis shows that among patients treated with RT alone, the outcome with respect to survival and local control appears to correlate with the biological effective

dose, BED_{10}. We found that patients treated with definitive RT had an average BED_{10} of 92 Gy_{10}. Surviving patients had an average BED_{10} of 98 Gy_{10} compared to an average BED_{10} of 84 Gy_{10} in diseased patients.

In contrast, for the group of patients treated with RT and surgery differences in biological effective dose, BED_{10} did not seem to influence overall survival. There are several possible reasons for this finding. BED is an additive quantity which will not be appropriate when a part of the target is removed by surgery. Furthermore, the combined treatment modalities lead to very prolonged overall treatment time especially for patients receiving both pre-and post-operative RT thus the relevance of the conclusions of the BED calculations could be questionable. Moreover, evaluation of BED correlations to survival and local control in patients that undergoes surgery is difficult to interpret since these parameters also are depending on the outcome of surgery and not only on the RT given.

We also performed calculations of BED considering tumour proliferation during treatment. We found a decrease of BED of approximately 30 Gy when the proliferation was taken into account. A lower BED when taking proliferation into account might be explained in the following way: a certain amount of the delivered dose is "wasted" on counteracting the effect of proliferation due to the increased overall treatment time.

Our finding that the outcome appears to correlate with radiation dose for the group of patients treated with RT only is in agreement with the results reported by Sood *et al*[157]. In that report a trend for better local control for patients with $BED_{10} > 89$ Gy_{10} to point A was found in the analyzed 49 patients with cervical cancer stage I-III treated with EBRT and HDR brachytherapy. There are however, reports showing no dose response correlations between the biologically effective dose to the tumour and outcome of RT[158 159]. In a review of 24 studies on cervical cancer patients treated with EBRT and HDR brachytherapy by Petereit and Pearcy in which the BED was calculated retrospectively by the authors, no relation between dose and local control was found[158]. These inconclusive findings may address the question of the validity of the LQ model, however, there are several additional factors that might be related to the diverging results. The composition of the studied patient populations with respect to factors that will have an impact on survival such as clinical stage, age, and co-morbidity varies between studies. Moreover, since the physical dose and its radiobiological counterpart, the BED, are only correctly defined at the prescription point due the heterogeneity of dose within the target, differences in technique of BT as well as in differences in definition of the prescription point, may all be of importance for the diverging results obtained in different studies.

Regarding side effects in our study, late toxicity from bladder and rectum were recorded in 25 out of 171 patients (15%), of which five patients showed complications from both organs. Grade 2 and grade 3 complications were found in 23 and 2 patients, respectively. In patients treated with RT alone we observed a late rectal complication rate of 30% (13/43) with two patients showing grade 3 complications. For the bladder the complication rate was 14% (6/43). Late complication rates between 0.6 and 25 % are reported for the bladder and rectum after RT for cervical carcinoma[48 160 161 66 64 49]. When comparing studies with respect to complication rates it is important to point out that differences in definitions of late complications as well as follow-up schedules may have an impact on the reported frequencies.

For the patients undergoing RT only, we found an average BED_3 of 86 Gy_3 for rectum and 76 Gy_3 for the bladder. Patients with rectal complications had an average

BED_3 of 83Gy_3 and the average BED_3 was 88 Gy_3 for patients with bladder complications. We did not find a correlation between BED_3 and complications either for the rectum or the bladder. Other studies analyzing BED in relation to complications have reported various thresholds BED_3 for late rectal complications such as BED_3 of 110 Gy_3[162], BED_3 of 125 Gy_3[163] and BED_3 of 143 Gy_3[164]. In our study we had only two patients with average $BED_3 \geq 131$ Gy_3 and none of them were recorded with any late rectal complication.

Our average BED_3 for the rectum is lower than reported threshold BED_3 from others which may explain our result of no correlation between BED_3 and complications from the rectum. The low median BED_3 in our study might lead to expecting a low level of complication probability which would not totally agree to our results with 25% grade 2 and 5% grade 3 rectal complications. This may be related to the rather high median age among the group of patients treated with RT only. It has been shown that high age as well as co-morbidity increases the risk for late complications[162 69]. Another factor of importance could be the large portion of patients treated with the manual technique among patients treated with RT only. The manual technique was associated with higher dosimetric uncertainties due to un-fixated applicators and longer treatment time compared to treatment with the after loading technique. This stresses the difficulties in comparing studies with various techniques for BT, from different time periods or with different stage compositions of the patient populations.

Moreover, it should be mentioned that the predictions of complication rates based on correlations with the BED for the organs at risk are subject of discussion due to the intrinsic limitations of the BED concept which does not take into account the volume effects and the dose heterogeneity within the organ.

In conclusion, we found a correlation between biological effective dose (BED) and survival and local control in patients treated with RT only but not for patients treated with RT and surgery. No correlation was found between BED and late toxicity from bladder and rectum.

5 CONCLUSIONS AND FUTURE PERSPECTIVES

The overall aim of this thesis was to study the treatment outcome for patients with cervical cancer treated with preoperative intracavitary radiotherapy (ICRT). In addition, clinical, dosimetric and molecular factors of importance for response to radiotherapy (RT) were explored.

In paper I we analysed the treatment results of 121 patients with cervical cancer stage IB-IIA treated with preoperative ICRT. We found a 5-year disease specific survival rate of 87% for stage IB and 75% for stage IIA which compares well with survival rates reported for other treatment regimens such as primary surgery and definitive RT. No serious late complications were recorded either from the bladder or the rectum. We found that pathologic complete tumour remission (pCR) after preoperative ICRT had a strong impact on survival. In patients with node negative disease and pCR the 5-year survival rate was 98% compared to 64% in cases with non-pCR. A marked difference in survival between cases with pCR and non-pCR was also found in patients with large tumours with a 5-year survival rate of 96% in cases with pCR compared to 40% in non-pCR cases. These results indicate that the use of preoperative ICRT contributes to treatment outcome. However, preoperative ICRT in the treatment of cervical cancer is controversial and it is still unclear which patients will benefit from this type of treatment. The ultimate way to answer this question is to perform a randomised trial between preoperative ICRT and primary surgery. Such a study is ongoing in Hungary, but there are, to our knowledge, no published results so far. Hopefully the Hungarian study will within a few years give an answer to the question of which patients should be selected for preoperative ICRT.

Nevertheless, a possibility to predict the response to radiotherapy on a pre-treatment tumour biopsy would be of great importance when deciding treatment strategy for the individual patient. A number of molecular markers have previously been studied, such as EGFR and IGFR, with the purpose to find predictive biomarkers for radiosensitivity. In paper II we examined if the DNA-DSB signalling proteins DNA-PKcs, Ku70, Ku86, p53, p21 and Mdm-2 can be used as predictive markers for RT response in cervical cancer. We did not find that the expression of DNA-PK complex proteins in primary tumour specimens can predict RT response. To further investigate the role of the DNA-PK complex proteins for RT response we analyzed the protein expression in pre- and post-treatment tumour specimens in paper III. We found that residual tumours showed an increased frequency of tumour cells positive for DNA-PK complex proteins compared to the frequency in the primary tumour. This result may be interpreted as that radiation causes a selection pressure allowing cells with high DNA-PK expression to survive radiotherapy. Another possibility is that RT induces an upregulation of the DNA-PK proteins in the radioresistant parts of the tumour. In this thesis we have studied only some of the proteins that are involved in the NHEJ pathway. Further studies are warranted to explore the specific role of the DNA-PK complex proteins for radiotherapy response as well as their change in activity during the radiation course.

Treatment planning for radiotherapy is individual for each patient and it is important to be able to compare and evaluate the influence of various treatment schedules on the outcome. We have in paper IV evaluated biologic effective dose (BED) according to the linear quadratic model with respect to survival, local control and late toxicity in patients treated either with radiotherapy and surgery or with radiotherapy alone. We found that BED cannot be used as a predictive factor for the outcome in patients treated with pre-and postoperative radiotherapy or for the late side effects but does correlate with the control of the tumour in patients treated with RT alone. Then again, this thesis is based on patients treated several years ago and due to the development of the treatment methods for both EBRT and BT further studies are needed to evaluate BED for the currently used treatment techniques.

In conclusion we have found that treatment outcome with respect to survival after preoperative intracavitary radiotherapy in early stage cervical cancer is comparable with other treatment regimens. Pathologic complete remission is a strong prognostic factor which indicates that preoperative ICRT contributes to the treatment outcome. Biologic effective dose calculated according to the linear quadratic model does not predict the outcome in patients treated with radiotherapy and surgery but correlates with treatment outcome for patients treated with RT only. The frequency of the DNA-damage repair proteins DNA-PKcs, Ku70 and Ku86, detected by immunohistochemistry in the primary tumour tissue, cannot be used as a predictive biomarker for radiotherapy response. Residual tumours after intracavitary radiotherapy show an increased frequency of tumour cells positive for DNA-PK complex proteins compared to the frequency in the primary tumour suggesting that these proteins have a role in resistance to radiotherapy of cervical carcinoma.

6 ACKNOWLEDGEMENTS

I wish to express my sincere gratitude to everyone who have supported me and contributed to this thesis. In particular, I would like to thank:

Rolf Lewensohn, my main supervisor, for introducing me into the field of molecular research, for your support and for constructive discussions and criticism and for providing a stimulating scientific environment.

KristinaViktorsson, my co-supervisor, for your positive spirit and to have encouraged me when I needed it most, for scrutinising my manuscript and for having patience with my untrained computer mind.

Lena Kanter and **Jurate Skikuniene** for co-authorship with interesting discussions and for generously sharing your knowledge in the field of pathology with me.

Iuliana Toma-Dasu, co-author, for your engaged and supportive collaboration and your expertise in radiobiology.

Anna-Karin Ågren-Cronqvist, my friend and former co-supervisor, for initiating this project together with me, for your profound knowledge and for all good talks.

Åsa Holgersson, co-author, for enthusiastic and spirited collaboration.

Bo Nilsson, Fredrik Granath and **Ronny Westberg** for guiding me through the winding paths of statistics.

Anders Nilsson, for pedagogic and inspiring discussions that have contributed to me finalizes this thesis.

Bo Frankendal, my former co-supervisor, for interesting discussions and fruitful collaboration.

Elisabeth Åvall-Lundqvist, co-author, for valuable clinical advices and supportive discussions.

Margareta Rodensjö, for skilful assistance with the immunohistochemistry studies.

Marianne Langéen, for your positive mind and for helping me with all sorts of practical work connected with this thesis.

Christina von Gertten for valuable assistance when I struggled with the computer programmes.

Mariann Iiristo, for encouragement and support, giving me the possibility to complete this thesis.

Roger Henriksson, for providing me the opportunity to do my research.

My colleagues, for friendship and kind support, for taking over my clinical workload and for discussions about important things in life.

Eva Lindblad, for all your extra work, sparing no effort to find solutions for my patients when I was doing my research.

Finally I wish to thank my beloved family **Robert**, **Peter, Lisa** and **Fredrik** for care and support, for our lively and loudly discussions and all good laughs.

These studies were supported by grants from the Swedish Cancer Society, the Stockholm Cancer Society, the European Commission, the research fund of the Stockholm County Council and funds of the Karolinska Institutet.

7 REFERENCES

1 Ferlay J, Shin HR, Bray F et al. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *International journal of cancer Journal international du cancer*. 2010.

2 Klint A, Tryggvadottir L, Bray F et al. Trends in the survival of patients diagnosed with cancer in female genital organs in the Nordic countries 1964-2003 followed up to the end of 2006. *Acta oncologica*. 2010;49: 632-43.

3 The National Board of Health and Wellfare. *Cancer incidence in Sweden 2008.* Stockholm: Socialstyrelsen 2009.

4 Bosch FX, Lorincz A, Munoz N et al. The causal relation between human papillomavirus and cervical cancer. *Journal of clinical pathology*. 2002;55: 244-65.

5 Bosch FX, Manos MM, Munoz N et al. Prevalence of human papillomavirus in cervical cancer: a worldwide perspective. International biological study on cervical cancer (IBSCC) Study Group. *Journal of the National Cancer Institute*. 1995;87: 796-802.

6 Walboomers JM, Jacobs MV, Manos MM et al. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. *The Journal of pathology*. 1999;189: 12-9.

7 Munoz N, Bosch FX, de Sanjose S et al. Epidemiologic classification of human papillomavirus types associated with cervical cancer. *The New England journal of medicine*. 2003;348: 518-27.

8 Tjalma WA, Van Waes TR, Van den Eeden LE, Bogers JJ. Role of human papillomavirus in the carcinogenesis of squamous cell carcinoma and adenocarcinoma of the cervix. *Best Pract Res Clin Obstet Gynaecol*. 2005;19: 469-83.

9 Green J, Berrington de Gonzalez A, Sweetland S et al. Risk factors for adenocarcinoma and squamous cell carcinoma of the cervix in women aged 20-44 years: the UK National Case-Control Study of Cervical Cancer. *British journal of cancer*. 2003;89: 2078-86.

10 Vaccarella S, Herrero R, Dai M et al. Reproductive factors, oral contraceptive use, and human papillomavirus infection: pooled analysis of the IARC HPV prevalence surveys. *Cancer Epidemiol Biomarkers Prev*. 2006;15: 2148-53.

11 Meeuwis KA, van Rossum MM, van de Kerkhof PC et al. Skin cancer and (pre)malignancies of the female genital tract in renal transplant recipients. *Transplant international : official journal of the European Society for Organ Transplantation*. 2010;23: 191-9.

12 Munoz N, Kjaer SK, Sigurdsson K et al. Impact of human papillomavirus (HPV)-6/11/16/18 vaccine on all HPV-associated genital diseases in young women. *Journal of the National Cancer Institute*. 2010;102: 325-39.

13 Pecorelli S, Zigliani L, Odicino F. Revised FIGO staging for carcinoma of the cervix. *International journal of gynaecology and obstetrics: the official organ of the International Federation of Gynaecology and Obstetrics*. 2009;105: 107-8.

14 Vizcaino AP, Moreno V, Bosch FX et al. International trends in the incidence of cervical cancer: I. Adenocarcinoma and adenosquamous cell carcinomas. *International journal of cancer Journal international du cancer*. 1998;75: 536-45.

15 Bergstrom R, Sparen P, Adami HO. Trends in cancer of the cervix uteri in Sweden following cytological screening. *British journal of cancer*. 1999;81: 159-66.

16 Smith HO, Tiffany MF, Qualls CR, Key CR. The rising incidence of adenocarcinoma relative to squamous cell carcinoma of the uterine cervix in the United States--a 24-year population-based study. *Gynecologic oncology*. 2000;78: 97-105.

17 Quinn MA, Benedet JL, Odicino F et al. Carcinoma of the cervix uteri. FIGO 6th Annual Report on the Results of Treatment in Gynecological Cancer. *Int J Gynaecol Obstet*. 2006;95 Suppl 1: S43-103.
18 Lee YN, Wang KL, Lin MH et al. Radical hysterectomy with pelvic lymph node dissection for treatment of cervical cancer: a clinical review of 954 cases. *Gynecologic oncology*. 1989;32: 135-42.
19 Perez CA, Grigsby PW, Camel HM et al. Irradiation alone or combined with surgery in stage IB, IIA, and IIB carcinoma of uterine cervix: update of a nonrandomized comparison. *International journal of radiation oncology, biology, physics*. 1995;31: 703-16.
20 Perez CA, Grigsby PW, Nene SM et al. Effect of tumor size on the prognosis of carcinoma of the uterine cervix treated with irradiation alone. *Cancer*. 1992;69: 2796-806.
21 Eifel PJ, Morris M, Wharton JT, Oswald MJ. The influence of tumor size and morphology on the outcome of patients with FIGO stage IB squamous cell carcinoma of the uterine cervix. *International journal of radiation oncology, biology, physics*. 1994;29: 9-16.
22 Barillot I, Horiot JC, Pigneux J et al. Carcinoma of the intact uterine cervix treated with radiotherapy alone: a French cooperative study: update and multivariate analysis of prognostics factors. *International journal of radiation oncology, biology, physics*. 1997;38: 969-78.
23 Lambin P, Kramar A, Haie-Meder C et al. Tumour size in cancer of the cervix. *Acta oncologica*. 1998;37: 729-34.
24 Sevin BU, Lu Y, Bloch DA et al. Surgically defined prognostic parameters in patients with early cervical carcinoma. A multivariate survival tree analysis. *Cancer*. 1996;78: 1438-46.
25 Samlal RA, van der Velden J, Ten Kate FJ et al. Surgical pathologic factors that predict recurrence in stage IB and IIA cervical carcinoma patients with negative pelvic lymph nodes. *Cancer*. 1997;80: 1234-40.
26 Hellebrekers BW, Zwinderman AH, Kenter GG et al. Surgically-treated early cervical cancer: Prognostic factors and the significance of depth of tumor invasion. *International journal of gynecological cancer : official journal of the International Gynecological Cancer Society*. 1999;9: 212-9.
27 Rutledge TL, Kamelle SA, Tillmanns TD et al. A comparison of stages IB1 and IB2 cervical cancers treated with radical hysterectomy. Is size the real difference? *Gynecologic oncology*. 2004;95: 70-6.
28 Landoni F, Maneo A, Colombo A et al. Randomised study of radical surgery versus radiotherapy for stage Ib-IIa cervical cancer. *Lancet*. 1997;350: 535-40.
29 Sedlis A, Bundy BN, Rotman MZ et al. A randomized trial of pelvic radiation therapy versus no further therapy in selected patients with stage IB carcinoma of the cervix after radical hysterectomy and pelvic lymphadenectomy: A Gynecologic Oncology Group Study. *Gynecologic oncology*. 1999;73: 177-83.
30 Rotman M, Sedlis A, Piedmonte MR et al. A phase III randomized trial of postoperative pelvic irradiation in Stage IB cervical carcinoma with poor prognostic features: follow-up of a gynecologic oncology group study. *International journal of radiation oncology, biology, physics*. 2006;65: 169-76.
31 Rogers L, Siu SS, Luesley D et al. Adjuvant radiotherapy and chemoradiation after surgery for cervical cancer. *Cochrane Database Syst Rev*. 2009: CD007583.
32 Morris M, Eifel PJ, Lu J et al. Pelvic radiation with concurrent chemotherapy compared with pelvic and para-aortic radiation for high-risk cervical cancer. *The New England journal of medicine*. 1999;340: 1137-43.
33 Rose PG, Bundy BN, Watkins EB et al. Concurrent cisplatin-based radiotherapy and chemotherapy for locally advanced cervical cancer. *The New England journal of medicine*. 1999;340: 1144-53.
34 Peters WA, 3rd, Liu PY, Barrett RJ, 2nd et al. Concurrent chemotherapy and pelvic radiation therapy compared with pelvic radiation therapy alone as adjuvant therapy after radical surgery in high-risk early-stage cancer of the cervix. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 2000;18: 1606-13.

35 Vale CL, Tierney JF, Davidson SE et al. Substantial improvement in UK cervical cancer survival with chemoradiotherapy: results of a Royal College of Radiologists' audit. *Clinical oncology*. 2010;22: 590-601.
36 Green JA, Kirwan JJ, Tierney J et al. Concomitant chemotherapy and radiation therapy for cancer of the uterine cervix. *Cochrane Database Syst Rev*. 2005: CD002225.
37 Calais G, Le Floch O, Chauvet B et al. Carcinoma of the uterine cervix stage IB and early stage II. Prognostic value of the histological tumor regression after initial brachytherapy. *International journal of radiation oncology, biology, physics*. 1989;17: 1231-5.
38 Gerbaulet AL, Kunkler IH, Kerr GR et al. Combined radiotherapy and surgery: local control and complications in early carcinoma of the uterine cervix--the Villejuif experience, 1975-1984. *Radiother Oncol*. 1992;23: 66-73.
39 Gerdin E, Cnattingius S, Johnson P, Pettersson B. Prognostic factors and relapse patterns in early-stage cervical carcinoma after brachytherapy and radical hysterectomy. *Gynecologic oncology*. 1994;53: 314-9.
40 Mundt AJ, Waggoner S, Herbst A, Rotmensch J. Preoperative intracavitary brachytherapy in early-stage cervical carcinoma. *American journal of clinical oncology*. 1999;22: 73-7.
41 Resbeut MR, Alzieu C, Gonzague-Casabianca L et al. Combined brachytherapy and surgery for early carcinoma of the uterine cervix: analysis of extent of surgery on outcome. *International journal of radiation oncology, biology, physics*. 2001;50: 873-81.
42 Haie-Meder C, Chargari C, Rey A et al. DVH parameters and outcome for patients with early-stage cervical cancer treated with preoperative MRI-based low dose rate brachytherapy followed by surgery. *Radiother Oncol*. 2009;93: 316-21.
43 Piver MS, Rutledge F, Smith JP. Five classes of extended hysterectomy for women with cervical cancer. *Obstetrics and gynecology*. 1974;44: 265-72.
44 Bergmark K, Avall-Lundqvist E, Dickman PW et al. Lymphedema and bladder-emptying difficulties after radical hysterectomy for early cervical cancer and among population controls. *Int J Gynecol Cancer*. 2006;16: 1130-9.
45 de Kroon CD, Gaarenstroom KN, van Poelgeest MI et al. Nerve sparing in radical surgery for early-stage cervical cancer: yes we should! *International journal of gynecological cancer : official journal of the International Gynecological Cancer Society*. 2010;20: S39-41.
46 Lanciano RM, Won M, Coia LR, Hanks GE. Pretreatment and treatment factors associated with improved outcome in squamous cell carcinoma of the uterine cervix: a final report of the 1973 and 1978 patterns of care studies. *International journal of radiation oncology, biology, physics*. 1991;20: 667-76.
47 Perez CA, Grigsby PW, Chao KS et al. Tumor size, irradiation dose, and long-term outcome of carcinoma of uterine cervix. *International journal of radiation oncology, biology, physics*. 1998;41: 307-17.
48 Montana GS, Fowler WC. Carcinoma of the cervix: analysis of bladder and rectal radiation dose and complications. *International journal of radiation oncology, biology, physics*. 1989;16: 95-100.
49 Perez CA, Grigsby PW, Lockett MA et al. Radiation therapy morbidity in carcinoma of the uterine cervix: dosimetric and clinical correlation. *International journal of radiation oncology, biology, physics*. 1999;44: 855-66.
50 Portelance L, Chao KS, Grigsby PW et al. Intensity-modulated radiation therapy (IMRT) reduces small bowel, rectum, and bladder doses in patients with cervical cancer receiving pelvic and para-aortic irradiation. *International journal of radiation oncology, biology, physics*. 2001;51: 261-6.
51 Mundt AJ, Mell LK, Roeske JC. Preliminary analysis of chronic gastrointestinal toxicity in gynecology patients treated with intensity-modulated whole pelvic radiation therapy. *International journal of radiation oncology, biology, physics*. 2003;56: 1354-60.
52 Taylor A, Powell ME. Conformal and intensity-modulated radiotherapy for cervical cancer. *Clinical oncology*. 2008;20: 417-25.

53 Forsell G. Erfenheter om radiumbehandling av underlivskräfta vid Radiumhemmet i Stockholm 1910-1913. *Hygiea*. 1914;76: 1170-80.
54 Gerbaulet A, Ash DM. *The GEC ESTRO handbook of brachytherapy*. Belgium: [European Society for Therapeutic Radiology and Oncology] 2002.
55 Walstam R. Remotely-controlled afterloading radiotherapy apparatus. (A preliminary report). *Phys Med Biol*. 1962;7: 225-8.
56 Tod M, Meredith WJ. Treatment of cancer of the cervix uteri, a revised Manchester method. *The British journal of radiology*. 1953;26: 252-7.
57 Potter R, Haie-Meder C, Van Limbergen E et al. Recommendations from gynaecological (GYN) GEC ESTRO working group (II): concepts and terms in 3D image-based treatment planning in cervix cancer brachytherapy-3D dose volume parameters and aspects of 3D image-based anatomy, radiation physics, radiobiology. *Radiotherapy and oncology : journal of the European Society for Therapeutic Radiology and Oncology*. 2006;78: 67-77.
58 Lindegaard JC, Tanderup K, Nielsen SK et al. MRI-guided 3D optimization significantly improves DVH parameters of pulsed-dose-rate brachytherapy in locally advanced cervical cancer. *International journal of radiation oncology, biology, physics*. 2008;71: 756-64.
59 Potter R, Dimopoulos J, Georg P et al. Clinical impact of MRI assisted dose volume adaptation and dose escalation in brachytherapy of locally advanced cervix cancer. *Radiotherapy and oncology : journal of the European Society for Therapeutic Radiology and Oncology*. 2007;83: 148-55.
60 ICRU. ICRU report 38. Dose and volume specifications for reporting intracavitary therapy in gynecology. In: ICRU, ed.: International Comission on Radiation Units and Measurements 1985.
61 Hareyama M, Sakata K, Oouchi A et al. High-dose-rate versus low-dose-rate intracavitary therapy for carcinoma of the uterine cervix: a randomized trial. *Cancer*. 2002;94: 117-24.
62 Stewart AJ, Viswanathan AN. Current controversies in high-dose-rate versus low-dose-rate brachytherapy for cervical cancer. *Cancer*. 2006;107: 908-15.
63 Viani GA, Manta GB, Stefano EJ, de Fendi LI. Brachytherapy for cervix cancer: low-dose rate or high-dose rate brachytherapy - a meta-analysis of clinical trials. *Journal of experimental & clinical cancer research : CR*. 2009;28: 47.
64 Eifel PJ, Levenback C, Wharton JT, Oswald MJ. Time course and incidence of late complications in patients treated with radiation therapy for FIGO stage IB carcinoma of the uterine cervix. *International journal of radiation oncology, biology, physics*. 1995;32: 1289-300.
65 Perez CA, Breaux S, Bedwinek JM et al. Radiation therapy alone in the treatment of carcinoma of the uterine cervix. II. Analysis of complications. *Cancer*. 1984;54: 235-46.
66 Lanciano RM, Martz K, Montana GS, Hanks GE. Influence of age, prior abdominal surgery, fraction size, and dose on complications after radiation therapy for squamous cell cancer of the uterine cervix. A patterns of care study. *Cancer*. 1992;69: 2124-30.
67 Hanks GE, Herring DF, Kramer S. Patterns of care outcome studies results of the national practice in cancer of the cervix. Wiley Subscription Services, Inc., A Wiley Company 1983:959-67.
68 Eifel PJ, Jhingran A, Bodurka DC et al. Correlation of smoking history and other patient characteristics with major complications of pelvic radiation therapy for cervical cancer. *J Clin Oncol*. 2002;20: 3651-7.
69 Chon BH, Loeffler JS. The effect of nonmalignant systemic disease on tolerance to radiation therapy. *Oncologist*. 2002;7: 136-43.
70 Shrivastav M, De Haro LP, Nickoloff JA. Regulation of DNA double-strand break repair pathway choice. *Cell Res*. 2008;18: 134-47.
71 Li X, Heyer WD. Homologous recombination in DNA repair and DNA damage tolerance. *Cell Res*. 2008;18: 99-113.
72 Hefferin ML, Tomkinson AE. Mechanism of DNA double-strand break repair by non-homologous end joining. *DNA repair*. 2005;4: 639-48.

73 Lavin MF, Shiloh Y. The genetic defect in ataxia-telangiectasia. *Annual review of immunology*. 1997;15: 177-202.
74 Rotman G, Shiloh Y. ATM: a mediator of multiple responses to genotoxic stress. *Oncogene*. 1999;18: 6135-44.
75 Branzei D, Foiani M. Regulation of DNA repair throughout the cell cycle. *Nat Rev Mol Cell Biol*. 2008;9: 297-308.
76 Mahaney BL, Meek K, Lees-Miller SP. Repair of ionizing radiation-induced DNA double-strand breaks by non-homologous end-joining. *Biochem J*. 2009;417: 639-50.
77 Dynan WS, Yoo S. Interaction of Ku protein and DNA-dependent protein kinase catalytic subunit with nucleic acids. *Nucleic acids research*. 1998;26: 1551-9.
78 Hartley KO, Gell D, Smith GC et al. DNA-dependent protein kinase catalytic subunit: a relative of phosphatidylinositol 3-kinase and the ataxia telangiectasia gene product. *Cell*. 1995;82: 849-56.
79 Shiloh Y. ATM and related protein kinases: safeguarding genome integrity. *Nature reviews*. 2003;3: 155-68.
80 Mimori T, Hardin JA. Mechanism of interaction between Ku protein and DNA. *The Journal of biological chemistry*. 1986;261: 10375-9.
81 DeFazio LG, Stansel RM, Griffith JD, Chu G. Synapsis of DNA ends by DNA-dependent protein kinase. *The EMBO journal*. 2002;21: 3192-200.
82 Weterings E, Chen DJ. DNA-dependent protein kinase in nonhomologous end joining: a lock with multiple keys? *The Journal of cell biology*. 2007;179: 183-6.
83 Ma Y, Pannicke U, Schwarz K, Lieber MR. Hairpin opening and overhang processing by an Artemis/DNA-dependent protein kinase complex in nonhomologous end joining and V(D)J recombination. *Cell*. 2002;108: 781-94.
84 Ahnesorg P, Smith P, Jackson SP. XLF interacts with the XRCC4-DNA ligase IV complex to promote DNA nonhomologous end-joining. *Cell*. 2006;124: 301-13.
85 Chang C, Biedermann KA, Mezzina M, Brown JM. Characterization of the DNA double strand break repair defect in scid mice. *Cancer research*. 1993;53: 1244-8.
86 Singleton BK, Priestley A, Steingrimsdottir H et al. Molecular and biochemical characterization of xrs mutants defective in Ku80. *Molecular and cellular biology*. 1997;17: 1264-73.
87 Allalunis-Turner MJ, Zia PK, Barron GM et al. Radiation-induced DNA damage and repair in cells of a radiosensitive human malignant glioma cell line. *Radiation research*. 1995;144: 288-93.
88 Lees-Miller SP, Godbout R, Chan DW et al. Absence of p350 subunit of DNA-activated protein kinase from a radiosensitive human cell line. *Science (New York, NY*. 1995;267: 1183-5.
89 Johnson N, Shapiro GI. Cyclin-dependent kinases (cdks) and the DNA damage response: rationale for cdk inhibitor–chemotherapy combinations as an anticancer strategy for solid tumors. *Expert Opin Ther Targets*. 2010;14: 1199-212.
90 Zetterberg A, Larsson O, Wiman KG. What is the restriction point? *Curr Opin Cell Biol*. 1995;7: 835-42.
91 Levine AJ, Oren M. The first 30 years of p53: growing ever more complex. *Nature reviews Cancer*. 2009;9: 749-58.
92 Waning DL, Lehman JA, Batuello CN, Mayo LD. Controlling the Mdm2-Mdmx-p53 Circuit. *Pharmaceuticals (Basel)*. 2010;3: 1576-93.
93 Sancar A, Lindsey-Boltz LA, Unsal-Kacmaz K, Linn S. Molecular mechanisms of mammalian DNA repair and the DNA damage checkpoints. *Annu Rev Biochem*. 2004;73: 39-85.
94 Udayakumar T, Shareef MM, Diaz DA et al. The E2F1/Rb and p53/MDM2 pathways in DNA repair and apoptosis: understanding the crosstalk to develop novel strategies for prostate cancer radiotherapy. *Seminars in radiation oncology*. 2010;20: 258-66.

95 Petitjean A, Mathe E, Kato S et al. Impact of mutant p53 functional properties on TP53 mutation patterns and tumor phenotype: lessons from recent developments in the IARC TP53 database. *Hum Mutat*. 2007;28: 622-9.
96 Fujita M, Inoue M, Tanizawa O et al. Alterations of the p53 gene in human primary cervical carcinoma with and without human papillomavirus infection. *Cancer research*. 1992;52: 5323-8.
97 Helland A, Holm R, Kristensen G et al. Genetic alterations of the TP53 gene, p53 protein expression and HPV infection in primary cervical carcinomas. *The Journal of pathology*. 1993;171: 105-14.
98 Busby-Earle RM, Steel CM, Williams AR et al. p53 mutations in cervical carcinogenesis--low frequency and lack of correlation with human papillomavirus status. *British journal of cancer*. 1994;69: 732-7.
99 Benjamin I, Saigo P, Finstad C et al. Expression and mutational analysis of P53 in stage IB and IIA cervical cancers. *American journal of obstetrics and gynecology*. 1996;175: 1266-71.
100 Harima Y, Harima K, Shikata N et al. Bax and Bcl-2 expressions predict response to radiotherapy in human cervical cancer. *Journal of cancer research and clinical oncology*. 1998;124: 503-10.
101 Scheffner M, Werness BA, Huibregtse JM et al. The E6 oncoprotein encoded by human papillomavirus types 16 and 18 promotes the degradation of p53. *Cell*. 1990;63: 1129-36.
102 Scheffner M, Huibregtse JM, Vierstra RD, Howley PM. The HPV-16 E6 and E6-AP complex functions as a ubiquitin-protein ligase in the ubiquitination of p53. *Cell*. 1993;75: 495-505.
103 Tommasino M, Accardi R, Caldeira S et al. The role of TP53 in Cervical carcinogenesis. *Hum Mutat*. 2003;21: 307-12.
104 Williams JR, Zhang Y, Zhou H et al. Genotype-dependent radiosensitivity: clonogenic survival, apoptosis and cell-cycle redistribution. *International journal of radiation biology*. 2008;84: 151-64.
105 Barendsen GW. Dose fractionation, dose rate and iso-effect relationships for normal tissue responses. *International journal of radiation oncology, biology, physics*. 1982;8: 1981-97.
106 Douglas BG, Fowler JF. Letter: Fractionation schedules and a quadratic dose-effect relationship. *The British journal of radiology*. 1975;48: 502-4.
107 Fowler JF. The linear-quadratic formula and progress in fractionated radiotherapy. *The British journal of radiology*. 1989;62: 679-94.
108 Thames HD, Bentzen SM, Turesson I et al. Time-dose factors in radiotherapy: a review of the human data. *Radiotherapy and oncology : journal of the European Society for Therapeutic Radiology and Oncology*. 1990;19: 219-35.
109 Steel GG. *Basic clinical radiobiology for radiation oncologists*. London: Edward Arnold 1993.
110 Deschavanne PJ, Fertil B. A review of human cell radiosensitivity in vitro. *International journal of radiation oncology, biology, physics*. 1996;34: 251-66.
111 Thames HD, Jr., Withers HR, Peters LJ, Fletcher GH. Changes in early and late radiation responses with altered dose fractionation: implications for dose-survival relationships. *International journal of radiation oncology, biology, physics*. 1982;8: 219-26.
112 Dorr W, Hendry JH. Consequential late effects in normal tissues. *Radiother Oncol*. 2001;61: 223-31.
113 Withers HR. 4 R´s of Radiotherapy. New York: Academic Press, Inc. 1975:241- 71.
114 Williams JR, Zhang Y, Zhou H et al. Overview of radiosensitivity of human tumor cells to low-dose-rate irradiation. *International journal of radiation oncology, biology, physics*. 2008;72: 909-17.
115 Pettersson F, ed. *Annual report on the results of treatment in gynecological cancer. Twentieth volume statements of results obtained in patients treated in 1979 to 1981, inclusive 5-year survival up to 1986.* Stockholm: International Federation of Gynecology and Obstetrics 1988.

116 Chassagne D, Sismondi P, Horiot JC et al. A glossary for reporting complications of treatment in gynecological cancers. *Radiother Oncol*. 1993;26: 195-202.
117 Withers HR, Maciejewski B, Taylor JM, Hliniak A. Accelerated repopulation in head and neck cancer. *Front Radiat Ther Oncol*. 1988;22: 105-10.
118 Gasinska A, Fowler JF, Lind BK, Urbanski K. Influence of overall treatment time and radiobiological parameters on biologically effective doses in cervical cancer patients treated with radiation therapy alone. *Acta oncologica*. 2004;43: 657-66.
119 Bianchi C, Botta F, Conte L et al. Biological effective dose evaluation in gynaecological brachytherapy: LDR and HDR treatments, dependence on radiobiological parameters, and treatment optimisation. *Radiol Med*. 2008;113: 1068-78.
120 Einhorn N, Patek E, Sjoberg B. Outcome of different treatment modalities in cervix carcinoma Stage IB and IIA. Observations in a well-defined Swedish population. *Cancer*. 1985;55: 949-55.
121 Atlan D, Touboul E, Deniaud-Alexandre E et al. Operable Stages IB and II cervical carcinomas: a retrospective study comparing preoperative uterovaginal brachytherapy and postoperative radiotherapy. *International journal of radiation oncology, biology, physics*. 2002;54: 780-93.
122 Sundfor K, Trope CG, Kjorstad KE. Radical radiotherapy versus brachytherapy plus surgery in carcinoma of the cervix 2A and 2B--long-term results from a randomized study 1968-1980. *Acta oncologica (Stockholm, Sweden)*. 1996;35 Suppl 8: 99-107.
123 Delgado G, Bundy B, Zaino R et al. Prospective surgical-pathological study of disease-free interval in patients with stage IB squamous cell carcinoma of the cervix: a Gynecologic Oncology Group study. *Gynecologic oncology*. 1990;38: 352-7.
124 Pilleron JP, Durand JC, Lenoble JC. Carcinoma of the uterine cervix, stages I and II, treated by radiation therapy and extensive surgery (1000 cases). *Cancer*. 1972;29: 593-6.
125 Rampone JF, Klem V, Kolstad P. Combined treatment of stage Ib carcinoma of the cervix. *Obstetrics and gynecology*. 1973;41: 163-7.
126 Marziale P, Atlante G, Le Pera V et al. Combined radiation and surgical treatment of stages IB and IIA and B carcinoma of the cervix. *Gynecologic oncology*. 1981;11: 175-83.
127 Bonar LD. Results of radical surgical procedures after radiation for treatment of invasive carcinoma of the uterine cervix in a private practice. *American journal of obstetrics and gynecology*. 1980;136: 1006-8.
128 Lambin P, Gerbaulet A, Kramar A et al. A comparison of early effects with two dose rates in brachytherapy of cervix carcinoma in a prospective randomised trial. *Eur J Cancer*. 1994;30A: 312-20.
129 Jacinto AA, Castilho MS, Novaes PE et al. Preoperative external beam radiotherapy and reduced dose brachytherapy for carcinoma of the cervix: survival and pathological response. *Radiat Oncol*. 2007;2: 9.
130 Grigsby PW, Perez CA, Chao KS et al. Lack of effect of tumor size on the prognosis of carcinoma of the uterine cervix Stage IB and IIA treated with preoperative irradiation and surgery. *International journal of radiation oncology, biology, physics*. 1999;45: 645-51.
131 Lee CM, Shrieve DC, Zempolich KA et al. Correlation between human epidermal growth factor receptor family (EGFR, HER2, HER3, HER4), phosphorylated Akt (P-Akt), and clinical outcomes after radiation therapy in carcinoma of the cervix. *Gynecologic oncology*. 2005;99: 415-21.
132 Pillai MR, Jayaprakash PG, Nair MK. Tumour-proliferative fraction and growth factor expression as markers of tumour response to radiotherapy in cancer of the uterine cervix. *J Cancer Res Clin Oncol*. 1998;124: 456-61.
133 Noordhuis MG, Eijsink JJ, Ten Hoor KA et al. Expression of epidermal growth factor receptor (EGFR) and activated EGFR predict poor response to (chemo)radiation and survival in cervical cancer. *Clinical cancer research : an*

official journal of the American Association for Cancer Research. 2009;15: 7389-97.
134 Kim TJ, Lee JW, Song SY et al. Increased expression of pAKT is associated with radiation resistance in cervical cancer. *British journal of cancer*. 2006;94: 1678-82.
135 Stronati L, Gensabella G, Lamberti C et al. Expression and DNA binding activity of the Ku heterodimer in bladder carcinoma. *Cancer*. 2001;92: 2484-92.
136 Hosoi Y, Watanabe T, Nakagawa K et al. Up-regulation of DNA-dependent protein kinase activity and Sp1 in colorectal cancer. *International journal of oncology*. 2004;25: 461-8.
137 Bartkova J, Horejsi Z, Koed K et al. DNA damage response as a candidate anti-cancer barrier in early human tumorigenesis. *Nature*. 2005;434: 864-70.
138 Wilson CR, Davidson SE, Margison GP et al. Expression of Ku70 correlates with survival in carcinoma of the cervix. *British journal of cancer*. 2000;83: 1702-6.
139 Gorgoulis VG, Vassiliou LV, Karakaidos P et al. Activation of the DNA damage checkpoint and genomic instability in human precancerous lesions. *Nature*. 2005;434: 907-13.
140 Halazonetis TD, Gorgoulis VG, Bartek J. An oncogene-induced DNA damage model for cancer development. *Science*. 2008;319: 1352-5.
141 Taccioli GE, Gottlieb TM, Blunt T et al. Ku80: product of the XRCC5 gene and its role in DNA repair and V(D)J recombination. *Science*. 1994;265: 1442-5.
142 Kirchgessner CU, Patil CK, Evans JW et al. DNA-dependent kinase (p350) as a candidate gene for the murine SCID defect. *Science*. 1995;267: 1178-83.
143 Jeggo PA, Taccioli GE, Jackson SP. Menage a trois: double strand break repair, V(D)J recombination and DNA-PK. *Bioessays*. 1995;17: 949-57.
144 Sirzen F, Nilsson A, Zhivotovsky B, Lewensohn R. DNA-dependent protein kinase content and activity in lung carcinoma cell lines: correlation with intrinsic radiosensitivity. *Eur J Cancer*. 1999;35: 111-6.
145 Dittmann K, Mayer C, Fehrenbacher B et al. Radiation-induced epidermal growth factor receptor nuclear import is linked to activation of DNA-dependent protein kinase. *The Journal of biological chemistry*. 2005;280: 31182-9.
146 Friesland S, Kanter-Lewensohn L, Tell R et al. Expression of Ku86 confers favorable outcome of tonsillar carcinoma treated with radiotherapy. *Head & neck*. 2003;25: 313-21.
147 Komuro Y, Watanabe T, Hosoi Y et al. Prediction of tumor radiosensitivity in rectal carcinoma based on p53 and Ku70 expression. *J Exp Clin Cancer Res*. 2003;22: 223-8.
148 Harima Y, Sawada S, Miyazaki Y et al. Expression of Ku80 in cervical cancer correlates with response to radiotherapy and survival. *American journal of clinical oncology*. 2003;26: e80-5.
149 Mukherjee G, Freeman A, Moore R et al. Biologic factors and response to radiotherapy in carcinoma of the cervix. *Int J Gynecol Cancer*. 2001;11: 187-93.
150 Ebara T, Mitsuhashi N, Saito Y et al. Prognostic significance of immunohistochemically detected p53 protein expression in stage IIIB squamous cell carcinoma of the uterine cervix treated with radiation therapy alone. *Gynecologic oncology*. 1996;63: 216-8.
151 Schneider J, Rubio MP, Rodriguez-Escudero FJ et al. Identification of p53 mutations by means of single strand conformation polymorphism analysis in gynaecological tumours: comparison with the results of immunohistochemistry. *Eur J Cancer*. 1994;30A: 504-8.
152 Ishikawa H, Mitsuhashi N, Sakurai H et al. The effects of p53 status and human papillomavirus infection on the clinical outcome of patients with stage IIIB cervical carcinoma treated with radiation therapy alone. *Cancer*. 2001;91: 80-9.
153 Kim CY, Tsai MH, Osmanian C et al. Selection of human cervical epithelial cells that possess reduced apoptotic potential to low-oxygen conditions. *Cancer research*. 1997;57: 4200-4.

154 Shintani S, Mihara M, Li C et al. Up-regulation of DNA-dependent protein kinase correlates with radiation resistance in oral squamous cell carcinoma. *Cancer science*. 2003;94: 894-900.
155 Bernstein C, Bernstein H, Payne CM, Garewal H. DNA repair/pro-apoptotic dual-role proteins in five major DNA repair pathways: fail-safe protection against carcinogenesis. *Mutat Res*. 2002;511: 145-78.
156 Niibe Y, Nakano T, Ohno T et al. Relationship between p21/WAF-1/CIP-1 and apoptosis in cervical cancer during radiation therapy. *International journal of radiation oncology, biology, physics*. 1999;44: 297-303.
157 Sood B, Garg M, Avadhani J et al. Predictive value of linear-quadratic model in the treatment of cervical cancer using high-dose-rate brachytherapy. *International journal of radiation oncology, biology, physics*. 2002;54: 1377-87.
158 Petereit DG, Pearcey R. Literature analysis of high dose rate brachytherapy fractionation schedules in the treatment of cervical cancer: is there an optimal fractionation schedule? *International journal of radiation oncology, biology, physics*. 1999;43: 359-66.
159 Niibe Y, Kenjo M, Onishi H et al. High-dose-rate intracavitary brachytherapy combined with external beam radiotherapy for stage IIIb adenocarcinoma of the uterine cervix in Japan: a multi-institutional study of Japanese Society of Therapeutic Radiology and Oncology 2006-2007 (study of JASTRO 2006-2007). *Jpn J Clin Oncol*. 2010;40: 795-9.
160 Khoury GG, Bulman AS, Joslin CA. Long term results of Cathetron high dose rate intracavitary radiotherapy in the treatment of carcinoma of the cervix. *The British journal of radiology*. 1991;64: 1036-43.
161 Bachaud JM, Fu RC, Delannes M et al. Non-randomized comparative study of irradiation alone or in combination with surgery in stage Ib, IIa and "proximal" IIb carcinoma of the cervix. *Radiother Oncol*. 1991;22: 104-10.
162 Chen SW, Liang JA, Yang SN et al. The prediction of late rectal complications following the treatment of uterine cervical cancer by high-dose-rate brachytherapy. *International journal of radiation oncology, biology, physics*. 2000;47: 955-61.
163 Clark BG, Souhami L, Roman TN et al. The prediction of late rectal complications in patients treated with high dose-rate brachytherapy for carcinoma of the cervix. *International journal of radiation oncology, biology, physics*. 1997;38: 989-93.
164 Lee SW, Suh CO, Chung EJ, Kim GE. Dose optimization of fractionated external radiation and high-dose-rate intracavitary brachytherapy for FIGO stage IB uterine cervical carcinoma. *International journal of radiation oncology, biology, physics*. 2002;52: 1338-44.