UNIVERSITÀ DI SIENA 1240

UNIVERSITA' DI SIENA

DIPARTIMENTO DI MEDICINA MOLECOLARE E DELLO SVILUPPO

DOTTORATO DI RICERCA IN MEDICINA MOLECOLARE

CICLO XXXV

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TITOLO DELLA TESI:
NEOADJUVANT DOSE-DENSE CHEMOTHERAPY IN LOCALLY ADVANCED CERVICAL CANCER: FROM MOLECULAR TO CLINICAL PRACTICE

SETTORE SCIENTIFICO-DISCIPLINARE: med/40

DOTTORANDO

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ANNO ACCADEMICO: 2022/2023

Dedico questo lavoro alla mia famiglia che mi ha sempre sostenuto in ogni scelta.
Un ringraziamento speciale alla Prof.ssa Paola Piomboni e ai colleghi dell'Istituto Europeo di Oncologia, fondamentali per la costante crescita personale e lavorativa.

Summary

Neoadjuvant dose-dense chemotherapy in locally advanced cervical cancer: from molecular to clinical practice

1. Introduction

Cervical cancer is the second most commonly diagnosed cancer and the third leading cause of cancer death among women in less developed countries (1).

Etiology of cervical cancer

Persistent human papillomavirus (HPV) infection is the most important cause in the development of cervical cancer (2). HPV is detected in almost all the cervical cancers, particularly the oncogenic subtypes HPV 16 and 18. In a study featuring over 30,000 cervical cancers, IARC showed that of the most frequent HPV serotypes that lead to cervical malignancy (16, 18, 58, 33, 45, 31, 52, 35, 59, 39, 51, and 56), HPV 16 induces more than 50% of cervical cancers, while HPV 16 and 18 together lead to over 70% of cases across the globe (3). HPV serotypes 18 and 45 are implicated in the more aggressive cervical adenocarcinomas. The incidence of cervical cancer and its geographic variation appear to be related to the prevalence of HPV in the population and to the availability of screening (which allows for the detection and treatment of precancerous lesions). Three different HPV vaccines are licenced and available: the bivalent, the quadrivalent and the nine-valent, all active against HPV 16 and 18.

Vaccination against HPV may decrease the incidence of both squamous cell and adeno-carcinoma (4-5).

Other epidemiologic risk factors associated with cervical cancer are smoke, parity, early age of onset of sexual intercourse, larger number of sexual partners, history of sexually transmitted disease, autoimmune disease, chronic immunosuppression (6-7).

Cervical Carcinogenesis and molecular mechanisms of HPV

The cervix, which is located between the vagina and the uterus, is a canal characterized by simple columnar secretory epithelium, as opposed to the vaginal cavity, which is lined by stratified non-keratinizing squamous epithelium. The epithelia that line the endocervix and esocervix join at the transition zone, or squamocolumnar junction. The squamocolumnar junction is a crucial cytological landmark since it is the area that is the most vulnerable to HPV infection, and it is the place in which over 90% of lower genital tract malignancies initiate. HPV is recognized as inducing cervical dysplasia and cervical intraepithelial neoplasia (CIN), which typically develop into cervical cancer due to an ongoing infection with high-risk HPV. Since the transition zone includes two kinds of epithelial cells (glandular and squamous cells), two different forms of cancers can occur in the cervix. An unregulated rapid increase of glandular cells in the endocervix generates an adenocarcinoma in 10–20% of cases, although the incidence seems to be on the rise in recent years. A squamous cell malignancy is the cause of squamous cell carcinoma. The latter is much more frequent (occurring in 80–90% of cases) and is typically asymptomatic in its first stages, but can cause coital and pelvic pain and deviant vaginal bleeding and discharge as it progresses (8).

Cervical carcinogenesis is strongly associated with the events that happen in the life cycle of the virus. In a stratified squamous epithelium, the cells creating the basal layer act as stem cells, and thus they undergo cell division when they replace the cells released from the surface layer. When a basal cell divides via mitosis, two daughter cells are

created: one rises and changes into a terminally differentiated cell and the other cell stays in the basal layer to retain the pool of dividing cells. The initial targets of the virus are the basal cells that are vulnerable via microwounds. HPV virions proceed into the cells by interacting with certain receptors, such as alpha6 integrin, which binds HPV-16. Viral DNA replication starts in the basal layers, generating 50–100 copies of the genome in every cell. This is followed by the expression of the E1 and E2 proteins that are required for the replication procedure and for the separation of recently synthesized DNA, therefore guaranteeing that infected stem cells stay in the lesion for an extended period of time. The virus mostly uses host equipment to perform DNA replication, with the exception of the E1 helicase. Early gene products, such as E5–E7, are believed to produce a favorable environment for replication to take place by encouraging DNA replication in the host cell and halting apoptosis.

As the infected basal cells move up and differentiate, the viral late genes L1 and L2 are transcribed, thus prompting the vegetative stage of the life cycle distinguished by dramatic amplification of the genome (9).

It appears that control over the expression of late genes depends on the state of differentiation of the host cell. Once the cell reaches the outermost layer of the epithelium, the newly synthesized viral DNA is encapsulated to form new virions, which are released, and the life cycle is then repeated. As HPVs do not induce complete lysis in host cells, new virions are deposited in squames that are continuously shed. It is intriguing that the virus is greatly hidden from the host immune system, since the immunogenic virions are put together only in the outer portions of the epithelium. In addition, viral proteins E6 and E7 act to ensure that the infection remains asymptomatic by deactivating interferon regulatory factor (8).

Squamous epithelial cells infected by HPV undergo koilocytosis to become cells called koilocytes. Compared to normal cells, these cells have a larger, darker, and asymmetrically outlined nucleus encompassed by an area of transparent space, termed a perinuclear halo, and they appear to be vacuolated.

This alteration suggests minor cellular dysplasia and shows a highly replicative viral state. When the dysplasia is moderate or severe, the cells are small and multiply on the uppermost portion of the epithelium, creating a potentially carcinogenic lesion if it is severe.

While HPV is the greatest risk factor for cervical cancer, many researchers propose that the specific integration of viral DNA in the host cell does not frequently happen, and in the majority of the time, HPV infection is removed quite speedily by the immune system. While viral DNA can lead to fast neoplastic alteration of infected cells once it is incorporated, the existence of HPV DNA in the cell by itself is not enough to induce cancer, as additional genetic and epigenetic occurrences are likely needed.

Two main oncogenic protein products of the HPV virus are E6 and E7; they act by modifying the control of the cell cycle and by regulating apoptosis. The incorporation of viral DNA disrupts the activity of the E2 protein. The E2 protein is recognized as having the ability to repress the transcription of E6 and E7, and thus its interruption causes dysregulated expression of these oncoproteins. Combined, these proteins are able to immortalize cells, so that cells retain their mitotic ability to generate clones that also have the immortalized phenotype and do not experience terminal differentiation (Figure 1). In particular E6 disrupts the expression of miR-23b, miR-218, and miR-34a via p53 degradation and their expression is transactivated by the binding of p53 to consensus sites in the promoter regions, affecting the expression of cell cycle regulators,

such as E2, cyclin D1, CDK4, CDK6, E2F1, E2F3, E2F5, Bcl-2, SIRT1, p18, uPA, and LAMBD3. In the overexpression of miR-15/16 cluster byE7, E2F1 transactivates the c-Myb expression and represses the c-Myc expression, and then the microRNA cluster regulation is controlled by binding of c-Myc or c-Myb to promoter region of microRNA cluster. The increased expression of miR-15a/miR-16-1 induces the inhibition of cell proliferation, survival, and invasion. The down regulation of miR-203 by E7 is mediated by MAPK/PKC pathway (10).

The immune response is a key factor in the fight against HPV infection and cervical carcinogenesis. However, HPV is able to promote immune evasion through the expression of the E5 oncogene, which is responsible for modulation of several immune mechanisms, including antigen presentation and inflammatory pathways (11) (Figure 1).

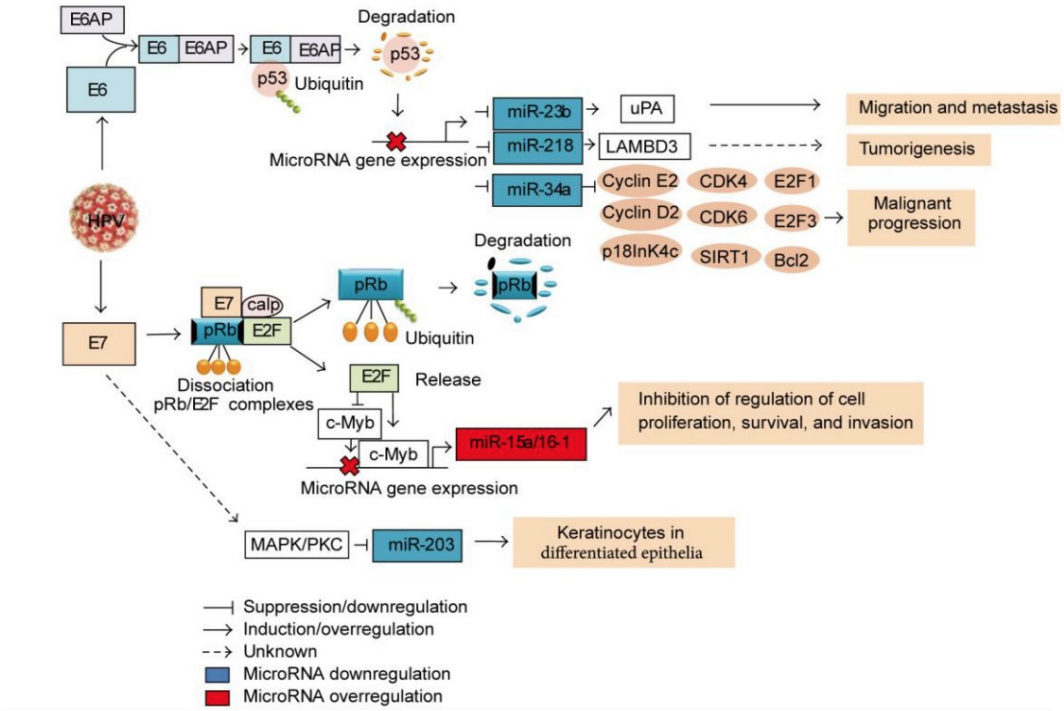

Figure 1. Molecular mechanism of HPV carcinogenesis.

Prognostic factors of cervical cancer

The major tumor-related prognostic factors are FIGO (International Federation of Gynecology and Obstetrics) stage, maximum tumor size, nodal involvement (number, size, location), pathological tumor type, depth of cervical stromal invasion, minimum thickness of uninvolved cervical stroma, presence or absence of lymphovascular space involvement (LVSI), presence or absence of distant metastases (12).

Pathology

The most common histologic types of cervical cancer are squamous cell carcinoma and adenocarcinoma, with a prevalence of approximately 80% and 20% respectively.

The histopathologic types described in the World Health Organization 2014 Tumors of the Female Reproductive Organs are: 1. Squamous cell carcinoma (keratinizing; non-keratinizing; papillary, basaloid, warty, verrucous, squamotransitional, lymphoepithelioma-like); 2. Adenocarcinoma (usual-type endocervical; mucinous – gastric type, mucinous – intestinal type, mucinous – signet ring; villoglandular, endometrioid); 3. Clear cell carcinoma; 4. Serous carcinoma; 5. Adenosquamous carcinoma; 6. Mesonephic carcinoma (13).

The pathological report is important to define the stage of the disease and the risk factors to consider for adjuvant treatment. The elements that should be reported are tumor site, tumor size, histologic type, grade, stromal invasion, surgical margins status, presence of LVSI, number of lymph nodes removed and positive ones (specifying if isolated tumor cells, micrometastasis or macrometastasis), parametrial involvement.

Screening

Although for many years the Papanicolaou (Pap) test has been the standard method for cervical cancer screening, it has some limits like low sensitivity, inadequate specimen

and subjective interpretation. The Pap test is also less effective in detection of adenocarcinoma of the cervix, because the endocervical canal is harder to sample.

Because of its higher sensitivity, HPV test has been introduced in screening programs, with improvement of the secondary prevention of cervical cancer (14).

Diagnosis

In majority of cases the diagnosis is made with cervical cytology or cervical biopsy, especially in asymptomatic early stage cervical cancer. Cone biopsy is recommended if simple cervical biopsy is inadequate to define pathological characteristics or to have an accurate assessment of microinvasive disease. Locally advanced disease is more often symptomatic, causing abnormal vaginal bleeding (also after coitus), discharge, pelvic pain and dyspareunia. If pelvic examination is difficult and/or painful for the patient, to detect vaginal/parametrial involvement it should preferably be done under anaesthesia by an interdisciplinary team including a gynaecologic oncologist and a radiation oncologist.

Imaging

After the diagnosis an appropriate imaging is recommended to complete the assessment of the diffusion of the disease and to guide treatment options.

Mandatory initial workup is pelvic magnetic resonance imaging (MRI), which can determine tumor size, degree of stromal penetrations, parametrial involvement, vaginal and uterine corpus extension with high accuracy (15). Endovaginal/ transrectal ultrasound is an option if performed by a properly trained sonographer.

Other radiologic imaging to consider consists of chest radiograph, computerized tomography (CT) to detect lymph nodes involvement and distant metastasis and positron emission tomography (PET), potentially much accurate in diagnosis of not

macroscopically enlarged lymph nodes or distance localization of disease (16). Cystoscopy and colonoscopy are recommended if bladder or rectal extension is suspected and/or documented on MRI or ultrasound.

Staging

FIGO staging classification 2009 was based mainly on clinical examination.

In 2018 the FIGO Gynecologic Oncology Committee determined that the staging classification needed revision to maintain unanimity worldwide, improving its utility and applicability. Imaging and pathological assessment of the pelvis and pelvic and para-aortic lymph nodes has been incorporated into the staging giving the clinician the flexibility to use the available resources. The major changes are that the size of the tumor can be assessed by clinical evaluation, imaging and/or pathological measurement, identification of lymph node metastasis should be done by imaging and/or pathological assessment, the method used for imaging and/or pathological technique examination should be recorded, the final stage is to be assigned after receiving all reports and the method of recording the size and assigning stage should be noted (17).

For stage IA the lateral extent of the lesion is no longer considered for staging. Diagnosis of stage IA1 and IA2 is made on microscopic examination of a cone biopsy specimen, which includes the entire lesion, or a trachelectomy or hysterectomy specimen. If the margins of the cone biopsy are positive for invasive cancer, the patient is allocated to stage IB1.

Clinically visible lesions and those with larger dimensions are allocated to Stage IB. Stage IB is characterized by stromal invasion ≥ 5 mm and is divided in 3 subgroups based on dimensions based on oncological data from fertility-sparing treatment: IB1 includes

tumors < 2.0 cm in greatest diameter, IB2 tumors of 2.0-4.0 cm and IB3 tumors ≥ 4.0 cm (18-19). Recurrence rates are significantly lower in patients whose tumors are less than 2.0 cm compared with those measuring 2.0–4.0 cm in their greatest dimension.

Since positive lymph nodes confer a worse prognosis, in the new staging system the presence of lymph nodes involvement is considered in the basis of imaging (r) or pathology (p) and is designed as stage IIIC, which can be IIIC1 or IIIC2 if only pelvic lymph nodes or para-aortic nodes are involved respectively (20) (Figure 2).

Stage I:

The carcinoma is strictly confined to the cervix (extension to the corpus should be disregarded).

- **IA** Invasive carcinoma that can be diagnosed only by microscopy with maximum depth of invasion ≤5 mm[a]
  - **IA1** Measured stromal invasion ≤3 mm in depth
  - **IA2** Measured stromal invasion >3 mm and ≤5 mm in depth
- **IB** Invasive carcinoma with measured deepest invasion >5 mm (greater than stage IA); lesion limited to the cervix uteri with size measured by maximum tumor diameter[b]
  - **IB1** Invasive carcinoma >5 mm depth of stromal invasion and ≤2 cm in greatest dimension
  - **IB2** Invasive carcinoma >2 cm and ≤4 cm in greatest dimension
  - **IB3** Invasive carcinoma >4 cm in greatest dimension

Stage II:

The cervical carcinoma invades beyond the uterus, but has not extended onto the lower third of the vagina or to the pelvic wall

- **IIA** Involvement limited to the upper two-thirds of the vagina without parametrial invasion
  - **IIA1** Invasive carcinoma ≤4 cm in greatest dimension
  - **IIA2** Invasive carcinoma >4 cm in greatest dimension
- **IIB** With parametrial invasion but not up to the pelvic wall

Stage III:

The carcinoma involves the lower third of the vagina and/or extends to the pelvic wall and/or causes hydronephrosis or non-functioning kidney and/or involves pelvic and/or paraaortic lymph nodes

- **IIIA** Carcinoma 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 (unless known to be due to another cause)
- **IIIC** Involvement of pelvic and/or paraaortic lymph nodes **(including micrometastases)**[c], irrespective of tumor size and extent (with r and p notations).[d]
  - **IIIC1** Pelvic lymph node metastasis only
  - **IIIC2** Paraaortic lymph node metastasis

Stage IV:

The carcinoma has extended beyond the true pelvis or has involved (biopsy proven) the mucosa of the bladder or rectum. A bullous edema, as such, does not permit a case to be allotted to stage IV

- **IVA** Spread of the growth to adjacent organs
- **IVB** Spread to distant organs

Figure 2: FIGO 2018 Classification

Primary treatment

The primary treatment of cervical cancer is either surgery or radiotherapy. Treatment strategy should aim for the avoidance of combining radical surgery and radiotherapy because of the highest morbidity after combined treatment.

Surgery is typically reserved for stage FIGO 2018 IA, IB1, IB2 and selected IIA1, concomitant chemoradiation (CCRT) is the primary treatment for stage IB3 to IVA. In stage IVA, there is a place for pelvic exenteration in selected cases.

Pelvic radiotherapy lead to ovarian failure in premenopause women. Ovarian transposition out of the pelvis can be considered to preserve hormonal function in women younger than 45 years old with squamous cervical carcinoma(21).

According to Querleu-Morrow classification, hysterectomies that can be performed for cervical cancer include extrafascial (type A), modified radical (type B) and radical hysterectomy (type C) (22). The 2017 modification of the Querleu-Morrow classification is recommended as a tool (23).

The type of radical hysterectomy should be based on the presence of prognostic risk factors (tumor size, maximum stromal invasion and LVSI) identified preoperatively, that categorize patients at high, intermediate and low risk.

For microinvasive cervical cancer (stage IA1) without LVSI the options are conization or simple trachelectomy (in fertility sparing approach) or extrafascial hysterectomy; if there is LVSI, modified radical hysterectomy along with bilateral pelvic lymph node dissection is the treatment of choice.

For patients with FIGO stage IA2, IB1, IB2 and IIA1 cervical cancer the treatment is radical hysterectomy with bilateral pelvic lymphadenectomy. In stage IB2 and IIA1 cervical cancer, surgery or radiotherapy can be chosen as the primary treatment depending on other patient factors and local resources, as both have similar outcomes.

According to guidelines, CCRT is preferred for patients with FIGO IB3-IIA2 disease; selected patients may be treated with radical hysterectomy or neoadjuvant chemotherapy followed by surgery.

Recent data support the sentinel lymph node (SNL) biopsy instead of systematic pelvic lymphadenectomy in early stage cervical cancer, in terms of feasibility and safety with best detection results in tumors of less than 2 cm (24). Tracer is injected directly into the cervix. The procedure should be done only in centres with enough expertise and training. Sentinel nodes should be detected on both sides (25). The SENTICOL trial showed the power of SNL mapping to identify unusual lymph drainage patterns.

For stage IVB disease (metastatic) the primary treatment is platinum-based chemotherapy; individualized external beam radiation therapy (EBRT) may be considered for control of pelvic disease or other symptoms.

Adjuvant treatment

Adjuvant treatment after surgery depends on stage of the disease and final pathological characteristics. Observation is appropriate for stage IA2, IB and IIA1 according to FIGO 2018 with negative margins of the specimen and no additional risk factor based on "Sedlis Criteria" (26) (Figure 3).

LVSI	Stromal Invasion	Tumor Size (cm) (determined by clinical palpation)
+	Deep 1/3	Any
+	Middle 1/3	≥2
+	Superficial 1/3	≥5
-	Middle or deep 1/3	≥4

LVSI: Lymphovascular space invasion

Figure 3: Sedlis Criteria

For stage IA2, IB and IIA1 disease with large tumor, deep stromal invasion and/or LVSI pelvic EBRT with or without concurrent chemotherapy is recommended.

Pelvic EBRT with concurrent chemotherapy with or without vaginal brachytherapy is recommended for patients with positives nodes (stage IIIC FIGO 2018), positive or close

margins and /or positive parametrium (stage IIB FIGO 2018). Vaginal brachytherapy may be indicated in patients with positive or close vaginal margins.

Surveillance

Surveillance is based on the patient risks of recurrence and should be modulated depending on the case. Physical examination is recommended every 3 (high-risk disease) to 6 (low-risk disease) months for the first 2 years, every 6-12 months for subsequent 3-5 years, and then annually.

For patients with stage I disease, follow up imaging should be performed if clinical examination or symptoms suggest recurrent/metastatic disease.

For patients with stage II or greater disease, PET/CT or CT should be performed within 3 to 6 months after the end of primary treatment. Additional imaging should be guided by clinical indicators for recurrent or metastatic disease.

Neoadjuvant chemotherapy and locally advanced cervical cancer

Patients with International Federation of Gynecology and Obstetrics (FIGO) stages IB2 to IVA disease are considered to have locally advanced cancer (LAAC).

Radiotherapy is the primary treatment, although definitive surgery can also be performed in patients with stage IB2 or IIA disease (27).

Exclusive CCRT, since 1999, has been representing the standard treatment of LAAC (FIGO stage IB2-IVA) patients. However, neoadjuvant chemotherapy (NACT) followed by radical surgery (RS) has been employed in the treatment of LAAC patients and is a valid alternative for investigation (28-38). The use of NACT has been considered an attractive approach to improve disease control and reduce disabling treatments such as radiotherapy especially in young women who can benefit from the maintenance of ovarian hormonal function and vaginal tissue trophism.

NACT followed by radical hysterectomy has achieved satisfactory results in cervical cancer, with tumor size reduction and down staging, increased operability rate with free surgical margins, decreased incidence of lymph node and parametrial involvement, and better control of distant metastases (39-51).

Encouraging results were reported from different pilot studies that used this approach. In addition, NACT plus RS is also employed in case of unavailability of radiotherapy units/equipments (52).

The long-term complications after radiotherapy, castration of the patients, the poor control of metastatic disease, and the lack of possibility for CCRT in less developed countries contribute to the use of NACT followed by surgery (53).

Several chemotherapeutic agents have been tested as NACT in cervical cancer, with cisplatin, paclitaxel, and ifosfamide considered among the most active drugs. An Italian group showed in the SNAP (Studio Neo-Adjuvante Portio) 01 trial that TIP (paclitaxel, ifosfamide, and cisplatin) resulted in a higher response rate than IP, without a statistically significantly different effect on overall survival (42).

The SNAP-02 trial of Lissoni et al compared TP with TIP and showed TIP to be the most active (25% vs 43% pathological optimal response rate) (47). However, TIP also has an important higher morbidity compared with TP (neutropenia in 26% and 76%, respectively) (47). Recently, it has been shown that 3-weekly paclitaxel carboplatin has similar efficacy compared with 3-weekly paclitaxel-cisplatinum in recurrent cervical cancer (54). In the meantime, Mori et al showed in 2010 promising results for weekly paclitaxel 60 mg/m2 and carboplatin AUC2 (55). Sahili et al observed excellent clinical (89%) and pathological response rates with dose-dense paclitaxel-carboplatin in patients operated on (no tumor on pathology or invasion of G3 mm in 50% of the

patients) (53). This compared with the 84% clinical response (CR or PR) seen in TIP (56). These results are also comparable with those seen by Mori et al (87% objective response rate). The main difference between this study and that of Mori et al is the dose of carboplatin. Sahili et al used a mean weekly dose of paclitaxel 60 mg/m2 and carboplatin (AUC 2.7) or paclitaxel 80 mg/m2 and carboplatin (AUC 4) d1,8 every 3 weeks, whereas Mori et al used the same paclitaxel dose but a carboplatin dose of AUC2.

The use of neo-adjuvant chemotherapy in LACC is strongly debated. The present study aimed to assess the role of neoadjuvant platinum/paclitaxel based dose-dense chemotherapy in LACC (stage FIGO IB2-IVA) patients treated at a tertiary referral center.

Molecular mechanisms of chemotherapy

Patients and clinicians should weigh the risks and benefits of different treatment options. Chemotherapy treatments aims to deliver a planned course of each drug based on a curative goal. The toxicity of chemotherapy often necessitates dose reductions, at the possible expense of efficacy and the resulting risk of treatment failure from dose reductions and delays has serious repercussions. Dose intensity is a measure of chemotherapy delivery that looks at the amount of drug delivered per unit time (measured as mg/m2/wk). The relative dose intensity of a single-drug regimen can be expressed as the ratio of its dose intensities in test and standard regimens. Higher dose intensity can be delivered by escalating the dose per-cycle or by reducing the intervals between cycles, known as dose density.

Two well-established hypotheses on tumor growth help to explain the value of dose intensification. The Goldie-Coldman hypothesis addresses the spontaneous development of drug-resistant cells following exposure of the tumor to cytotoxic agents (57). Drug-resistant mutations arise at a measurable frequency. The larger the tumor

burden, the more likely a mutation will occur. This hypothesis predicts that early introduction of dose-intensive alternating agents is most likely to prevent a large number of resistant clones, increasing efficacy. A basic premise of the Norton-Simon hypothesis is that “chemotherapy results in a rate of regression of tumor volume that is proportional to the rate of growth for an unperturbed tumor that size”, that is, small tumors grow faster than larger ones (58). Nonexponential Gompertzian kinetics apply, which posit that cytoreductive therapy will lead to an increase in the regrowth between cycles. Subsequent chemotherapy must be delivered sequentially in the shortest possible intervals to be most effective. In this Gompertzian model, the regrowth of cancer cells is a function of cytoreduction, such that the greater the tumor cell killing, the faster is the regrowth. Thus, there is a clear rationale for increasing the dose intensity of chemotherapy.

Regarding chemotherapy regimen adopted, Carboplatin and Paclitaxel are widely used for the treatment of ovarian cancer. However, many scientific evidences have shown effective results also on cervical cancer and other gynaecological oncological diseases.

Carboplatin (1,1-cyclobutyldicarboxylate), is a derivative of cisplatin and one of the main platinum-based drugs (59). This antineoplastic drug is usually classified as alkylating agent, although does not alkylate DNA. The main target of carboplatin is DNA, to which it binds efficiently, thereby inhibiting replication and transcription and inducing cell death. The nature of these DNA adducts affects a number of transduction pathways and triggers apoptosis or necrosis in tumor cells. The adducts formed by this compound can be monoadducts or intra and interchain diadducts (Figure 4).

The linkage between DNA and carboplatin can produce lesions in DNA. Crosslinking between strands of DNA (interstrand cross-linking; ISC) is the most cytotoxic effect,

because it inhibits the process of DNA replication, causing changes that generate errors in replication, with the accumulation of cells in G2/M phase and the induction of apoptosis. Alkylation of a single strand of DNA can be repaired easily, but cross-linked inter strands such as those produced by bifunctional alkylating agents require more complex mechanisms of repair.

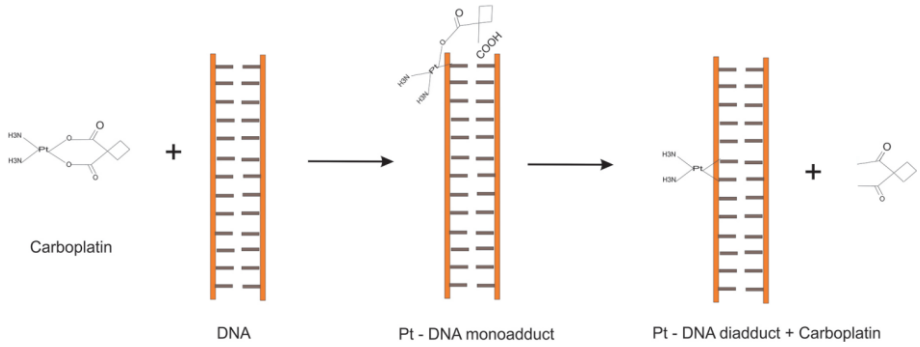

Figure 4: Carboplatin interactions with DNA

The manner whereby platinum drugs enter cells has traditionally been attributed to simple passive diffusion. However, some studies suggest that a number of mechanisms of uptake and efflux are active in the process, and altered regulation of these transporters is responsible for the reduced accumulation of drugs in resistant cells. Anticancer drugs based on platinum, such as cisplatin, oxaliplatin and carboplatin, are captured by cells, followed by binding to DNA and cytotoxicity.

Platinum uptake varies widely among different cell types and different types of tissues, and is a factor in the sensitivity and resistance of tumors.

Transporters of metals such as copper transporters, i.e. CTR1, ATP7A and ATP7B, have been of particular interest in the study of drugs based on platinum. A significant influence of the carrier CTR1 has been observed in mediating the influx of carboplatin,

while ATP7B and ATP7A are known to be mediators of copper removal from the cell (Figure 5).

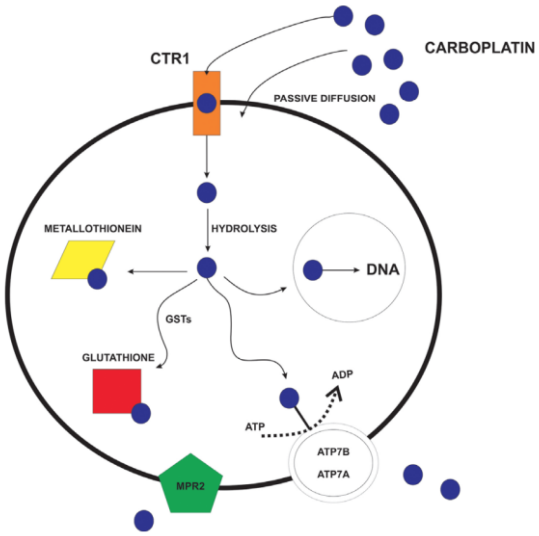

Figure 5: Carboplatin molecular mechanisms

Paclitaxel (trade name Taxol) is a tricyclic diterpenoid compound naturally produced in the bark and needles of Taxus brevifolia (60). Because of its unique anticancer mechanism, it is already one of the most successful and widely used natural anticancer drugs. In 1979, it was reported that paclitaxel promotes the assembly of microtubules, structures that consist of repeating subunits composed of α/β-tubulin heterodimers. During the prophase, microtubules form a spindle to pull the chromosomes towards the poles. During later stages, they depolymerize and the spindle structure dissolves. Both exposure to cold temperatures and exposure to calcium ions trigger the depolymerization of microtubules. Paclitaxel binds to and stabilizes microtubules, and paclitaxel-bound microtubules resist depolymerization, even upon treatment under cold temperatures or with calcium ions. In particular, Paclitaxel interferes with normal microtubular functioning by increasing the polymerization of alpha and beta monomers tubulin (paclitaxel binds to the β subunit of tubulin) and thus determining the formation

of highly stable microtubular structures. This negatively affects the cellular function as the shortening and lengthening of microtubules (defined "dynamic instability") is indispensable for their function. This implies an inhibition of the cellular ability to use its cytoskeleton in a flexible way and, therefore, an inhibition of cell mitosis. Therefore, paclitaxel treatment blocks the progression of mitosis.

2. Objectives

The primary endpoint of the study is to evaluate the feasibility of dose-dense neoadjuvant chemotherapy (NACT) with carboplatin and paclitaxel followed by radical surgery in locally advanced cervical cancer (LACC), avoiding detrimental treatments such as radiation therapy especially in young patients.

Secondary endpoints are the analysis of predictors of response to dose-dense at imaging before surgery and of receiving radiotherapy after surgery.

Tertiary endpoint is the analysis of follow-up and possible relapses.

3. Materials and methods

This study was conducted on 82 patients with a diagnosis of locally advanced cervical cancer (LAAC) that underwent to dose-dense Carboplatin and Paclitaxel based neoadjuvant chemotherapy (NACT) followed by radical surgery or exclusive chemoradiotherapy (CCRT) after assessment of the multidisciplinary committee of gynecologic oncology at the European Institute of Oncology (Milan), from July 2014 to December 2022. All records were retrospectively collected after obtaining approval by the Institutional Review Board at European Institute of Oncology (IEO).

Pre-treatment evaluation included history, physical examination, vaginal-pelvic examination, colposcopy, biopsy, complete blood tests, abdominal-pelvic computed tomography (CT) scan, Magnetic Resonance Imaging (MRI) and/or or positron emission tomography (PET). Further investigations were carried out when indicated.

The clinical staging was performed according to the system adopted by FIGO. In all patients the FIGO stage according to the 2009 classification was revised and all cases were also converted according to the new FIGO stage classification 2018.

The diagnosis of cervical carcinoma was histologically confirmed in all patients before NACT. The histologic types included were squamous and adenocarcinoma usual-type. Adenosquamous and special adenocarcinoma as endometrioid, mucinous NOS (not otherwise specified), mucinous signet ring, mucinous intestinal, mucinous gastric type, clear cell and serous carcinoma were excluded.

Patients and tumours characteristics were retrospectively collected from electronic medical records.

Chemotherapy was administered in dose-dense NACT regimen with a weekly dose of Taxol (80 mg/m2) plus CBDCA AUC 2 or Taxol (60mg/m2) plus CBDCA AUC 2.7 every week for 6 to 9 cycles.

Physical and vaginal-pelvic examination and abdominal-pelvic CT scan and/or MRI were repeated after the completion of NACT. When suspicious lymph nodes involvement or distant metastases were found at MRI or CT scan, a total body PET-FDG was performed. Response to NACT was determined using the response evaluation criteria in solid tumors (RECIST) version 1.1 (61).

After the completion of NACT, radiologic imaging of all patients were discussed by a multidisciplinary team to decide for further adjuvant therapies and patients were

divided into 4 groups (progression disease, stable disease, partial response and complete response). Post-NACT treatment consisted of type B-C radical hysterectomy with pelvic lymphadenectomy or definitive concurrent chemo-radiotherapy.

The patients scheduled for definitive CCRT received external beam RT 50.4 Gy (in 25-28 fractions) concurrent with CDDP 40 mg/m2 weekly plus intracavitary brachytherapy. Physical and vaginal-pelvic examination and abdominal-pelvic CT scan were repeated 8 to 12 weeks after the completion of RT.

For patients who underwent radical surgery, pathologic response was evaluated. Complete pathological response of the patients who underwent surgery was defined as the complete disappearance of the tumor in the cervix with negative nodes; optimal partial response was defined as persistent residual disease with <=3 mm stromal invasion including in situ carcinoma on the surgical specimen and negative nodes; and suboptimal partial response consisted of persistent residual disease with >3 mm stromal invasion on the surgical specimen and negative nodes (intra-cervical residual disease), or positive nodes with positive or negative parametria and/or surgical margins (extracervical residual disease with positive nodes), or positive parametria and/or surgical margins with negative nodes (extra-cervical residual disease with negative nodes). Overall, optimal response rate was the sum of complete and optimal partial response rates.

Adjuvant treatment included either systemic chemotherapy, radiation alone, or chemoradiation according to the final pathology report (residual macroscopic disease, close margins, and parametrial and/or lymph-node involvement, or in case they met the Sedlis criteria). Post-operative management was individually established after multidisciplinary committee of gynecologic oncology.

Postoperative radiotherapy was administered as a whole pelvic external-beam radiation with or without additional vaginal brachytherapy. We reported whether patients received any adjuvant treatment after surgery, including radiotherapy alone or combined with chemotherapy, with or without brachytherapy; the date of the end of treatment was recorded.

Our follow-up schedule included physical examination every 3–4 months for the first 2 years, then every 6-12 months for 3–5 years. Vaginal cytology was performed every 12 months. A total body CT scan was also performed annually for 3 years in asymptomatic patients. MRI or CT and laboratory tests were performed based on symptoms suggestive of recurrence or morbidity. When CT scan or MRI were suspicious but not conclusive for recurrence, a PET/CT was performed.

All patients medical records were reviewed up to the last available follow-up at our institution. In case the patient did not have a recent follow-up visit, she was contacted by the medical stuff.

Patient and tumor variables were summarized as number (percentage), mean (SD), or median (interquartile range [IQR]), as appropriate. Baseline characteristics including age, histologic findings, grade, cervical stroma invasion, and adjuvant therapy (chemotherapy vs all others) were compared between the radiologic complete/partial response and stable/progressive disease cohorts using the 2-sample t test, the Wilcoxon rank sum test, and the c2 test or Fisher exact test as appropriate. All calculated P values were 2- sided, and $P < 0.05$ was considered statistically significant. Statistical analysis was done using JMP software.

4. Results

From 2014 to 2022, a total of 82 patients were triaged to NACT dose-dense followed by surgery for locally advanced cervical cancer.

Baseline characteristics are described in Table 1. The median age of the patients was 41 years (range, 26 - 66 years) and all patients had a previous biopsy with histological analysis.

The median largest tumor diameter was 42,8 mm (standard deviation 11,33).

Squamous carcinoma was the most frequent (n=65, 79,3%), followed by adenocarcinoma usual type (n=17, 20,7%).

Tumour grading was distributed as following: G1 in 5 patients (6,1%), G2 in 29 (35,4%) and G3 in 24 (29,2%); while in 24 patients the grade was not reported.

According to FIGO stage 2018, the distribution of disease at diagnosis was the following: 2 patient with stage IB1 (2,4%), 20 stage IB2 (24,3%), 37 stage IB3 (45,1%), 3 stage IIA1 (3,6%), 2 stage IIA2 (2,4%), 6 stage IIB (7,3%), 12 patients with stage IIIC1 (14,6%). Radiologic evaluation showed in 42 patients (51,2%) disruption of stromal ring, in 36 patients (43,9%) no disruption, while in 4 patients this data was not reported. All patients with stage IB1 and IB2 were suspect for disruption of the stromal ring.

Regarding chemotherapy regimens, 52 patients (63,4 %) were scheduled for Carboplatin AUC 2+ Paclitaxel 80 mg/m2, 24 patients (29,2%) for Carboplatin AUC 2,7 + Paclitaxel 60 mg/m2, while in 6 patients the data was not reported. All patients received at least 5 cycles of NACT, 28 patients (34%) completed nine cycles of NACT while none of the patients discontinued the therapy due to severe side effects.

After NACT all cases were discussed by the multidisciplinary team of IEO for indication to either surgery or exclusive chemoradiotherapy. Radiological evaluation according to

the RECIST 1.1 criteria after NACT showed 11 (13,4%) patients with complete response, 48 (58,5%) with partial response, 19 (23%) with stable disease and 4 (4,9%) with progressive disease. Predictors of response to dose-dense at imaging are described in Table 3.

Thirteen patients (15,8%) were judged not suitable for surgery because of stable/progressive disease or in case of partial response when the tumour reduction was deemed insufficient to obtain negative margin. 12 patients were treated with exclusive combined chemoradiation with brachytherapy while 1 patients only with chemoradiation.

All the remaining 69 (84,1%) women with complete and partial response to neoadjuvant chemotherapy underwent surgery. The median time from the last chemotherapy to surgery was 26 days (range, 15-58 days).

Among these patients, 52 (75,3%) were operated by laparotomy and 17 (24,6%) by robotically assisted minimally invasive surgery. As shown in Table 2: radical hysterectomy type C1 with pelvic lymphadenectomy was performed in 50 cases (72.4%), radical hysterectomy type B2 with pelvic lymphadenectomy in 14 (20%) patients while radical hysterectomy type B1 with pelvic lymphadenectomy in 5 (7,2%) patients. Pelvic sentinel node biopsy was performed in 22 patients (31,8%) and bilateral oophorectomy in 40 (8%).

Pathologic evaluation of the surgical specimen showed a pathologic complete response with no residual disease to chemotherapy in 14 (20,3%) of the 69 women submitted to surgery. Whereas in 21 cases (30%) was observed a deep stromal invasion, in 19 cases (27,5%) middle stromal invasion and in 15 (21,5%) superficial stromal invasion.

Lymphovascular space invasion (LVSI) was present in 12 cases (LVSI diffuse n=6, 50% and focal n=5, 41,7%).

After surgery all cases were discussed by a multidisciplinary team for indication to adjuvant therapy, based on final histopathological analysis.

Adjuvant treatment after surgery was indicated in presence of 1 or more risk factors (lymph nodes involvement, lymphovascular space invasion, parametrial involvement, positive or close surgical margins and stromal invasion). Predictors of receiving radiotherapy after surgery are reported in Table 4.

In our series, of 69 patients who have undergone to surgery, 43 patients (62,3%) did not receive any adjuvant treatment, while 17 (24,6%) underwent chemoradiation, 2 (2,89%) radiotherapy alone and 7 (10%) chemotherapy alone. In particular, 50 (72,5%) did not receive radiation after surgery.

This is an important finding, particularly as it relates to young patients who could be spared the long-lasting adverse effects of radiotherapy.

The median follow-up time among patients who did not experience disease recurrence was 52 months (range 6-94). During the follow-up after surgery a total of 12 (17,3%) patients reported a recurrence of disease. At the time of the last follow-up, 60 (86,9%) were NED (no evidence of disease), 2 (2.9%) were AWD (alive with disease) and 7 (10%) were DOD (death of disease). Progression free survival (PFS) at 36 months was 84% and at 60 months 79%.

5. Discussion

Neoadjuvant dose-dense chemotherapy followed by radical surgery could be an alternative treatment to exclusive chemoradiation in LAAC patients.

To our knowledge, the present is the largest series of patients who underwent neoadjuvant chemotherapy in a tertiary referral center reported in the literature.

82 patients were initially triaged to underwent surgery after NACT. Among them, after chemotherapy surgery was possible in 69 patients (84,14%), while 12 patients (14,6%) underwent to chemoradiation with brachytherapy and one patients only chemoradiation because of stable/progressive disease or in case of partial response when the tumour reduction was deemed insufficient to obtain negative margin. Among 69 patients, 50 patients (72,5%) did not perform radiation after surgery.

If we consider overall 82 patients, we can conclude that dose-dense NACT spared radiotherapy in 60.9% of patients.

This is a first important finding, particularly as it relates to young patients who could be spared the long-lasting adverse effects of radiotherapy.

In fact, radiotherapy has deleterious effects particularly in young patients as it causes iatrogenic menopause, damages the genital organs causing severe atrophy, vaginal stenosis, urinary and intestinal disorders, as well as the possible severe complications such as entero-genito-urinary fistulas.

Unfortunately 19 patients (23%) required adjuvant radiation, resulting in trimodality treatment (NACT, surgery and chemoradiation), which is detrimental to the patients due to the related side effects.

From our results, combination of Carboplatin and Paclitaxel in weekly administrations showed a radiological complete response or partial response rate of 71,9% and a stable disease or progression disease rate of 27,9%.

This second finding highlights the efficacy of the combination based on Carboplatin and Paclitaxel on cervical cancer as well as for other gynecological tumours. It is important

to underline that none of the patients had to suspend the treatment due to side effects. Furthermore, the statistical analysis showed that there were no significant differences between the two chemotherapy protocols (CBDCA AUC2+ PTX80 and CBDCA AUC2,7+PTX60) and number of cycles.

Even if the two histotypes squamous cell and adenocarcinoma share common pathogenetic mechanisms related to HPV infection, they are very different from the histological point of view. Adenocarcinoma often presents as a multifocal tumor and is more difficult to diagnose and study, however it has shown even better response rates compared to squamous cell (complete or partial response of 82.5% vs 69.3% respectively), even if without statistically significant differences.

Even the differences between grading, FIGO staging at diagnosis, stromal ring disruption did not show statistically significant differences in terms of response to chemotherapy.

After surgery, the pathological examination showed an optimal response with no residual disease in 20,3% and superficial stromal invasion of 21,7% of patients who underwent to surgery after NACT. These data, are similar to those reported from other studies, confirming the hypothesis of the potential role of neoadjuvant chemotherapy in reducing tumor volume, making feasible radical surgery. Mori et al reported that NACT with TAX (60 mg/m2) plus CBDCA (AUC2) weekly for six cycles obtained a clinical complete response and partial response in 2 and 24, respectively, of 30 patients with stage Ib2-IIIb cervical cancer, with an overall response rate of 86.7% (55). Twenty-eight patients underwent radical hysterectomy followed by adjuvant RT in 13 cases with high–risk factors. Similarly, Park et al noted a response rate of 31% in woman with FIGO IB2-IIB treated with 3 cycles of 10 day cisplatin and paclitaxel prior to surgery (62). In the phase II study of McCormack et al, 46 patients with stage IB2-IVA disease underwent

NACT with TAX (80 mg/m2) and CBDCA (AUC2) weekly for six cycles followed by CCRT (63). Overall response rate was 70% after NACT and 85% 3 months after CCRT. Gadducci et al, who utilized the same regimen adopted in the current study, reported the OPR rate of 21.4%, a figure to considered with caution considering the very small sample series (48).

In the study of Singh et al, TAX (60 mg/m2) plus CBDCA (AUC2) weekly for six cycles achieved a complete response and a partial response in 2 and 17, respectively, of 28 patients with stage IIB-IVA disease, with an overall response rate of 67.8% (64). Twenty-four patients received CCRT, 23 (82.1%) achieved a complete response, and 22 complete responders were still in complete response at a median follow-up of 12 months (range, 7 to 24 months).

In the end, analyzing predictors factors of receiving radiotherapy after surgery, none of the parameters considered, including surgical approach and type of radical hysterectomy showed statistically significant differences (Table 4).

The median follow-up time among patients who did not experience disease recurrence was 52 months (range 6-94). During the follow-up after surgery only 12 (17,3%) patients reported a recurrence of disease and at the time of the last follow-up, 60 (86,9%) were NED (no evidence of disease), 2 (2.9%) were AWD (alive with disease) and 7 (10%) were DOD (death of disease). Progression free survival (PFS) at 36 months was 84% and at 60 months 79%.

This study has some limitations. First, its retrospective, nonrandomized design may have introduced bias inherent in that design. An additional limitation is the low number of patients that underwent to surgery after NACT, which did not allow us to perform

survival study and a multivariate analysis. Lastly, the small number of patients included in each stage group did not allow us to stratify the analysis by stage.

Although the chemoradiation remains the gold standard of treatment for LAAC, neoadjuvant chemotherapy could represent an alternative option of treatment, making radical surgery possible in many cases previously inoperable. Therefore, especially in young patients, the alternative regimen of neoadjuvant chemotherapy instead of exclusive chemoradiotherapy could be offered, avoiding many detrimental side effects and improving the quality of life of these patients. However, patients should be informed on the risk of receiving trimodality treatment (NACT, surgery and radiotherapy) and on the absence of long-term survival data.

Baseline characteristics	Total patients (n = 82)
Age (years) (SD)	41 (9,18)
Histotype	
Squamous	65 (79,3%)
Adenocarcinoma	17 (20,7%)
Tumor grading	
1	5 (6.1%)
2	29 (35.4%)
3	24 (29.2%)
Unknown	24 (29,2%)
Stage FIGO 2018 at diagnosis	
IB1	2 (2,4%)
IB2	20 (24,3%)
IB3	37 (45,1%)
IIA1	3 (3,6%)
IIA2	2 (2,4%)
IIB	6 (7,3%)
IIIC1	12 (14,6%)
Mean tumour largest diameter (mm) (SD)	42,8 (11,33)
Disruption of stromal ring	
No	36 (43,9%)
Unknown	4 (4,9%)
Yes	42 (51,2%)
Chemotherapy regimen	
CBDCA 2 + PTX 80	52 (63,4 %)
CBDCA 2,7 + PTX 60	24 (29,2%)
Unknown	6 (7,3%)
Number cycles dose dense	
5	6 (7,3%)
6	39 (48%)
7	4 (4,8%)
8	4 (4,8%)
9	28 (34%)
Unknown	1 (1,2%)
Response at imaging to dose-dense	
Complete response	11 (13,4%)
Partial response	48 (58,5%)
Stable disease	19 (23%)
Progression	4 (4,9%)

Performed treatment after dose-dense	
Surgery	69 (84,1%)
CHT-RT+ Brachytherapy	12 (14,6%)
CHT-RT	1 (1,2%)
Stage FIGO 2018 final after surgery	
IB1	2 (2,4%)
IB2	17 (20,7%)
IB3	28 (34%)
IIA1	2 (2,4%)
IIA2	2 (2,4%)
IIB	6 (7,3%)
IIIC1	25 (30%)

Table 1: Baseline characteristics of patients

Surgery characteristics	Total patients undergoing surgery (N 69)
Route of hysterectomy	
Open	52 (75,3%)
Robotic-assisted	17 (24,6%)
Type of surgery	
Radical B1 + LND	5 (7,2%)
Radical B2 + LND	14 (20%)
Radical C1 + LND	50 (72,4%)
SLN performed	
No	47 (68,1%)
Yes	22 (31,8%)
Oophorectomy performed	
No	29 (42%)
Yes	40 (58%)
Histopatological specimens	
LVSI	
No	57 (82,6%)
Yes	12 (17,4%)
Diffuse	6 (50%)
Focal	5 (41,7%)
Unknown	1 (8,3%)
Stromal invasion	
No residual disease	14 (20,3%)
Superficial 1/3	15 (21,7%)
Middle 1/3	19 (27,5%)
Deep 1/3	21 (30%)
Mean tumour largest diameter (mm) (SD)	13 (13,42)

Table 2. Surgery characteristics

	No response to imaging (stable/progression) N 23	Response to imaging (complete/partial) N 59	P-value
Age years (SD)	40,39 (1,92)	41,4 (1,20)	0,68
Histotype			0,37
Squamous	20 (30,77%)	45 (69,23%)	
Adenocarcinoma	3 (17,65%)	14 (82,35%)	
Grading			0,56
1	1 (20 %)	4 (80%)	
2	6 (20,69%)	23 (79,3%)	
3	9 (37,50%)	15 (62,50%)	
Unknown	7 (29,17%)	17 (70,83%)	
Largest tumour diameter (SD)	44,13 (10,67)	42,37 (11,63)	0,51
Disruption of stromal ring			0,43
No	11 (30,56%)	25 (69,44%)	
Yes	12 (28,57%)	30 (71,43%)	
Unknown	0	4 (100%)	
Stage FIGO 2018 at diagnosis			0,11
IB1	0	2 (100%)	
IB2	4 (20%)	16 (80%)	
IB3	7 (18,92)	30 (81%)	
IIA1	1 (33,33)	2 (66,67%)	
IIA2	1 (50%)	1 (50%)	
IIB	3 (50%)	3 (50%)	
IIIC1	7 (58,33%)	5 (41,67%)	
Days from diagnosis to start dose-dense (SD)	49 (4,5)	43,9 (2,82)	0,31
Regimen of dose-dense			1
CBDCA2-PTX 80	14 (26,92%)	38 (73,08%)	
CBDCA2.7-PTX 60	7 (29,17%)	17 (70,83%)	

Table 3. Univariate analysis of predictors of response to dose-dense at imaging (n=82)

	RT after surgery NO N=50	RT after surgery YES N=19	P-value
Age years (SD)	41,2 (1,26)	41,05 (1,05)	0,94
Histotype			0,53
Squamous	39 (75%)	13 (25%)	
Adenocarcinoma	11 (64,71%)	6 (35,29%)	
Grading			0,37
1	4 (100%)	0	
2	15 (62,50%)	9 (37,50%)	
3	15 (78,95%)	4 (21,05%)	
Unknown	16 (72,73%)	6 (27,27%)	
Largest tumour diameter (SD)	43 (10,8)	39 (11,2)	0,26
Disruption of stromal ring			0,44
No	21 (70%)	9 (30%)	
Yes	25 (71,43%)	10 (28,57%)	
Unknown	4 (100%)	0	
Stage FIGO 2018 at diagnosis			0,79
IB1	1 (50%)	1 (50%)	
IB2	12 (66,67%)	6 (33,33%)	
IB3	26 (76,47%)	8 (23,53%)	
IIA1	2 (66,67%)	1 (33,33%)	
IIA2	1 (100%)	0	
IIB	2 (50%)	2 (50%)	
IIIC1	6 (85,71%)	1 (14,29%)	
Days from diagnosis to start dose-dense (SD)	46 (20,4)	42,7 (22)	0,56
Regimen of dose-dense			1
CBDCA 2-PTX 80	34 (75,56%)	11 (24,44%)	
CBDCA 2.7-PTX 60	14 (73,68%)	5 (26,32%)	
Response at imaging			**0,0001**
Complete/partial	49 (84,48%)	9 (15,52%)	
Progression/stable	1 (9,09)	10 (90,09%)	
Route of hysterectomy			0,76
Open	37 (71,15%)	15 (28,85%)	
Robot assisted	13 (76,47%)	4 (23,53%)	
Type of hysterectomy			0,39

Radical B1 Radical B2 Radical C1	4 (80%) 12 (85,7%) 34 (68%)	1 (20%) 2 (14,29%) 16 (32%)	
Oophorectomy No Yes	 23 (79,31%) 27 (67,50%)	 6 (20,69%) 13 (32,50%)	0,41

Table 4: Univariate analysis of predictors of receiving radiotherapy after surgery

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