

# Correlation of CTR1, ERCC-1, and HSP70 expressions with cisplatin response in cervical cancer stage IIB

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**Correlation of CTR1, ERCC-1, and HSP70 expressions with cisplatin response in cervical cancer stage IIB**

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ABSTRACT

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**Objectives:** This study aimed to determine the resistance of cisplatin (chemotherapy drugs) at three point areas including expression of CTR1, ERCC1, and HSP70 in cervical cancer patients.

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**Materials and Methods:** This research used a cross sectional approach. The population in this study were patients with stage IIB of cervical carcinoma. The study sample were patients with stage IIB of cervical carcinoma according to the FIGO classification. patients received cisplatin chemotherapy treatment (50 mg/m<sup>2</sup> 4 times), had good kidney and liver function. The independent variables in this study were the expression of CTR1, ERCC1, and HSP70 and the dependent variable of this study was the response to cisplatin therapy in cervical cancer. Primary data was obtained through MRI examination after the administration of cisplatin 50 mg/m<sup>2</sup> every week. The parameters of this study included the expression of CTR1, ERCC1 and HSP70. Data obtained were analyzed using Wilcoxon rank test, Spearman test and categorical regression with a significance level of p <0.05.

**Results:** The treatment of cisplatin therapy in cervical cancer patients had no significant correlation between the expression of CTR1 and ERCC1, but in the expression of HSP70 there was a significant negative correlation which means that the higher the expression of HSP70, the worse the response of therapy.

**Conclusion:** This study showed that HSP70 expression can be used as an indicator in treatment of cisplatin therapy in cancer patients through MRI examination.

**Keywords:** Chemotherapy; cisplatin; cervical cancer; MRI

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ABSTRAK

**Tujuan:** Penelitian ini bertujuan untuk menentukan resistensi cisplatin (obat kemoterapi) pada tiga titik termasuk ekspresi CTR1, ERCC1, dan HSP70 pada pasien kanker serviks.

**Bahan dan Metode:** Penelitian ini menggunakan pendekatan cross sectional. Populasi dalam penelitian ini adalah pasien dengan karsinoma serviks stadium IIB. Sampel penelitian adalah pasien dengan karsinoma serviks stadium IIB menurut klasifikasi FIGO, pasien yang menerima pengobatan kemoterapi cisplatin (50 mg/m<sup>2</sup> 4 kali), memiliki fungsi ginjal dan hati yang baik. Variabel independen dalam penelitian ini adalah ekspresi CTR1, ERCC1, dan HSP70 dan variabel dependen dari penelitian ini adalah respon terhadap terapi cisplatin pada kanker serviks. Data primer diperoleh melalui pemeriksaan MRI setelah pemberian cisplatin 50 mg/m<sup>2</sup> setiap minggu. Parameter penelitian ini termasuk ekspresi CTR1, ERCC1 dan HSP70. Data yang diperoleh dianalisis menggunakan uji Wilcoxon rank, uji Spearman dan regresi kategorikal dengan tingkat signifikansi p <0,05.

**Hasil:** Pengobatan terapi cisplatin pada pasien kanker serviks tidak memiliki hubungan yang signifikan antara ekspresi CTR1 dan ERCC1, tetapi dalam ekspresi HSP70 ada hubungan negatif yang signifikan yang berarti bahwa semakin tinggi ekspresi HSP70, semakin buruk respon dari terapi.

**Simpulan:** Penelitian ini menunjukkan bahwa ekspresi HSP70 dapat digunakan sebagai indikator dalam pengobatan terapi cisplatin pada pasien kanker melalui pemeriksaan MRI.

**Kata kunci:** Kemoterapi; cisplatin; kanker serviks; MRI

## INTRODUCTION

Nowadays, cervical cancer is one of the most common cancers in women. From the type of histopathology, there are 3 main types of histopathology of cervical cancer: squamous cell carcinoma, adenocarcinoma and adenosquamous.<sup>1</sup> Cervical cancer is the fourth cancer that leading cause of death in the worldwide.<sup>2</sup> In Indonesia cervical cancer is still in the first position followed by breast cancer, ovarian cancer and skin cancer.<sup>3</sup>

Most cervical cancer patients in Indonesia take the medical treatment when they have reached an advanced stage (stage IIB and above). The choice of treatment in advanced stage patients that is in accordance with the standards of the world gynecological obstetric organization is chemoradiation, which is a combination the treatment of radiation and chemotherapy every week as a radiosensitizer.<sup>4</sup> Alternatively, neoadjuvant chemotherapy can be given in patient with stage IIB and then post-chemotherapy evaluation is performed to assess operability and surgery will be performed if possible.<sup>4</sup> If it is considered possible to proceed with surgery, a radical hysterectomy and lymphadenectomy of lymph node are performed according to the standard of cervical cancer surgery. Whereas in patients who experience chemotherapy resistance, the choice of treatment is radiation. In patients who are resistant to chemotherapy, treatment of neoadjuvant chemotherapy causes the treatment of radiation as the main therapy to be delayed.<sup>5</sup>

The aim of neoadjuvant chemotherapy in cervical cancer is to reduce tumor size so that it becomes easier to do surgery and eliminate distant micrometastasis. Until now there is still a debate about the treatment of neoadjuvant chemotherapy in cervical cancer.<sup>6</sup> The treatment of neoadjuvant chemotherapy has a disadvantage, if resistance occurs then tumor proliferation will experience acceleration and will turn cancer cells into radioresistant.<sup>7</sup> Giving a treatment neoadjuvant chemotherapy to stage IB2-IIIB cervical cancer can cause a cervical cancer patient to receive 3 therapeutic modalities: chemotherapy, surgery and radiation with greater side effects than being directly given chemoradiation, however in developing countries with limited radiotherapy facilities, giving chemotherapy neoadjuvant continued with surgical procedure is one of options.<sup>8</sup> Based on the fact that chemotherapy can improve survival rate in the respondent group and have a negative impact on the non-respondent group, it is necessary to predict the sensitivity of cervical cancer to chemotherapy.

Cisplatin is platinum group chemotherapy and is the main choice of chemotherapy in cervical cancer. Giving cisplatin monotherapy on a weekly basis as neoadjuvant in cervical cancer provides a response rate of 85%.<sup>9</sup> In accordance with the guidelines for diagnosis and therapy of gynecological oncology at the Regional General Hospital (RSUD) Dr. Soetomo Surabaya, Indonesia in stage IIB cervical cancer patients were given cisplatin based neoadjuvant chemotherapy and the response after chemotherapy were assessed, if there was a response then the treatment will be continued with surgery and if there was no response then continued with radiation.

Cisplatin is a platinum group that works with the main target of Deoxyribonucleid acid (DNA). The main mechanism of cisplatin in killing cancer cells is by damaging DNA. DNA damage will trigger the apoptosis process which aims to kill cancer cells.<sup>10</sup> Apoptosis is a programmed cell death mechanism triggered by a series of cellular processes. Apoptosis is a normal process and is one of the defense mechanisms activated by cell damage due to mutations and diseases. Various stimuli and conditions can trigger the apoptotic process, both physiological and pathological conditions, one of the triggers of apoptosis is the administration of chemotherapy.<sup>11</sup> The process of apoptosis activation can occur through intrinsic and extrinsic pathways. The intrinsic pathway starts from the formation of holes in the mitochondrial membrane that are affected by bax and then transmembrane cytochrome c will move towards the cytosol and join the Apoptotic protease activating factor 1 (Apaf-1) activates caspase-9, caspase-3 and proteasome forms. The extrinsic pathway is triggered by the death receptor bond on the cell surface. Some known death receptors include tumor necrosis factor- $\alpha$  (TNF $\alpha$ ) and Fas receptor. Three or more molecules Fas join cytoplasmic death domains form binding sites as adapters, FADD (Fas-associated death domain). The next process is the formation of active caspase-8 which will trigger the caspase activation sequence and activate the mediator enzyme in the execution phase.<sup>12</sup>

Research on cancer cells shows that chemotherapy induces the apoptotic process. Any changes that cause disruption of apoptosis will affect the sensitivity of chemotherapy and barriers to activation of apoptosis causing resistance to chemotherapy.<sup>11</sup> One of the inhibitors of apoptosis is some heat shock protein (HSP). Heat shock protein, which was first discovered in 1962, is a group of proteins that express when cells experience various types of stress. Heat shock protein is a chaperone that functions to help proteins reach a functional form, helps interact with one protein with another protein and prevents proteins from being

misfolded. Chaperone plays a role in helping various types of processes: folding proteins, transport of proteins through membranes, modulation of protein functions and regulation of protein degradation.<sup>13</sup> Heat shock proteins in mammals are categorized into large molecular weight HSPs and low molecular weight HSPs. Included in high molecular weight HSPs are: HSP90, HSP70 and HSP60, while low molecular weight HSP is HSP27.

The main target of cisplatin is DNA, resistance can occur in pre-target, target and post-target. Resistance that occurs in the pre-target can be either an influx or cisplatin efflux into the cell, whereas the disturbance that occurs in the cisplatin target is related to the DNA repair process, the better the DNA repair activity, the cells become increasingly resistant to cisplatin. Post-target disorders are disorders of the apoptotic process. Until now there have been no clinical studies that have seen resistance to cisplatin in terms of 3 places: pre-target, target and post-target and are associated with the therapeutic response of cervical cancer to cisplatin. Existing research, usually only see from one side. This research aimed to determine the chemotherapy resistance at 3 points, that is pre-target by observing at the CTR1 expression which pumps cisplatin into the cell, on the target by observing at ERCC1 which describes DNA repair activity and at post-target by observing the HSP70 as one of the inhibiting factors apoptosis. Responses to chemotherapy were assessed by MRI to determine changes in tumor volume before and after chemotherapy. This study aimed to determine the correlation of CTR1, ERCC1 and HSP70 with the response of cisplatin therapy in cervical cancer.

## MATERIALS AND METHODS

### Study and sample

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This study was an observational analytic with cross sectional approach. The study was conducted in the Division of Gynecology Oncology/Obstetrics and Gynecology Department of the Airlangga University FK/RSUD Dr. Soetomo Surabaya, Indonesia. The subjects of the study were patients with stage IIB cervical carcinoma that undergo treatment in the Gynecological Oncology Division of the Department of Obstetrics and Gynecology at the Airlangga University/RSUD Dr. Soetomo Surabaya, Indonesia as well as patients who went to the gynecology oncology clinic of the Department of Obstetrics and Gynecology Airlangga University/RSUD Dr. Soetomo Surabaya, Indonesia that meets the inclusion criteria. Before being included in the study, patients received an explanation of this study and signed informed consent to take part in

the study. The tools and materials used in the research are gynecological table, speculum, biopsy forceps, formalin, stationery, pelvic MRI examination using 1.5 Tesla MRI units (OPTIMA MR360, GE Medical System) with fast spin-echo (FSE) and body phased-array coil. T1WI axial, coronal, sagittal + contrast properties (used Gadolinium contrast with a dose of 0.1 ml/kgBB (maximum 10ml) manually administered intravenously), Data collection sheet, painting kit for HSP70: Anti-Hsp70 Mouse Monoclonal Antibody (2A4), Painting kit for ERCC1: ERCC1 Ab-2 (Clone 8F1) Mouse Monoclonal Antibody - Thermo, and painting kit for CTR1: SLC31A1/CTR1 NOVUS antibody. Then for the immunohistochemical examination materials were 10% formalin, 95% alcohol, 80% and 70%, pH 6.0 buffer citrate, 0.3% H<sub>2</sub>O<sub>2</sub>, Hematoxylin Meyer, Secondary Antibodies, CTR1 Antibodies, ERCC1 Antibodies and HSP70 Antibodies.

In the study 42 consecutive samples were obtained that met the inclusion criteria, and there were no exclusion criteria. On the trip there were 1 patient who did not continue treatment, so a total of 41 study samples were evaluated. Samples were taken consecutively which met the inclusion criteria and there were no exclusion criteria. The inclusion criteria used in this study are as follows: (1) Patients with stage IIB cervical carcinoma patients according to FIGO classification; (2) Patient who got cisplatin chemotherapy treatment 50 mg/m<sup>2</sup> 4 times; (3) Patients with good function of kidney and liver; (4) Patients are willing and allow chemotherapy to be completed; and (5) Patients have never received chemotherapy and radiation before, while the exclusion criteria used are: (1) Patients refuse to be the respondent of the study; and (2) Patients get other alternative treatments and drop out criteria that are used as follows: (1) Patients do not continue treatment before the treatment schedule is complete; (2) The patient does not do the control; and (3) The patient passed away.

### The variable of the study

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The variables used in the study consist of independent variables and dependent variables. The independent variable were the expression of CTR1, expression of ERCC1 and expression of HSP70 while the dependent variable was the response to cisplatin therapy in cervical cancer. Sampling was carried out with the following steps: (1) Clean mucus in the cervical area and surrounding areas with cotton; (2) Perform a biopsy; (3) Specimens obtained are included in formalin solution and (4) the specimen labelled with the patient identity.

The sample tube along with the inspection request form were inserted into a styrofoam box without the need for a cooler. The sample was then sent to the Pathology



Laboratory of Faculty of Medical, Universitas Airlangga, Surabaya, and the results were obtained within 1 week.

### Procedure

Patient in gynecology oncology department of RSUD Dr. Soetomo Surabaya with IIB cervical cancer from anatomical pathology and clinical stage was included as the study sample. If the preparations could be borrowed, a re-reading of the preparation was done to ensure the diagnosis. If the preparation was difficult to get, repeat biopsy was performed. Then immunohistochemical examination of HSP70, ERCC1 and CTR1 was carried out. Furthermore, the patient was given an MRI examination to measure cervical and parametric volume. The patient was given cisplatin based neoadjuvant chemotherapy of 50 mg/m<sup>2</sup> every week as much as 4x. Then 2-3 weeks after chemotherapy, a MRI is re-examined to measure tumor volume and compared before and after chemotherapy.

### Histopathology examination

#### Hematoxylin eosin Staining

The tissue specimen was placed on the glass object and deparaffinization carried out. The tissue were put into xylol 3 times, 2 minutes each and then put in 100% ethanol 3 times, 1 minute each, then put in ethanol 95% 2 times, 1 minute each and then 90%, 80% and 70% ,1 minute each. After ethanol immersion, the specimen was washed with water for 5 minutes and put in hematoxylin 6 minutes then rinse the water and put in acid alcohol 3-5 dip and rinse the water. After rinsing with water, dip it in ammonia and put it in eosin solution, 95% ethanol twice, xylol twice and then hanging procedure carried out and observing it with a microscope.

#### Immunohistochemical examination

Immunohistochemical staining aimed to detect CTR1, ERCC1 and HSP70. Equipment (antibody kits) used in this study include CTR1 (SLC31A1/CTR1 NOVUS Antibody), ERCC1 (Ab-2 (Clone 8F1) Mouse Monoclonal Antibody - Thermo), HSP70 (Anti-Hsp70 Mouse monoclonal antibody (2A4)). In tissue that had been sliced, deparaffinization was carried out by inserting tissue incisions into xylol 3 times each 5 minutes and then soak in alcohol 95%, 80%, 70% @ 5 minutes, then soak in Tris pH 7.6 for 5 minutes, then soak in the pH 6.0 buffer citrate in the microwave with a low level for 10 minutes and then soak it in Tris for 5 minutes. Then 100µl of the primary antibody dilution was applied 100 times for 1 hour at room temperature

and then washed with Tris for 5 minutes and then dripped with 100µl of secondary antibody for 30 minutes at room temperature. Wash with Tris for 5 minutes and drip 100µl DAB (20µl DAB + 1 ml chromogen substrate) for 10 minutes. Wash with distilled water. Soak in Hematoxylin Meyer for 5 minutes. Wash with aquadest and soak in alcohol 70%, 80%, 95% @ 5 minutes then soak in xylol 1x @ 5 minutes. Air dried and mounted with Entelan + cover with glass cover and then observed under a microscope.

### Measurement

Cervical cancer IIB was stated by the results of cervical biopsy and stage IIB determined based on clinical examination referring to the stage of cervical cancer determination by FIGO. Determination of the stage of cervical cancer was done by conducting a vaginal examination and examination in the rectum. Immunohistochemistry staining of CTR1 expression was assessed based on immunohistochemical examination using the SLC31A1/CTR1 NOVUS Antibody kit and CTR1 expression degrees were measured.<sup>14</sup> ERCC1 expression was assessed based on immunohistochemistry by using the ERCC1 Ab-2 (Clone 8F1) Mouse monoclonal-Thermo kit and measurement of ERCC1 expression also carried out.<sup>15</sup> HSP70 expression was assessed based on immunohistochemical examination using the Anti-Hsp70 Mouse monoclonal antibody kit (2A4) and measurement of expression level.<sup>16</sup> After cisplatin chemotherapy 50 mg/m<sup>2</sup>, 4 times at 1-week interval was administered. Changes in tumor volume measured by MRI use elliptical formulas.<sup>17</sup> Clinical responses were assessed after 3 series of cisplatin chemotherapy and evaluated using MRI at 2 weeks post chemotherapy.

### Ethical clearance

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Ethical clearance was obtained from the ethics committee of the RSUD Dr. Soetomo Surabaya, Indonesia. The confidentiality of patients in this study will be maintained, initials will be used in the patient list. This research was confidential for academic purposes.

### Data analysis

The results of the study were tabulated and presented in tables, graphs and diagrams as well as statistical analysis using the SPSS 21 program. Treatment responses were calculated statistically using the Wilcoxon rank test. The correlation between HSP70, ERCC1 and CTR1 expression with therapeutic response was calculated statistically using Spearman correlation analysis. Categorical regression analysis was used to

analyze the correlation of several independent variables (HSP70, ERCC1 and CTR1) with therapeutic responses. Probability was considered statistically significant if  $p < 0.05$  with a confidence interval of 95% was obtained.

## RESULTS AND DISCUSSION

### Patient demographic data

61 Table 1. The Characteristics of the patients

15 Category	Frequency	Percentage
<b>Age</b>		
31-40 years	11	26.8
41-50 years	18	43.9
>50 yeras	12	29.3
Total	41	100.0
<b>Parity</b>		
0 person	3	7.3
1-2 person	16	39.0
3-4 person	17	41.5
>4 person	5	12.2
Total	41	100.0
<b>Histopathology</b>		
Squamous cell carcinoma	24	58.5
Adenocarcinoma	13	31.7
Adenosquamous	4	9.8
Total	41	100.0

In terms of age, the highest frequency was found in the age group of 41-50 years that consist of 18 patients (43.9%). In terms of parity, most patients have 3-4 children, as many as 17 patients (41.5%). In terms of the type of histopathology, most common type was squamous cell carcinoma (58.5%) followed by adenocarcinoma (31.7%) and Adenosquamous (9.8%).

### CTR1, ERCC1 and HSP70 expression

Table 2. The results of CTR1, ERCC1 and HSP70 expression

Variable	Category	Frequency	Percentage
CTR1	Low	8	19.5%
	High	33	80.5%
ERCC1	Low	14	34.1%
	High	27	65.9%
HSP70	Low	19	46.3
	High	22	53.7

The results of the analysis to determine the correlation of expression of CTR1, ERCC1 and CTR1 with tumor volume showed no association between the initial tumor volume before therapy with HSP70 expression ( $p = 0.940$ ), ERCC1 ( $p = 0.180$ ), or CTR1 ( $p = 0.521$ ).

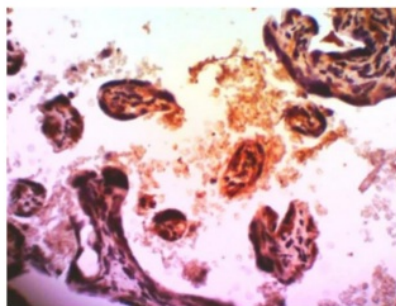


Figure 1. Positive control of CTR1

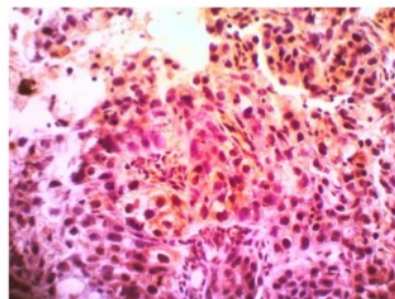


Figure 2. Low CTR1 expression

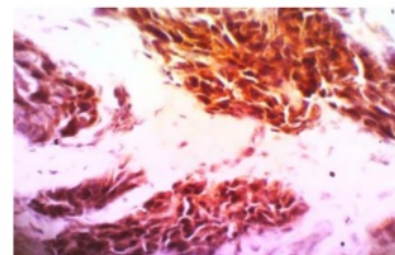


Figure 3. High CTR1 expression

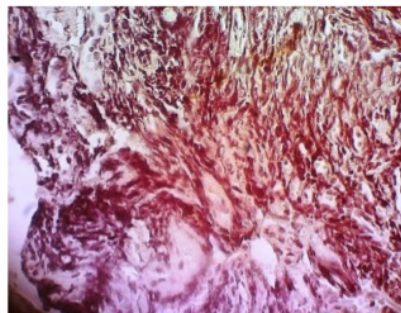


Figure 4. Positive control of ERCC1



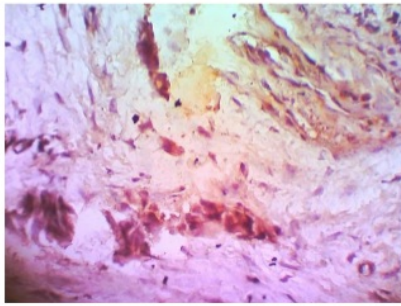


Figure 5. Low ERCC1 expression

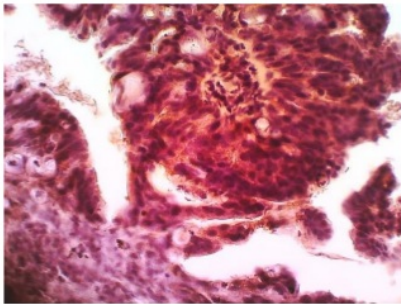


Figure 6. High ERCC1 expression

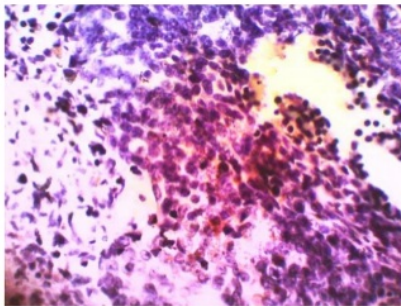


Figure 7. Positive control of HSP70

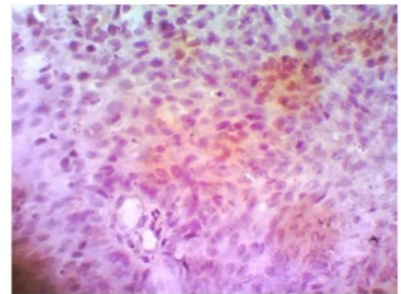


Figure 8. Low HSP70 expression

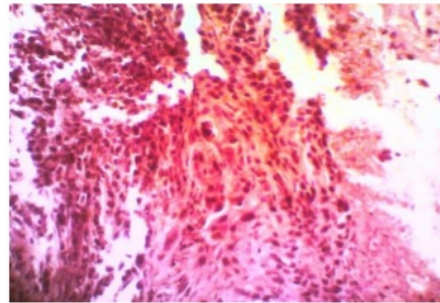


Figure 9. High HSP70 expression

CTR1 expression was not significantly different between the three histopathological type: squamous cell carcinoma, adenocarcinoma and adenosquamous. ERCC1 expression was not significantly different between the three histopathological types: squamous cell carcinoma, adenocarcinoma and adenosquamous and HSP70 expression did not differ significantly between the three histopathological types: squamous cell carcinoma, adenocarcinoma and adenosquamous

#### Tumor volume

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Tumor volume was measured before cisplatin chemotherapy using MRI and after 4 series of cisplatin chemotherapy, MRI was re-examined to determine tumor volume after chemotherapy. Table 4 shows a significant difference in tumor size before and after Cisplatin therapy ( $p < 0.05$ ). In general, after the tumor size is reduced, there are some patients who show an increase in tumor size.

Table 5 shows that most patients showed a positive response (a decrease in tumor size) to cisplatin therapy, and only 4 people (9.8%) showed a negative response (enlarged tumor size).

#### Responses to therapy

If the tumor volume was categorized according to the RECIST Criteria, then the therapeutic response results are obtained as shown in Table 6. In this table most patients showed partial response to cisplatin therapy, and only 9 people (21.9%) showed stable or progressive disease. None of the patients achieved complete response. Table 7 shows the therapeutic response based on RECIST does not differ between the three histopathological type: squamous cell carcinoma, adenocarcinoma and adenosquamous.

**Expressions of CTR1, ERCC1, HSP70 and histopathological types**

Table 3. Expressions of CTR1, ERCC1, HSP70 and histopathological types

Histopathology	CTR1 Expression		Total
	Low	High	
12 Squamous cell carcinoma	5 (20.8%)	5 (20.8%)	24 (100.0%)
Adenocarcinoma	2 (15.4%)	11 (84.6%)	13 (100.0%)
Adenosquamous	1 (25.0%)	3 (75.0%)	4 (100.0%)
Total	8 (19.5%)	33 (80.5%)	41 (100.0%)
Exact p = 1.000			
Histopathology	ERCC1 Expression		Total
	Low	High	
12 Squamous cell carcinoma	9 (37.5%)	15 (62.5%)	24 (100.0%)
Adenocarcinoma	4 (30.8%)	9 (69.2%)	13 (100.0%)
Adenosquamous	1 (25.0%)	3 (75.0%)	4 (100.0%)
Total	14 (34.1%)	27 (65.9%)	41 (100.0%)
Exact p = 0.893			
Histopathology	HSP70 Expression		Total
	Low	High	
12 Squamous cell carcinoma	11 (45.8%)	13 (54.2%)	24 (100.0%)
Adenocarcinoma	7 (53.8%)	6 (46.2%)	13 (100.0%)
Adenosquamous	1 (25.0%)	3 (75.0%)	4 (100.0%)
Total	19 (46.3%)	22 (53.7%)	41 (100.0%)
Exact p = 0.651			

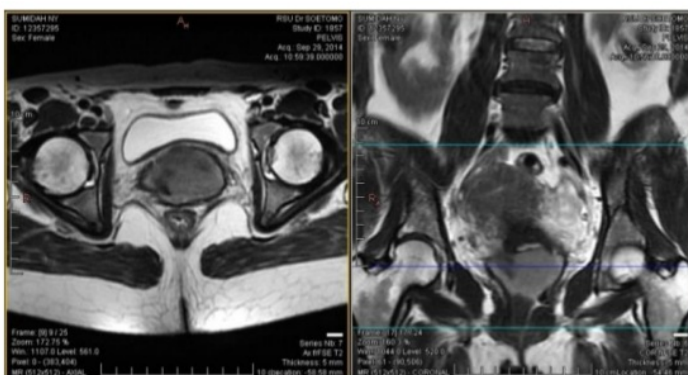


Figure 10. MRI examination results before chemotherapy

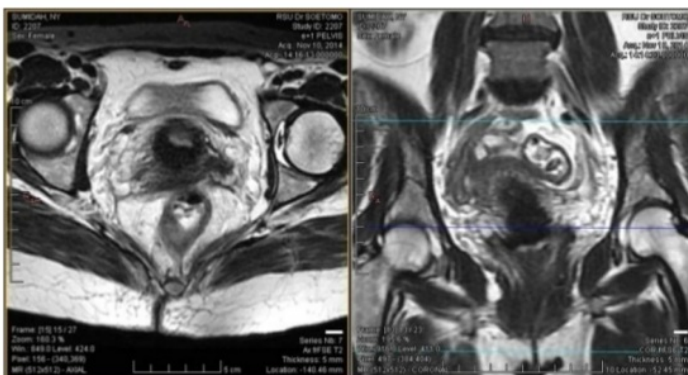


Figure 11. MRI examination results after chemotherapy



Table 4. Tumor volume before and after therapy

Measurement	Tumor volume					p*
	Mean	SD	Median	Min	Max	
Before Tx (mm)	64.99	66.72	44.79	2.35	276.23	0.000
After Tx (mm)	38.82	75.83	15.74	0.28	422.11	
Reduction (%)	48.46	35.66	53.02	-52.81	92.26	

\*Wilcoxon Signed Rank Test

Table 5. Frequency of tumor volume before and after therapy

Response to therapy	Frequency	%
Reduced	37	90.2
Enlarge	4	9.8
Total	41	100.00

Table 6. Frequency of therapeutic response based on RECIST criteria

Response to therapy	Frequency	%
Partial Response	32	78.0
Stable Disease	6	14.6
Progressive Disease	3	7.3
Total	41	100.0

Table 7. RECIST therapy responses according to histopathology

Histopathology	Response to Therapy			Total
	Partial	Stable	Progressive	
Squamous cell carcinoma	18 (75.0%)	3 (12.5%)	3 (12.5%)	24 (100.0%)
Adenocarcinoma	11 (84.6%)	2 (15.4%)	0 (0.0%)	13 (100.0%)
Adenosquamous	3 (75.0%)	1 (25.0%)	0 (0.0%)	4 (100.0%)
Total	32 (78.0%)	6 (14.6%)	3 (7.3%)	41 (100.0%)

Exact p = 0.695

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**Correlation between expression of CTR1, ERCC1, HSP70 and cisplatin therapy response**

Table 8. Expressions of CTR1, ERCC1, HSP70 and therapy response based on RECIST

CTR1 Expression	Response to therapy			Total
	Partial	Stable	Progressive	
Low	5 (62.5%)	2 (25.0%)	1 (12.5%)	8 (100.0%)
High	27 (81.8%)	4 (12.1%)	2 (6.1%)	33 (100.0%)
Total	32 (78.0%)	6 (14.6%)	3 (7.3%)	41 (100.0%)
Exact p = 0.618				
ERCC1 Expression	Response to Therapy			Total
	Partial	Stable	Progressive	
Low	13 (92.9%)	1 (7.1%)	0 (0.0%)	14 (100.0%)
High	19 (70.4%)	5 (18.5%)	3 (11.1%)	27 (100.0%)
Total	32 (78.0%)	6 (14.6%)	3 (7.3%)	27 (100.0%)
Exact p = 0.245				
HSP70 Expression	Response to Therapy			Total
	Partial	Stable	Progressive	
Low	19 (100.0%)	0 (0.0%)	0 (0.0%)	19 (100.0%)
High	13 (59.1%)	6 (27.3%)	3 (13.6%)	22 (100.0%)
Total	32 (78.0%)	6 (14.6%)	3 (7.3%)	41 (100.0%)
Exact p = 0.003				

Table 9. Results of the analysis of the correlation between CTR1, ERCC1 and HSP70 expressions with therapeutic response

Step	Independent Variable	Beta Coefficient	Sig.
1	HSP70	0.442	0.001
	ERCC1	0.092	0.454
	CTR1	-0.083	0.662
2	HSP70	0.459	0.000
	ERCC1	0.094	0.433
3	HSP70	0.493	0.000

Dependent variable: therapeutic response based on RECIST criteria

Table 8 shows that most patients with high CTR1 expression and low CTR1 expression show partial response to therapy. Then all patients with low ERCC1 expression showed partial response or stable disease to cisplatin therapy, none of which showed progressivity. On the other hand, all patients who show progressive disease are present in patients with high ERCC1 expression. While the HSP70 expression table shows all patients with low HSP70 expression showed partial response to therapy. On the other hand, all patients who show stable or progressive disease are present in patients with high HSP70 expression.

**Correlation between expression of HSP70, ERCC1, and CTR1 with therapeutic response**

Categorical analysis shows that the dominant variable that influences the therapeutic response is the expression of HSP70. The higher the expression of HSP70, the worse the response to therapy. This study showed that histopathology does not affect the expression of CTR1, ERCC1 and HSP70 and HSP70 expression is a factor that influences the response of therapy. The samples analyzed consisted of 24 squamous cell carcinomas (58.5%), 13 adenocarcinomas (31.7%) and 4 adenosquamous (9.8%). Expression of HSP 70, ERCC1 and CTR1 did not differ in the three histopathological type: cell carcinoma squamous, adenocarcinoma and adenosquamos, with p values of 0.651, 0.893 and 1.000 respectively.

Research conducted on 417 cervical cancer patients given chemotherapy without distinguished histopathology types showed a response in 14.6% of patients who received chemotherapy for cisplatin, vincristine and bleomycin and there was a response in 22.4% of patients who received chemotherapy for carboplatin-paclitaxel. The response categories in this study were only assessed through internal examination, without using an imaging diagnostic tool.<sup>19</sup> Studies showed that there is no difference in response to single cisplatin chemotherapy between various histopathological type. This is in accordance with the study conducted by Lina He which showed no difference in short-term effectiveness in terms of therapeutic response to squamous cell carcinoma or squamous cell carcinoma.<sup>20</sup>

At a cancer treatment center that has good radiation facilities, the treatment modality for stage IIB cervical cancer is the treatment of chemoradiation in the form of a combination of radiation and chemotherapy as a radiosensitizer. Research comparing neoadjuvant chemotherapy and chemoradiation in cervical cancer shows that neoadjuvant chemotherapy in stage IIB cervical cancer does not provide benefits in terms of survival, compared to being given chemoradiation directly without neoadjuvant chemotherapy.<sup>21</sup> But at a cancer treatment center with limited radiation facilities, one way to treat stage IIB cervical cancer is by giving a treatment of neoadjuvant chemotherapy followed by surgery or radiation. The treatment of neoadjuvant chemotherapy has advantages in terms of recurrence rates and survival rates compared to those without neoadjuvant chemotherapy.<sup>22</sup> In Dr. Soetomo Hospital, radiation facilities are limited, according to the guidelines for the management of cervical cancer, the neoadjuvant chemotherapy was given to stage IIB cervical cancer, chemotherapy given is a single drug using cisplatin. Several studies have shown the effectiveness of single cisplatin administration in cervical cancer.<sup>23-25</sup> Our research proved the effectiveness of single cisplatin as neoadjuvant chemotherapy in stage IIB cervical cancer. 37 patients (90.2%) had decreased tumor volume after cisplatin administration from an average of 64.99cm<sup>3</sup> to 38.82 cm<sup>3</sup>, this was statistically significant.

The most widely used assessment of therapeutic response is according to RECIST criteria (response evaluation criteria in solid tumors). From this study, no patient experienced complete response, the maximum was a partial response. From 41 patients, 32 people experienced a partial response (78%), 6 people experienced stable disease (14.6%) and 3 people experienced an increase in tumor volume (7.3%). When analyzed by histopathological type, the therapeutic response based on RECIST criteria in the three types of histopathology did not differ significantly, according to the study conducted by Lina He.<sup>20</sup> Several previous studies on single cisplatin in cervical cancer performed on stage IV cervical cancer, recurrent cervical cancer or persistent cervical cancer proved that the administration

of single cisplatin to stage IIB cervical cancer effectively reduced tumor volume.<sup>23,24</sup>

Research on CTR1 in cervical cancer is very limited, one of the studies conducted shows that administration of copperchelator (copper binder) will increase the death of cancer cells due to cisplatin, this is likely with a decrease in systemic levels of copper due to copper chelator causing CTR1 work to pump copper into cells increase, while pumping cisplatin into cells.<sup>26</sup> Almost all studies on CTR1 have been carried out rather than using clinical specimens in humans but, the first study in human clinical specimens was carried out by Kim in lung cancer which showed that in lung cancer with negative CTR 1 had intracellular cisplatin concentrations and a low therapeutic response.<sup>27</sup>

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The results of this study indicated that there is no statistically significant correlation between CTR1 and the therapeutic response although it appears in the results of the study that in the group with high CTR1 expression there was a decrease in tumor size in 90.2% of patients, compared to the group with low CTR1 expression that had a response decreased tumor size in 75% of patients. In the research we conducted showed that high CTR1 was not related to therapeutic response so it might be concluded that the amount of cisplatin that enter the body was not related to the response to cisplatin administration. The accumulation of cisplatin in cells influenced by CTR1, other than that can also be affected by ATP7B which carries cisplatin out of cells.<sup>28</sup> The results of this study indicate the role of other factors other than CTR1 in the accumulation of cisplatin in cervical cancer cells, one possibility is CTR2. Research conducted by Blair on ovarian cancer shows the role of CTR2 in addition to CTR1 on uptake of cisplatin.<sup>29</sup> The results of this study open the opportunity to determine the correlation of CTR2 with the response of cisplatin therapy to cervical cancer. Our study did not analyze whether high CTR1 expression affected the cisplatin concentration in cells, because the concentration of cisplatin in cells in our study was not measured, further research was needed to see the correlation of CTR1 expression with cisplatin concentration in cervical cancer cells and determine whether the concentration of cisplatin in cervical cancer cells associated with therapeutic response.

The research we conducted showed positive ERCC1 expression in all samples, where the sample used was stage IIB cervical cancer. This is consistent with a study conducted by Hasegawa that obtained positive ERCC1 expression in all cervical cancer adenocarcinoma stage I-II<sup>30</sup> and research conducted by Park on cervical cancer in squamous cell carcinoma and non-squamous type also showed positive ERCC1 expression in all

samples.<sup>31</sup> Previous ERCC1 studies on cervical cancer to determine the correlation between ERCC1 and the chemotherapy response to cervical cancer showed that low ERCC1 had a better response to chemotherapy.<sup>31</sup> In our study, the measure of therapeutic response using MRI to see a decrease in tumor size and response based on RECIST criteria and chemotherapy used was single cisplatin, whereas in previous studies, response ratings were categorized based on responder and non-responder and the chemotherapy used was a combination of cisplatin and etoposide, not monotherapy with cisplatin.

Previous research to see the expression of ERCC1 in cervical cancer showed the same thing with this study, in the squamous type there were 88.9% high ERCC1 expression and 11.1% low ERCC1 expression, whereas in the non-squamous type it was 28.6% with low ERCC1 expression and 71.4% with high ERCC1 expression, but statistically this difference was not significant with  $p = 0.102$ .<sup>31</sup> Other studies have shown that the cervix with low ERCC1 expression has the possibility of cervical pre-cancerous lesions or cervical cancer greater than the cervix with high ERCC1 expression. It can be explained that the role of ERCC1 is in the repair DNA mechanism, if ERCC1 is high, DNA repair works better, DNA damaged by HPV infection can be repaired, and vice versa.<sup>32</sup>

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Our study showed that all patients with low ERCC1 expression have a positive therapeutic response (tumor size decreases) and on the other hand, all patients who show a negative therapeutic response (enlarged tumor size) are present in patients with high ERCC1 expression. If the response is categorized according to RECIST criteria, the results of our study show that all patients with low ERCC1 expression showed partial response or stable disease to cisplatin and none showed progressive disease, on the other hand, all patients who showed progressive disease were present in patients with High ERCC1 expression.

Statistical tests to analyze the correlation of ERCC1 expression with therapeutic response showed insignificant results. although it looks clinically meaningful. The results of this study suggested that there is a DNA repair mechanism in addition to nucleotide excision repair which plays a role in repairing DNA damaged by cisplatin in cervical cancer cells. Another mechanism for repairing DNA damage by cisplatin is through mismatch repair pathway (MMR), deficiency of the MMR mechanism causing cells to become resistant to chemotherapy.<sup>33,34</sup> Another possibility, in cervical cancer, is the mechanism of cell death without DNA damage, namely through reactive oxygen species (ROS) as suggested by Florea and Brozovic.<sup>35,36</sup> In vitro studies conducted show that



cisplatin can induce ROS formation which can trigger cell death without DNA damage.<sup>37</sup>

Assessment of HSP70 expression was carried out by assessing immunohistochemical preparations and assessed through scores and then determined by the final result of expression. Statistical calculations with the Kruskal-Wallis test to determine whether there is a correlation between HSP70 scores shows a value of  $p = 0.233$  which means there is no significant difference in HSP70 scores on the three types of histopathological features: squamous cell carcinoma, adenocarcinoma and adenosquamous. HSP70 expression was not influenced by histopathology type.

In this study, the assessment of HSP70 was performed only on stage IIB cervical cancer, not on other stages, and the results of HSP70 expression assessment showed 46.3% low expression and 53.7% high expression from all samples of 41 study samples: 24 carcinomas squamous cell, 13 adenocarcinoma and 4 adenosquamous. When viewed from each histopathological, the adenosquamous type had high HSP70 expression, 3 of 4 adenosquamous samples showed high HSP70 expression (75%). While the most common type is squamous cell carcinoma, showing 13 results with high expression (54.2%) and 11 with low expression (45.8%). However, the statistical calculation shows that the differences in HSP70 expression in the three histopathological were not significant. Several HSP70 studies on cervical cancer were previously performed on squamous cell carcinoma with positive HSP70 expression results. Research on expression in adenocarcinoma and adenosquamous has not been done, possibly considering squamous cell carcinoma more than adenocarcinoma and adenosquamous. Cervical adenocarcinoma originates from endocervical tissue which is a columnar epithelium, research on HSP70 expression in the columnar epithelium is found in HSP70 studies in endometrial cancer which shows HSP70 expression in 52% of endometrial cancers.<sup>38</sup>

In the study there were also patients who had high HSP70 expression but also had a good therapeutic response. It is suspected that cisplatin, which is a non-specific chemotherapy, not only reacts with DNA but also with proteins in cells, causing cisplatin to also be able to induce apoptosis directly on the caspase activation pathway or execution path.<sup>10</sup>

The HSP70 study in pre-cervical cancer lesions showed that the further pre-cervical cancer lesions the higher the expression of HSP70, this is thought to be related to the pathogenesis of cervical cancer involving oncogenic HPV infection. Cellular stress occurs due to HPV infection until there is a carcinogenesis process and an

increase in HSP70 which is a protective protein.<sup>39</sup> HPV infection also occurs in cervical cancer types of adenocarcinoma, the world multicenter data shows the main types of HPV in adenocarcinoma are HPV 16, 18 and 45.<sup>40</sup> In this study there was HSP70 expression in adenocarcinoma type cervical cancer probably because cellular adenocarcinoma also occurs due to HPV infection and carcinogenesis. Previous studies were not carried out on adenocarcinoma-type.

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The results of our research analysis showed a negative correlation between HSP70 expression and decreased tumor volume size, the lower HSP70 expression, the greater the reduction in tumor volume after chemotherapy. HSP70 expression was assessed from the score, indicating that at a low expression score, the tumor volume was smaller. When assessed from the results of HSP70 expression, cervical cancer with low HSP70 expression shows 100% of reduced tumor volume after single cisplatin chemotherapy. If the therapeutic response is categorized according to the RECIST criteria, the results of the analysis show that cervical cancer with low HSP70 expression has a positive therapeutic response, in this case a partial response or in other words the volume of the tumor after chemotherapy shrinks. Whereas on the other hand, all patients who have a response to therapy for stable disease and progressive disease are found in the cervical cancer group with high HSP70 expression.

Heat Shock Protein 70 is a powerful apoptosis that can inhibit the apoptosis process at several stages.<sup>41</sup> The results of this study indicated that there is a correlation between HSP70 expression and therapeutic response, possibly through the role of apoptosis. The mechanism of cisplatin in eliminating cancer cells is through the process of apoptosis.<sup>42</sup> The molecular mechanism of chemotherapy inducing apoptosis is not yet clear. After cisplatin enters the cell and binds to DNA and other molecules and cellular stress responses will occur and trigger cellular effector system activation and apoptosis.<sup>43</sup>

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The results of this study proved that HSP70 plays a role in the sensitivity of cervical cancer to cisplatin. Cervical cancer with a low HSP70 score has a better response to cisplatin than cervical cancer with a high HSP70 score. This can be explained because the nature of HSP70 as an anti-apoptosis hence if its expression is high it will inhibit the process of apoptosis while cell death due to cisplatin requires the role of apoptosis. Conversely, if HSP70 expression is low, the resistance to apoptosis is also smaller so that cell death due to cisplatin can be more effective.

Several studies on HSP70 have been conducted, such as a study conducted by Kim et al. Which compared HSP70 expression in pre-cervical cancer lesions and cervical cancer.<sup>16</sup> Castle et al.<sup>39</sup> and a study conducted by Park et al. assessed the correlation between HSP70 expression and p53 expression and estrogen receptor on cervical cancer.<sup>44</sup> Some of these studies do not link HSP70 with therapeutic response. Other research is the research that we have done at Dr. Hospital. Soetomo and Roy to assess the operability of cervical cancer associated with HSP70 expression turned out to show no correlation between HSP70 expression and operability of cervical cancer.<sup>45</sup> There are differences in methodology in the study with this study. In previous studies, the assessment was not based on tumor volume but based on a vaginal examination using the hand to assess operability, the examination was subjective and did not describe the actual response because there was no radiological examination to measure tumor size or volume. The research we are currently doing proves that HSP70 is associated with a therapeutic response to cisplatin where the response of therapy is measured by MRI so that changes in tumor volume can be known to be more accurate.

The correlation analysis of each expression of CTR1, ERCC1 and HSP70 with therapeutic response showed only HSP70 which had a significant correlation. The higher the expression of HSP70, the worse the response to therapy. Analysis to see the correlation of CTR1, ERCC1 and HSP70 expressions with therapeutic response showed that HSP70 was the dominant factor affecting the response of therapy.

The research conducted was the first study to analyze pre-target, target and post-target factors against the cisplatin response to cervical cancer. From the analysis, the factors that significantly correlated with the therapeutic response were HSP70 which was an anti apoptosis, whereas the results of the analysis of pre-target factors (CTR1) and factors in the target area (ERCC1) did not show a significant correlation with the response of therapy. DNA damage that occurs as a result of cisplatin will cause the cell cycle to stop at the checkpoint from the cell cycle, giving the opportunity for DNA to be repaired, ensuring that the damaged DNA does not replicate. If DNA damage cannot be repaired, the cell will undergo a process of death through the process of apoptosis.<sup>46</sup> The results of the analysis show that ERCC1 and CTR1 are not significantly associated with therapeutic response, indicating the possibility that the most important role in the treatment response of cervical cancer to cisplatin is the post-target factor which in this case is the apoptosis process, while the accumulation of cisplatin in cells in cervical cancer does not play a role in the response,

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there are other mechanisms that might play a role in the accumulation of cisplatin in cervical cancer cells other than CTR1.

### Limitation

This study had several limitations, and our hope is that this limitation is an opportunity to conduct further research in the future, some of these limitations are: (1) This study did not observe HSP70, ERCC1 and CTR1 expression at post-chemotherapy; (2) This study did not evaluate the concentration of cisplatin in cells; and (3) In this study not all molecular biology factors that can influence the therapeutic response are homogeneous.

### CONCLUSION

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Studies show that there was no correlation between CTR1 expression and ERCC1 expression on the response to cisplatin therapy in cervical cancer. Then there was a negative correlation between the expression of HSP70 and the response of cisplatin therapy to cervical cancer. From CTR1, ERCC1 and HSP70 expressions, HSP70 expression was a factor that influences the response of therapy

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