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**PREDICTING THE RESPONSE OF GYNAECOLOGICAL TUMOURS TO
CHEMOTHERAPY**

A thesis submitted for the Degree of

Doctor of Philosophy

By

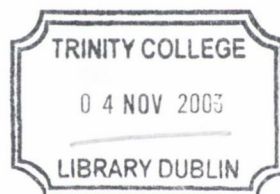
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2003



THESIS

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DECLARATION

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Sharon A. O'Toole

Sharon A. O'Toole

Dedicated to

My late brother, Fr. Declan O'Toole (MHM)

Martyred in Uganda 2002

R.I.P.

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Peer-Reviewed Articles

O'Toole SA, Sheppard BL, McGuinness E, Gleeson NC, Bonnar J. Serous papillary adenocarcinomas of the ovary display heterogeneity in their response to chemotherapy. *Int J Gynecol Cancer* 2001; 11(5): 365-71.

O'Toole SA, Sheppard BL, McGuinness EP, Gleeson NC, Yoneda M, Bonnar J. The MTS assay as an indicator of chemosensitivity/resistance in malignant gynaecological tumours. *Cancer Detect Prev* 2003; 27(1): 47-54.

Abstracts

O'Toole S. Chemosensitivity Testing of Gynaecological Tumours. Proceedings of the 1st International Postgraduate Student Conference. Dublin, 1998.

O'Toole SA. Cell Culture Drug Sensitivity/Resistance Testing Of Malignant Gynaecological Tumours. Proceedings of the Irish Gynaecological Oncology Society. Dublin 1999.

O'Toole S., Bonnar J., Sheppard BL., McGuinness E. Cell Culture Drug Sensitivity/Resistance Testing of Gynaecological Tumours. *Ir. J. Med. Sc.* 1999; 168(Suppl 3): 35.

Sharon A. O'Toole MSc, Brian L. Sheppard DPhil, Eamon P.J. McGuinness MD, Noreen C. Gleeson MD, Misaho Yoneda MD, John Bonnar MD. The MTS assay as an indicator of chemosensitivity / resistance in malignant gynaecological tumours. Proceedings of the 5th International Symposium on Predictive Oncology and Therapy, Geneva, Switzerland. (2000).

Cell Culture drug sensitivity/resistance testing of malignant gynaecological tumours. O'Toole S, Sheppard B.L., Bonnar J, McGuinness E, Gleeson N. British Journal of Obstetrics and Gynaecology 2000; 107(6): 818-819.

SA. O'Toole, BL. Sheppard, EPJ. McGuinness, NC. Gleeson, M. Yoneda, J. Bonnar. Determining the response of gynaecological tumours to chemotherapy using an MTS cytotoxicity assay in combination with an explant culture technique. British Journal of Cancer 2002; 86(Suppl 1): S46.

O'Toole SA, Sheppard BL, McGuinness EPJ, Gleeson NC, Bonnar J. Predicting the response of gynaecological tumours to chemotherapy. Anticancer Research 2003 (In Press).

SUMMARY

The aim of this study was to use an *in vitro* (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulphophenyl)-2H-tetrazolium, inner salt) (MTS) assay in combination with an explant culture technique to determine the response of ovarian, endometrial and cervical tumours to various cytotoxic drugs and correlate this with the *in vivo* response. Three candidate markers, CD31, VEGF (vascular endothelial growth factor) and MDR1 (multidrug resistance 1) were assessed to determine if they could predict the response to chemotherapy in ovarian cancers or act as prognostic factors.

Tumour samples were taken at surgery and cultured using the explant technique. Cells were reseeded and incubated with various concentrations of chemotherapy drugs. The MTS cytotoxicity assay was carried out to ascertain the response to the drugs and correlated retrospectively to the clinical outcome. Ovarian cancers and normal ovarian specimens were examined immunohistochemically for expression of CD31, VEGF and MDR1.

Tumours of similar stage and grade displayed heterogeneity in their responses to the same drugs. One hundred and thirty nine of 144 tumours yielded chemosensitivity data, giving an evaluability rate of 96.5%. Of these, 76, mainly ovarian specimens, were available for *in vitro-in vivo* correlations. *In vitro* sensitivity was associated with clinical response in 29 of 39 patients. *In vitro* resistance was associated with progressive disease in 36 of 37 patients. The association between *in vitro* and *in vivo* response, as measured by Chi-Squared was highly significant ($p < 0.0001$). *In vitro* response remained an independent prognostic indicator in multivariate analysis using Cox Regression for progression free survival ($p < 0.05$) but not for overall survival.

Immunohistochemical analysis was carried out on 79 ovarian specimens. Increased staining with CD31 was associated with decreased PFS ($p<0.01$) and OS ($p<0.01$) in univariate but not multivariate analysis. CD31 offered no indication as to which patients might best respond to chemotherapy. Quantifying VEGF proved to be a valuable independent prognostic indicator in PFS ($p<0.05$) and OS ($p<0.0001$) but fails to provide an adequate measure for resolving the response to chemotherapy drugs in ovarian cancer. A significant correlation ($p<0.0001$) was detected between CD31 and VEGF suggesting VEGF plays a role in angiogenesis. MDR1 expression was detected in previously untreated ovarian tumours. MDR1 failed to act as a prognostic marker or as a predictor of response to chemotherapy. No association was found between MDR1 and CD31 or VEGF.

Based on the results of this retrospective study a randomised prospective trial using the explant technique in combination with the MTS assay should be carried out to validate chemosensitivity / resistance testing. The *in vitro* assay proved more valuable than the candidate markers in predicting the response to chemotherapy. Further studies on angiogenic markers and multidrug resistance need to be carried out to elucidate the role in ovarian cancer.

ABBREVIATIONS

ABC	ATP-Binding cassette
AJCC	American Joint Committee on Cancer
APES	3-Aminopropyltriethoxysilane
ATP	Adenosine Triphosphate
Balb/c	Bagg albino breed/colour
C	Cisplatin
°C	Degrees celcius
CAM	Cell adhesion molecule
C + P	Cisplatin and Paclitaxel
Cb	Carboplatin
CD31	CD31 Antigen
CD34	CD34 Antigen
CHO	Chinese hamster ovary
CIN	Cervical intraepithelial neoplasia
cm ²	centimetre squared
CO ₂	Carbon Dioxide
Cum	Cumulative

CX	Cervix
D	Doxorubicin
DAB	3,3'-diaminobenzidine
D & C	Dilation and curettage
DiSC	Differential Staining Cytotoxicity
DMEM	Dulbecco's Modified Eagle Medium
DMSO	Dimethyl sulfoxide
DPX	distyrene, plasticizer (tricresyl phosphate) and xylene
EDTA	Ethylenediaminetetra-acetic acid
EN	Endometrium
FC	Fetal Clone
FCA	Fluorescent cytoprint assay
FIGO	Federation of International Gynecology and Obstetrics
g	gramme
G	Grade
G1	Gap 1
G2	Gap 2
GOG	Gynecologic Oncology Group
HCl	Hydrochloric acid

HPV	Human papilloma virus
HRP	Horseradish peroxidase
HTCA	Human tumour clonogenic assay
IC50	Inhibitory concentration 50
is	in situ
kDa	kilo Dalton
L	Litre
LLETZ	Large loop excision of the transformation zone
LTF	Lost to follow up
M	Mitosis
MCF-7	human Caucasian mammary breast cancer
MDR	Multidrug Resistance
mg	milligramme
ml	millilitre
mM	milli molar
MTS	3-(4,5–dimethylthiazol-2- yl)-5-(3-carboxymethoxyphenyl)-2-(4 - sulfophenyl) - 2H - tetrazolium, inner salt
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MVD	Microvessel Density

N/A	Not applicable
NC	Not cultured
ND	Not documented
NE	Not evaluable
NCI	National Cancer Institute
OD	Optical Density
OS	Overall survival
OV	Ovary
P	Paclitaxel
PAP	Papanicolaou
PECAM-1	Platelet endothelial cell adhesion molecule-1
PFS	Progression free survival
Pgps	P-glycoproteins
PGY1	P-glycoprotein-1
PMS	phenazine methosulfate
PPC	Peak plasma concentration
Prev	Previous
Prog	Progression
rpm	Revs per minute

RPMI	Roswell Park Memorial Institute
S	Synthesis
T	Topotecan
TE	Trypsin EDTA
TB	Tris Buffer
TBS	Tris Buffer Saline
TNM	Tumour, Node, Metastasis
U	Unknown
UICC	Union Internationale Centre le Cancer
μl	Microlitre
μm	Micrometre
VEGF	Vascular endothelial growth factor
VPF	Vascular permeability factor

TABLE OF CONTENTS

Declaration	i
Acknowledgements	iii
Publications Arising from Thesis	v
Summary	vii
Abbreviations	ix
 Chapter 1 Introduction	
1.1 Cancer	1
1.2 Malignant versus Benign	3
1.3 Carcinogenesis	4
1.4 Tumour Angiogenesis	5
1.5 Cancer Grade	6
1.6 Staging of Cancer	7
1.7 Correction of Abnormalities	8
1.8 Cancer Treatment	9
1.9 Gynaecological Cancer	18
1.10 Predicting Chemotherapy Response	57
1.11 <i>In vitro</i> Determination of Drug Response	58
1.12 Chemosensitivity/Resistance Testing of Malignant Tumours of the Ovary, Cervix and Endometrium	65

1.13 Potential Predictors of Response to Chemotherapy	70
1.14 Angiogenesis in Ovarian Carcinomas	72
1.15 Angiogenesis and Chemotherapy Response	74
1.16 Multidrug Resistance (MDR)	76
1.17 P-glycoprotein	76
1.18 MDR And Chemotherapy	78
1.19 MDR in Ovarian Cancer	78
1.20 Aims of study	80

Chapter 2 Patients, Materials and Methods

2.1 Patients	82
2.2 Specimen Collection	93
2.3 Explant Culture Technique	94
2.4 Trypsinisation	96
2.5 MTS Cytotoxicity Assay	96
2.6 Paraffin Embedding Of Tumour Specimens	101
2.7 3-Aminopropyltriethoxysilane (APES) Coated Slides	101
2.8 Haematoxylin and Eosin Staining of Sections	102
2.9 Immunostaining of Tissue Sections	103
2.10 Quantification of Staining	107
2.11 Statistics	108

Chapter 3 Heterogeneity in the response of ovarian tumours to cytotoxic drugs

3.1 Culture of Ovarian Biopsies	112
3.2 <i>In Vitro</i> Effects of Chemotherapy on Ovarian Specimens	116

Chapter 4 Heterogeneity in the response of cervical and endometrial tumours to cytotoxic drugs

4.1 Culture of Cervical and Endometrial Specimens	151
4.2 <i>In Vitro</i> Effect of Chemotherapy on Cervical Cancers	154
4.3 <i>In Vitro</i> Effect of Chemotherapy on Endometrial Cancers	168

Chapter 5 *In Vitro In Vivo* Correlations

5.1 <i>In Vitro In Vivo</i> Correlations	191
5.2 Tumour Grade and survival	204
5.3 Tumour Stage and survival	205
5.4 Previous Chemotherapy and survival	207
5.5 <i>In vitro</i> response to chemotherapy and survival	208
5.6 <i>In vivo</i> response to chemotherapy and survival	210
5.7 Patient Age and survival	211
5.8 Multivariate Analysis	213

Chapter 6 Potential prognostic markers and predictors of response to chemotherapy in ovarian cancers

6.1 CD31 Results	216
6.2 VEGF Results	233
6.3 MDR Results	240
6.4 Correlation between CD31, VEGF and MDR	246
6.5 Tumour Details and Patient Outcome	247
6.6 Multivariate Analysis	258

Chapter 7 Discussion

7.1 Heterogeneity in response to chemotherapy in ovarian cancers	261
7.2 Heterogeneity in response to chemotherapy in cervical and endometrial cancers	267
7.3 <i>In vitro- in vivo</i> correlations	271
7.4 Potential prognostic factors and predictors of response to chemotherapy in ovarian cancer	277

Chapter 8 Conclusions and recommendations for further research

8.1 Conclusions	292
8.2 Recommendations for future research	295

References	296
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List of Figures

Figure 1.1. Outline of the Cell Cycle 1

Figure 1.2. Outline of the female reproductive system 18

Figure 1.3. Gross appearance of an ovarian papillary cystadenocarcinoma 23

Figure 1.4. Microscopic view of a moderately differentiated serous papillary
cystadenocarcinoma. 26

Figure 1.5. Gross appearance of a cervical squamous cell carcinoma 34

Figure 1.6. Management of abnormal cervical cytology 37

Figure 1.7. Microscopic view of a well differentiated squamous cell carcinoma of the
cervix..... 40

Figure 1.8. Gross appearance of an endometrial adenocarcinoma. 49

Figure 1.9. Microscopic view of a poorly differentiated endometrial
Adenocarcinoma. 51

Figure 2.1 Typical Plate Set-up..... 100

Figure 2.2 Typical Response Curve.....101

Figure 3.0 Explant culture of ovarian adenocarcinoma 113

Figure 3.1 Effect of chemotherapy on a Stage I Grade 1 Adenocarcinoma of the
Ovary.....116

Figure 3.2 Effect of Cisplatin on Stage I Grade 2 Ovarian Adenocarcinomas.....117

Figure 3.3 Effect of Paclitaxel on Stage I Grade 2 Ovarian Adenocarcinomas.....118

Figure 3.4 Effect of Cisplatin & Paclitaxel on Stage I Grade 2 Ovarian
Adenocarcinomas.....118

Figure 3.5 Effect of Topotecan on Stage I Grade 2 Ovarian Adenocarcinomas.....119

Figure 3.6 Effect of Cisplatin on Stage III Grade 2 Ovarian Adenocarcinomas.....120

Figure 3.6(a) Effect of Cisplatin on Stage III Grade 2 Ovarian Adenocarcinomas.120

Figure 3.7 Effect of Paclitaxel on Stage III Grade 2 Ovarian Adenocarcinomas...121

Figure 3.7(a)Effect of Paclitaxel on Stage III Grade 2 Ovarian Adenocarcinomas.121

Figure 3.8 Effect of Cisplatin & Paclitaxel on Stage III Grade 2 Ovarian
Adenocarcinomas.....122

Figure 3.8(a) Effect of Cisplatin & Paclitaxel on Stage III Grade 2 Ovarian
Adenocarcinomas.....122

Figure 3.9 Effect of Topotecan on Stage III Grade 2 Ovarian Adenocarcinomas...123

Figure 3.9(a) Effect of Topotecan on Stage III Grade 2 Ovarian
Adenocarcinomas.....123

Figure 3.10 Effect of Cisplatin on Stage III Grade 3 Ovarian Adenocarcinomas...124

Figure 3.10(a) Effect of Cisplatin on Stage III Grade 3 Ovarian
Adenocarcinomas.....125

Figure 3.11 Effect of Paclitaxel on Stage III Grade 3 Ovarian Adenocarcinomas..125

Figure 3.12 Effect of Cisplatin & Paclitaxel on Stage III Grade 3 Ovarian
Adenocarcinomas.....126

Figure 3.13 Effect of Topotecan on Stage III Grade 3 Ovarian Adenocarcinomas.126

Figure 3.14 Effect of Cisplatin on Stage IV Grade 2 Ovarian Adenocarcinomas...127

Figure 3.15 Effect of Paclitaxel on Stage IV Grade 2 Ovarian Adenocarcinomas..128

Figure 3.16 Effect of Cisplatin & Paclitaxel on Stage IV Grade 2 Ovarian
Adenocarcinomas.....128

Figure 3.17 Effect of Topotecan on Stage IV Grade 2 Ovarian Adenocarcinomas.129

Figure 3.18 Effect of Cisplatin on Stage IV Grade 3 Ovarian Adenocarcinomas...130

Figure 3.19 Effect of Paclitaxel on Stage IV Grade 3 Ovarian Adenocarcinomas..131

Figure 3.20 Effect of Cisplatin & Paclitaxel on Stage IV Grade 3 Ovarian Adenocarcinomas.....131

Figure 3.21 Effect of Topotecan on Stage IV Grade 3 Ovarian Adenocarcinomas.132

Figure 3.22 Effect of Cisplatin on Clear cell Adenocarcinomas of the Ovary.....133

Figure 3.23 Effect of Paclitaxel on Clear cell Adenocarcinomas of the Ovary.....134

Figure 3.24 Effect of Cisplatin & Paclitaxel on Clear cell Adenocarcinomas of the Ovary.....134

Figure 3.25 Effect of Topotecan on Clear cell Adenocarcinomas of the Ovary.....135

Figure 3.26 Effect of Chemotherapy on a Stage IB Mucinous Adenocarcinoma of the Ovary.....136

Figure 3.27 Effect of Chemotherapy on a Stage IIIB Grade 2 Mucinous Adenocarcinoma of the Ovary.....137

Figure 3.28 Effect of Chemotherapy on a Stage III Grade 3 Mucinous Adenocarcinoma of the Ovary.....138

Figure 3.29 Effect of Chemotherapy on a Stage IV Grade 3 Mucinous Adenocarcinoma of the Ovary.....139

Figure 3.30 Effect of Chemotherapy on a Grade 3 Adenocarcinoma of the Peritoneum.....140

Figure 3.31 Effect of Chemotherapy on a Stage I Grade 3 Endometrioid Adenocarcinoma of the Ovary.....141

Figure 3.32 Effect of Chemotherapy on a Stage II Grade 1 Endometrioid Adenocarcinoma of the Ovary.....142

Figure 3.33 Effect of Chemotherapy on a Stage II Transitional Cell Carcinoma of the Ovary.....143

Figure 3.34 Effect of Cisplatin on Mucinous Borderline Carcinomas of the Ovary.....144

Figure 3.35 Effect of Paclitaxel on Borderline Mucinous Carcinomas of the Ovary.....144

Figure 3.36 Effect of Cisplatin & Paclitaxel on Borderline Mucinous Tumours of the Ovary.....145

Figure 3.37 Effect of Topotecan on Borderline Mucinous Carcinomas of the Ovary.....146

Figure 3.38 Effect of Cisplatin on Borderline Serous Tumours of the Ovary.....147

Figure 3.39 Effect of Paclitaxel on Borderline Serous Tumours of the Ovary.....147

Figure 3.40 Effect of Cisplatin & Paclitaxel on Borderline Serous Carcinomas of the Ovary.....148

Figure 3.41 Effect of Topotecan on Borderline Serous Carcinomas of the Ovary..149

Figure 4.0 (a) Cervical culture showing fibroblast growth..... 153

Figure 4.0 (b) Endometrial culture *in vitro*..... 153

Figure 4.1 Effect of Cisplatin on Stage I Grade 2 Cervical Squamous carcinomas.154

Figure 4.2 Effect of Paclitaxel on Stage I Grade 2 Cervical Squamous carcinoma.155

Figure 4.3 Effect of Cisplatin & Paclitaxel on Stage I Grade 2 Cervical Squamous Carcinomas.....155

Figure 4.4 Effect of Fluorouracil on Stage I Grade 2 Cervical Squamous Carcinomas.....156

Figure 4.5 Effect of Cisplatin on Stage I Grade 3 Cervical Squamous Carcinomas.....157

Figure 4.6 Effect of Paclitaxel on Stage I Grade 3 Cervical Squamous Carcinomas.....157

Figure 4.7 Effect of Cisplatin & Paclitaxel on Stage I Grade 3 Cervical Squamous
Carcinomas.....158

Figure 4.8 Effect of Topotecan on Stage I Grade 3 Cervical Squamous
Carcinomas.....158

Figure 4.9 Effect of Cisplatin on Stage II Grade 2 Cervical Squamous
Carcinomas.....159

Figure 4.10 Effect of Paclitaxel on Stage II Grade 2 Cervical Squamous
Carcinomas.....160

Figure 4.11 Effect of 4-Hydroxycyclophosphamide on Stage II Grade 2 Cervical
Squamous Carcinomas.....160

Figure 4.12 Effect of Cisplatin on Stage II Grade 3 Cervical Squamous
Carcinoma.....161

Figure 4.13 Effect of Doxorubicin on Stage II Grade 3 Cervical Squamous
Carcinomas.....162

Figure 4.14 Effect of Cisplatin & Doxorubicin on Stage II Grade 3 Cervical
Squamous Carcinomas.....162

Figure 4.15 Effect of Chemotherapy on a Stage IV Grade 3 Cervical Squamous
Carcinoma.....163

Figure 4.16 Effect of Chemotherapy on a Stage I Grade 2 Adenocarcinoma of the
Cervix.....164

Figure 4.17 Effect of Chemotherapy on a Stage II Grade 2 Adenocarcinoma of the
Cervix.....165

Figure 4.18 Effect of Cisplatin on Adenosquamous Carcinomas of the Cervix.....166

Figure 4.19 Effect of Chemotherapy on a Grade 3 Recurrent Squamous Carcinoma
of the Cervix.....167

Figure 4.20 Effect of Chemotherapy on a Stage I Grade 1 Endometrioid
Adenocarcinoma of the Endometrium.....168

Figure 4.21 Effect of Cisplatin on Stage I Grade 2 Endometrioid
Adenocarcinomas.....169

Figure 4.22 Effect of Paclitaxel on Stage I Grade 2 Endometrioid
Adenocarcinomas.....169

Figure 4.23 Effect of Cisplatin & Paclitaxel on Stage I Grade 2 Endometrioid
Adenocarcinomas.....170

Figure 4.24 Effect of Topotecan on Stage I Grade 2 Endometrioid
Adenocarcinomas.....171

Figure 4.25 Effect of Cisplatin on Stage III Grade 2 Endometrial
Adenocarcinomas.....172

Figure 4.26 Effect of Paclitaxel on Stage III Grade 2 Endometrial
Adenocarcinomas.....172

Figure 4.27 Effect of Cisplatin & Paclitaxel on Stage III Grade 2 Endometrial
Adenocarcinomas.....173

Figure 4.28 Effect of Topotecan on Stage III Grade 2 Endometrial
Adenocarcinomas.....174

Figure 4.29 Effect of Cisplatin on Stage III Grade 3 Endometrial Adenosquamous
Carcinomas.....175

Figure 4.30 Effect of Paclitaxel on Stage III Grade 3 Endometrial Adenosquamous
Carcinomas.....175

Figure 4.31 Effect of Cisplatin & Paclitaxel on Stage III Grade 3 Endometrial
Adenosquamous Carcinomas.....176

Figure 4.32 Effect of Topotecan on Stage III Grade 3 Endometrial Adenosquamous
Carcinomas.....176

Figure 4.33 Effect of Chemotherapy on a Stage I Grade 1 Endometrial
Adenocarcinoma.....177

Figure 4.34 Effect of Cisplatin on Stage I Grade 2 Endometrial
Adenocarcinomas.....178

Figure 4.35 Effect of Paclitaxel on Stage I Grade 2 Endometrial
Adenocarcinomas.....179

Figure 4.36 Effect of Cisplatin & Paclitaxel on Stage I Grade 2 Endometrial
Adenocarcinomas.....179

Figure 4.37 Effect of Topotecan on Stage I Grade 2 Endometrial
Adenocarcinomas.....180

Figure 4.38 Effect of Chemotherapy on a Stage II Grade 3 Endometrial
Adenocarcinoma.....181

Figure 4.39 Effect of Chemotherapy on a Stage III Grade 3 Endometrial
Adenocarcinoma.....182

Figure 4.40 Effect of Cisplatin on a Stage IV Grade 2 Endometrial
Adenocarcinoma.....182

Figure 4.41 Effect of Chemotherapy on a Stage IV Grade 3 Endometrial
Adenocarcinoma.....183

Figure 4.42 Effect of Cisplatin on an Endometrial stromal sarcoma (1st Biopsy)...184

Figure 4.43 Effect of Chemotherapy on a Recurrent Endometrial stromal sarcoma
(2nd Biopsy).....185

Figure 4.44 Effect of Chemotherapy on an Endometrial stromal sarcoma.....185

Figure 4.45 Effect of Cisplatin on an Endometrial Adenosarcoma.....186

Figure 4.46 Effect of Cisplatin on Recurrent Endometrial Leiomyosarcomas.....	187
Figure 4.47 Effect of Paclitaxel on Recurrent Endometrial Leiomyosarcomas.....	187
Figure 4.48 Effect of Cisplatin & Paclitaxel on Recurrent Endometrial Leiomyosarcomas.....	188
Figure 4.49 Effect of Chemotherapy on a Stage III Mixed Mullerian Tumour of the Endometrium.....	189
Figure 5.1 PFS in relation to tumour grade.....	204
Figure 5.2 OS in relation to tumour grade.....	205
Figure 5.3 PFS in relation to tumour stage.....	206
Figure 5.4 OS in relation to tumour stage.....	206
Figure 5.5 PFS in relation to previous chemotherapy.....	207
Figure 5.6 OS in relation to previous chemotherapy.....	208
Figure 5.7 PFS in relation to <i>in vitro</i> response to chemotherapy.....	209
Figure 5.8 OS in relation to <i>in vitro</i> chemotherapy response.....	209
Figure 5.9 PFS in relation to <i>in vivo</i> chemotherapy response	210
Figure 5.10 OS in relation to <i>in vivo</i> chemotherapy response	211
Figure 5.11 PFS in relation to age category.....	212
Figure 5.12 OS in relation to age category.....	213
Figure 6.1 Positive Control for CD31 Expression	217
Figure 6.2 Negative Control for CD31 Staining.....	218
Figure 6.3 CD31 Staining in an Ovarian Adenocarcinoma	218
Figure 6.4 PFS in relation to CD31 expression	228
Figure 6.5 OS in relation to CD31 expression.....	231
Figure 6.6 Positive Control for VEGF Staining.....	233
Figure 6.7 Negative Control for VEGF Staining	234

Figure 6.8 VEGF Staining in an Ovarian Adenocarcinoma	234
Figure 6.9 PFS in relation to VEGF expression.....	237
Figure 6.10 OS in relation to VEGF expression.....	238
Figure 6.11 Positive Control for MDR Staining	240
Figure 6.12 Negative Control for MDR Staining.....	241
Figure 6.13 MDR Staining in an Ovarian Adenocarcinoma.....	241
Figure 6.14 PFS in relation to MDR expression.....	244
Figure 6.15 OS in relation to MDR	245
Figure 6.16 PFS in relation to Pathology.....	247
Figure 6.17 OS in relation to Pathology.....	248
Figure 6.18 PFS in relation to tumour grade.....	249
Figure 6.19 OS in relation to tumour grade.....	249
Figure 6.20 PFS in relation to tumour stage.....	250
Figure 6.21 OS in relation to FIGO stage.....	251
Figure 6.22 PFS in relation to previous chemotherapy.....	252
Figure 6.23 OS in relation to previous chemotherapy.....	252
Figure 6.24 PFS in relation to <i>in vitro</i> chemotherapy response	254
Figure 6.25 OS in relation to <i>in vitro</i> response to chemotherapy.....	254
Figure 6.26 PFS in relation to <i>in vivo</i> response to chemotherapy.....	255
Figure 6.27 OS in relation to <i>in vivo</i> response to chemotherapy.....	256
Figure 6.28 PFS in relation to patient age.....	257
Figure 6.29 OS in relation to patient age.....	257

List of Tables

Table 1.1 Chemotherapy drugs	13
Table 1.2. Histological classification of ovarian tumours according to FIGO	24
Table 1.3 Histopathologic grades (G) of ovarian cancers.....	26
Table 1.4 Staging of carcinoma of the ovary: FIGO nomenclature (Rio de Janeiro 1988).....	27
Table 1.5 Carcinoma of the ovary: Stage grouping for ovarian cancer	29
Table 1.6 Classification of tumours of the cervix	38
Table 1.7. Histological degree of differentiation	40
Table 1.8: Carcinoma of the cervix uteri: FIGO nomenclature (Montreal, 1994).....	41
Table 1.9: Carcinoma of the cervix uteri: stage grouping for cervical cancer.....	43
Table 1.10 Histological classification of endometrial neoplasms	50
Table 1.11 Histological grading of endometrial carcinomas.....	50
Table 1.12 FIGO surgical staging system for uterine corpus carcinoma (Rio de Janeiro, 1988).....	52
Table 1.13 Carcinoma of the corpus uteri: stage grouping for endometrial cancer....	53
Table 2.1 Ovarian specimens.....	83
Table 2.2 Cervical specimens	88
Table 2.3 Endometrial specimens	90
Table 2.4 Peak plasma concentration (PPC) of cytotoxic drugs.....	99
Table 3.1 Ovarian specimens with <i>in vitro</i> results.....	114
Table 4.1 Cervical specimens with <i>in vitro</i> results	151
Table 4.2 Endometrial specimens with <i>in vitro</i> results.....	152
Table 5.1 <i>In vitro</i> and <i>in vivo</i> results and patient outcome	193

Table 5.2 *In vitro-In vivo* correlations201

Table 5.3 Log rank and quartiles for progression free survival202

Table 5.4 Log rank and quartiles for overall survival.....203

Table 5.5 Cox regression analysis for PFS214

Table 5.6 Cox regression analysis for OS.....214

Table 6.1 Specimens stained for CD31, VEGF and MDR216

Table 6.2 Staining scores and follow-up.....219

Table 6.3 Log rank and quartiles for progression free survival229

Table 6.4 Log rank and quartiles for overall survival.....230

Table 6.5 Cox regression analysis for PFS258

Table 6.6 Cox regression analysis for OS.....259

CHAPTER 1

INTRODUCTION

1.1 Cancer

There are over two hundred types of cells in the human body, which are assembled into a variety of tissue types. The four main tissue groups include the general supporting tissues, collectively known as the mesenchyme; the tissue-specific cells – epithelium; the “defence” cells – the reticuloendothelial system; and the nervous system. Each tissue has its own specific cells (epithelial cells), which maintain the structure and function of the individual tissue. The specific cells are grouped in organs, which have a standard pattern. In normal development and growth there is a precise mechanism that allows individual organs to reach a fixed size and if a tissue is injured the surviving cells in most organs begin to grow and replace the damaged cells. The method by which cells increase in number is similar in all somatic cells, and involves the growth of all cell components, leading eventually to division of the cell into two new cells. Cell division is fundamental in the body to replace cells that are lost by wear and tear or by programmed cell death. Four stages are usually recognised in the cell cycle: G_1 -S- G_2 - M^1 (Fig. 1.1).

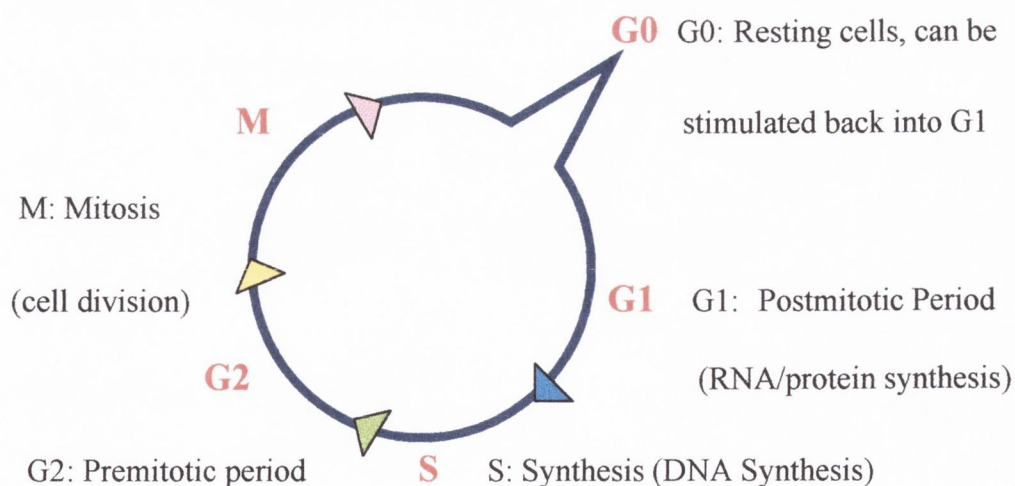


Figure 1.1. Outline of the Cell Cycle

The most dramatic stage of the cell cycle is the M phase. M is the stage of mitosis in which the nuclear envelope breaks down, the contents of the nucleus condense into visible chromosomes, and the cell's microtubules reorganise to form the mitotic spindle that will eventually separate the chromosomes. As mitosis proceeds, the cell seems to pause briefly in a state called metaphase, in which the chromosomes are aligned on the mitotic spindle, ready for segregation. The separation of the duplicated chromosomes marks the beginning of anaphase, when the chromosomes move to the poles of the spindle and decondense to form intact nuclei. The cell is then pinched in two by a process called cytokinesis, which is viewed as the end of the M phase.

The time that elapses between one M phase and the next is known as interphase. Replication of the nuclear DNA occupies only a portion of interphase known as the S (synthesis) phase of the cell cycle. The interval between the completion of mitosis and the beginning of DNA synthesis is called the G_1 phase (G =gap). This phase enables the cell to grow and to produce all the necessary proteins for DNA synthesis. G_2 is a second gap period between the end of DNA synthesis and the beginning of mitosis. This allows the cell to ensure that DNA replication is complete; the cell undergoes growth and protein synthesis priming it to be able to divide. Cells in G_1 , if they are not committed to cell replication, can pause in their progress around the cycle and enter a specialised resting state, often called G_0 , where they can remain indefinitely before resuming proliferation.

The cell cycle is a highly regulated process with two major control points: one towards the end of G_1 , known as the restriction point, and the other at the initiation of mitosis. Feedback from downstream processes and signals from the environment can cause the cell to bypass certain specific checkpoints.

An abnormality in a cell's internal regulatory mechanisms results in uncontrolled growth and reproduction of the cell eventually forming a new growth, which is known as a tumour or neoplasia. Willis, 1952² described cancer as an “abnormal mass of tissue, the growth of which exceeds and is uncoordinated with that of the normal tissues and persists in the same excessive manner after cessation of the stimuli which evoked the change”.

1.2 Malignant versus Benign

A tumour can be classified as malignant or benign. In both cases, there is an abnormal aggregation and proliferation of cells. In the case of a malignant tumour, these cells behave more aggressively, acquiring properties of increased invasiveness. Ultimately, the tumour cells may even gain the ability to break away from the microscopic environment in which they originated, spread to another area of the body (with a very different environment, not normally conducive to their growth) and continue their rapid growth and division in this new location, in a process termed metastasis. Once malignant cells have metastasised, achieving cure is more difficult.

Benign tumours do not usually invade or metastasise, although they grow and increase in size. Depending on their location, they can be just as life threatening as malignant lesions e.g. a benign tumour in the brain can grow and occupy space within the skull, leading to increased pressure on the brain.

1.3 Carcinogenesis

Carcinogenesis is a multistage process. Initiation is the primary and essential step and is induced by a carcinogen (cancer causing agent). The carcinogen is thought to damage or destroy specific genes probably in the stem cell population of the tissue involved. Initiated cells remain latent and are harmless until acted upon by promoting agents. Promoting agents are not carcinogenic themselves but they do induce initiated cells to divide. Many agents will induce cell division but only promoters will induce tumour development so that although cell growth is necessary for tumour development there must be other factors involved. It is postulated that promoting agents may interfere with the process of differentiation that normally takes place when cells move from the dividing stem cell population into functioning and usually non-dividing cells. Even though these promoting stimuli are acting on the cells they may still be sensitive to the normal growth-inhibiting factor in the body so that the final outcome depends on the balance between the factors and the extent of the changes in the initiated cells. Tumour progression is the development by a tumour of changes in one or more characters in its constituent cells and is usually towards greater malignancy.

Tumour dissemination is a complex process where the eventual outcome depends on the result of a number of interactions between tumour cells and host cells. Following tumour growth and development there are five major steps involved in metastasis:

1. Invasion and infiltration of surrounding normal host tissue with penetration of small lymphatic or vascular channels
2. Release of neoplastic cells into the circulation

3. Survival in the circulation
4. Arrest in capillary beds of distant organs
5. Penetration of the lymphatic or blood vessel walls and growth of the disseminated tumour cells

1.4 Tumour Angiogenesis

Growth and metastatic dissemination of solid tumours requires vascular support for nutritive supply and access ^{3 4}. For a tumour to get much larger than 1mm³ a process of angiogenesis or neovascularisation must occur ^{5 6}. Angiogenesis refers to the formation of new capillary blood vessels from pre-existing microvessels by remodeling of the primary plexus. The angiogenic process begins with endothelial cell proliferation and migration into the primary vascular network, and leads to vascularisation of previously avascular tissues and organs as well as to growth and remodeling of the initially homogenous capillary plexus to form a new microcirculation. Angiogenesis is a developmental process that occurs during embryogenesis and is down regulated in the healthy adult ⁷. In adults, angiogenesis is linked to physiological conditions such as ovarian and endometrial alterations during the menstrual cycle and to pathological conditions such as wound healing and tumour growth^{8 9}. The onset of this vascular phase marks a period of more rapid growth, local invasion and ultimately metastasis of solid tumours^{10 11 12 13}.

1.5 Cancer Grade

Each cell in the body derives from one original cell. As this cell reproduces into more cells and forms a fetus, they differentiate into different tissue types. Differentiated cells "stick together" in well-defined ways within the body, to form the various tissues. A series of changes takes place after a tissue cell is initiated but the rate at which this occurs depends on changes in the host. Most physical and chemical cancer inducing agents are very highly reactive and when they react with DNA in the affected cell they usually damage many other sites as well as the relatively few that are thought to control neoplastic transformation. Thus the same agent may produce tumours in a given organ that differ greatly from each other, depending on the specific genes that have been altered or lost. If the genes responsible for normal structure are more severely damaged, the resulting tumour cells have fewer normal properties. At the other extreme the cells may have lost almost all the normal properties of the cell from which they have arisen. The loss of normal characteristics is known as dedifferentiation or anaplasia. The pathologist can grade tumours by making an approximate assessment of the degree of structural dedifferentiation by examining sections of tumours under the microscope. The more differentiated a cancer cell looks, the closer it resembles cells belonging to its organ of origin. The more undifferentiated a cell is, the less "normal" it looks under a microscope. In some cancers, grade can affect the cancer stage and/or impact on treatment decisions.

1.6 Staging of Cancer

Once a diagnosis of cancer is made, it is important that the stage of disease be assessed. Stage generally refers to the degree to which the cancer has spread beyond its original location. Lower stages of cancer (stages I and II) are generally more confined to their site or region of origin than more advanced stages (III or IV).

The concept of a classification scheme that would encompass all aspects of cancer in terms of primary tumour (T), regional lymph nodes (N), and distant metastasis (M) was first introduced by the Union Internationale Centre le Cancer (UICC), in 1958 for worldwide use.

Different cancer types are staged in different ways, according to a complex series of rules. While there are subtle differences in the staging rules for different cancer types, a physician generally needs to look at three things:

1. **"T" stage (tumour stage)**, defined according to the size of the tumour itself;
2. **"N" stage (nodal stage)**, defined according to the number of lymph nodes which contain cancer; and
3. **"M" stage (stage of metastatic disease)**, defined according to the presence (or absence) of cancer that has spread into other organs or parts of the body.

The T, N, and M stages have many nuances within them, and each is subdivided (T1, T2, T3, etc.) Different combinations of T, N, and M combine under the staging rules to determine whether a patient is stage I, II, III, or IV. In some cancers, there are even additional stages to account for unique situations (Stage V, for instance). It should

also be noted that some cancers are staged with different staging systems, but the TNM system is the most common.

Ultimately, the goal of staging is threefold:

1. To help select the most appropriate therapy for the patient;
 2. To help predict a patient's prognosis; and
 3. To help future patients by assessing the response to therapy and tracking that along with the treatment responses of others with the same stage of cancer.
- This is necessary so that physicians can determine which treatments work best for which patients, so that less effective treatments can be discarded.

Staging, while important for most cancers, does not tell the whole story. Other factors such as tumour biology, the patient's overall medical condition, and the skills and resources available to the health care team can all impact on the ultimate outcome.

1.7 Correction of Abnormalities

The cell has many inbuilt mechanisms for correcting cellular defects or for initiating apoptosis in abnormal cells. Ideally reversing the abnormality should treat a cancerous cell, however, despite significant advances in this area our knowledge is insufficient to effectively treat cancerous cells in this way. The problem is exacerbated by the fact that cells including non-cancerous ones naturally acquire mutations as they reproduce. Rapidly reproducing groups of cells mutate at an even higher rate. The end result is that a single tumour may be the result of an accumulation of defects and even if we understood the mechanism for proliferative

tendency within a tumour not all cells in a particular tumour are the same. For this reason the most effective treatments involve the killing or removal of tumour cells.

1.8 Cancer Treatment

The treatment of cancer is a very complex subject and not all parts of it apply to all types of cancer.

Surgery

Surgery is often the first step in the treatment of cancer. The objective of surgery varies. Sometimes it is used to remove as much of the evident tumour as possible, or at least to "debulk" it. Depending on the cancer type and location, surgery may also provide some symptomatic relief to the patient. Not all tumours are amenable to surgery. Some may be located in parts of the body that make them impossible to remove completely e.g. tumours in the brainstem, a tumour which has grown in and around a major blood vessel. In these cases, the extent of surgery is limited due to the high risk associated with tumour removal.

Radiation Therapy

Radiation therapy is another commonly used weapon in the fight against cancer. Radiation kills cancer by damaging the DNA within the tumour cells. The radiation is "applied" in two possible ways. The first, and most common, involves pointing a

beam of radiation at the patient in a highly precise manner, focusing on the tumour. Another technique for delivering radiation to cancer cells is to place radioactive implants directly in a tumour or body cavity. This is called internal radiotherapy. In this treatment, the radiation dose is concentrated in a small area, and the patient stays in the hospital for a few days.

Chemotherapy

Chemotherapy is another common form of cancer treatment. Essentially, it involves the use of medications, usually given by mouth or injection, which specifically attack rapidly dividing cells, such as those found in a tumour, throughout the body. This makes chemotherapy useful in treating cancers that have already metastasised, as well as tumours that have a high chance of spreading through the blood system but are not evident beyond the primary tumour. Unfortunately, other cells in the human body that also normally divide rapidly, such as the lining of the stomach and hair, are also affected by chemotherapy. For this reason, some, though not all, chemotherapy agents induce nausea or hair loss. Chemotherapy treatment often involves the use of more than one agent at a time. There are many different chemotherapy combinations, each used for different types and stages of cancers.

History of Chemotherapy

Systemic treatment of cancer had its roots in the work of Paul Ehrlich, who coined the word chemotherapy. Ehrlich's use of rodent models of infectious diseases to develop antibiotics led George Clowes, at Roswell Park Memorial Institute in Buffalo, NY, in the early 1900s, to develop inbred rodent lines that could carry transplanted tumours that could be used to screen potential anticancer drugs and provide the foundation for mass screenings¹⁴. The possible role in treating illness was discovered when the bone marrow suppressive effect of nitrogen mustard was noted following an explosion during World War II^{15 16}. The first anticancer drugs were introduced to clinical practice in 1943 for the treatment of haematopoietic neoplasms such as Hodgkin's disease and lymphocytic lymphomas^{14 17}. Since that time, the search for drugs effective against cancer has continued, and the goal of treatment with chemotherapy has evolved from relief of symptoms to cure.

There are four ways chemotherapy is generally used¹⁸:

1. As an **induction** treatment for advanced disease for which no alternative treatment exists¹⁹
2. As an adjunct to local methods of treatment. **Adjuvant** chemotherapy denotes the use of systemic treatment after the primary tumour has been treated by an alternative method, such as surgery and radiotherapy. The selection of an adjuvant treatment program for a particular patient is usually based on response rates in separate groups of patients with advanced cancers of the same histological type.

3. As the primary treatment for patients who present with localised cancer.

Primary (neoadjuvant) is used for localised cancer for which there is an alternative but less than completely effective local treatment^{20 21}.

4. By direct installation into body cavities or by site-directed perfusion of specific regions of the body most affected by the cancer.

Principles of Treatment

An understanding of the normal cell cycle and the behaviour of malignant or cancerous cells are necessary in order to comprehend how chemotherapy destroys cancer cells. Almost all chemotherapy agents currently available kill cancer cells by affecting DNA synthesis or function, a process that occurs through the cell cycle. Each drug varies in the way this occurs within the cell cycle.

Chemotherapeutic Agents

The major categories of chemotherapy agents are alkylating agents, antimetabolites, plant alkaloids, antitumour antibiotics and steroid hormones. Each drug is categorised according to their effect on the cell cycle and cell chemistry. Examples of drugs from each category, which are used to treat gynaecological cancers, can be seen in Table 1.1.

Table 1.1 Chemotherapy drugs

Chemotherapy Class	Drug
Alkylating agents	Cyclophosphamide, Cisplatin, Carboplatin, Ifosfamide, Mechlorethamine, Melphalan, Chlorambucil, Thiotepa, Nitrosoureas, Dacarbazine
Antimetabolites	Fluorouracil, Cytarabine, Mercaptopurine, Hydroxurea, Methotrexate, 5-Azacytidine, Thioguanine
Antitumour Antibiotics	Mitomycin, Doxorubicin, Dactinomycin, Daunorubicin, Pliamycin, Bleomycin
Vinca Alkaloids	Vincristine, Vinblastine
Hormones	Oestrogens, Progestins, Medroxyprogesterone, Tamoxifen, Fluxymesterone, Androgens, Megestrol, Dexamethasone.
Other	Procarbazine, Mitotane, Streptozotocin, asparaginase, Altretamine, Paclitaxel, Topotecan.

Alkylating agents kill cells by directly attacking DNA. These drugs all possess alkyl groups, which replace hydrogen groups of a variety of cellular molecules and compounds such as nucleic acids, proteins and amino acids. They form cross-links of guanine bases of adjacent DNA strands, which in turn interfere with the separation of chromosomal pairs during the mitotic phase. Alkylation also affects DNA at the level of transcription and RNA at the level of translation. Alkylating agents may be used in the treatment of chronic leukaemias, Hodgkin's disease, lymphomas, and certain carcinomas of the lung, breast, prostate and ovary. Nitrosoureas act similarly to alkylating agents and are usually classed with them. They also inhibit changes necessary for DNA repair and thus the cells undergo apoptosis. These agents cross the blood-brain barrier and are therefore used to treat brain tumours, lymphomas, multiple myeloma and malignant melanoma²².

Antimetabolites interfere with DNA and RNA synthesis to prohibit cell replication. They do this either by deceiving cells into including them in certain metabolic passages necessary for the synthesis of RNA or DNA, so that a false genetic message is transmitted and the cell cannot be replicated; or, by blocking enzymes essential for nucleic acid synthesis²³. All drugs in this category affect the cell during the synthesis ("S") phase of the cell cycle. Antimetabolites may be used in the treatment of acute and chronic leukaemias, choriocarcinoma, and some tumours of the gastrointestinal tract, breast and ovary.

Antitumour antibiotics are a diverse group of compounds. In general, they act by forming complexes with DNA, thus inhibiting transcription and translation²⁴. These agents are widely used in the treatment of a variety of cancers.

Plant (vinca) alkaloids are anti-tumour agents derived from plants. These drugs act specifically in the metaphase (“M”) phase of the cell cycle by blocking cell division during mitosis. They bind to microtubular proteins, which are a vital part of the contractile protein in the mitotic spindle formed at the time of cell division and thereby block cell division by causing mitotic arrest²³. They are commonly used in the treatment of acute lymphoblastic leukaemia, Hodgkin's and non-Hodgkin's lymphomas, neuroblastomas, Wilms' tumour, and cancers of the lung, breast and testes.

Steroid hormones are useful in treating some types of tumours. Once they are inside the cell they bind to their receptor and penetrate the nucleus and bind to chromatin. This alters the patterns of gene transcription and thereby the newly synthesised messenger RNA¹. Eventually these messenger RNAs are translated into new proteins responsible for hormonally induced antitumour effects by modulating the activity of transcription factors. Steroid-induced effects therefore depend on the presence of steroid receptors within the cell. This class includes adrenocorticosteroids, oestrogens, antioestrogens, progesterones, and androgens.

In addition, two antineoplastic drugs exist with mechanisms of action, which do not permit inclusion on the above broad categorisation. Paclitaxel and Topotecan are commonly used in the treatment of gynaecological malignancies.

Paclitaxel

Paclitaxel (Taxol[®]) was first isolated from the bark of the Western yew tree in 1971²⁵. Paclitaxel acts as a promoter of tubulin polymerisation and stabilises microtubules to depolymerisation, unlike the vinca alkaloids, which induce microtubule disassembly^{26 27}. Paclitaxel alters the normal equilibrium between the tubulin dimers and microtubules and therefore disrupts cell division, i.e. stabilisation of the microtubules interferes with the G2 and M phases of the cell cycle and those cellular activities involving microtubules²⁸. Following its introduction into clinical trials, the drug was approved for treatment of cisplatin-refractory ovarian cancer in 1992^{29 30}.

Topotecan

Topotecan (Hycamtin[®]) is the first substance in a new group of cytotoxic agents, the topoisomerase I (topo I) inhibitors, that has been authorised for clinical use. Its probable mechanism of action is to stabilise the complex of topo I and cleaved DNA formed during the replication, ultimately leading to irreversible DNA double-strand breaks³¹. Topotecan is indicated for metastatic carcinoma of the ovary after the failure of initial or subsequent chemotherapy³².

Combination Chemotherapy

Combination Chemotherapy was first used in 1952 by Skipper *et al.*³³ to treat leukaemia. Following this an alkylating agent and an antimetabolite were used to treat ovarian cancer in 1962³⁴. Combination therapy combines agents that differ in both the way they act and their side effects. This is done to achieve maximum tumour effect with minimal side effects. Because tumour cells have different biological characteristics (heterogeneity), combining drugs may effectively eliminate cancer cells' resistance to a single agent. Drugs used in combination must fulfil the following:

- Have activity against the cancer being managed
- Should not share the same organ toxicities
- Optimal dose schedule should be possible for each drug
- Toxicity of the combination should allow frequent cycles
- Synergistic combinations are preferred over those possessing additive effects

Such treatment is based on a firm theoretical rationale that grew out of studies of cellular kinetics, biochemical drug action, drug interactions, drug resistance, and tumour heterogeneity³⁵.

1.9 Gynaecological Cancer

Gynaecological cancers are malignancies of the female reproductive system (Fig. 1.2) and include cervical cancer, ovarian cancer and endometrial cancer, all of which are separate diseases. Malignant tumours of the ovary, cervix and endometrium account for about 20% of cancers in women and for a large proportion of deaths in the female³⁶.

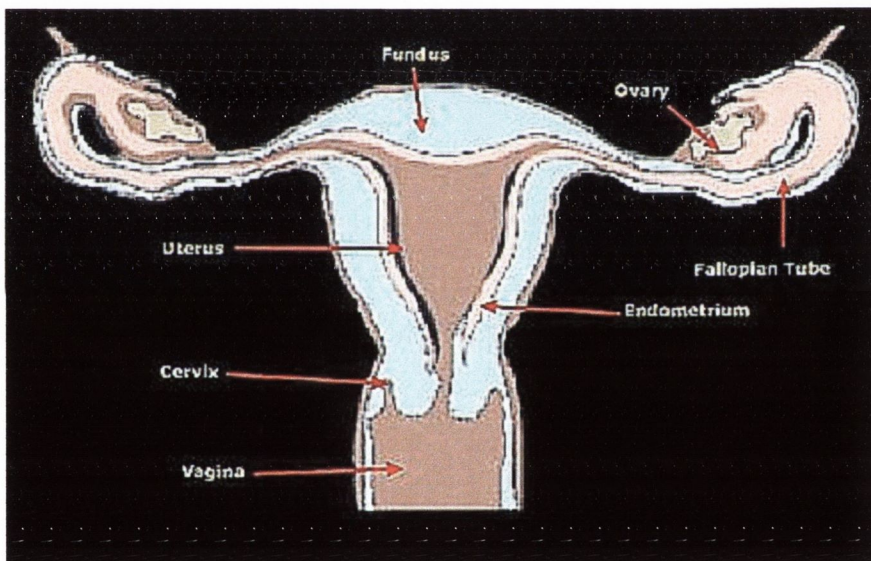


Figure 1.2. Diagram of the female reproductive system

1.9.1 Ovarian cancer

No organ in the human body is the site of origin or metastasis for a greater range of tumours than the ovary. The ovary contains a variety of cell types, including some which, given appropriate stimuli, can develop into any tissue in the body. This mixture of cells is subjected throughout reproductive life to repeat cycles of follicle growth and rupture followed by corpus luteum growth and regression. The vasculature and anatomical position of the ovaries predispose them to metastasis from the reproductive organs and gastrointestinal cancer.

Epidemiology

Ovarian cancer is one of the most common cancers in the female and the leading cause of death from gynaecological malignancy in the western world³⁷. About 190,000 cases of ovarian cancer occur worldwide each year³⁸. Ovarian cancer accounts for 3% of female cancers in Ireland with about 300 new cases each year³⁹. Mortality rates are only slightly lower than incidence rates and are a reflection of the poor prognosis. It has long been recognised that ovarian cancer is more common in the industrialised world, Japan being an exception⁴⁰.

Although there is a better understanding of the various histological subtypes of epithelial ovarian cancer, their frequency, distributions and behaviour, progress has been slow in determining precursor lesions, screening tests, early warning signs, etc. for this malignancy. This disease does not present with any consistent early warning signs or symptoms so that the vast majority of patients are diagnosed with stage III or IV disease⁴¹.

Peak age group at presentation is 50-70 years. The overall survival remains low at 28% to 35%^{42 43}. Most of the survivors come from stages I and II with only 10% surviving with advanced disease⁴⁴.

Aetiology

The aetiology for ovarian cancer remains obscure. The risk for the disease appears to increase with age and is approximately twice as common in nulliparous as compared to parous women. An increased risk has also been suggested for women who have a late age at first birth, early menarche and late menopause; some protection is afforded by higher parity. This has led to the concept of 'incessant ovulation' as put forth by Fathalla⁴⁵ in the 1970s as a basic mechanism to explain these observations. It is suggested that repeated ovulations produce tears on the surface epithelium and that the ultimate repair and regeneration process may subject the cells to influences that ultimately lead to incorporation of surface epithelium into the subsurface areas of the ovary with eventual transformation into malignant epithelium.

Of importance is the fact that several studies^{46 47 48 49} have indicated that oral contraceptive use is protective against ovarian cancer with the incidence being reduced by approximately 40% in continuous users and to an even greater extent in individuals who have been long-term users of these medications. Thus, on a population scale, the widespread use of combined oral contraceptive medication has probably been the major determinant of the recent favourable decrease of ovarian cancer rates noted in some western countries⁵⁰.

Five to ten percent of all ovarian cancers are genetically determined. When two first-degree relatives have had ovarian cancer before the age of 50, a woman has a lifetime

risk of 1:3 of developing ovarian cancer⁵¹. The strongest risk factors currently known for inherited predisposition to breast and ovarian cancer are mutations in BRCA1 and BRCA2 genes^{52 53 54}. BRCA1 gene has been localised to chromosome 17q⁵⁵, and BRCA2 to chromosome 13q12-13⁵⁶. Sporadic ovarian tumours are the end result of a complex pathway involving multiple oncogenes and tumour suppressor genes, including HER2/neu, K-ras, p53, BRCA1 and additional tumour suppressor genes on chromosome 17⁵⁷.

Other risk factors include diet⁵⁸, environmental factors, talc^{59 60} and ionising radiation⁶¹.

Diagnosis

Because ovarian cancer is often asymptomatic in its early stages, 70% or more patients present with widespread (stage III or IV) disease at the time of diagnosis. Not until the ovarian mass begins to encroach on other viscera or there is intra-abdominal spread do symptoms occur. At that time, patients most commonly report vague abdominal pain, abdominal swelling, dyspepsia, urinary frequency, constipation, and weight change. Pelvic discomfort, low back pain, and vaginal bleeding may also occur. The lack of significant symptomatology in the early stages of ovarian cancer leads to a low survival rate. During the past ten years studies of possible screening tools for detection in asymptomatic postmenopausal women have been investigated using the tumour marker CA-125, pelvic ultrasound and bimanual pelvic examinations. Of the three, only CA-125 has proved useful⁶².

Histopathology of ovarian neoplasms

Over 90% of ovarian cancers arise from the epithelial monolayer on the ovarian surface or in its inclusion cysts⁶³. Malignant serous cystadenocarcinomas are the commonest (Fig. 1.3) and account for 40-50% of malignant ovarian neoplasms⁶⁴. The histological typing of surface epithelial ovarian tumours is complex and difficult due to their diverse morphology and plethora of cell types; therefore, their classification has engendered considerable controversy over the past few decades⁶⁵. The task forces of the Federation of International Gynecology and Obstetrics (FIGO) endorse the histological typing of ovarian tumours as presented by the World Health Organisation⁶⁶, and recommend that all ovarian epithelial tumours be subdivided according to a simplified version. The types of tumours classified are as follows: serous, mucinous, endometrioid, clear cell (meso-nephroid), undifferentiated, and unclassified. Table 1.2 shows the FIGO classification. Histological grading of ovarian cancers is shown in Table 1.3. The FIGO staging for primary carcinoma of the ovary is shown in Table 1.4. Table 1.5 shows the UICC staging of ovarian cancer.



Figure 1.3. Gross appearance of an ovarian papillary cystadenocarcinoma mostly composed of solid tissue and has invaded outside of the ovary, with papillations seen over the surface.

Table 1.2. Histological classification of ovarian tumours according to FIGO

- **Serous tumours**

- Benign serous cystadenomas

- Of borderline malignancy: serous cystadenomas with proliferating activity of the epithelial cells and nuclear abnormalities, but with no infiltrative destructive growth (carcinomas of low potential malignancy)

- Serous cystadenocarcinomas

- **Mucinous tumours**

- Benign mucinous cystadenomas

- Of borderline malignancy: mucinous cystadenomas with proliferating activity of the epithelial cells and nuclear abnormalities, but with no infiltrative destructive growth (carcinomas of low potential malignancy)

- Mucinous cystadenocarcinomas

- **Endometrioid tumours**

- Benign endometrioid cystadenomas

- Endometrioid tumours with proliferating activity of the epithelial cells and nuclear abnormalities, but with no infiltrative destructive growth (carcinomas of low potential malignancy)

- Endometrioid adenocarcinomas

- **Clear cell tumours**

- Benign clear cell tumours
- Clear cell tumours with proliferating activity of the epithelial cells and nuclear abnormalities, but with no infiltrative destructive growth (low potential malignancy)
- Clear cell cystadenocarcinomas

- **Brenner tumours**

- Benign Brenner
- Borderline malignancy
- Malignant

- **Transitional cell tumours**

- **Undifferentiated carcinomas**

A malignant tumour of epithelial structure that is too poorly differentiated to be placed in any other group.

- **Mixed epithelial tumours**

these tumours are composed of two or more of the five major cell types of common epithelial tumours (types should be specified).

- Cases with intraperitoneal carcinoma in which the ovaries appear to be incidentally involved and not the primary origin should be labelled as extra-ovarian peritoneal carcinoma.

Table 1.2. Histological classification of ovarian tumours according to FIGO

- **Serous tumours**

- Benign serous cystadenomas

- Of borderline malignancy: serous cystadenomas with proliferating activity of the epithelial cells and nuclear abnormalities, but with no infiltrative destructive growth (carcinomas of low potential malignancy)

- Serous cystadenocarcinomas

- **Mucinous tumours**

- Benign mucinous cystadenomas

- Of borderline malignancy: mucinous cystadenomas with proliferating activity of the epithelial cells and nuclear abnormalities, but with no infiltrative destructive growth (carcinomas of low potential malignancy)

- Mucinous cystadenocarcinomas

- **Endometrioid tumours**

- Benign endometrioid cystadenomas

- Endometrioid tumours with proliferating activity of the epithelial cells and nuclear abnormalities, but with no infiltrative destructive growth (carcinomas of low potential malignancy)

- Endometrioid adenocarcinomas

- **Clear cell tumours**

- Benign clear cell tumours
- Clear cell tumours with proliferating activity of the epithelial cells and nuclear abnormalities, but with no infiltrative destructive growth (low potential malignancy)
- Clear cell cystadenocarcinomas

- **Brenner tumours**

- Benign Brenner
- Borderline malignancy
- Malignant

- **Transitional cell tumours**

- **Undifferentiated carcinomas**

A malignant tumour of epithelial structure that is too poorly differentiated to be placed in any other group.

- **Mixed epithelial tumours**

these tumours are composed of two or more of the five major cell types of common epithelial tumours (types should be specified).

- Cases with intraperitoneal carcinoma in which the ovaries appear to be incidentally involved and not the primary origin should be labelled as extra-ovarian peritoneal carcinoma.

Table 1.3 Histopathologic grades (G) of ovarian cancers

Gx - Grade cannot be assessed

G1 - Well differentiated

G2 - Moderately differentiated e.g. Fig. 1.4

G3 - Poorly

G4 - Undifferentiated

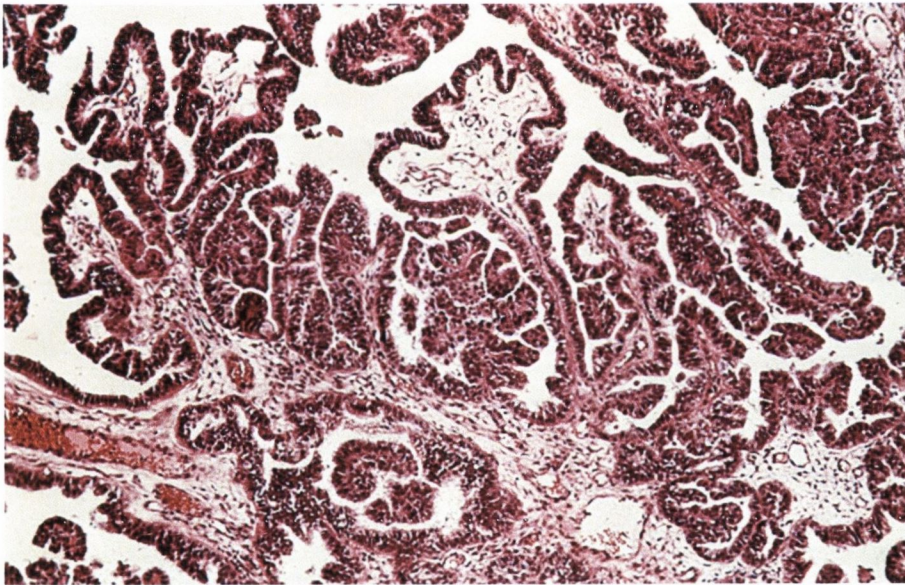


Figure 1.4. Microscopic view of a moderately differentiated serous papillary cystadenocarcinoma.

Table 1.4 Staging of Carcinoma of the ovary: FIGO nomenclature
(Rio de Janeiro 1988)

Stage I	Growth limited to the ovaries
IA	Growth limited to one ovary; no ascites present containing malignant cells. No tumour on the external surface; capsule intact
IB	Growth limited to both ovaries; no ascites present containing malignant cells. No tumour on the external surfaces; capsules intact
IC ^a	Tumour either Stage IA or IB, but with tumour on surface of one or both ovaries, or with capsule ruptured, or with ascites present containing malignant cells, or with positive peritoneal washings
Stage II	Growth involving one or both ovaries with pelvic extension
IIA	Extension and/or metastases to the uterus and/or tubes
IIB	Extension to other pelvic tissues
IIC ^a	Tumour either Stage IIA or IIB, but with tumour on surface of one or both ovaries; or with capsule(s) ruptured; or with ascites present containing malignant cells or with positive peritoneal washings
Stage III	Tumour involving one or both ovaries with histologically confirmed peritoneal implants outside the pelvis and/or positive retroperitoneal or inguinal nodes. Superficial liver metastases equal Stage III. Tumour is limited to the true pelvis, but with histologically proven malignant extension to small bowel or omentum

- IIIA Tumour grossly limited to the true pelvis, with negative nodes, but with histologically confirmed microscopic seeding of abdominal peritoneal surfaces, or histologically-proven extension to small bowel or mesentery
- IIIB Tumour of one or both ovaries with histologically confirmed implants, peritoneal metastasis of abdominal peritoneal surfaces, none exceeding 2 cm in diameter; nodes are negative
- IIIC Peritoneal metastasis beyond the pelvis > 2 cm in diameter and/or positive retroperitoneal or inguinal nodes
- Stage IV** Growth involving one or both ovaries with distant metastases. If pleural effusion is present, there must be positive cytology to allot a case to Stage IV. Parenchymal liver metastasis equals Stage IV

^a In order to evaluate the impact on prognosis of the different criteria for allotting cases to Stage IC or IIC, it would be of value to know if rupture of the capsule was spontaneous, or caused by the surgeon; and if the source of malignant cells detected was peritoneal washings, or ascites.

Table 1.5 Carcinoma of the ovary: stage grouping for ovarian cancer

FIGO		UICC	
Stage	T	N	M
I	T1a	N0	M0
I	T1b	N0	M0
I	T1c	N0	M0
II	T2a	N0	M0
II	T2b	N0	M0
II	T2c	N0	M0
III	T3a	N0	M0
III	T3b	N0	M0
III	T3c	N0	M0
	any T	N1	M0
IV	any T	any N	M1

Treatment of ovarian cancer

Epithelial ovarian cancer is a highly metastatic disease, with disseminated intraperitoneal metastases present in the 80% of cases at diagnosis^{67 68}. Surgery is normally required to make the diagnosis.

Surgery

The purpose of surgery is threefold:

1. To stage the disease accurately, allowing better choices of adjuvant therapy and a better assessment of prognosis
2. To remove completely, if possible or reduce tumour bulk to nodules of less than 1cm and perform total hysterectomy, bilateral adnexectomy and omentectomy. In a few stage I young patients conservative therapy may be justifiable
3. To relieve or prevent bowel obstruction caused by tumour

This form of surgery is referred to as cytoreductive or debulking surgery. Primary surgery to remove as much disease as possible followed by adjuvant chemotherapy is the current standard of care for this disease^{69 70 71}.

Chemotherapy

Ovarian cancer is one of the few malignancies where chemotherapy can lead to improved patient survival. The majority of ovarian cancers present in advanced stages (III or IV) and are treated with systemic chemotherapy; despite an initial 70-80% response rate, current therapy is frequently followed by recurrence which is often resistant to chemotherapy, as demonstrated by the 5-20% long-term survivors. Approximately one-third of tumours do not respond to initial chemotherapy, but it is uncertain whether the mechanisms responsible for intrinsic drug resistance are similar to the more commonly observed acquired resistance to multiple chemotherapeutic agents⁷². Prognosis is related to clinical stage, histological grade, tumour type, and the amount of unresectable residual tumour at the time of cytoreductive surgery, and response to postoperative adjuvant chemotherapy.

Seligman *et al.*⁷³ first described the beneficial effects of alkylating agents in advanced ovarian cancer in 1952. Treatment was initially restricted to palliative use in patients with advanced disease or those with recurrence following surgery and radiotherapy. In the 1960s chemotherapy was incorporated into adjuvant treatment of ovarian cancer. Since 1996, the combination of cisplatin and paclitaxel has been shown to prolong survival in comparison to previous regimens containing cisplatin and cyclophosphamide⁷¹. Carboplatin has now replaced cisplatin in combination with paclitaxel and has similar efficacy but reduced toxicity⁷⁴. Chemotherapeutic agents have been developed for advanced ovarian cancer and combined to enhance effectiveness and minimise drug resistance. Frequently the patient with advanced disease receives chemotherapy and responds with the disease regressing and becoming clinically undetectable and with a marked amelioration of symptoms and

improvement in her quality of life. Unfortunately, the disease recurs 18-24 months later and is treated either by second-line chemotherapy or by second surgical tumour resection followed by chemotherapy; however, response rates of only 20% to 40% are observed⁷¹. Only a minority of the patients with advanced disease are alive and disease free after 4-7 years^{75 76}.

Radiation

There has been a longstanding interest in irradiation, either external or intraperitoneal, for ovarian cancer. Radiation oncologists point out that pelvic irradiation alone is inadequate treatment; even for Stage I since the entire peritoneal cavity is at risk⁷⁷.

1.9.2 Cervical cancer

In 1928, Babes⁷⁸ and Papanicolaou⁷⁹ described methods for detecting cervical malignancy by exfoliative cytology, the latter's name being synonymous with cervical cytology. Emphasis on the early detection of cervical cancer was accompanied by an increasing interest in its origins. In 1886, Williams⁸⁰ described "the earliest condition of cervical cancer" that is now termed carcinoma in situ. Schauenstein,⁸¹ in 1908, expressed the view that invasive cervical cancer was preceded by an intraepithelial phase of growth. In the 1960s, Richart⁸² integrated the different approaches into a simple terminology with both clinical and pathological relevance. On the basis of a variety of complementary observations, he demonstrated that the precursors of cervical cancer formed a continuum, which he had termed "cervical intraepithelial neoplasia" (CIN).⁸³ CIN is generally divided into CIN 1, corresponding to mild dysplasia, CIN 2 corresponding to moderate dysplasia, and CIN 3, which encompasses severe dysplasia and carcinoma in situ. The time required for a lesion to evolve from a low grade to a high grade or to progress eventually to invasive cancer is not known and impossible to determine by direct observation.

Epidemiology

Globally, cervical cancer continues to be the second most common form of malignancy in women, with approximately 450000 cases estimated annually. It is also the leading cause of death from malignancy in developing countries. Generally the highest recorded rates in the world are in sub-Saharan Africa, Central and South America and some regions of South-East Asia. In many developed countries,

particularly Western Europe and North America, the incidence and mortality rates for this disease have been gradually reduced over the past 30-40 years³⁸. In Ireland cervical cancer accounts for 9% of female cancers, including carcinoma in situ³⁹. The majority of cervical cancers are squamous cell. Figure 1.5 shows an example of the gross appearance of a squamous cell carcinoma of the cervix. Adenocarcinoma constitutes 8-26% of all epithelial malignancies of the uterine cervix. Reports from the USA and Norway have shown an increasing incidence in cervical adenocarcinoma, particularly among younger women^{84 85}.



Figure 1.5. Gross appearance of a cervical squamous cell carcinoma that is still limited to the cervix (stage I).

Aetiology

Cervical cancer has been one of the most studied of all human malignancies and a vast literature exists on its epidemiology and possible aetiology. A clear association has been shown between a woman's risk of developing cervical cancer and the number of sexual partners she or her partner had. This association strongly suggests a sexually transmitted agent in the aetiology of cervical neoplasia and viruses have been considered amongst the most likely candidates^{86 87 88 89 90 91 92}. Currently human papillomavirus (HPV), particularly certain subtypes- namely HPV 16 and 18, are thought to be major causative factors of this disease^{93 94 95}. The disease seems to be more common in the lower socio-economic groups.

Oral contraceptives and high multiparity have been shown in some studies to be associated with an increased risk of developing cervical cancer^{87 96}. However, a recent review of cervical cancer and the use of hormonal contraceptives suggests that although long term use of contraceptives is associated with increased risk of cervical cancer, the public health implications depend largely on the extent to which this risk remains after cessation of hormonal contraceptives. This cannot be evaluated properly from published data⁹⁷.

Smoking is an added risk factor: a chemical inhaled in the smoke may influence the immune cells in the cervix and alters their protective action against the papilloma virus. An independent positive association with squamous cell carcinoma of the cervix and smoking has been reported⁹⁸.

Diagnosis

The Papanicolaou smear (Pap test) is an effective screening tool, identifying those women who need colposcopic examination (a colposcope allows the detailed examination of the cervix and vagina, by magnification), biopsy and further study. A flow diagram illustrating the management of abnormal cervical cytology is shown in Figure 1.6. Invasive cervical cancer is usually staged when the biopsy is taken. Staging can be defined as the clinical estimation of the extent of disease which is important in that it directly relates to prognosis and provides an approximation of tumour size and configuration for treatment planning.

Histopathology of cervical carcinomas

A classification of tumours of the cervix is shown in Table 1.6 and the histological grading of cervical carcinomas in Table 1.7. The current staging system for carcinoma of the cervix is the 1992 American Joint Committee on Cancer (AJCC) and the 1994 FIGO clinical staging system as shown in Table 1.8. The FIGO staging and the TNM staging are in Table 1.9.

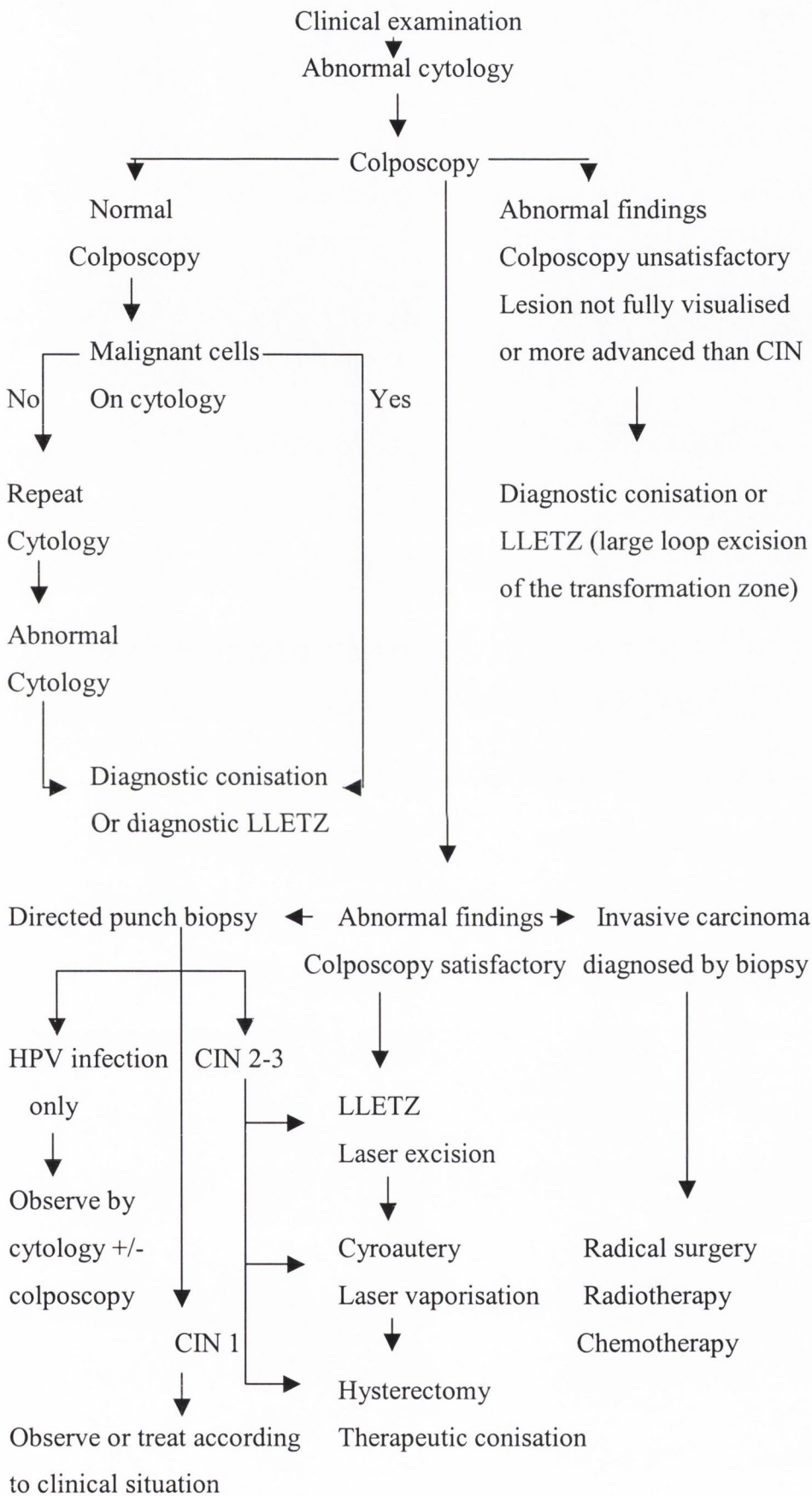


Figure 1.6. Management of abnormal cervical cytology

Table 1.6 Classification of tumours of the cervix

Epithelial Tumours and Related Lesions

Squamous lesions

- Squamous intraepithelial lesions (dysplasia-carcinoma in situ; cervical intraepithelial neoplasia [CIN])
 - CIN 1 (mild dysplasia)
 - CIN 2 (moderate dysplasia)
 - CIN 3 (severe dysplasia)
- Squamous cell carcinoma
 - Keratinising
 - Nonkeratinising
 - Verrucous
 - Warty
 - Papillary
 - Lymphoepithelioma-like

Glandular lesions

- Glandular intraepithelial lesions
 - Dysplasia
 - Adenocarcinoma in situ
- Adenocarcinoma
 - Mucinous
 - Endocervical type
 - Intestinal type
 - Endometrioid

- Clear cell
- Serous
- Mesonephric

Other epithelial tumours

- Adenosquamous carcinoma
- Glassy cell carcinoma
- Adenoid cystic carcinoma
- Adenoid basal carcinoma
- Carcinoid tumour
- Small cell carcinoma
- Undifferentiated carcinoma

Mesenchymal tumours

- Leiomyosarcoma
- Endocervical stromal sarcoma
- Embryonal rhabdomyosarcoma

Mixed epithelial and mesenchymal tumours

- Adenosarcoma
- Carcinosarcoma (malignant mixed mullerian tumour)

Miscellaneous tumours

- Malignant melanoma
- Lymphoma
- Tumours of germ cell type

Secondary tumours

Table 1.7. Histological degree of differentiation

Gx	Grade cannot be assessed
G1	Well differentiated e.g. Fig. 1.7
G2	Moderately differentiated
G3	Poorly
G4	Undifferentiated

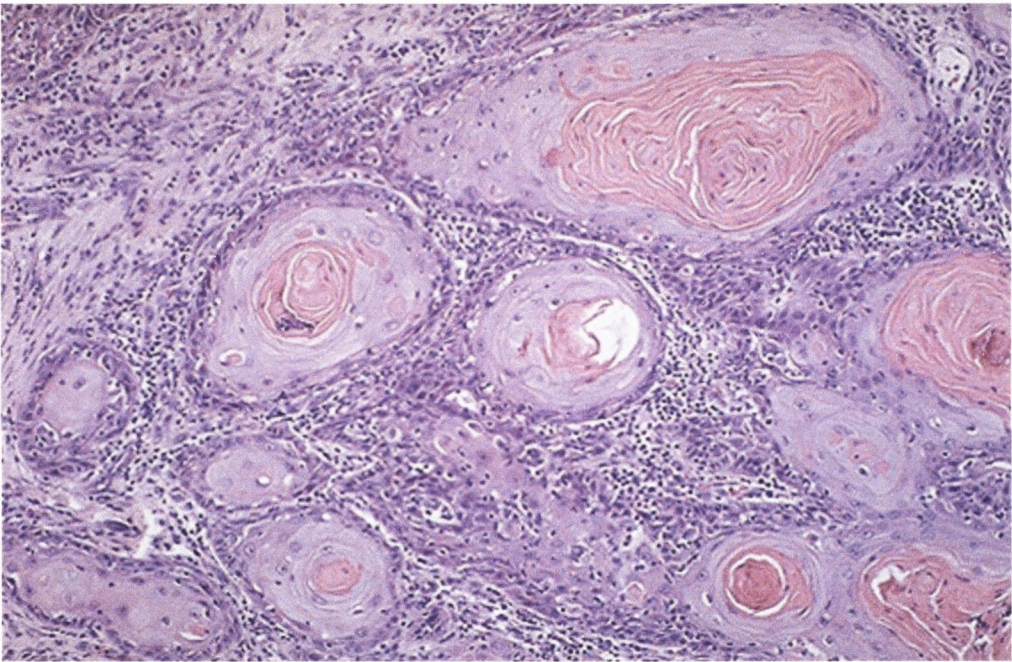


Figure 1.7. Microscopic view of a well differentiated squamous cell carcinoma of the cervix

Table 1.8: Carcinoma of the cervix uteri: FIGO nomenclature (Montreal, 1994)

Stage 0	Carcinoma in situ, cervical intraepithelial neoplasia Grade III
Stage I	The carcinoma is strictly confined to the cervix (extension to the corpus would be disregarded) Invasive carcinoma, which can be diagnosed only by microscopy. All macroscopically visible lesions – even with superficial invasion - are allotted to Stage IB carcinomas. Invasion is limited to a measured stromal invasion with a maximal depth of 5.0 mm and a horizontal extension of not > 7.0 mm. Depth of invasion should not be > 5.0 mm taken from the base of the epithelium of the original tissue - superficial or glandular. The involvement of vascular spaces - venous or lymphatic - should not change the stage allotment
IA1	Measured stromal invasion of not > 3.0 mm in depth and extension of not > 7.0 mm
IA2	Measured stromal invasion of > 3.0 mm and not > 5.0 mm with an extension of not > 7.0 mm Clinically visible lesions limited to the cervix uteri or preclinical cancers greater than Stage IA.
IB1	Clinically visible lesions not > 4.0 cm
IB2	Clinically visible lesions > 4.0 cm
Stage II	Cervical carcinoma invades beyond the uterus, but not to the pelvic wall or to the lower third of the vagina

IIA	No obvious parametrial involvement
IIB	Obvious parametrial involvement
Stage III	The carcinoma has extended to the pelvic wall. On rectal examination, there is no cancer-free space between the tumour and the pelvic wall. The tumour involves the lower third of the vagina. All cases with hydronephrosis or nonfunctioning kidney are included, unless they are known to be due to another cause
IIIA	Tumour involves lower third of the vagina, with no extension to the pelvic wall
IIIB	Extension to the pelvic wall and/or hydronephrosis or nonfunctioning kidney
Stage IV	The carcinoma has extended beyond the true pelvis, or has involved (biopsy-proven) the mucosa of the bladder or rectum. A bullous oedema, 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

Table 1.9: Carcinoma of the cervix uteri: stage grouping for cervical cancer

Stage	T	N	M
0	T in situ(is)	N0	M0
IA	T1a	N0	M0
IB	T1b	N0	M0
IC	T1c	N0	M0
IIA	T2a	N0	M0
IIB	T2b	N0	M0
IIIA	T3a	N0	M0
IIIB	T3b	N0	M0
IIIC	T1	N1	M0
	T2	N1	M0
	T3a	N1	M0
	T3b	N1	M0
IVA	T4	any N	M0
IVB	any T	any N	M1

Treatment of cervical cancer

Treatment for cancer of the cervix depends on the stage of the disease, the size of the tumour, patient's age, the overall condition and the desire to have children. Three kinds of treatment used are surgery, radiation therapy and chemotherapy. With the use of the Papanicolaou smear, cervical carcinoma is mostly diagnosed early, when the disease is still confined to the uterine cervix.

Surgery

Radical hysterectomy with pelvic lymphadenectomy is the treatment of choice in patients with stage IB large cell squamous cancers or adenocarcinomas 3cm or less in diameter⁹⁹. Cervical carcinoma spreads to the regional pelvic and aortic lymph nodes. Five-year survival is reduced from 90% in patients with negative nodes, to 64% and 41% with pelvic and aortic node metastasis respectively¹⁰⁰. Optimal management of patients with lymph node metastasis remains an unsolved problem.

Radiation

Radiation is an effective treatment method for patients with all stages of cervical cancers. Specifically it is the treatment of choice in patients with stage IB cervical cancer who are not candidates for radical surgery and in all patients with disease at a more advanced stage. Many practitioners use adjuvant radiotherapy following radical hysterectomy and pelvic node dissection for patients with positive nodes or at a high risk for a pelvic recurrence of tumour¹⁰¹.

Chemotherapy

Chemotherapy is the therapeutic method of choice for patients with extrapelvic metastases as well as for those previously treated with surgery and irradiation who have recurrent tumour⁹⁹. One of the difficulties is the relative drug resistance of cervical carcinoma. Cisplatin has significant activity in squamous cell carcinoma of the cervix with response rates of 38%¹⁰². Paclitaxel and cisplatin have been shown to be highly active in advanced and recurrent squamous cell carcinoma of the cervix and the Gynecologic Oncology Group (GOG) are conducting a phase III randomised study comparing the combination of paclitaxel and cisplatin with cisplatin alone¹⁰³. Chemotherapy agents have been combined with irradiation for primary treatment of cervical cancer in a number of settings¹⁰⁴. The chemotherapeutic agents that have been used have, for the most part, been radiation sensitisers. Recently, randomised trials comparing cisplatin-based chemoradiation to radiation alone showed a significant reduction in the risk of recurrence and death with chemoradiation^{105 106}. This reduction of 30-50% led the National Cancer Institute (NCI) to release a Clinical Announcement stating a strong consideration should be given to the incorporation of concurrent chemotherapy with radiation for patients who require radiation therapy for the management of cervical cancer¹⁰⁷.

1.9.3 Endometrial Cancer

Historically, descriptions of endometrial carcinoma were accompanied by the recognition of lesions, which could precede or develop into cancer. These associated endometrial lesions appeared to constitute a premalignant phase of this disease analogous to the relationship of CIN to invasive cervical cancer. Cystic hyperplasia could precede adenomatous hyperplasia and when accompanied by atypia of glandular cells, becomes atypical adenomatous hyperplasia. This could progress to carcinoma *in situ* and ultimately endometrial carcinoma¹⁰⁸.

Epidemiology

Cancer of the endometrium or corpus cancer is a common form of pelvic malignancy in women and is most frequent in postmenopausal women. Primary uterine cancer is classified and separated into cancers of the cervix and uterine body, the latter most commonly endometrial carcinomas, and the less common uterine sarcomas. There appears to be an inverse relationship geographically in the frequency of endometrial cancer and cervical cancer. In the highly industrialised countries of the West, a higher frequency of endometrial cancer tends to occur, while developing countries have a higher incidence of cancer of the cervix with a lower incidence of endometrial tumours¹⁰⁹. Japan has been an exception in this respect¹¹⁰. The incidence of endometrial cancer worldwide is about 170,000³⁸. In Ireland there are about 200 new cases each year³⁹. Between 75 and 80% of women diagnosed with endometrial carcinoma are postmenopausal, and the mean age at diagnosis is about 60 years^{111 112}.

Survival rates from endometrial cancer fall after age 60 years at diagnosis. However, for all ages over 60, survival is 20% higher than for cervical cancer¹¹³.

Aetiology

Conditions associated with prolonged unopposed oestrogen stimulation of the endometrium have been identified as major risk factors for this disease^{114 115}. As described earlier carcinogenesis consists of a first phase of initiation followed by various phases of promotion. With a few exceptions hormones act as promoters or cocarcinogens¹¹⁶. Generally hormones enhance the rate of initiation and development of tumours induced by all of the proven classes of initiators such as chemical carcinogens, viruses and ionising radiation. Hormones could exert their influence in promotion, in accelerating tumour growth or in sensitising target cells to initiating agents. While there is a striking example of tumour promotion in the human it is surprising that oestrogens rarely induce endometrial cancer in experimental animals. The difference could be of some importance because it could imply that induction of tumours by the sex hormones is not simply by evoking changes in cell proliferation and mitosis, other factors are almost certainly involved. Endogenous oestrogen may also contribute to endometrial cancer in patients with polycystic ovary syndrome¹¹⁷. Patients with ovarian dysgenesis have also been reported to be at high risk for endometrial cancer because of their need for long-term supplemental oestrogen beginning at an early age¹¹⁸. Obesity¹¹⁹, nulliparity^{120 121} and late menopause¹²² are all variants of normal anatomy or physiology that have been associated with endometrial carcinoma. Cigarette smoking may be protective against endometrial cancer^{123 124} but the potential risk reduction is obviously outweighed by lung cancer

and cardiovascular morbidity and mortality. Diet¹²⁵, diabetes mellitus¹²⁶ or hypertension¹²⁷ have also been frequently associated with endometrial cancer. A family history has been found in 12 to 28% of cases¹²⁸. Use of combined oral contraceptives is associated with a decrease in the risk of developing endometrial cancer and there also appears to be a residual protective effect that persists long after discontinuation of oral contraceptive use¹²⁹.

Diagnosis

The most common presenting symptom of endometrial cancer is vaginal bleeding in the postmenopausal woman: an endometrial carcinoma is found in 14% of these cases¹³⁰. The complaint of pelvic pain is a poor prognostic indicator since it tends to occur late in the course of the disease. Endometrial carcinoma can occasionally be detected by cervical cytology, but the yield of this method is low. Historically, fractional dilation and curettage (D&C) has been the definitive diagnostic procedure in evaluating patients with bleeding problems. Currently, endometrial biopsy by suction catheter (pipelle biopsy) is frequently used, so that the need for a formal D and C has decreased considerably. Hysteroscopy and hystero-graphy are also used as adjuvant techniques for diagnosing endometrial carcinoma and establishing the extent of the disease.

Histopathology of endometrial carcinomas

Carcinoma of the endometrium occurs in a number of subtypes, each varying in its propensity for myometrial invasion and metastasis. Adenocarcinoma is the most

common variety of subtype¹³¹. The gross appearance of an endometrial adenocarcinoma is shown in Figure 1.8. Table 1.10 shows the classification of endometrial neoplasms. Other less common malignant uterine tumours include uterine sarcomas, stromal sarcomas, adenosarcomas and carcinosarcomas. Histological grades of endometrial cancers are shown in Table 1.11. The FIGO staging system was revised in 1988 and changed to one based on surgical rather than clinical staging and is shown in Table 1.12. This compares to the UICC staging shown in Table 1.13.



Figure 1.8. Gross appearance of an Endometrial Adenocarcinoma. Irregular masses of white tumour are seen over the surface of this uterus that has been opened anteriorly. The cervix is at the bottom of the picture.

Table 1.10 Histological classification of endometrial neoplasms

- Endometrioid carcinoma
- Adenocarcinoma
- Adenocanthoma (adenocarcinoma with squamous metaplasia)
- Adenosquamous carcinoma (mixed adeno-carcinoma and squamous cell carcinoma)
- Mucinous adenocarcinoma
- Papillary serous adenocarcinoma
- Clear cell adenocarcinoma
- Adenosquamous carcinoma
- Undifferentiated carcinoma
- Mixed carcinoma

Table 1.11 Histological grading of endometrial carcinomas

Gx - Grade cannot be assessed

G1 - Well differentiated

G2 - Moderately differentiated

G3 - Poorly (Fig. 1.9)

G4 - Undifferentiated

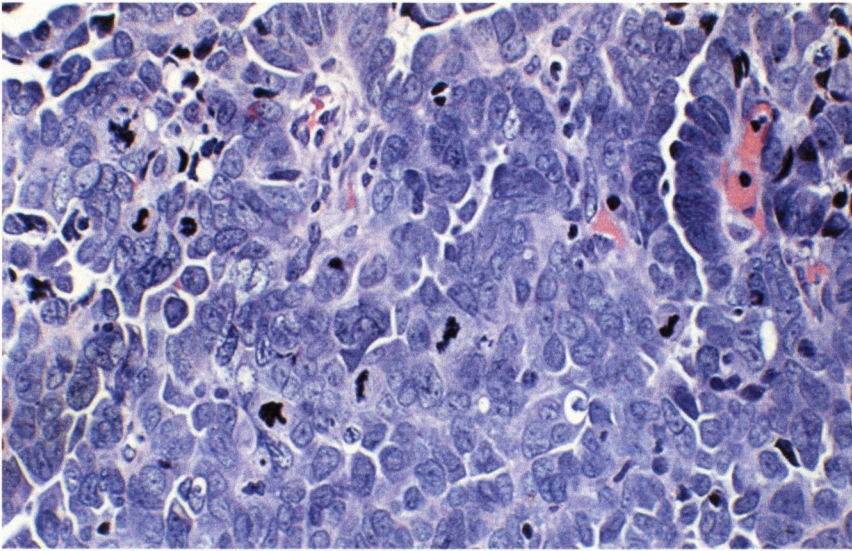


Figure 1.9. Microscopic view of a poorly differentiated endometrial Adenocarcinoma.

Table 1.12 FIGO surgical staging system for uterine corpus carcinoma (Rio de Janeiro, 1988)

Stage I	Confined to the uterine corpus
IA	Tumour limited to the endometrium
IB	Invasion to less than half of the myometrium
IC	Invasion equal to or more than half of the myometrium
Stage II	Uterine cervix involved
IIA	Endocervical glandular involvement only
IIB	Cervical stromal invasion
Stage III	Pelvic extension
IIIA	Tumour invades the serosa of the corpus uteri and/or adnexa and/or positive cytological findings
IIIB	Vaginal metastases
IIIC	Metastases to pelvic and/or paraaortic lymph nodes
Stage IV	Extrapelvic extension
IVA	Tumour invasion of bladder and/or bowel mucosa
IVB	Distant metastases, including intra-abdominal metastasis and / or inguinal lymph nodes

Table 1.13 Carcinoma of the corpus uteri: stage grouping for endometrial cancer

FIGO		UICC	
Stage	T	N	M
0	Tis	N0	M0
IA	T1a	N0	M0
IB	T1b	N0	M0
IC	T1c	N0	M0
IIA	T2a	N0	M0
IIB	T2b	N0	M0
IIIA	T3a	N0	M0
IIIB	T3b	N0	M0
IIIC	T1	N1	M0
	T2	N1	M0
	T3a	N1	M0
	T3b	N1	M0
IVA	T4	any N	M0
IVB	any T	any N	M1

Treatment of Endometrial Cancer

The true extent of endometrial carcinoma can only be ascertained after exploratory laparotomy, so the primary approach to almost all patients with Stages I and II disease should be surgical. Seventy-five percent of all cases of endometrial cancer are diagnosed while the disease is still confined to the uterus. Endometrial cancer has been considered a relatively low-grade malignancy with a 5-year survival of 67% for all stages and about 76% for stage I disease¹³².

Surgery

Surgery is the recommended treatment for cancer of the endometrium. The operation may be a total abdominal hysterectomy and bilateral salpingo-oophorectomy or a radical hysterectomy. Pelvic lymph nodes are usually taken for histological examination. Low risk patients with Grade 1 tumours limited to the uterine body with myometrial invasion of no more than 50% and negative nodes need no adjuvant therapy and have a high rate of survival. Tumour grade, myoinvasion, extent of spread, and positive lymph nodes are the most common risk factors for recurrence¹³³.

Radiation

Vaginal vault or pelvic radiation are frequently used in women with extension of the disease to the lower uterine segment or stage II disease. Whole abdominal radiation following surgical therapy may be beneficial for patients with disease confined to the

abdomen and single sites of residual disease no larger than 2cm in its greatest diameter¹³⁴.

Chemotherapy

Primary adjunctive chemotherapy is reserved for metastatic disease, such as intra-abdominal tumour spread, or for the small number of patients with primary distant metastases. A wide range of chemotherapeutic agents, such as platinum, anthracyclines and taxanes have been used in endometrial cancer but results have generally been disappointing^{135 136 137}. Studies are underway to examine other chemotherapeutic agents. In 1998, the GOG initiated a phase 3 trial in patients with advanced or recurrent endometrial cancer that compared their “gold standard” regimen of cisplatin and doxorubicin with a program of cisplatin, doxorubicin and paclitaxel. The 3-drug combination produced a higher response rate, 57% vs 33% but there was no difference in overall survival¹³⁸. For the present the standard combination chemotherapy treatment programs for endometrial cancer include cisplatin plus doxorubicin and carboplatin plus paclitaxel.

Hormonal Therapy

Hormonal therapy may still have a role as a minimally toxic treatment. Response rates are in the 20-25% range, but not of long duration. Oestrogens promote the disease after earlier phases of hyperplasia and preneoplasia and these are all countered by progesterone or synthetic progestins. After an initial response, the tumours may become resistant to continuous progestin administration¹³⁹. Two factors

appear to be of value in predicting which patients will most likely respond to progestins: histological grade and hormone receptor status. Patients with well differentiated tumours are more likely to be positive for oestrogen and progesterone receptors than those with poorly differentiated neoplasms. Receptor positivity appears to correlate with a better response to progestins, a response rate of 77% was observed compared to 9% among the tumours negative for both receptors¹⁴⁰.

1.10 Predicting Chemotherapy Response

Cancer chemotherapy for the treatment of most malignant tumours has a variable patient response. Factors such as cell type, degree of differentiation, cell cycle stage and oncogene-induced alterations all influence the effectiveness. Early detection of ovarian cancer is difficult and the only therapeutic means to improve the prognosis of a patient are radical surgery, with minimal residual disease after debulking, and treatment with effective chemotherapy. For many years, the selection of chemotherapeutic regimens has remained empirical. These treatments are based on previous trials of how patients with similar disease have responded to certain regimens of treatment, and not, as in bacteriological infections, based on individualised *in vitro* sensitivity studies⁴¹. This “blanket” approach will result in a large number of cancer patients receiving treatment where the tumours are not sensitive to the chemotherapy used. Besides factors such as immune system and nutritional status, a patient’s drug response depends on whether the tumour is sensitive to cytotoxic treatment. The diversity of pathological processes and resistance mechanisms make it difficult to select the most appropriate therapy for an individual patient on a purely empirical or epidemiological basis. Thus, it would seem important to determine the individual patient’s chemosensitivity profile to allow selection of the appropriate cytotoxic agents. This should lead to improved quality of life of the patient, to reduced costs and to improved outcome.

Clinicians have tried to improve chemotherapy treatment by clinical trials^{141 142 143 144}, using different drugs, drug dosages, schedules, combinations and different routes of administration but 5-year survival rates for these women remain unchanged. In ovarian cancer only the development of new drugs (e.g. paclitaxel) and the use of

drug combinations have resulted in better treatment response¹⁴⁵. Increasing treatment intensity, i.e. giving higher doses over shorter time periods, was aimed at preventing the development of drug resistance. However, drug side effects in patients have limited these attempts. Recent developments in managing side effects such as myelosuppression with growth factors promise to improve tolerance to treatment and possibly overall treatment results¹⁴⁶.

In an attempt to improve the 5-year survival and the treatment response, scientists have sought to develop an *in vitro* assay system to determine the potential activity of chemotherapy agents for a given patient prior to their administration. By eliminating ineffective agents, the patient is spared toxic treatment without benefit, while the selection of agents active *in vitro* may increase the probability of response. Several other approaches have also been made in determining chemosensitivity/resistance of tumours; these include the use of prognostic markers such as angiogenesis markers and multidrug resistance markers.

1.11 *In vitro* Determination of Drug Response

In vitro drug response stems from the work of Ehrlich and Pasteur who in the 1870s¹⁴⁷ evaluated the effect of agents of microbial and synthetic origin on the growth of cultured microbes. Fleming's discovery of penicillin in 1928¹⁴⁸ introduced the modern era of culture and sensitivity testing. The subsequent discoveries of bacterial antibiotic resistance mechanisms paved the way for translating this approach to oncology. In 1954 Black and Speer¹⁴⁹ compared clinical outcomes with the response of tumours *in vitro* in a succinate dehydrogenase-dependent dye reduction assay system. This small study suggested that the predictive accuracy of their test was

good in regard to resistance but weak in regard to sensitivity and so further developmental work was needed.

To study drug response / resistance in individual patients would require a sensitive, reproducible method that can be used on fresh tumour tissue. All *in vitro* drug sensitivity assays essentially require four stages:

1. Isolation of cells: solid tumours require excision and disaggregation (mechanical or enzymatic) to liberate tumour cells.
2. Incubation of cells with drugs.
3. Assessment of cell survival: The choice of assay end points to evaluate drug effects distinguishes between the various methods.
4. Interpretation of the result.

Various approaches have been used to develop *in vitro* models of drug action on tumours *in vivo*.

Clonogenic Assay

The clonogenic assay was the second significant attempt to develop a reliable *in vitro* drug response method^{150 151 152 153 154}. Originally developed by Puck and Marcus¹⁵⁰ in the mid 1950s to assess the impact of radiation on tumour cell growth, it was applied to testing the effects of drugs on human tumours in the mid 1970s^{150 151}. The clonogenic assay and its derivatives evaluate the ability of chemotherapeutic agents to inhibit tumour stem cell proliferation in agar. The assay is based on the finding that

cancer cells proliferate readily in agar, an anchorage-independent environment, whereas the nontransformed cells in each tumour remain viable but do not proliferate readily^{150 155}. The first report by Hamburger and Salmon¹⁵¹ on the human tumour clonogenic assay (HTCA) raised the expectations of oncologists that *in vitro* chemosensitivity testing can predict curative chemotherapy selection. This high expectation was in part due to the implied theoretical concept that the clonogenic cells are identical to the stem cells of a given tumour, which needs to be eradicated for a patient to be cured. The validity of this theory has been seriously questioned¹⁵⁶. The colony-forming assay has several problems making it impractical for routine clinical use^{157 158}. One major drawback of the HTCA is its low percentage of successful assays of approximately 40-70%^{159 160 161 162}. This low evaluability rate was due to poor tumour growth and the large number of cells required for the assay. There is also the difficulty of completely dissociating tumour specimens into single cells^{158 163 164}. If cell clumps are plated initially, they can be misread as colonies.

Thymidine Incorporation Assay

Tritiated thymidine uptake can also be assessed in the agar-based clonogenic assay system and was developed by Kern *et al.*¹⁶⁵ to eliminate the problem of true colony growth versus a clump of cells plated at the outset^{166 167}. In contrast to the clonogenic assay, small clumps are preferred to maintain cell-cell interactions. Tumour suspensions are exposed to drug for 5 days, with tritiated thymidine added during the final 48 hours of the assay to label proliferating cells. Determination of drug action is based on a comparison of the incorporation of labelled thymidine by untreated controls with incorporation by the groups treated with different drugs. Incorporation

of thymidine is determined after liquefying the agar-cell suspension, harvesting the cells onto glass-fiber filters with a microharvester, and counting the radioactivity with a liquid scintillation counter. Difficulties exist in the measurement of thymidine over a short incubation period^{168 169}. The proliferation capacity of cells with longer doubling times, or a low S-phase, will not be detected with a short incubation period, potentially causing false-positive and false-negative estimates of drug action for some tumours. The stress of tumour disaggregation and plating can cause a lag in cell proliferation immediately after the culture is set up, leading to a decrease in thymidine incorporation unrelated to drug action.

Adenosine Triphosphate luminescence (ATP) Assay

ATP levels decrease immediately with cell death and changes in levels after drug exposure can be determined by bioluminescence. ATP is extracted with trichloroacetic acid. Luciferin-luciferase is added to acid-neutralised cytosols to react with ATP and produce light. Quantification of the light produced, using a luminometer has been shown to directly correspond with the number of viable cells^{170 171 172}. The assay is reproducible, reliable and very sensitive¹⁷³. However, one drawback is the interference of stromal cells in the final signal.

Differential Staining Cytotoxicity (DiSC) Assay

The DiSC assay is the principle drug sensitivity assay, which relies on structural integrity of cells^{174 175 176}. This system was originally developed by Weisenthal *et al.*¹⁷⁷ for haematological malignancies and is based on the ability of most viable cells

to exclude dyes *in vitro*. Cells are incubated with drugs for 4 days. Dead cells are stained in suspension with fast green dye with or without nigrosin, and duck red blood cells are added as an internal counting standard. The specimen is cytocentrifuged to deliver discs of cells onto microscope slides. Live cells are then stained with haematoxylin-eosin or Romanowsky stain. The endpoint of this test is the morphological identification of tumour cell cytotoxicity compared with the internal control of fixed duck erythrocytes. Most of the data using the DiSC assay were collected from patients with haematological neoplasms; very few with solid tumours were reported¹⁷⁷.

Fluorescence Assays

A number of drug sensitivity assays use the conversion of nonfluorescent fluorescein monoacetate or diacetate to fluorescein by nonspecific cytosolic esterases to determine cell survival. One of these is a modified explant assay that uses the fluorescein monoacetate system, the fluorescent cytoprint assay (FCA)^{178 179 180 181}. In this assay viable microorgans of 50 or more cells are prepared and identified with the transient but nontoxic effect of fluorescein monoacetate. Microorgans are immobilised in a cellulose-collagen matrix in 24-well tissue culture plates for 24 hours and then incubated with drugs for an additional 48 hours. Photographs (the fluorescent cytoprint) before and after drug incubation are compared to identify drug-induced cytotoxicity; an additional photograph is taken after 96 hours to determine cell-cycle-dependent cytotoxicity.

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) Assay

Improvements in the succinate dehydrogenase assay developed by Black and Speer¹⁸² have evolved into the tetrazolium dye MTT assay. The assay¹⁸³ depends on cellular reductive capacity to metabolise the MTT dye to a highly coloured formazan product. The MTT assay provides a simple, rapid, semiautomated technology that is readily reproducible between laboratories. It is less laborious than other techniques. The disadvantages are that it may be susceptible to changes in enzymatic activity, pH, cellular-ion concentrations, and cell-cycle variation¹⁸⁴. In addition, the MTT formazan dye begins to lose colour within hours and is affected by the grade of dimethyl sulfoxide (DMSO) that is required to solubilise the formazan crystals.

3-(4,5 – dimethylthiazol - 2 - yl) - 5 - (3 - carboxymethoxyphenyl) - 2 - (4 - sulfophenyl) - 2H - tetrazolium, inner salt (MTS) Assay

A modified version of the MTT assay known as the MTS assay has been developed. The CellTitre 96® AQueous Non-Radioactive Cell Proliferation Assay is a colorimetric method for determining the number of viable cells in proliferation or chemosensitivity assays. It is composed of solutions of a novel tetrazolium compound MTS and an electron coupling agent (phenazine methosulfate; PMS). MTS is bio-reduced by living cells into a formazan that is soluble in tissue culture medium¹⁸⁵. The absorbance of the formazan at 490nm can be measured directly from 96 well assay plates without additional processing¹⁸⁶. The conversion of MTS into the aqueous soluble formazan is accomplished by dehydrogenase enzymes found in metabolically active cells. The quantity of formazan product is directly proportional

to the number of living cells in culture. One of the advantages of the MTS assay is safety; it is non radioactive and requires no volatile organic solvent to solubilise the formazan product, unlike MTT. The solubilisation process with MTT can be troublesome and error prone as was exemplified in the first example of a related MTT microculture tetrazolium assay ¹⁸⁷ and many subsequent studies as reviewed in Marshall *et al.*, 1995¹⁸⁸. The assay can be performed in a 96 well plate with no washing or cell harvesting. The assay is fast and is supplied as ready to use stable, frozen sterile solutions. It is flexible as the plates can be read and returned to the incubator for further colour development, unlike MTT. Malich *et al.*¹⁸⁹ found it to be an economic, rapid, sensitive and specific *in vitro* cytotoxicity assay.

Mothersill Outgrowth Assay

The Mothersill outgrowth assay was first developed as a rapid short term *in vitro* method for determining the differential radiation response of both normal and tumour tissue from the same patient. The assay is based on explant culture, which keeps the three-dimensional architecture of the tumour intact during incubation in an attempt to solve the perceived technical difficulties inherent in single cell suspension assays. Poor colony forming efficiency is avoided with this assay, as it does not concentrate solely on the clonogenic ability of the tumour cells but rather takes into account a heterogeneous tumour cell population, which should be truer to the *in vivo* situation¹⁹⁰. In this system cell-to-cell contacts stay partially in effect, as the cells grow out from a central initial population to form a confluent monolayer they are in constant contact with neighbouring cells. In the clonogenic assay single cells are

seeded and thus cell-to-cell contact is not an influencing factor in response to treatment.

1.12 Chemosensitivity/Resistance Testing of Malignant Tumours of the Ovary, Cervix and Endometrium

The majority of the work carried out to date on chemosensitivity testing in gynaecological malignancies has been on ovarian carcinomas with few reports on cervical and endometrial cancers. This is mainly due to the fact that radiotherapy has been used successfully for the treatment of locally advanced cervical cancer but radiocurability had been limited by tumour size. For many decades, invasive cervical cancer has been considered more or less chemoresistant and chemotherapy has been limited to patients presenting with overt metastatic disease or those suffering from pelvic recurrences, which could not be advised to secondary local treatments. In the last decade the role of multiple modality treatment including neoadjuvant, concurrent and adjuvant chemotherapy with either surgery or radiation therapy as part of an initial treatment plan have been evaluated in randomised clinical trials^{104 105 106}. Chemosensitivity in cervical cancer has been examined using cell lines but very little published work exists using fresh tumour tissue. Evaluability rates and specimen numbers in these studies were low^{191 192}. The development of effective systemic therapy for endometrial cancer has been slow because of the relatively high cure rate of 67%¹³² but efforts have been made to evaluate hormonal therapy, single agent chemotherapy and combination chemotherapy. Chemotherapy for endometrial cancer is reserved for patients who develop a recurrence or present with advanced disease and chemosensitivity testing in endometrial cancers has been limited^{191 192 193}.

One of the reasons chemosensitivity testing has not been incorporated into standard oncology therapy has been the lack of a good drug sensitivity assay. *In vitro* testing of solid tumours has been difficult for various reasons including the low growth rate of tumours *in vitro*, and the low viability of tumour cells after disaggregation required for plating cells in culture¹⁹⁴. Various assay endpoints have been used to assess the effects of drugs on cancer cells. Each assay endpoint has its advantages and disadvantages based on ease of use, reproducibility, precision and success rate. The evaluability rates for examining ovarian cancers *in vitro* have varied from 40% to 90% depending on the assay used.

Eight ovarian tumours were first examined for chemosensitivity using the HTCA in 1977¹⁵¹. Following this numerous retrospective studies were carried out¹⁵⁹. Two hundred ovarian tumour specimens were submitted for HTCA from 106 patients. Of the 115 samples that grew in culture, only 62% had sufficient plating efficiency to yield significant drug results. Of the 106 patients in this study, therefore, only 57 had evaluable *in vitro* results. In addition, 34 patients were treated with other means, and only 23 (22%) received one of the three drugs tested. The accuracy of predicting sensitivity ranged from 40-75% and of resistance, from 80-99%¹⁹⁵. In a prospective study by the Southwest Oncology Group, a response rate of 28% was observed when patients with refractory ovarian cancer were treated according to HTCA results compared with 11% in patients treated according to clinician's choice¹⁹⁶. The evaluability rate was 44% and no significant difference in survival was observed between the two groups. A small group of 19 gynaecological cancer patients were treated with single agent therapy prospectively chosen on the basis of HTCA, giving a sensitive predictive accuracy of 90% and a resistant predictive accuracy of 100%¹⁹⁷.

The overall growth rate was 88.4% for this study. A study examining the colony-forming assay was found to be 36% evaluable in cervical cancers and 35% evaluable in endometrial cancers¹⁹¹.

The thymidine incorporation assay has been used to examine chemosensitivity in 52 ovarian cancers: an evaluability rate of 92% was reported¹⁶⁵. Another study using this technique on 7 ovarian carcinomas had an evaluability of 75%¹⁶⁷. The Kern assay¹⁶⁵, using suprapharmacological drug concentrations evaluates drug resistance. In 46 patients with ovarian cancer, only 58% of patients whose tumours had 75% or more inhibition of thymidine incorporation had a clinical response¹⁶⁶. The evaluability rates were reported to be approximately 80%. These radiolabelled assays measure the effect of cytotoxic drugs only in those cells that are actively synthesising DNA. Tumours *in vivo* have a relatively small fraction of cells undergoing DNA synthesis of 5-10%¹⁵⁶. Khoo *et al.*¹⁹² examined seven uterine carcinomas and six cervical carcinomas using the ³H Thymidine incorporation assay but none of these were correlated to the clinical outcome.

Ninety-seven out of 108 ovarian cancers were successfully examined in one study for chemosensitivity using the ATP assay yielding an evaluability rate of 90%¹⁹⁸. Cisplatin resistance in ovarian cancers was examined using an ATP assay¹⁹⁹, which had an 87% success rate. Cisplatin results were presented for 81 specimens from 70 untreated patients and 33 specimens from refractory patients. The assay had >90% accuracy for cisplatin resistance. Konecny *et al.*²⁰⁰ have recently evaluated 93 fresh human primary epithelial ovarian cancer specimens using the ATP assay. Evaluability for this study was 89% and clinical correlations were carried out in 38

patients. The assay demonstrated a sensitivity of 95%, specificity of 44%, positive predictive value of 66% and a negative predictive value of 89%. The evaluability rate for a study of chemosensitivity using the ATP assay in a small group of cervical and endometrial tumours was 68%¹⁹⁸.

Evaluability rates of 73-95% have been reported using the FCA¹⁸¹. In 101 cases, including 31 ovarian tumours, the assay predicted sensitivity in 86% and resistance in 91%. The reported advantage of this assay is that 'microorgans' maintain cell-to-cell contact, which is postulated to result in a more normal response to chemotherapeutic agents. There are, however, no separate untreated controls as the cytoprint prior to drug exposure is used as 'internal control'. It is therefore difficult to determine if the tumour cells are dying in response to the drug treatment or because they are unable to maintain viability.

Clinical correlations have been carried out in a study of 37 FIGO stage III-IV ovarian adenocarcinoma patients whose tumours were examined using the MTT assay²⁰¹. Eleven out of 17 (65%) patients in the sensitive group had a complete response to therapy compared to 3 out of 20 (15%) in the resistant group. The 3-year cumulative survival was 36% in the sensitive group compared to 24% in the resistant group. On analysing the data from ovarian cancer patients treated with a platinum drug, the MTT assay accurately predicted the response in 18 of 25 cases²⁰². In another study there was a true prediction of complete response in 60% of cases and of resistance in 90% of cases²⁰³. The MTT assay has been used in endometrial cancers to compare the chemosensitivity of irinotecan and cisplatin²⁰⁴. A group of 24 endometrial cancers have recently been examined using the MTT assay in an attempt to evaluate a

chemotherapeutic index for this cancer and the assay had an 81.3% predictive accuracy¹⁹³.

The MTS assay has been used in several studies in preference to the MTT assay^{205, 206, 207, 208} but it has not been used to evaluate the response of malignant tumours to cytotoxic drugs.

The Mothersill outgrowth assay was used to examine the *in vitro* response of malignant tumours of the ovary, cervix and endometrium to radiation and cytotoxic drugs²⁰⁹. Five tumours out of 7 (71% evaluability) were cultured and counting the number of grid squares covered by the cells assessed growth response. Three of the patients had presented with recurrences and the tumours displayed resistance *in vitro* to the drugs initially received. The study was too small for meaningful follow up.

Chemosensitivity in cell lines has confirmed heterogeneity of the *in vitro* drug response in morphologically similar neoplasms²¹⁰. Sevin *et al.*²¹¹ showed heterogeneity of drug response in fresh human ovarian carcinomas but they did not limit this to similar stages and grades of tumours. Our intention was to evaluate this new assay combination of explant technique and MTS assay in gynaecological cancers and to improve the evaluability rate of previous techniques. We sought to determine how accurate the procedure of chemotherapy selection is by examining tumours of similar stage and grade. The most important outcome is how the *in vitro* results correlate with the *in vivo* response of the patient. For an *in vitro* test to be incorporated into routine procedure a significant increase in patient survival would have to be observed for assay directed chemotherapy regimens.

1.13 Potential Predictors of Response to Chemotherapy

Angiogenesis

As indicated earlier, growth of tumours, both primary and metastatic is dependent on angiogenesis^{3 4 5}; thus any increase in tumour mass must be accompanied by an increase in capillary formation to supply the tumour mass¹⁰. Various growth factors have been shown to stimulate angiogenesis in physiological and pathological conditions, including neoplastic disease. Among these, vascular endothelial growth factor (VEGF) has been shown to play a major role in the proliferation and migration of endothelial cells, providing nourishment to the growing tumours and allowing the tumour cells to establish continuity with the host vasculature²¹².

VEGF

Vascular endothelial growth factor (VEGF)²¹³, also known as vascular permeability factor (VPF)²¹⁴ or vasculotropin²¹⁵ is a homodimeric 32-42kDa, heparin-binding glycoprotein that was purified originally on the basis of its vascular permeability enhancing activity and was shown subsequently to be a potent mitogen for endothelial cells^{216 217}. Five VEGF forms have been identified in mammals, resulting from alternative splicing of the single VEGF gene: VEGF₁₂₁, VEGF₁₄₅, VEGF₁₆₅, VEGF₁₈₉, VEGF₂₀₆^{218 219}. VEGF₁₆₅ is the predominant molecular species produced by a variety of normal and transformed cells. This factor was first identified in the culture supernatant and the ascites of rodent tumours²¹⁴. More recently, VEGF has

been found to be present in the malignant effusions of human ovarian, breast and lung tumours²²⁰.

Expression of VEGF can be measured immunohistochemically using the DAKO Envision™ + System (DakoCytomation, Carpinteria, California) staining technique. The system is based on a horseradish peroxidase (HRP) labelled polymer, which is conjugated with secondary antibodies. The labelled polymer does not contain avidin or biotin. Consequently, non-specific-staining resulting from endogenous avidin-biotin activity in liver, kidney, lymphoid tissues and cryostat sections is eliminated or significantly reduced. Oncogene supplies a mouse monoclonal antibody, which recognises VEGF₁₂₁, VEGF₁₆₅, VEGF₁₈₉ and VEGF₂₀₆.

Assessment of angiogenesis

Microvessel density (MVD) counting techniques have been widely used to assess the degree of angiogenesis in tumours. A variety of endothelial cell markers can be used to highlight blood vessels immunohistochemically. The most commonly used markers include Platelet endothelial cell adhesion molecule-1 PECAM-1 or CD31, CD34 and Factor VIII-related antigen. CD31 has been proposed to be the standard for microvessel study as it has been found to be more sensitive and a specific marker for endothelial cells^{221 222 223}.

CD31

Human CD31 also known as hec7 or endoCAM is a 130kDa type I transmembrane glycoprotein that belongs to the cell adhesion molecules (CAM), or C2-like subgroup

of the immunoglobulin superfamily^{224 225 213}. CD31 is expressed on the surface of endothelial cells, platelets, monocytes, myeloid cells and selective T-cell subsets²²⁶. CD31 mediates cell to cell adhesion in many tissue types and plays an important role in a number of endothelial cell functions including migration, angiogenesis, vascular wound healing and transmigration of leukocytes across the endothelium in inflammatory responses^{227 228 229}. CD31 has been largely used for immunohistochemical analysis on formalin- fixed, paraffin-embedded tissue sections to assess tumour angiogenesis²³⁰.

Expression of CD31 can be measured immunohistochemically using the DAKO Envision™ + System staining technique as described in the detection of VEGF. Oncogene supplies a mouse monoclonal antibody, which recognises a 100kDa glycoprotein in endothelial cells and a 130kDa glycoprotein in platelets. This antibody reacts with endothelial cells in normal tissue and in benign and malignant proliferations²²⁹.

1.14 Angiogenesis in Ovarian Carcinomas

Ovarian cancer is unusual in that it spreads early locally into the pelvis and then to abdominal serosal surfaces and may invade into retroperitoneal and pelvic lymph nodes, but invades organ parenchyma late. Neovascularisation has been demonstrated to be an early and key event in the progression of numerous cancers and is necessary to provide vascular and lymphatic conduits before invasion can begin. VEGF mRNA is markedly up regulated in the vast majority of human tumours, including those of the ovary (VEGF₁₆₅ and VEGF₁₂₁ isoforms)^{231 232}. Angiogenesis has been correlated

with prognosis in patients with advanced ovarian carcinoma, and significant differences in vascular pattern have been observed between benign and malignant lesions^{233 234}. Previous immunohistochemical studies^{234 235 236} reported significantly greater VEGF expression in malignant ovarian neoplasms than in cystadenomas suggesting a strong correlation between VEGF-induced angiogenesis and aggressiveness of ovarian serous tumours. Studies using multivariate statistical analyses to assess the potential prognostic value of VEGF expression in ovarian carcinoma are limited. In one study using multivariate analysis in early stage ovarian carcinomas, VEGF mRNA overexpression was found to be the strongest independent prognostic indicator²³⁷. Another study reported that only disease stage was a significant prognostic factor and VEGF expression was not an independent factor²³⁸.

Expression of CD31 was reported significantly higher in ovarian carcinomas than in borderline and benign tumours²³⁹. Conflicting evidence surrounds the correlation of MVD to the clinical outcome of the patient²⁴⁰. One study carried out by Gasparini *et al.*²⁴¹ reported microvessel density to be an independent predictor of overall survival in univariate analysis; however it failed to attain statistical significance in the multivariate analysis. In another study Hollingsworth *et al.*²⁴² identified higher average microvessel count as an independent prognostic factor in patients with stage III-IV epithelial ovarian cancer. Other studies contradicted the putative association between increased MVD and poor prognosis in ovarian tumours²²¹.

Studies addressing a relationship between VEGF and MVD are limited^{243 244 245 246}. In one study a significant association was found between MVD and VEGF²⁴³. In contrast the other three studies found no correlation between MVD and VEGF

expression. The influence of VEGF expression and MVD in ovarian cancer still remains controversial.

1.15 Angiogenesis and Chemotherapy Response

There is very little published data on the relationship between the tumour level of VEGF or CD31 and the efficacy of response to chemotherapy. Any data published remains contradictory. A study carried out by Takiuchi *et al.*²⁴⁷ on gastric carcinomas reported that VEGF-positive tumours showed a significantly higher response rate to cisplatin and fluorouracil than did VEGF-negative tumours. They concluded that the correlation between VEGF and chemotherapy response might be explained by the drug delivery through angiogenesis and vascular permeability. Volm *et al.*²⁴⁸ observed a similar pattern with VEGF and anti-Factor VIII in the response of non-small cell lung cancer to doxorubicin and proposed that hypoxia may be the reason for the interrelationships because some drugs and radiation require oxygen to be maximally cytotoxic²⁴⁹. A study carried out on breast cancer²⁵⁰ reported conflicting evidence to these results and demonstrated that high tumour levels of VEGF predicts poor response to systemic therapy in advanced breast cancer. In relation to ovarian cancer only one published study was found which correlated CD31 and response to platinum based chemotherapy²⁴¹. In multivariate analysis the degree of vascularisation was significantly associated with the lack of pathological response. Considering the degree of vascularisation of a primary cancer and the response to conventional anticancer treatment, the onset of angiogenesis should gradually make a tumour more accessible to drugs, and thus more responsive. Several studies have indicated that this, however, is not the case and that neovascularisation facilitates

tumour growth via both a perfusion effect that enhances the intratumoral delivery of nutrients and oxygen and by a paracrine effect including the direct production of growth factors and cytokines from endothelial cells²⁵¹.

1.16 Multidrug Resistance (MDR)

The term “multidrug resistance” refers to a special form of clinical resistance of various tumours against a wide range of chemotherapeutic agents and remains a major obstacle in the treatment of cancer patients²⁵². Medical oncologists are all too familiar with the phenomenon of tumour recurrence following an initial clinical response to chemotherapeutic drugs. Determining mechanisms of drug resistance is critical to the development of rational therapeutic strategies to overcome or prevent drug resistance. One mechanism of resistance, which has been well characterised, is that of multidrug resistance. Tumour cells that have the MDR phenotype are generally resistant to such natural products as the vinca alkaloids, anthracyclines and podophylotoxins. This form of drug resistance has consistently been associated with increased expression of a 170-kilodalton molecular weight plasma protein termed P-glycoprotein²⁵³. P-glycoprotein association with MDR was first described in colchicine resistant Chinese hamster ovary (CHO) cells and in actinomycin D resistant Syrian hamster cells²⁵⁴.

1.17 P-glycoprotein

P-glycoproteins (P-gps) are a homologous family of integral membrane proteins and function as an energy-dependent drug efflux pump that reduces intracellular drug accumulation, thereby conferring resistance to many different drugs. The P-gp family is in turn part of a superfamily of ATP-Binding Cassette (ABC)-transporters. P-gps contain four domains, two transmembrane domains and two ATP-binding domains located in the cytoplasm. In prokaryotes separate genes usually encode these

domains. P-glycoprotein is encoded by a small family of closely related genes, and of the two genes in humans, only the *mdr1* gene (also known as PGY1) causes the MDR phenotype²⁵⁵. MDR1 is a single copy gene located on the long arm of chromosome 7, it is greater than 100kb in length and is composed of 27 introns with 28 exons^{256 257}. P-glycoprotein is expressed in a large number of normal tissues including kidney, adrenal glands, large intestine, and liver indicating that it is involved in normal physiological functions including detoxification and transport of lipophilic molecules. Tumours arising from tissues normally expressing P-glycoprotein may be intrinsically resistant to chemotherapeutic agents or, alternatively, tumours that were initially responsive to chemotherapy may develop multidrug resistance during the treatment regimen and subsequently not respond to therapy^{258 259}.

Several methods have been developed for the detection of the MDR phenotype, aiming for its recognition either at the mRNA level, the protein expression, or the function of the transporters^{260 261}. As MDR1 levels in the clinical samples are generally low the sensitivity of these methods is a key issue. An added confounding factor is the fact that P-glycoprotein is expressed in certain normal tissue such as adrenal gland, kidney, colon, liver and rectum²⁶², so detection methods must be able to discriminate between normal and neoplastic tissue. P-glycoprotein can be measured immunohistochemically. The antibody JSB-1 reacts with a conserved cytoplasmic epitope of the plasma membrane-associated 170-180 kDa glycoprotein, the expression of which is strongly correlated with the degree of multidrug resistance derived MDR cell lines and human MDR cell lines including cell lines derived from lung, ovaries and B cell lymphomas. The antibody has been selected for optimal

performance in immunocytological and immunohistochemical staining techniques²⁶³

264 265 266

1.18 MDR And Chemotherapy

Multidrug resistance of tumours is a major obstacle for successful cancer chemotherapy. MDR1, one of the key molecules in MDR, has been shown to bind anti cancer drugs, to be an ATPase, and to be localised in the plasma membrane of MDR cells²⁶⁷. The expression of MDR1 was found to be elevated in intrinsically drug-resistant cancers as well as in some tumours that acquired drug resistance during chemotherapy²⁶². Salmon *et al.*²⁶⁸ first reported that positive immunostaining for P-gp predicts intrinsic cellular resistance of human cancer to doxorubicin. They showed that 12 out of 26 tumours (myeloma, lymphoma, breast cancer) stained positively and that all of the 12 positive tumours exhibited drug resistance *in vitro* to doxorubicin. Quite similar results were reported in renal cell carcinomas²⁶⁹ and non-small cell lung carcinomas²⁷⁰. In contrast Keith *et al.*²⁷¹ found only a weak correlation between the level of P-gp gene expression and resistance in breast cancer.

1.19 MDR in Ovarian Cancer

While increased levels of P-gp appear to play a role in the drug resistance phenotype of certain human cancers, evidence for the involvement of this protein in ovarian cancer is conflicting^{272 273}. Historically, it was the description by Bell in 1985²⁷² of P-glycoprotein in two of five patients with clinically resistant ovarian cancer that initiated much of the interest in screening for P-gp in human cancers. Low levels of

MDR1 expression were shown to serve as a marker of resistance to combination chemotherapy in ovarian cancer and small-cell lung cancer²⁷⁴. Kavallaris *et al.*²⁷⁵ reported that MDR1 gene expression predicts for disease progression in previously untreated ovarian tumours. P-gp was found to be an independent prognostic variable in untreated cases of advanced ovarian cancer²⁷⁶. In contrast an immunohistochemical study that examined P-glycoprotein in advanced ovarian carcinoma found no association with either survival or progression free survival²⁷⁷²⁷⁸. Van der Zee *et al.*²⁷⁹ also examined P-gp expression in advanced ovarian cancers and found that the frequency of P-gp staining in residual tumours after chemotherapy was higher in comparison to untreated tumours but P-gp could not predict response to chemotherapy adequately.

1.20 Aims of study

To evaluate the Mothersill-MTS assay as a predictor of chemotherapy response in ovarian, cervical and endometrial cancers

To correlate the *in vitro* response with the *in vivo* response and determine if an *in vitro* assay would improve patient survival

To investigate other potential predictors of prognosis in ovarian cancer

To examine if a correlation exists between the degree of vascularisation and response to chemotherapy in ovarian cancer

To examine if a correlation exists between the degree of staining with MDR and the response to chemotherapy

CHAPTER 2

PATIENTS, MATERIALS AND METHODS

2.1 Patients

Women with carcinoma of the ovary, cervix and endometrium diagnosed at St. James's Hospital, The Coombe Women's Hospital and The National Maternity Hospital (Holles St.) formed the study group. Normal and benign ovarian biopsy specimens were collected from women undergoing hysterectomies with uni/bilateral salpingo oophorectomies (removal of one or both ovaries) for suspected ovarian cancer, prolapse uterus, premenstrual tension syndrome, dysfunctional uterine bleeding or adenomyosis. Biopsy size was at the discretion of the physician and no specimen was considered unsuitable. Specimens were confirmed malignant or non malignant by histological examination. Clinical details can be seen in Tables 2.1 (Ovary), 2.2 (Cervix), 2.3 (Endometrium).

Table 2.1 Ovarian specimens

Number	Specimen Pathology	Grade	Stage	Age
OV1	Serous papillary adenocarcinoma	1	1	29
OV2	Serous papillary adenocarcinoma	1	IC	53
OV3	Serous papillary adenocarcinoma	2	IC	43
OV4	Serous papillary adenocarcinoma	2	IC	49
OV5	Serous papillary adenocarcinoma	3	II	40
OV6	Serous papillary adenocarcinoma	2	III	65
OV7	Serous papillary adenocarcinoma	2	III	61
OV8	Serous papillary adenocarcinoma	2	III	59
OV9	Serous papillary adenocarcinoma	2	IIIA	55
OV10	Serous papillary adenocarcinoma	2	IIIB	31
OV11	Serous papillary adenocarcinoma	2	IIIC	63
OV12	Serous papillary adenocarcinoma	2	IIIC	77
OV13	Serous papillary adenocarcinoma	2	IIIC	70
OV14	Serous papillary adenocarcinoma	2	IIIC	53
OV15	Serous papillary adenocarcinoma	2	IIIC	43
OV16	Serous papillary adenocarcinoma	2	IIIC	62
OV17	Serous papillary adenocarcinoma	2	IIIC	78
OV18	Serous papillary adenocarcinoma	2	IIIC	45
OV19	Serous papillary adenocarcinoma	2	IIIC	46
OV20	Serous papillary adenocarcinoma	2	IIIC	57
OV21	Serous papillary adenocarcinoma	2	IIIC	70
OV22	Serous papillary adenocarcinoma	2	IIIC	60
OV23	Serous papillary adenocarcinoma	2	IIIC	54
OV24	Serous papillary adenocarcinoma	2	IIIC	60
OV25	Serous papillary adenocarcinoma	2	IIIC	32
OV26	Serous papillary adenocarcinoma	2	IIIC	74

OV27	Serous papillary adenocarcinoma	2	IIIC	70
OV28	Serous papillary adenocarcinoma	2	IIIC	58
OV29	Serous papillary adenocarcinoma	2	IIIC	76
OV30	Serous papillary adenocarcinoma	2	IIIC	65
OV31	Serous papillary adenocarcinoma	3	III	43
OV32	Serous papillary adenocarcinoma	3	IIIC	57
OV33	Serous papillary adenocarcinoma	3	IIIC	64
OV34	Serous papillary adenocarcinoma	3	IIIC	43
OV35	Serous papillary adenocarcinoma	3	IIIC	76
OV36	Serous papillary adenocarcinoma	3	IIIC	70
OV37	Serous papillary adenocarcinoma	3	IIIC	66
OV38	Serous papillary adenocarcinoma	3	IIIC	65
OV39	Serous papillary adenocarcinoma	3	IIIC	66
OV40	Serous papillary adenocarcinoma	3	IIIC	84
OV41	Serous papillary adenocarcinoma	3	IIIC	76
OV42	Serous papillary adenocarcinoma	3	IIIC	64
OV43	Serous papillary adenocarcinoma	3	IIIC	36
OV44	Serous papillary adenocarcinoma	3	IIIC	54
OV45	Serous papillary adenocarcinoma	3	IIIC	48
OV46	Serous papillary adenocarcinoma	3	IIIC	48
OV47	Serous papillary adenocarcinoma	3	IIIC	55
OV48	Serous papillary adenocarcinoma	3	IIIC	61
OV49	Serous papillary adenocarcinoma	3	IIIC	59
OV50	Serous papillary adenocarcinoma	3	IIIC	73
OV51	Serous papillary adenocarcinoma	2	IV	61
OV52	Serous papillary adenocarcinoma	2	IV	50
OV53	Serous papillary adenocarcinoma	2	IV	45
OV54	Serous papillary adenocarcinoma	2	IV	60
OV55	Serous papillary adenocarcinoma	2	IV	58
OV56	Serous papillary adenocarcinoma	2	IV	39

OV57	Serous papillary adenocarcinoma	2	IV	78
OV58	Serous papillary adenocarcinoma	2	IV	29
OV59	Serous papillary adenocarcinoma	2	IV	57
OV60	Serous papillary adenocarcinoma	2	IV	43
OV61	Serous papillary adenocarcinoma	3	IV	47
OV62	Serous papillary adenocarcinoma	3	IV	49
OV63	Serous papillary adenocarcinoma	3	IV	47
OV64	Serous papillary adenocarcinoma	3	IV	60
OV65	Serous papillary adenocarcinoma	3	IV	74
OV66	Serous papillary adenocarcinoma	3	IV	62
OV67	Serous papillary adenocarcinoma	3	IV	50
OV68	Serous papillary adenocarcinoma	3	IV	54
OV69	Serous papillary adenocarcinoma	3	IV	58
OV70	Clear cell adenocarcinoma	3	ND	71
OV71	Clear cell adenocarcinoma	3	IC	62
OV72	Clear cell adenocarcinoma	3	II	69
OV73	Clear cell adenocarcinoma	3	II	71
OV74	Clear cell adenocarcinoma	3	IIIC	64
OV75	Clear cell adenocarcinoma	3	IIIC	85
OV76	Clear cell adenocarcinoma	3	IIIC	58
OV77	Clear cell adenocarcinoma	ND	IV	47
OV78	Mucinous adenocarcinoma	ND	1B	52
OV79	Mucinous adenocarcinoma	2	IIIC	47
OV80	Mucinous adenocarcinoma	3	III	41
OV81	Mucinous adenocarcinoma	3	IV	49
OV82	Primary peritoneal adenocarcinoma	3	IV	71
OV83	Endometrioid adenocarcinoma	3	IC	44

OV84	Endometrioid adenocarcinoma	1	II	66
OV85	Transitional cell carcinoma	ND	II	52
OV86	Adenosquamous	2	IIIC	40
OV87	Sarcoma	3	ND	61
OV88	Mixed mullerian	3	III	72
OV89	Mucinous cystadenoma	N/A	N/A	65
OV90	Borderline mucinous	N/A	I	46
OV91	Borderline mucinous	N/A	IA	68
OV92	Borderline mucinous	N/A	1C	50
OV93	Borderline mucinous	N/A	IIIC	72
OV94	Borderline mucinous	N/A	IIIC	75
OV95	Borderline serous	N/A	IA	35
OV96	Borderline serous	N/A	IA	47
OV97	Borderline serous	N/A	IA	62
OV98	Borderline serous	N/A	IC	63
OV99	Benign	N/A	N/A	45
OV100	Benign	N/A	N/A	43
OV101	Benign	N/A	N/A	64
OV102	Benign	N/A	N/A	71
OV103	Benign	N/A	N/A	67
OV104	Benign	N/A	N/A	45
OV105	Benign	N/A	N/A	52
OV106	Benign	N/A	N/A	76
OV107	Benign	N/A	N/A	50

OV108	Benign	N/A	N/A	36
OV109	Normal	N/A	N/A	45
OV110	Normal	N/A	N/A	75
OV111	Normal	N/A	N/A	46
OV112	Normal	N/A	N/A	71
OV113	Normal	N/A	N/A	45

Abbreviations: ND (Not Documented), N/A (Not applicable)

Table 2.2 Cervical specimens

Number	Specimen Pathology	Grade	Stage	Age
CX1	Squamous	2	I	71
CX2	Squamous	2	IB	44
CX3	Squamous	2	IB	51
CX4	Squamous	2	IB	37
CX5	Squamous	2	IB1	82
CX6	Squamous	2	IB2	40
CX7	Squamous	2	IB2	34
CX8	Squamous	3	IB	58
CX9	Squamous	3	IB	35
CX10	Squamous	3	IB	51
CX11	Squamous	3	IB	64
CX12	Squamous	3	IB	51
CX13	Squamous	3	IB	63
CX14	Squamous	3	IB1	84
CX15	Squamous	2	IIA	70
CX16	Squamous	2	IIA	75
CX17	Squamous	2	IIA	67
CX18	Squamous	2	IIB	61
CX19	Squamous	3	IIA	33
CX20	Squamous	3	IIB	33
CX21	Squamous	3	IIB	47
CX22	Squamous	3	IIB	42
CX23	Squamous	3	IV	33

CX24	Serous papillary adenocarcinoma	2	IB	30
CX25	Serous papillary adenocarcinoma	2	IIA	42
CX26	Adenosquamous	2	IB	47
CX27	Adenosquamous	3	IB	72
CX28	Adenosquamous	3	IIA	42
CX29	Adenosquamous	3	IIIA	70
CX30	Recurrent Squamous	3	IB	71

Table 2.3 Endometrial specimens

Number	Specimen Pathology	Grade	Stage	Age
EN1	Endometrioid adenocarcinoma	1	IB	72
EN2	Endometrioid adenocarcinoma	2	IB	60
EN3	Endometrioid adenocarcinoma	2	IB	42
EN4	Endometrioid adenocarcinoma	2	IB	67
EN5	Endometrioid adenocarcinoma	2	IC	80
EN6	Endometrioid adenocarcinoma	2	IIIC	39
EN7	Endometrioid adenocarcinoma	2	IIIC	74
EN8	Endometrioid adenocarcinoma	2	III	73
EN9	Adenosquamous carcinoma	3	III	77
EN10	Adenosquamous carcinoma	3	III	53
EN11	Adenosquamous carcinoma	3	IIIA	91
EN12	Adenosquamous carcinoma	3	IIIA	42
EN13	Serous papillary adenocarcinoma	1	IB	58
EN14	Serous papillary adenocarcinoma	2	IB	52
EN15	Serous papillary adenocarcinoma	2	IC	56
EN16	Serous papillary adenocarcinoma	3	IIB	53
EN17	Serous papillary adenocarcinoma	3	IIIC	72
EN18	Serous papillary adenocarcinoma	2	IV	53

EN19	Serous papillary adenocarcinoma	3	IV	57
EN20	Recurrent stromal sarcoma	2	ND	50
EN21	Stromal sarcoma	ND	ND	78
EN22	Adenosarcoma	3	ND	65
EN23	Leiomyosarcoma	3	IV	54
EN24	Leiomyosarcoma	ND	ND	39
EN25	Mixed mullerian	4	III	48

Abbreviations: ND (Not Documented)

MATERIALS AND METHODS

2.2 Specimen Collection

Transport Medium

Dulbecco’s Modified Eagle Medium (DMEM) (Gibco)

With 25mM Hepes, Sodium pyruvate, 1000mg/L glucose and pyroxidine

This was supplemented to give a final concentration of:

Fetal Clone III (FC III) (Hyclone)	10%
(Heat inactivated 30 min @ 56°C)	
Gentamicin (Gibco)	100µg/ml
Amphotericin (Gibco)	10µg/ml
L-Glutamine (Gibco)	2mM

10% Neutral Buffered Formalin

40% Formaldehyde solution (BDH)	100ml
Distilled Water	900ml
Sodium Dihydrogen Phosphate Monohydrate (BDH)	4g
Disodium Hydrogen Phosphate Anhydrous (BDH)	6.5g

The specimens were taken during surgery and split in two in theatre. One half was placed in sterile transport media for culture and the other half was placed in an empty sterile container for histological studies. Both specimens were brought to the laboratory and the part in transport media was processed for culturing. The specimen in the empty container was cut in two and 1 part placed in 10% neutral buffered formalin for paraffin embedding. The remaining sample was snap frozen with liquid nitrogen and stored at -80°C.

2.3 Explant Culture Technique

Tris Buffer (TB)

Trizma Base (Sigma)	1.39g
Trizma Hydrochloride	6.06g

Volume was adjusted to one litre with distilled water. pH was adjusted to 7.4.

Tris Buffered Saline (TBS)

9 Parts Sodium Chloride solution

1 Part TB

To make one litre

Sodium chloride solution (5.256g sodium chloride)	900ml
Tris Buffer	100ml

Dissociation buffer

Collagenase Type IV (Sigma)

Enzymatic digestive used in solution of TBS to digest explants prior to plating. 1% solution made in sterile TBS and filtered prior to use. Stored at -20°C.

Wash Buffer

Sterile TBS pH 7.4

Gentamicin	50µg/ml
Amphotericin	10µg/ml

Culture Medium

DMEM

FC III 10%

Gentamicin 50µg/ml

Amphotericin 2.5µg/ml

L-Glutamine 2mM

The Mothersill outgrowth assay¹⁹⁰, optimised to culture the gynaecological samples²⁸⁰ was used to culture the specimens. Tissue specimens were aseptically chopped into 1-2mm³ fragments using paragon sterile single use scalpels No. 22 blades (Maersk Medical Ltd.) and incubated for 30 mins at 37°C in dissociation buffer. This was then centrifuged at 1200rpm for 5 mins and the supernatant removed. Wash buffer was then added and the centrifugation repeated for 4 washes of 5 minutes each. The digested explants were then plated in 25cm² costar culture flasks (0.2µm vented filter cap) and 2mls of culture medium added. Care was taken not to dislodge the explants with the culture medium but to ensure sufficient media surrounded each explant. Flasks were incubated at 37°C and 5% CO₂. Cell growth was monitored after seven days and only flasks with epithelial cells were kept, flasks with fibroblasts were discarded.

2.4 Trypsinisation

Following establishment of sufficient cell growth, after 7-10 days, the flasks were trypsinised using Trypsin-Ethylenediaminetetra-acetic acid (EDTA) (TE) in HBSS without calcium and magnesium, with EDTA.4Na (Gibco). One ml of TE was used to rinse each flask for 30 seconds to remove any traces of media. The flasks were incubated at 37°C with 2mls of TE for 10mins. 2mls of media was added to each flask. The cell suspension was filtered through a 100µm sterile nylon cell strainer (Falcon) to remove the explants. The cells were examined for viability and counted using Trypan blue exclusion dye (0.4%) (Sigma).

2.5 MTS Cytotoxicity Assay

Celltiter 96[®] Aqueous non-radioactive cell proliferation assay (Promega).

The kit contains an MTS solution and a PMS solution. The PMS can be added to the MTS the first time the products are thawed and then aliquoted and stored at -20°C.

Cells, at a concentration of 1×10^5 cells / ml were stirred using a magnetic stirrer and 100µl of cells were seeded onto each well of a 96-well Nunclon TM Surface plate (Nunc). These were left to adhere overnight at 37°C and 5% CO₂. Chemotherapy drugs to be tested were frequently chosen by the clinician in an attempt to predict the *in vivo* sensitivity of the tumour to the drug. Drugs were tested which were likely to be administered *in vivo* or to which the patient had already received, to confirm resistance. 100µl of appropriate cytotoxic drugs were added at concentrations varying from 0.5 x peak plasma concentration (PPC) of the drug to 10 x PPC. The peak

plasma concentrations^{199 281} of the drugs are shown in Table 2.4. Drug concentrations were added in triplicate or quadruplicate. Control wells were incubated with 100µl of culture medium. Blank wells were incubated with media and drugs only, without cells. A typical plate setup can be seen in Figure 2.1. The plate was incubated for a further 72 hours. The cells were examined under a microscope to assess cell viability by eye. The MTS cytotoxicity assay was carried out by adding 40µl of the MTS solution to each well of the 96-well plate, including controls and blanks. This gave a concentration of 333µg/ml MTS and 25µM PMS in the assay. The plate was incubated at 37°C for a further 4 hours to allow the tetrazolium salt to be converted to a soluble formazan product. Optical density (OD) was then read at 490nm using a Dynex Technologies MRX[®] microplate reader. A reference wavelength of 650nm was used. Calculations were carried out using Revelations[®] for Windows, Version 3.04, 1997, Virginia, USA: Dynex Technologies Inc. The surviving fraction of cells was calculated for each assay by the formula: Percent surviving cells= OD test sample / OD control sample x 100. The PPC of the drugs was graphed against the % cell survival using Fig.P[®] for Windows, Version 2.98, 1999, Durham, USA: Fig.P Software Corporation. The concentration of most interest was 1 x PPC and a 50% or less cell survival was desirable at this concentration¹⁴⁵. A typical response curve is shown in Figure 2.2. The IC50 (inhibitory concentration 50 or concentration giving 50% cell kill) was calculated for the drugs and expressed as a factor of the PPC.

Retrospective *in vitro* – *in vivo* correlations were carried out. The patients' charts were monitored every 3-6 months during and after chemotherapy treatment to determine the *in vivo* patient response. All patients were followed up until they died or until January 2003. Sensitive was defined as having a good response to

chemotherapy and remaining disease free for at least 12 months. Exceptions to this were patients who had received neoadjuvant chemotherapy, which significantly reduced the size of their tumour. The neoadjuvant regimen was then tested *in vitro* to assess the response. In addition to this exception were patients who demonstrated a very good initial response to the chemotherapy but may have recurred within the year or those who responded to the chemotherapy but died from another cause.

Resistant was defined as a recurrence of the disease or progressive disease within 12 months. Recurrence was defined as demonstrable disease both by clinical and ancillary examinations. Exceptions to the resistant definition were patients with recurrent disease who had previous chemotherapy and had repeat surgical treatment. Other exceptions included patients sent to the hospice for palliative care where the only follow-up was date of death. If a patient had to change their chemotherapy regimen due to allergies this was taken into consideration when determining the *in vivo* response. Patients who had a recurrence of extensive disease shortly after the 12-month cut-off were also considered resistant.

Table 2.4 Peak plasma concentration (PPC) of cytotoxic drugs

Drug	Manufacturer	PPC (µg/ml)
Cisplatin	(David Bull Laboratories)	2.5
Paclitaxel	(Bristol-Myers Squibb)	6.8
Carboplatin	(David Bull Laboratories)	25
Topotecan	(Smithkline Beecham)	1.25
Fluorouracil	(David Bull Laboratories)	50
Doxorubicin	(David Bull Laboratories)	0.6
Mitomycin	(Kyowa)	0.52
Bleomycin	(Bristol-Myers Squibb)	2
Etoposide	(Bristol-Myers Squibb)	30
4-Hydroxycyclophosphamide	(Asta Medica)	1

Note: Liver enzymes activate cyclophosphamide so the alternative was to use the active metabolite of this drug (4-hydroxycyclophosphamide), which was kindly donated by the company.

C10	C1	P10	P1	CB10	CB1	T10	T1	D10	D1	C+P	MED
C10	C1	P10	P1	CB10	CB1	T10	T1	D10	D1	C+P	MED
C10	C1	P10	P1	CB10	CB1	T10	T1	D10	D1	C+P	MED
C10	C1	P10	P1	CB10	CB1	T10	T1	D10	D1	C+P	MED
C2	C.5	P2	P.5	CB2	CB.5	T2	T.5	D2	D.5	CB+P	BLANK
C2	C.5	P2	P.5	CB2	CB.5	T2	T.5	D2	D.5	CB+P	BLANK
C2	C.5	P2	P.5	CB2	CB.5	T2	T.5	D2	D.5	CB+P	BLANK
C2	C.5	P2	P.5	CB2	CB.5	T2	T.5	D2	D.5	CB+P	BLANK

Figure 2.1 Typical Plate Set-up

Abbreviations:

C: Cisplatin, 10 x PPC, 2 x PPC, 1 x PPC 0.5 x PPC

P: Paclitaxel

CB: Carboplatin

T: Topotecan

D: Doxorubicin

C+P: Cisplatin and Paclitaxel

CB+P: Carboplatin and Paclitaxel

MED: Culture medium

Blank: No cells, just culture medium and drugs

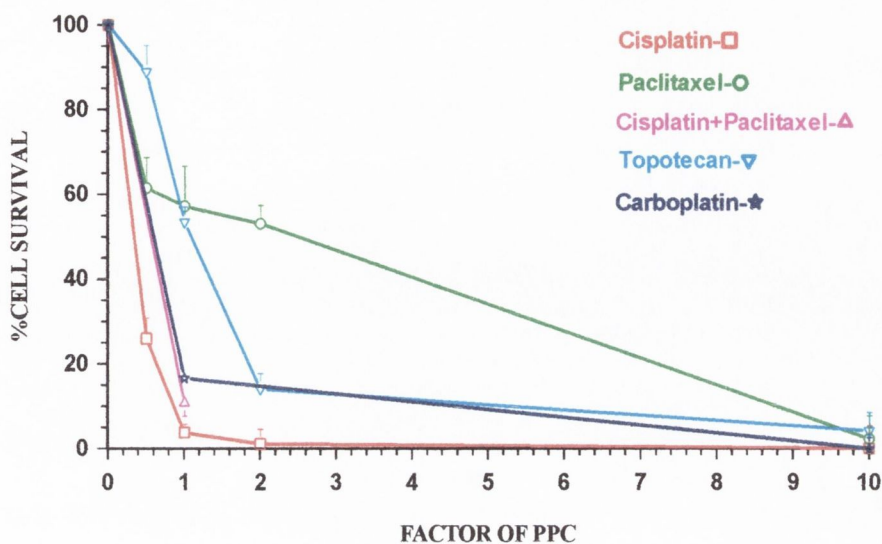


Figure 2.2 Typical Response Curve

2.6 Paraffin Embedding Of Tumour Specimens

Specimens were placed in 10% neutral buffered formalin for at least 48 hours. Specimens were processed overnight in a vacuum infiltration processor in the routine pathology laboratory. Specimens were dehydrated with alcohols, which were then removed by xylene solutions, and then specimens were impregnated with wax. The specimens were then embedded in paraffin wax.

2.7 3-Aminopropyltriethoxysilane (APES) Coated Slides

Slides (Menzel-Glaser) were washed in distilled water and rinsed in absolute alcohol and dried for one hour. A 2% solution of APES (Sigma) in absolute alcohol or acetone was made. Slides were dipped 5 times in APES solution, then dipped twice in

absolute alcohol / acetone, and twice in distilled water. Slides were dried at 60°C in a Gallenkamp[®] hotbox oven.

2.8 Haematoxylin and Eosin Staining of Sections

Sections were cut at 4µm and placed on APES coated slides. The sections were de-waxed and stained according to the following protocol:

1. Xylene (BDH) for 3 mins
2. Xylene for 3 mins
3. 100% Alcohol (BDH) for 3 mins
4. 100% Alcohol for 2 mins
5. 70% Alcohol, made in distilled water, for 3 mins
6. Distilled water for 3 mins
7. Mayer's Haemalum (BDH) for 1 min
8. Warm running tap water for 5 mins
9. 70% Alcohol (drop of Hydrochloric acid(HCl)) for 3 mins
10. Tap water for 5 mins
11. 1% (made in distilled H₂O) eosin Y (Sigma) for 2 mins
12. Tap water for 90 secs
13. 100% Alcohol for 2 mins
14. 100% Alcohol for 2 mins
15. Xylene for 3 mins
16. Slides were mounted in DPX (BDH) DPX is a mixture of distyrene (a polystyrene), a plasticiser (tricresyl phosphate), and xylene

2.9 Immunostaining of Tissue Sections

Immunohistochemistry staining kit (DAKO EnVision™+System, Peroxidase (3,3'-diaminobenzidine) (DAB), mouse) consisting of:

- Peroxidase block (0.03% hydrogen peroxide containing sodium azide)
- Labelled polymer (peroxidase labelled polymer conjugated to goat anti-mouse immunoglobulins in Tris-HCl buffer containing carrier protein and an anti-microbial agent)
- Buffered substrate (buffered substrate solution, pH 7.5, containing hydrogen peroxide and a preservative)
- DAB+ Chromogen solution
(1ml of buffered substrate and 1 drop of DAB + Chromogen are mixed thoroughly prior to use)

Monoclonal Primary Antibodies

CD31 Clone JC/70A CP50 (Oncogene)

Clone JC/70A is a mouse monoclonal antibody generated by immunising mice with a membrane preparation of a human spleen with hairy cell leukaemia and fusing Bagg albino breed/colour (BALB/c) splenocytes with p3-NS-1/Ag4-1 (NS1) mouse myeloma cells. Formulated using 0.5ml of tissue culture supernatant containing 0.1% sodium azide. Clone JC/70A recognises a 100kDa glycoprotein in endothelial cells and 130kDa glycoprotein in platelets.

VEGF(Ab-3) Clone 14-124 GF25 (Oncogene)

Clone 14-124 is a mouse monoclonal antibody generated by immunising BALB/c mice with a peptide from the N-terminus region of VEGF₁₆₅ and fusing with SP2/0. 100µg of purified antibody in 1.0ml of 0.05M sodium phosphate buffer containing 0.1% sodium azide and 0.2% gelatin. VEGF (Ab-3) recognises VEGF₁₂₁, VEGF₁₆₅, VEGF₁₈₉ and VEGF₂₀₆.

P170-Glycoprotein, Multidrug-resistance related, clone JSB-1 (Monosan Mon 9011)

Clone JSB-1 is a murine monoclonal antibody. It is supplied as concentrated tissue culture supernatant with approximately 250µg immunoglobulin/ml and 1% BSA and 0.1% sodium azide. The hybridoma cell line was obtained by fusion of lymph node cells from an immunised mouse (inbred Balb/C strain) with SP2/0 mouse myeloma cells. The culture medium was RPMI-1640, supplemented with Nutridoma-SR (Boehringer, Indianapolis, USA). The medium did not contain serum or added enzymes. The antibody JSB-1 reacts with a conserved cytoplasmic epitope of the plasma membrane-associated 170-180kD glycoprotein.

4µm sections on APES coated slides were dewaxed and stained according to the following protocol:

1. Xylene for 3 mins
2. Xylene for 3 mins
3. 100% alcohol for 3 mins
4. 100% alcohol for 2 mins

5. 70% alcohol for 3 mins
6. Distilled water for 3 mins
7. Sections were pretreated with a Tefal[®] pressure cooker
 - 1600mls of distilled water was placed in the pressure cooker
 - 15mls of antigen unmasking solution (Vector Laboratories, Burlingame, California) was added
 - This was brought to the boil on a Cookworks[®] double boiling ring without totally closing lid and using the 13lb pressure regulator (red)
 - Slides were placed in the pressure cooker ensuring no drying out occurred
 - The lid was closed and when all the air had been removed (steady steam, noise) the valve was closed (red line in line with the red arrow) and the pressure was allowed to build up
 - When correct pressure was achieved (strong hissing) the hot plate was turned off and the slides were incubated for 1 min
 - The pressure cooker was placed under cold water and then pressure released (turn regulator to open position)
 - The slides were transferred to cold running water to prevent the sections from drying out
 - Sections were placed in Tris buffered saline pH 7.4
8. Buffer was removed and each slide was wiped
9. Peroxidase block was applied to cover the specimen and incubated for 10 mins

10. Slides were rinsed with TBS and placed in a fresh buffer bath for 3 mins
11. Excess buffer was tapped off and the slides wiped
12. Primary antibody or negative control reagent (antibody diluent) was added to cover the specimen and incubated for 30 mins. Antibodies were diluted using 1% Bovine Serum Albumin (Sigma) made up in TBS. Dilutions were made up as follows:

CD31 was diluted 1:50.

VEGF was diluted 1:20.

MDR was diluted 1: 20.

13. Slides were rinsed gently with buffer and placed in a fresh buffer bath for 3 mins
14. Excess buffer was tapped off and the slides wiped
15. Labelled polymer was applied to cover the specimen for 30 mins
16. Slides were rinsed as in step 10 & 11 for 5 mins
17. DAB and substrate chromogen was applied to cover the specimen for 5-10mins
18. Slides were gently rinsed with distilled water (substrate chromogen waste was collected in hazardous materials container)
19. Slides were immersed in Mayer's Haemalum for 1 min
20. Slides were placed under warm running tap water for 5 mins

Slides were then washed in the following solutions:

21. 70% alcohol (drop of HCl) for 3 mins
22. 70% alcohol for 2 mins
23. 100% alcohol for 2 mins
24. 100% alcohol for 2 mins

25. Xylene for 3 mins

26. Slides were mounted in DPX

Positive control slides for CD31 were from an appendix section. Positive controls for VEGF and MDR were from a gastric tumour.

2.10 Quantification of Staining

It was decided to quantify all the sections by the same technique. Sections were scored from 0 to 2 based on the degree of staining. 0- no staining, 1- low degree of staining (< 50% of section), 2- high degree of staining (>50% of section stained).

Various methods exist for measurement of MVD and we used a semiquantitative grading method in preference to the vascular hot spot technique^{12 13}. The obvious advantage of MVD grading is its time efficiency but also we used the same technique for grading VEGF and MDR sections. Several studies have reported a positive correlation between quantitative and semiquantitative MVD scores^{13 282}.

2.11 Statistics

Cytotoxicity Assay

Treated wells were assayed at least in quadruplicate and results were expressed as mean percentage of surviving cells. Error bars represent the coefficient of variation (the standard deviation divided by the mean, multiplied by 100 to give a percentage) for the findings in the replicate wells. Coefficients of less than 12% were regarded as acceptable. An additional control was generated by means of the dose response curve. The sensitivity²⁸³ of the assay was calculated by $[(\text{true positives})/(\text{true positives} + \text{false negatives})]$. The specificity was calculated by $[(\text{true negative})/(\text{true negative} + \text{false positives})]$

Correlations

Statistics were performed using SPSS for Windows, Rel. 11.0.1. 2001. Chicago: SPSS Inc. The criterion of statistical significance applied was $p < 0.05$. For statistical evaluation, Pearson correlation, one-way ANOVA and Chi Square were used for univariate analysis.

Pearson correlation

The correlation between two variables reflects the degree to which the variables are related. Pearson correlation can be used to determine the association between two parametric continuous variables.

One-way Analysis of variance (ANOVA)

One-way ANOVA estimates the effects of an independent factor on a dependent or response variable in a set of parametric data. ANOVA can be used to determine if an association exists between a categorical and a continuous variable.

Chi-Square

Chi-Square is a non-parametric test of statistical significance for bivariate tabular analysis. Typically, the hypothesis tested with Chi-Square is whether or not two different samples are different enough in some characteristic or aspect of their behaviour that we can generalise from the samples that the populations from which the samples are drawn are also different in the behaviour or characteristic. Chi-Square can be used to determine if an association exists between two non-continuous variables.

Survival Curves

Survival analysis takes the survival times of a group of subjects and generates a survival curve, which shows how many of the members remain alive over time. Survival time is usually defined as the length of the interval between diagnosis and death, although other "start" events (such as surgery instead of diagnosis), and other "end" events (such as recurrence instead of death) are sometimes used. The major mathematical complication with survival analysis is that you usually do not have the luxury of waiting until the very last subject has died of old age; you normally have to

analyse the data while some subjects are still alive. Also, some subjects may have moved away, and may be lost to follow-up. In both cases, the subjects were known to have survived for some amount of time but you don't know how much longer they might ultimately have survived. Several methods have been developed for using this "at least this long" information to preparing unbiased survival curve estimates, the most common being the method of Kaplan and Meier²⁸⁴. The Kaplan-Meier procedure is a method of estimating time-to-event models in the presence of censored cases. Censored cases are cases for which the second event is not recorded. The Kaplan-Meier model is based on estimating conditional probabilities at each time point when an event occurs and taking the product limit of those probabilities to estimate the survival rate at each point in time. Progression free survival was determined as the survival time (months) from the date of surgery to the time of last follow-up for those with no evidence of disease, or the number of months to time of relapse for those with evidence of progressive disease or a recurrence after surgery and treatment. Patients that were still alive and free of disease were censored. Overall survival was measured in months from date of surgery, to date of last follow-up or death. Patients that were still alive were censored. Median or percentile values are quoted, where the 50th percentile refers to the survival time in months when 50% of the patients had an event. The log-rank was used for univariate analysis and a Cox proportional hazards²⁸⁵ regression model was used for multivariate evaluation of survival rates. Like Kaplan-Meier survival analysis, Cox Regression is a method for modelling time-to-event data in the presence of censored cases. However, Cox Regression allows you to include predictor variables (covariates) in your models to assess the effect of each predictor on the shape of the survival curve. The covariates can be categorical or continuous variables.

CHAPTER 3

HETEROGENEITY IN THE RESPONSE OF OVARIAN TUMOURS TO CYTOTOXIC DRUGS

3.1 Culture of Ovarian Biopsies

Chemosensitivity testing was carried out on 89 of the 113 ovarian specimens collected. A summary of these specimens is shown in Table 3.1. Fifteen (ov99-ov113) of the ovarian specimens were normal or benign samples and so no chemosensitivity testing was undertaken for these specimens. Nine of the ovarian samples (ov1, ov5, ov29, ov30, ov49, ov50, ov60, ov61, ov69) were mistakenly put into formalin in theatre and were not able to be cultured but were kept for paraffin embedding. Eighty-four of the 89 specimens were successfully cultured giving a 94.4% evaluability rate. Of the 5 (ov48, ov86, ov87, ov88, ov89) not evaluable it was found on histological examination that 2 of these (ov87 and ov 88) were not of ovarian origin and 1 was benign (ov89) so these were excluded from further calculations.

In some cases the tumour was small or the pathology was critical so the amount of tissue received was not sufficient to establish enough cultures. In these cases it was necessary to be selective in the choice of drugs tested and in the range of doses tested. Some of the specimens were examined only at the concentration of interest (1 x PPC) in this situation e.g. Fig. 3.23.

An example of an ovarian tumour growing in culture using the explant technique can be seen in fig 3.0. The dark section is the piece of tissue (explant) and the epithelial cells can be observed radiating out from the tumour tissue.

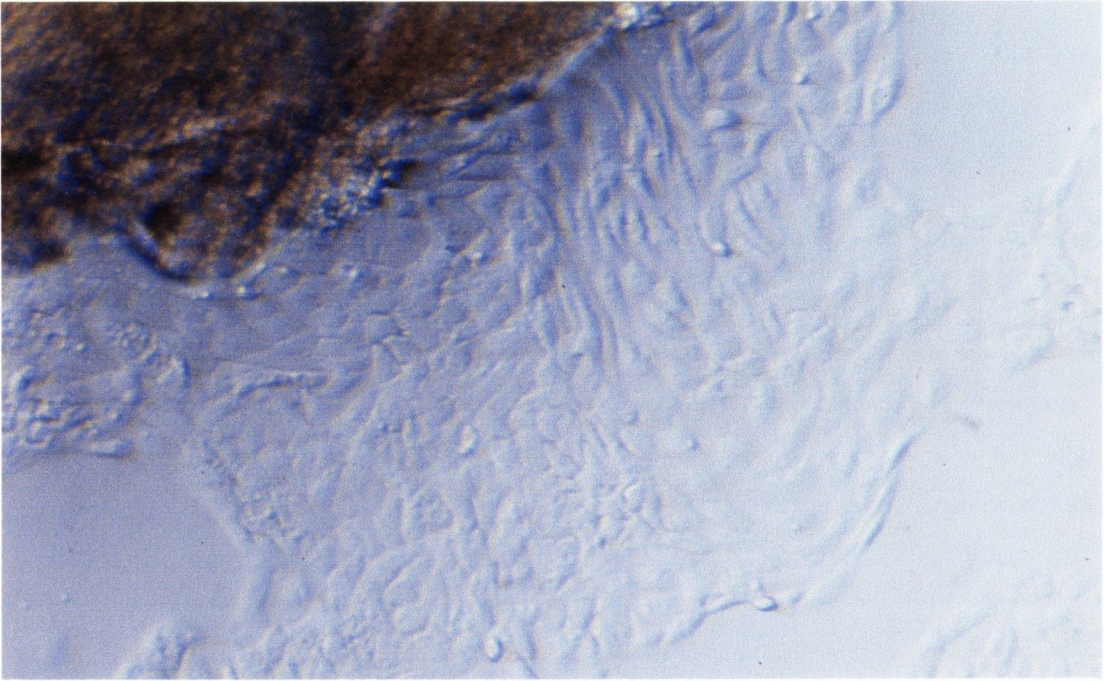


Figure 3.0 Explant culture of ovarian adenocarcinoma

Table 3.1 Ovarian specimens with *in vitro* results

<u>Tumour Type</u>	<u>Stage</u>	<u>Grade</u>	<u>Number</u>
Serous Papillary Adenocarcinoma	IC	1	1
Serous Papillary Adenocarcinoma	IC	2	2
Serous Papillary Adenocarcinoma	III	2	23
Serous Papillary Adenocarcinoma	III	3	17
Serous Papillary Adenocarcinoma	IV	2	9
Serous Papillary Adenocarcinoma	IV	3	7
Clear Cell Adenocarcinoma	ND	3	1
Clear Cell Adenocarcinoma	IC	3	1
Clear Cell Adenocarcinoma	II	3	2
Clear Cell Adenocarcinoma	IIIC	3	3
Clear Cell Adenocarcinoma	IV	ND	1
Mucinous Adenocarcinoma	IB	ND	1
Mucinous Adenocarcinoma	IIIC	2	1
Mucinous Adenocarcinoma	III	3	1
Mucinous Adenocarcinoma	IV	3	1
Primary Peritoneal Adenocarcinoma	IV	3	1
Endometrioid Adenocarcinoma	IC	3	1
Endometrioid Adenocarcinoma	II	1	1

Transitional Cell Carcinoma	II	ND	1
Borderline Mucinous	I	N/A	3
Borderline Mucinous	IIIC	N/A	2
Borderline Serous	I	N/A	4

Abbreviations: ND (Not Documented), N/A (Not Applicable)

3.2 *In Vitro* Effects of Chemotherapy on Ovarian Specimens

3.2.1 Effect of Chemotherapy on a Stage I Grade 1 Ovarian Adenocarcinoma (OV 2)

The response of a Stage I Grade 1 tumour can be seen in Fig. 3.1. This tumour was sensitive to single agent platinum and also to the platinum / paclitaxel combination. Mean survival rates varied from 3.8% for single agent cisplatin to 61.5% for paclitaxel at the PPC. The tumour responded to all the drugs at 10 x PPC. The concentrations which gave 50% kill (inhibitory concentrations -IC50s) for the individual drugs as a fraction of their peak plasma concentration were: Cisplatin (0.36 x PPC), Paclitaxel (2.49 x PPC), Topotecan (1.05 x PPC), Cisplatin & Paclitaxel (0.59 x PPC) and Carboplatin (0.6 x PPC).

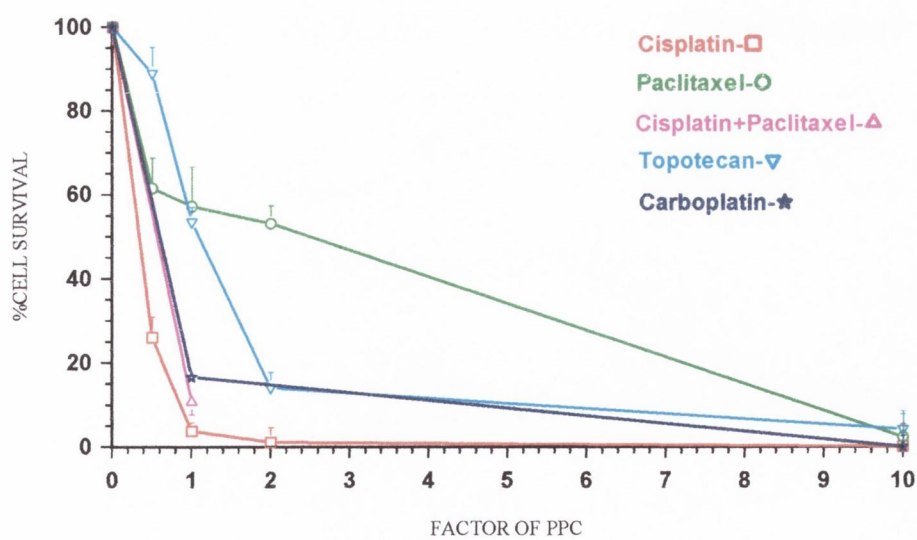


Fig. 3.1 Effect of Chemotherapy on a Stage I Grade 1 Adenocarcinoma of the Ovary

3.2.2 Effect of Chemotherapy on 2 Stage I Grade 2 Ovarian Adenocarcinomas (OV3, OV4)

Cisplatin: (Fig. 3.2) Two tumours were examined at the PPC, both responded to cisplatin. One tumour showed a 41.6% cell survival and the other 12.6%. Total tumour kill was observed at 10 x PPC for both tumours. The IC50 was 0.55 x PPC for one tumour and 0.85 x PPC for the other.

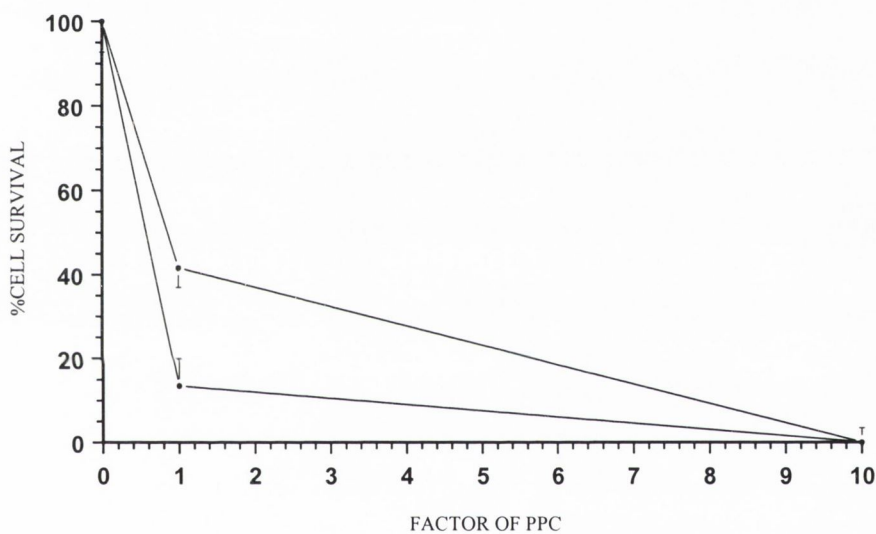


Fig. 3.2 Effect of Cisplatin on Stage I Grade 2 Ovarian Adenocarcinomas

Paclitaxel: (Fig. 3.3) The two tumours were examined with paclitaxel and one responded well at the PPC but the other was quite resistant with 100% cell survival. The IC50 was 0.9 x PPC for one and 6.5 x PPC for the other.

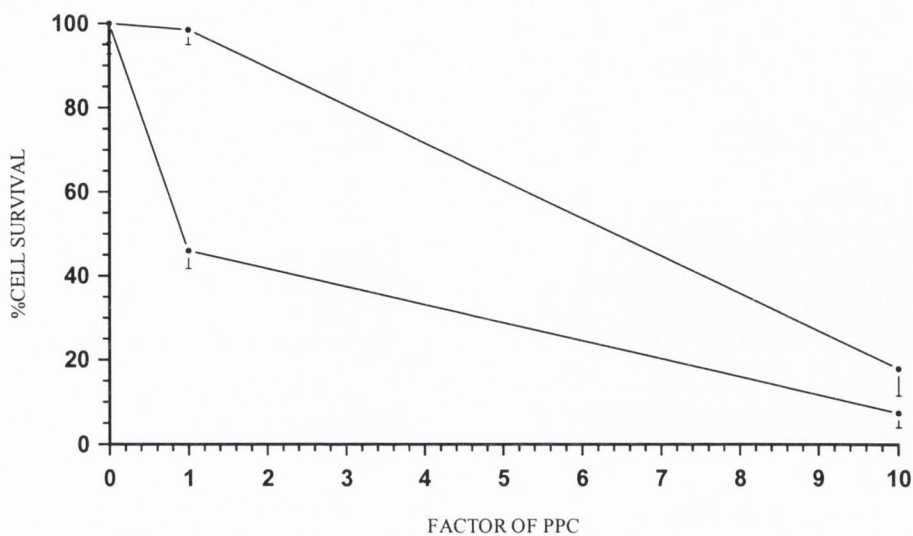


Fig. 3.3 Effect of Paclitaxel on Stage I Grade 2 Ovarian Adenocarcinomas

Cisplatin and Paclitaxel: (Fig. 3.4) One of the tumours responded at the PPC and the other just gave a borderline response of 48.1% kill. The IC₅₀ was 0.92 x PPC for the one that responded and it was greater than 1 x PPC for the other.

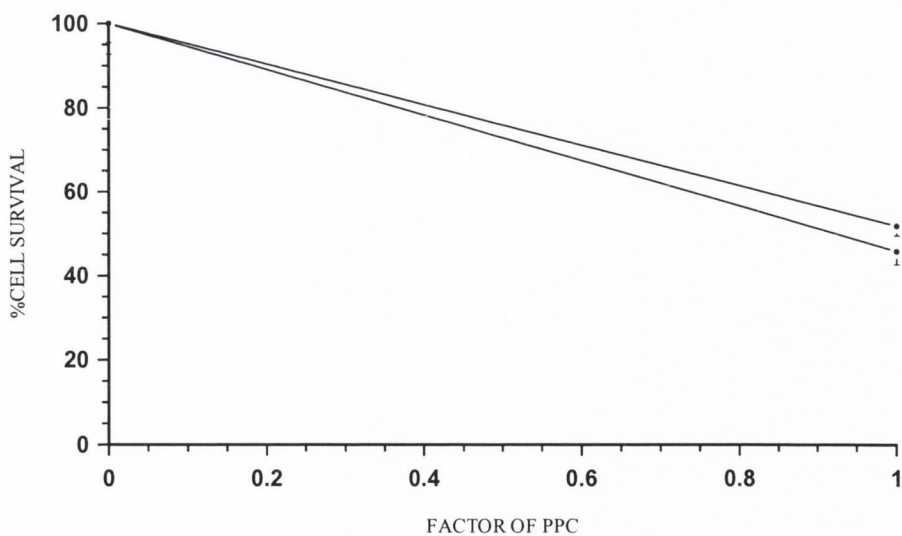


Fig. 3.4 Effect of Cisplatin & Paclitaxel on Stage I Grade 2 Ovarian Adenocarcinomas

Topotecan: (Fig. 3.5) The two tumours responded to this drug with IC50 values of 0.55 x PPC and 0.82 x PPC. One of the tumours responded really well at the PPC with only an 8.1% cell survival.

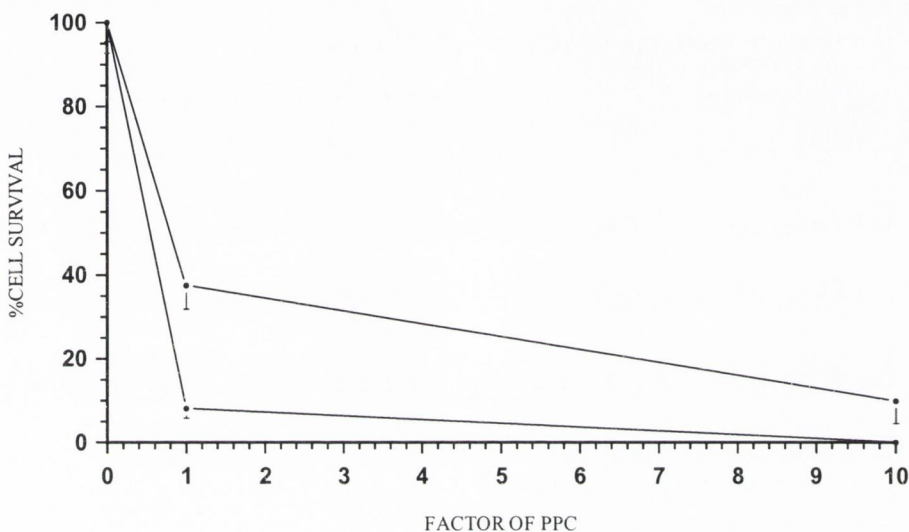


Fig. 3.5 Effect of Topotecan on Stage I Grade 2 Ovarian Adenocarcinomas

3.2.3 Effect of Chemotherapy on 23 Stage III Grade 2 Ovarian Adenocarcinomas (OV6-OV28)

Cisplatin: (Fig. 3.6) The 23 tumours examined varied in their response to cisplatin from 0% survival to 92% survival at the PPC. Some of the tumours displayed a 50% kill or greater at even 0.5 x PPC and 16 of the tumours showed a >50% kill at the PPC. All the tumours responded well at 10 x PPC. The IC50 in this category of tumours varied from 0.3 to 2.55 x PPC. The error bars have been excluded here for clarity. Fig. 3.6(a) shows a clearer picture of the response at the PPC.

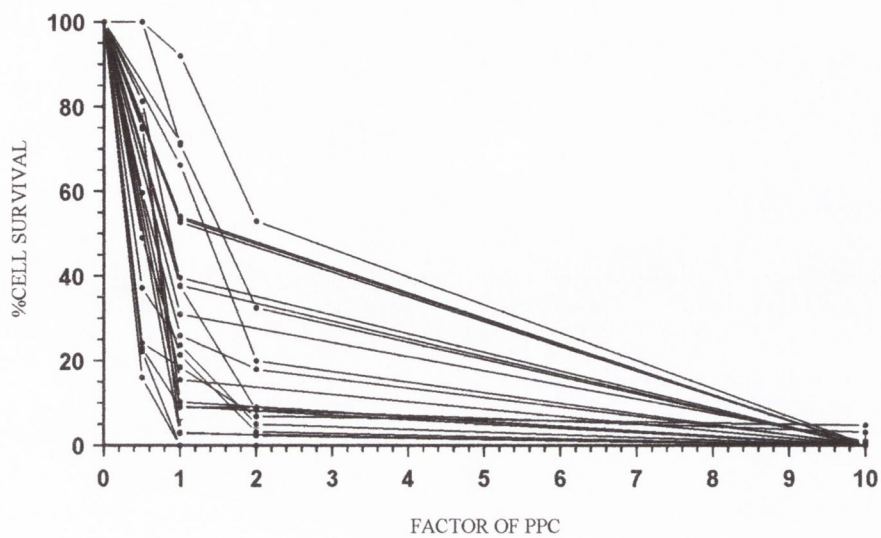


Fig. 3.6 Effect of Cisplatin on Stage III Grade 2 Ovarian Adenocarcinomas

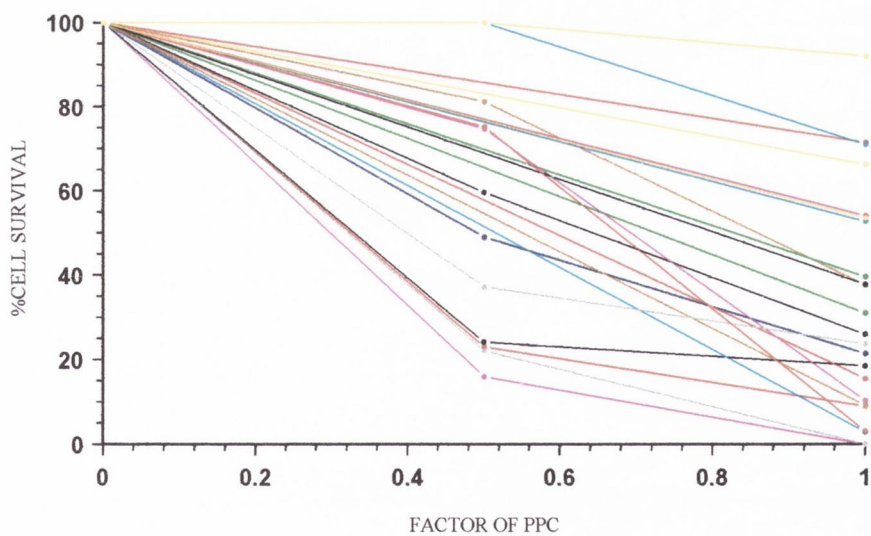


Fig. 3.6(a) Effect of Cisplatin on Stage III Grade 2 Ovarian Adenocarcinomas

Paclitaxel: (Fig. 3.7) The same tumours were examined with paclitaxel giving response rates varying from 8.3% cell survival to complete resistance at the PPC. Responses at 10 x PPC varied from 100% kill to 0% kill. The IC50 ranged from 0.41 x PPC to >10 x PPC. The individual responses are displayed in colour in Fig. 3.7(a).

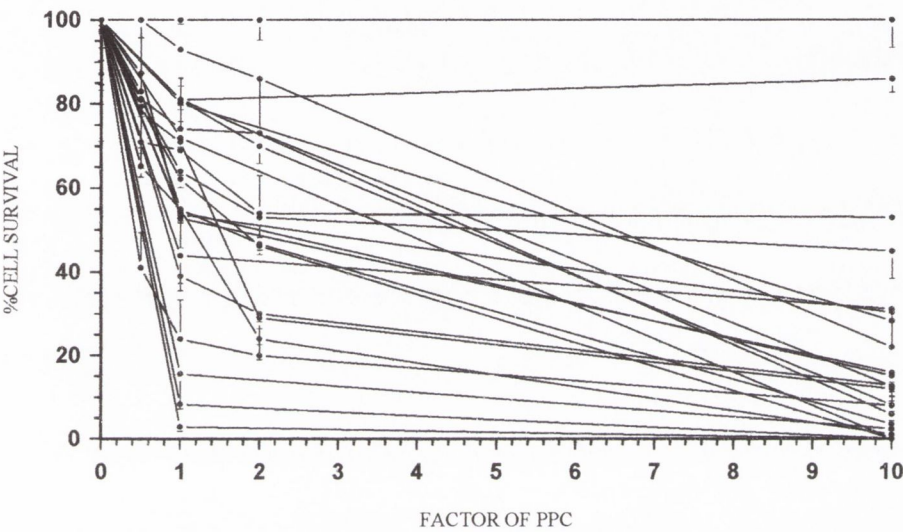


Fig. 3.7 Effect of Paclitaxel On Stage III Grade 2 Ovarian Adenocarcinomas

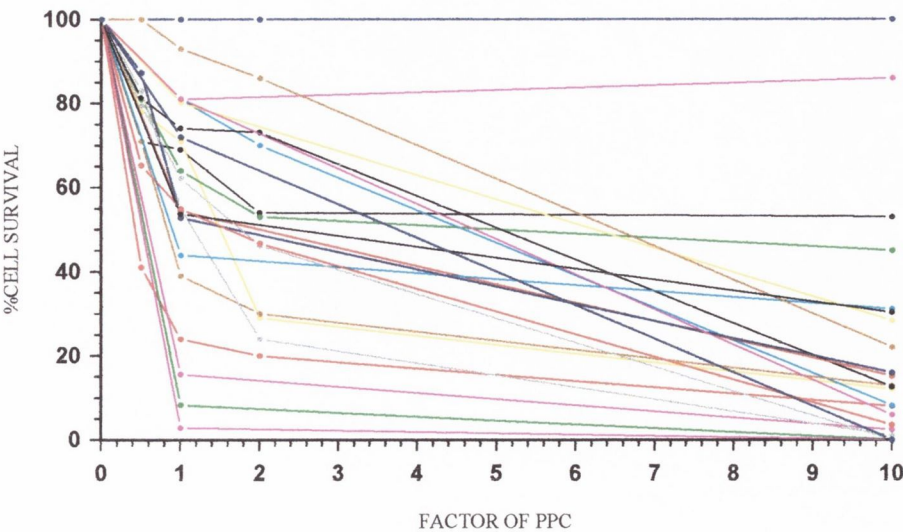


Fig. 3.7(a) Effect of Paclitaxel On Stage III Grade 2 Ovarian Adenocarcinomas

Cisplatin and Paclitaxel: (Fig. 3.8) Twenty-one of the tumours were examined with the combination of cisplatin and paclitaxel. Response rates varied from 8.3% survival to 90% survival at the PPC. This combination was only tested at the PPC. Fifteen out of the twenty-one gave a greater than 50% response at the PPC. The IC50 varied from 0.54 to >1 x PPC. A clearer picture can be seen in Fig. 3.8(a).

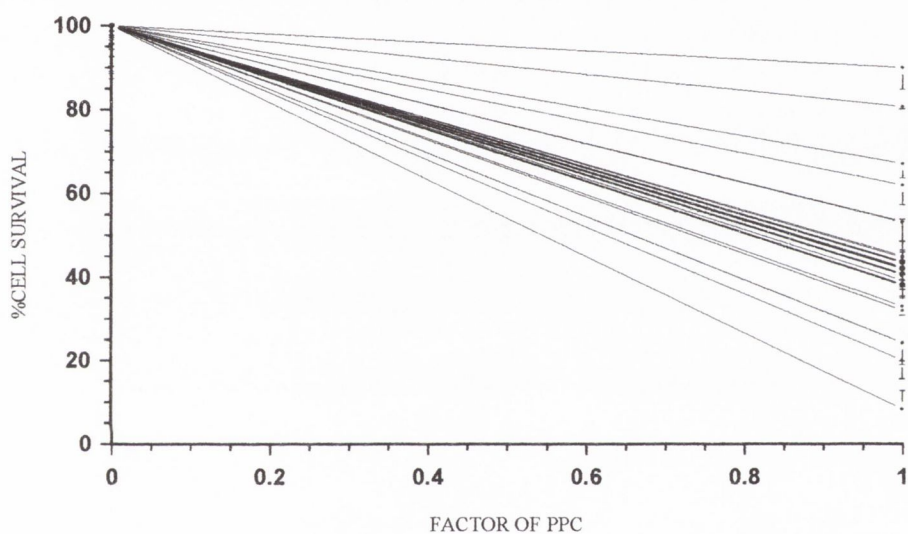


Fig. 3.8 Effect of Cisplatin & Paclitaxel on Stage III Grade 2 Ovarian Adenocarcinomas

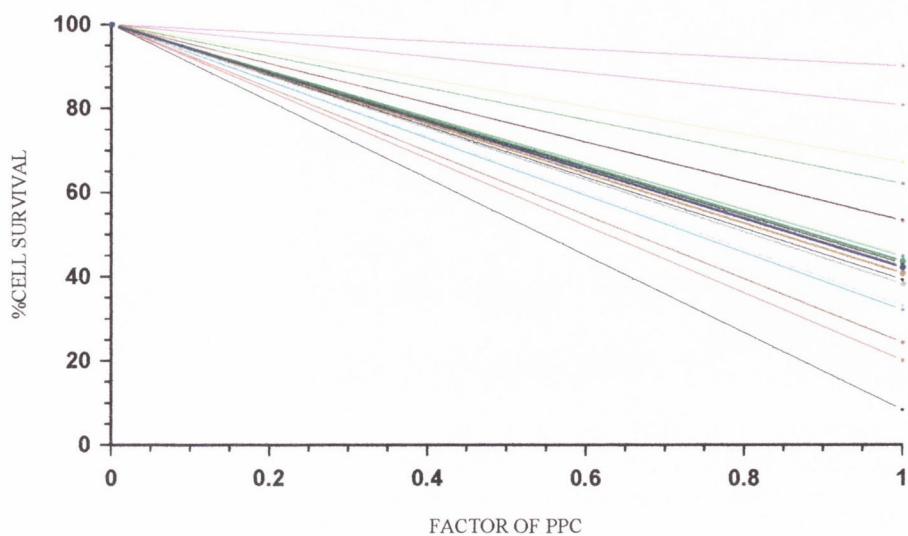


Fig. 3.8(a) Effect of Cisplatin & Paclitaxel on Stage III Grade 2 Ovarian Adenocarcinomas

Topotecan: (Fig. 3.9) Sixteen tumours were examined with topotecan and gave response rates varying from 3.8% cell survival to 68% cell survival at the PPC with 14 of the sixteen being sensitive at this concentration. The IC50 ranged from 0.38 x PPC to 2.82 x PPC. Fig. 3.9(a) shows this response in colour.

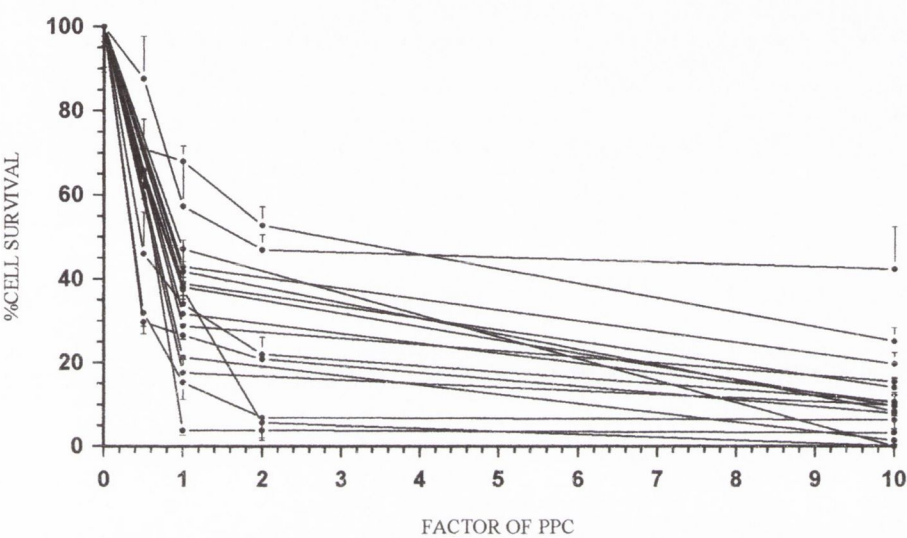


Fig. 3.9 Effect of Topotecan on Stage III Grade 2 Ovarian Adenocarcinomas

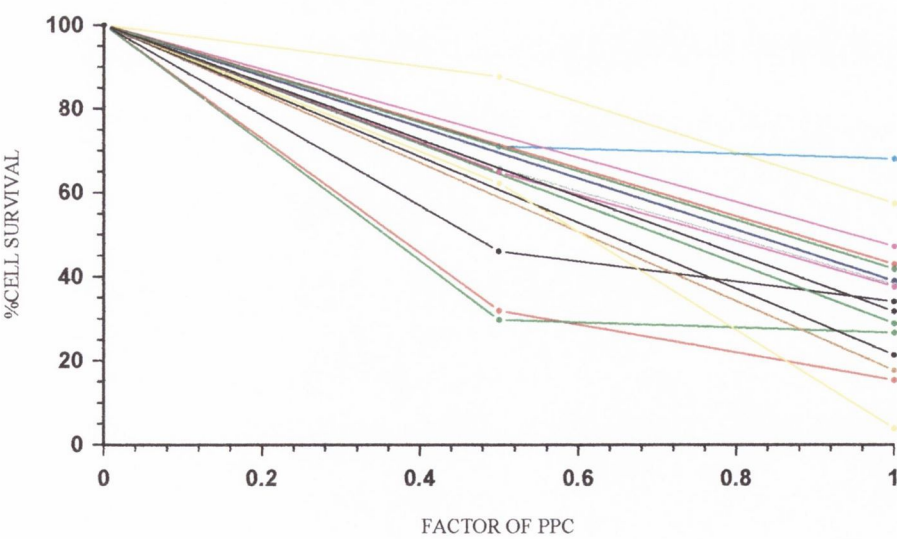


Fig. 3.9(a) Effect of Topotecan on Stage III Grade 2 Ovarian Adenocarcinomas

3.2.4 Effect of Chemotherapy on 17 Stage III Grade 3 Ovarian Adenocarcinomas (OV31-OV47)

Cisplatin: (Fig. 3.10) Seventeen tumours were examined in this group with eleven giving greater than 50% kill at the PPC and one displaying complete resistance at this concentration. One tumour was completely killed at 0.5 x PPC. All the tumours responded well at 10 x PPC. The IC50 in this category of tumours varied from 0.25 x PPC to 9.7 x PPC. A second biopsy from patient ov31 was received 5 months after the first and it displayed a similar pattern of resistance as the first biopsy. It was not included in the graphs as it was from the same patient.

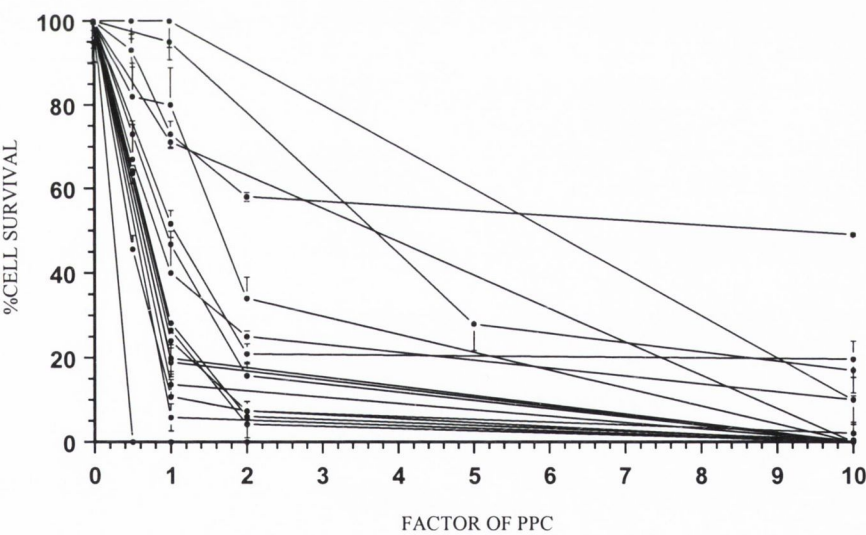


Fig. 3.10 Effect of Cisplatin on Stage III Grade 3 Ovarian Adenocarcinomas

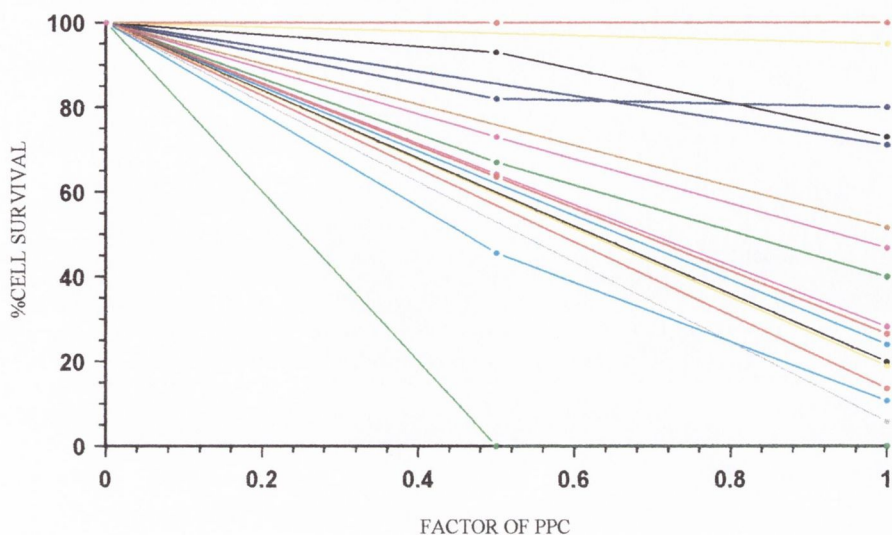


Fig. 3.10(a) Effect of Cisplatin on Stage III Grade 3 Ovarian Adenocarcinomas

Paclitaxel: (Fig. 3.11) Three out of the seventeen tumours gave a greater than 50% kill when examined with paclitaxel at the PPC while another four displayed complete resistance at this concentration. The IC₅₀ ranged from 0.39 x PPC to >10 x PPC.

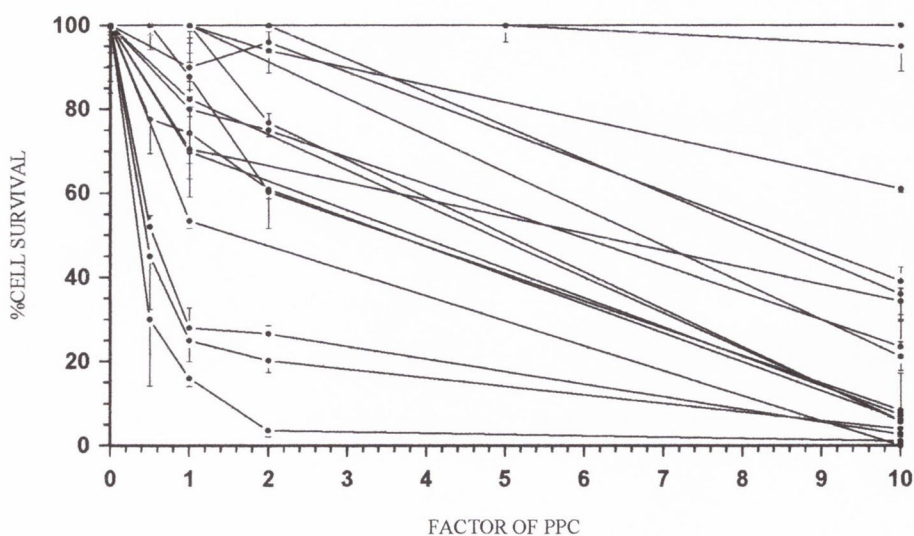


Fig. 3.11 Effect of Paclitaxel on Stage III Grade 3 Ovarian Adenocarcinomas

Cisplatin and Paclitaxel: (Fig. 3.12) The cisplatin / paclitaxel combination was tested on 13 of the tumours giving responses varying from 37% survival to 85% survival. The IC50 varied from 0.5 x PPC to greater than 1 x PPC.

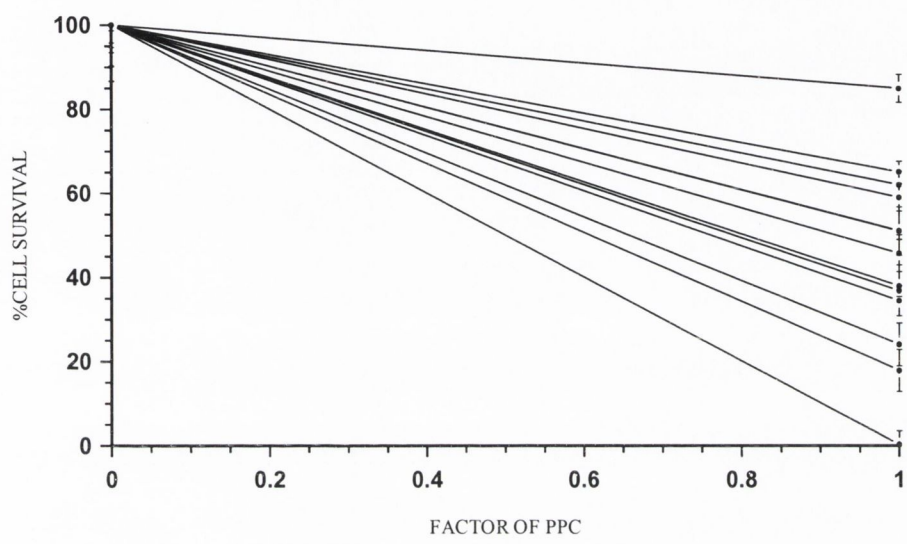


Fig. 3.12 Effect of Cisplatin & Paclitaxel on Stage III Grade 3 Ovarian Adenocarcinomas

Topotecan: (Fig. 3.13) Eleven tumours were examined with topotecan with six responding well at the PPC and three giving a good response at 0.5 x PPC. IC50 values ranged from 0.25 to greater than 10 x PPC.

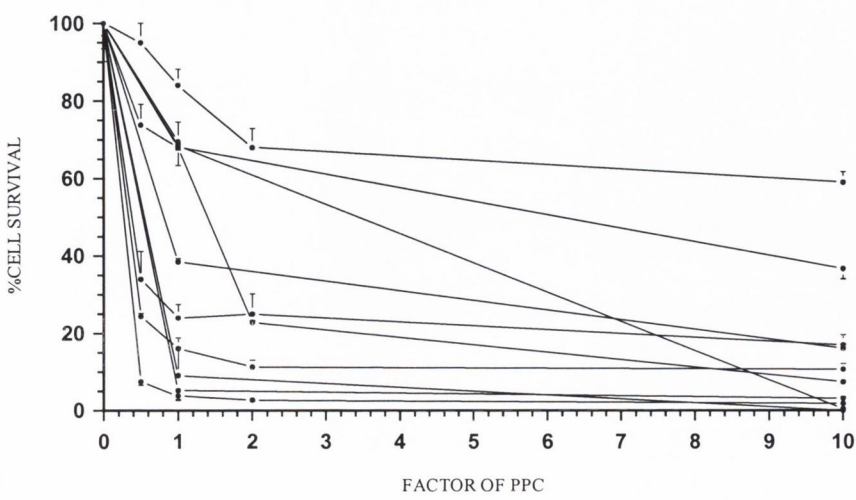


Fig. 3.13 Effect of Topotecan on Stage III Grade 3 Ovarian Adenocarcinomas

3.2.5 Effect of Chemotherapy on 9 Stage IV Grade 2 Ovarian Adenocarcinomas (OV51-OV59)

Cisplatin: (Fig. 3.14) Six of the nine tumours examined displayed a good response to cisplatin at the PPC and total cell kill was observed in five of these at 10 x PPC. The remaining three specimens were quite resistant with survival values of 84.6%, 96% and 97% at the PPC. The IC50 in this category of tumours varied from 0.41 to greater than 10 x PPC.

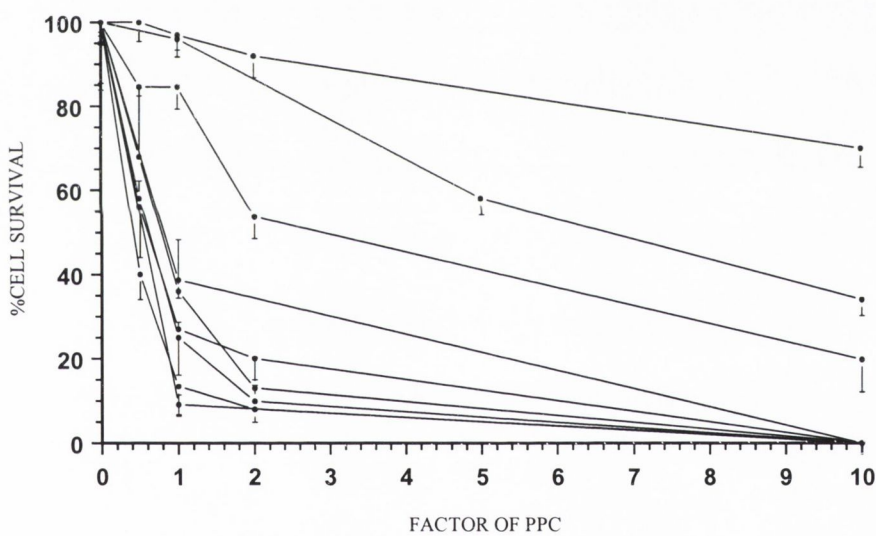


Fig 3.14 Effect of Cisplatin on Stage IV Grade 2 Ovarian Adenocarcinomas

Paclitaxel: (Fig. 3.15) Five of the nine tumours were sensitive to paclitaxel at the PPC. Response rates varied from 1.9% survival to 100% survival at this concentration. One of the tumours displayed complete resistance even at 10 x PPC. The IC50 ranged from 0.35 x PPC to >10 x PPC.

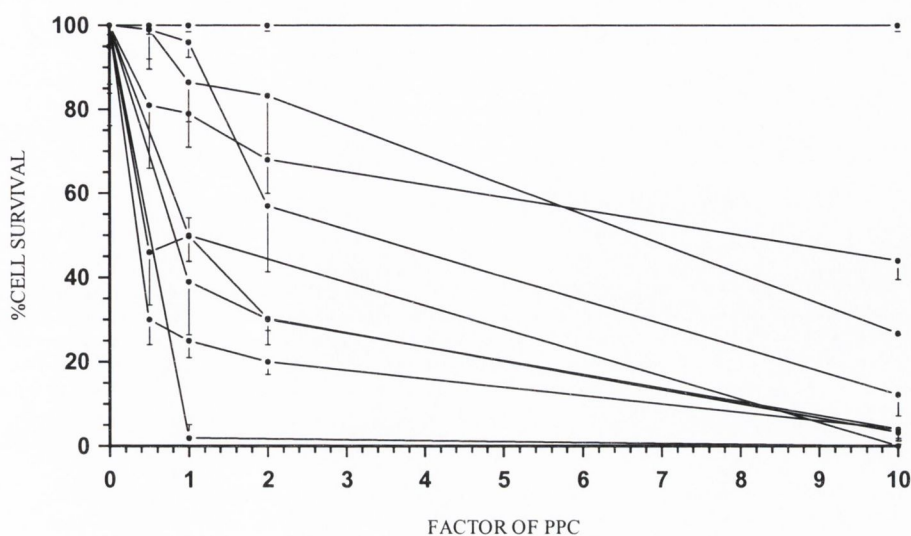


Fig 3.15 Effect of Paclitaxel on Stage IV Grade 2 Ovarian Adenocarcinomas

Cisplatin and Paclitaxel: (Fig. 3.16) The effect of the cisplatin / paclitaxel combination ranged from 20.2% cell survival to 100% cell survival at the PPC. The IC50 varied from 0.63 x PPC to greater than 1 x PPC.

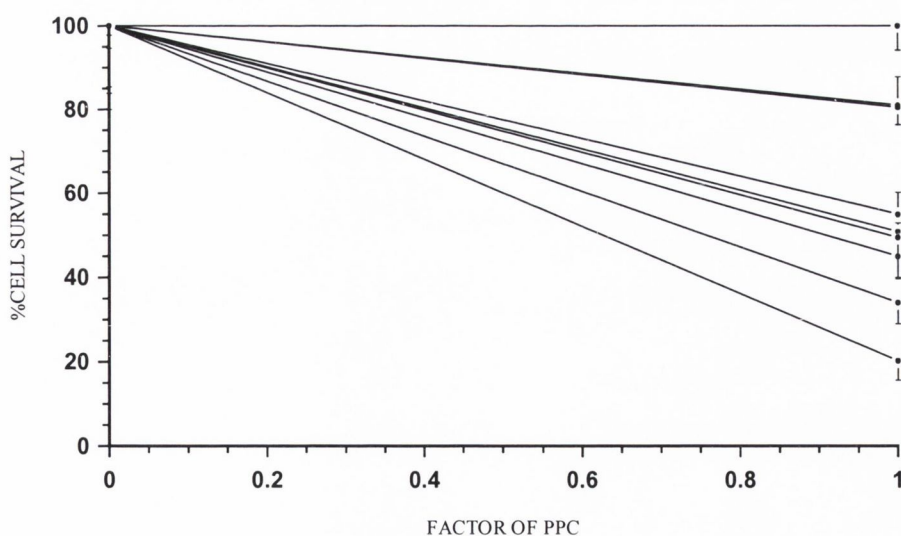


Fig 3.16 Effect of Cisplatin & Paclitaxel on Stage IV Grade 2 Ovarian Adenocarcinomas

Topotecan: (Fig. 3.17) Six of the tumours were examined with topotecan and four were sensitive at the PPC. Responses varied from 1.9% to 58.4% at the PPC. IC50 values ranged from 0.51 to 1.6 x PPC.

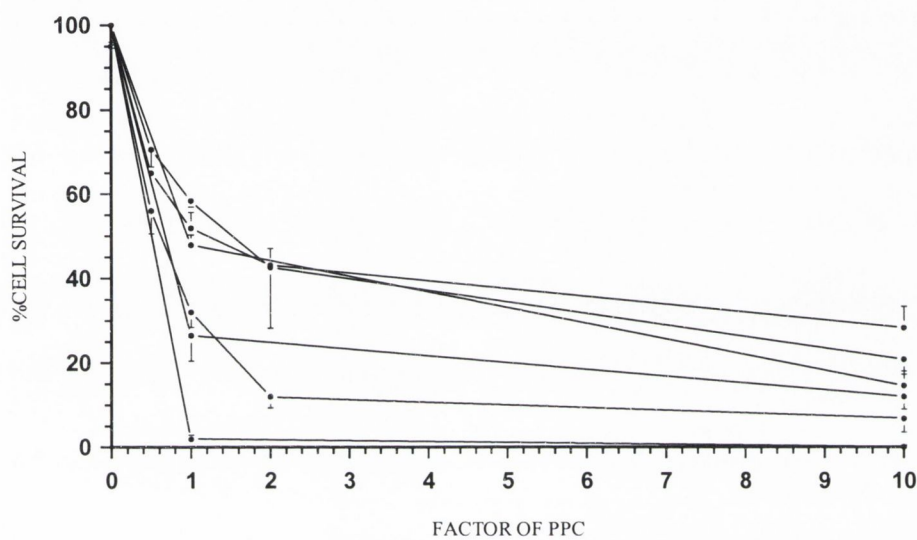


Fig 3.17 Effect of Topotecan on Stage IV Grade 2 Ovarian Adenocarcinomas

3.2.6 Effect of Chemotherapy on 7 Stage IV Grade 3 Ovarian Adenocarcinomas (OV62-OV68)

Cisplatin: (Fig. 3.18) Mean cell survival rates ranged from 3.3% to 86% for the seven tumours examined at the PPC of cisplatin. Five of these exceeded the 50% cell kill mark and at 10 x PPC the responses varied from 0% survival to 70% survival. The IC50 in this category of tumours varied from 0.48 to greater than 10 x PPC.

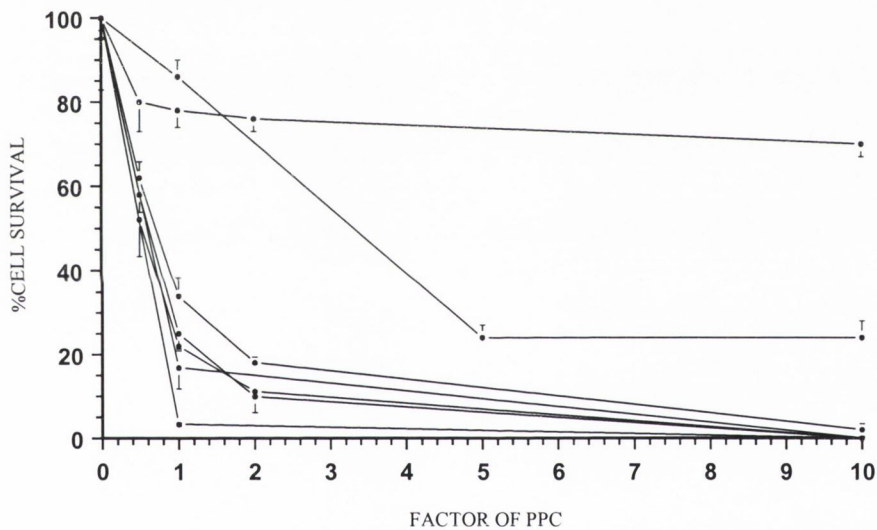


Fig 3.18 Effect Of Cisplatin On Stage IV Grade 3 Ovarian Adenocarcinomas

Paclitaxel: (Fig. 3.19) Mean survival rates varied from 39% survival to 96% survival when five of the seven tumours were examined with paclitaxel. At 10 x PPC one of these tumours was still quite resistant giving a 70% survival response. The IC50 ranged from 0.82 x PPC to >10 x PPC.

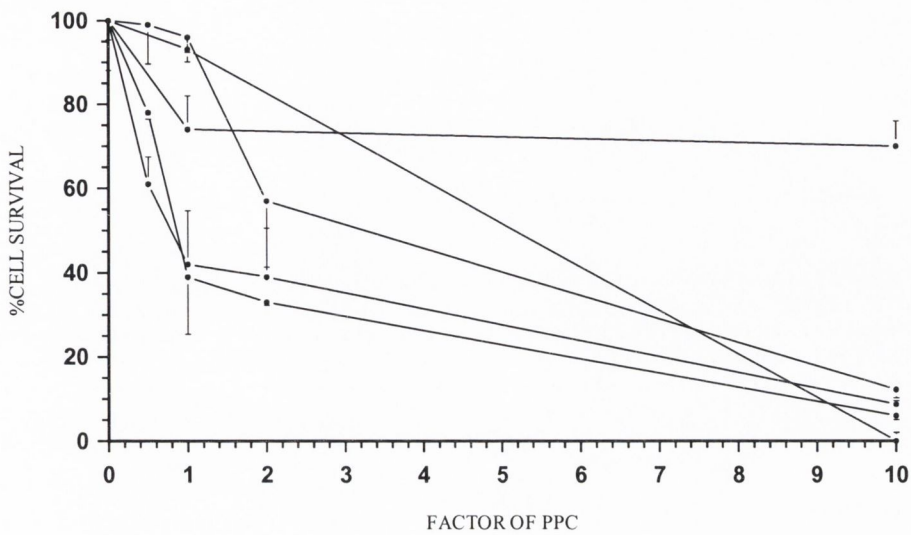


Fig 3.19 Effect Of Paclitaxel On Stage IV Grade 3 Ovarian Adenocarcinomas

Cisplatin and Paclitaxel: (Fig. 3.20) Survival rates of 28.9% to 55% were observed when these carcinomas were tested at the PPC of the cisplatin / paclitaxel combination. The IC50 varied from 0.7 x PPC to greater than 1 x PPC.

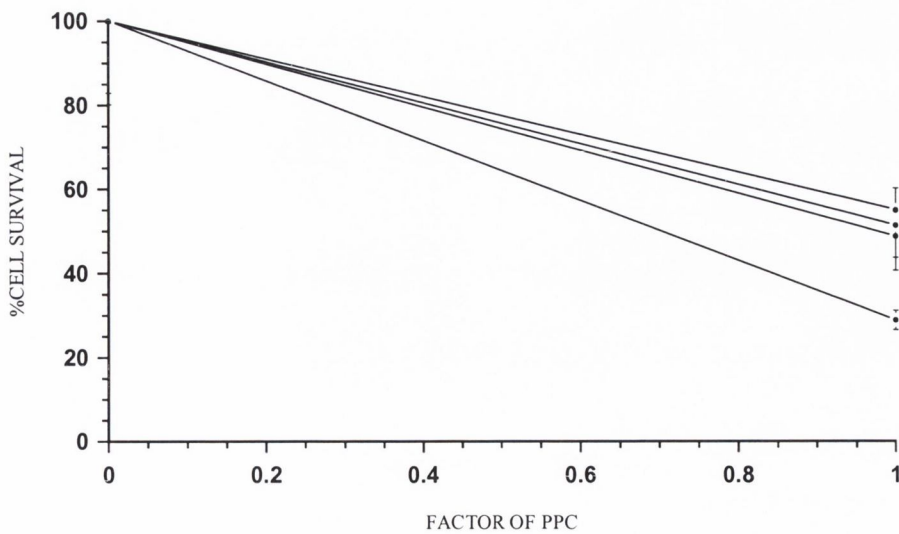


Fig 3.20 Effect of Cisplatin & Paclitaxel on Stage IV Grade 3 Ovarian Adenocarcinomas

Topotecan: (Fig. 3.21) All of the five tumours tested with topotecan were found to be sensitive at the PPC with response rates varying from total cell kill to 33% survival. Two of these responded well at 0.5 x PPC with mean survival rates of 16.03 and 17.3%. Mean survival rates at 10 x PPC ranged from 0% to 17%. IC50 values ranged from 0.31 to 0.61 x PPC.

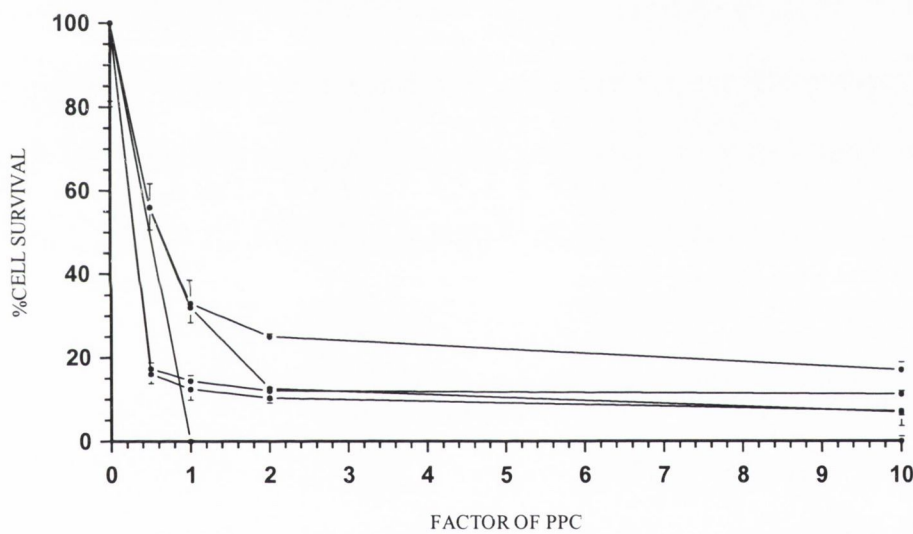


Fig 3.21 Effect Of Topotecan On Stage IV Grade 3 Ovarian Adenocarcinomas

3.2.7 Effect of Chemotherapy on 8 Clear cell Adenocarcinomas of the Ovary (OV70-OV77)

Cisplatin: (Fig. 3.22) Eight clear cell adenocarcinomas were examined with cisplatin and response rates varied from 12.2% survival to 99% survival at the PPC. Three of the tumours were sensitive at this concentration and all tumours were sensitive at 10 x PPC. The IC50 in this category of tumours varied from 0.59 to 8 x PPC.

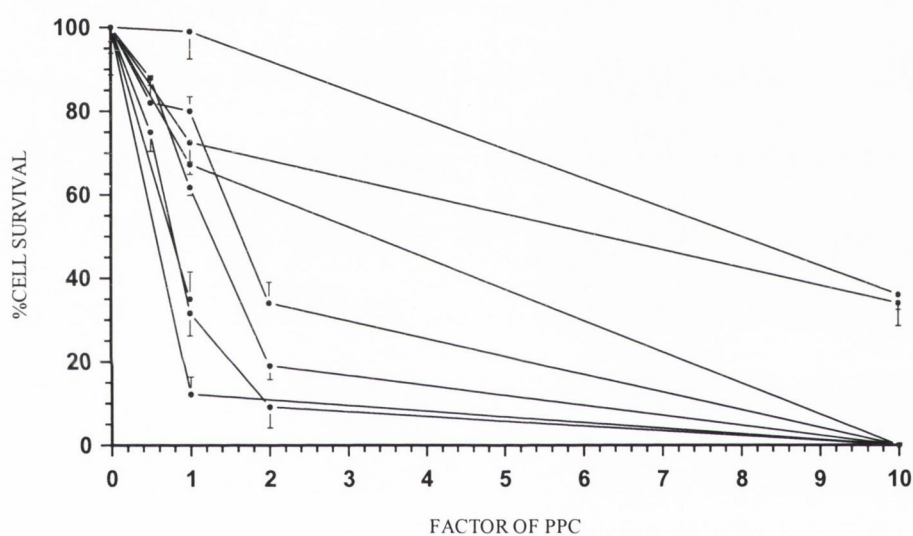


Fig. 3.22 Effect of Cisplatin on Clear Cell Adenocarcinomas of the Ovary

Paclitaxel: (Fig. 3.23). Seven of the tumours were examined with paclitaxel and mean survival rates varied from 35% to 100% at the PPC. At 10 x PPC two of the tumours remained quite resistant to the drug resulting in survival values of 73.8% and 81.2%. The IC50 ranged from 0.79 x PPC to >10 x PPC.

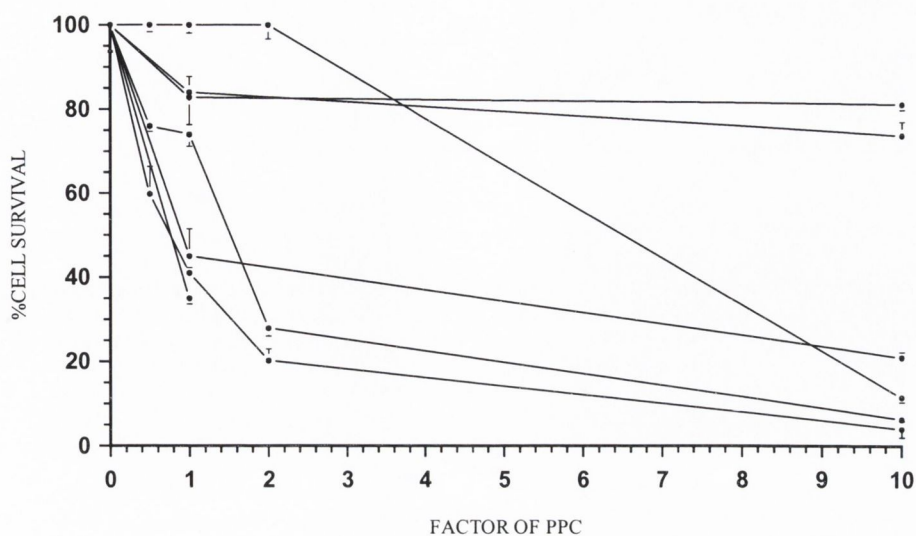


Fig. 3.23 Effect of Paclitaxel on Clear Cell Adenocarcinomas of the Ovary

Cisplatin and Paclitaxel: (Fig. 3.24) Survival rates of 29% to 72.9% were observed when these carcinomas were tested at the PPC of the cisplatin / paclitaxel combination. Four of the tumours were sensitive to the combination at the PPC. The IC50 varied from 0.71 x PPC to greater than 1 x PPC.

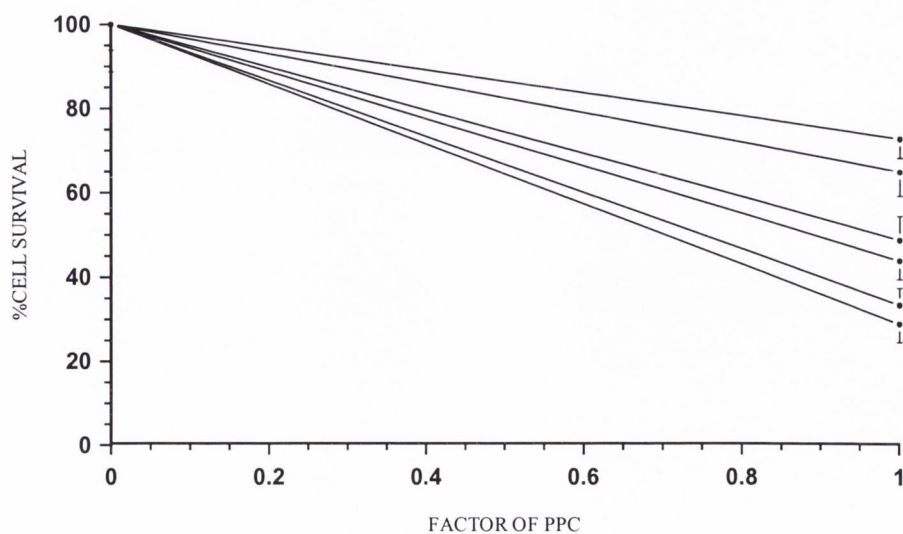


Fig. 3.24 Effect of Cisplatin & Paclitaxel on Clear Cell Adenocarcinomas of the Ovary

Topotecan: (Fig. 3.25) All of the five tumours tested with topotecan were found to be sensitive at the PPC with response rates varying from 24.7% to a borderline response of 49.8%. Two of these were sensitive at 0.5 x PPC. IC50 values ranged from 0.45 to 0.99 x PPC.

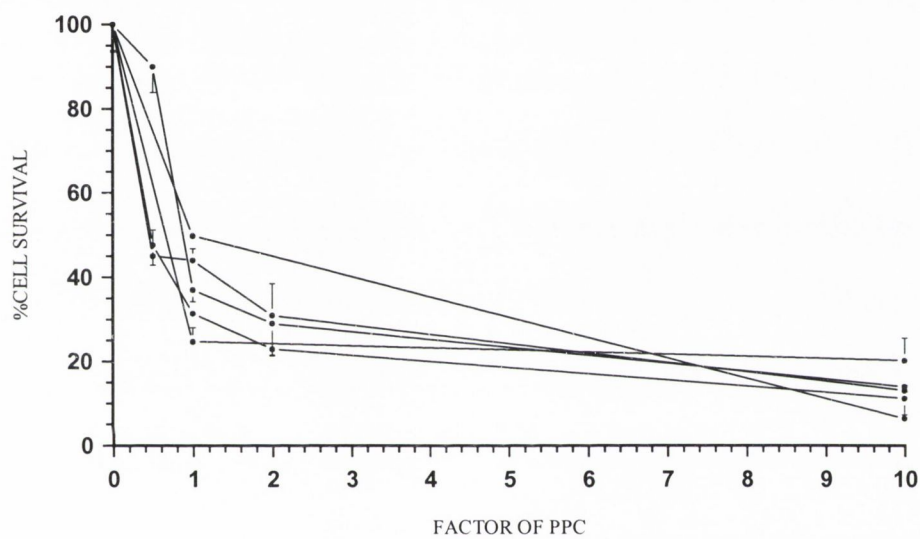


Fig. 3.25 Effect of Topotecan on Clear Cell Adenocarcinomas of the Ovary

3.2.8 Effect of Chemotherapy on a Stage IB Mucinous Adenocarcinoma of the Ovary (OV78)

The response of the mucinous adenocarcinoma can be seen in Fig. 3.26. The tumour was resistant to cisplatin and to paclitaxel at the PPC. The mean survival rate was 68.5% for cisplatin and 84.8% for paclitaxel. The IC50 for cisplatin was 5.8 x PPC and >10 x PPC for paclitaxel.

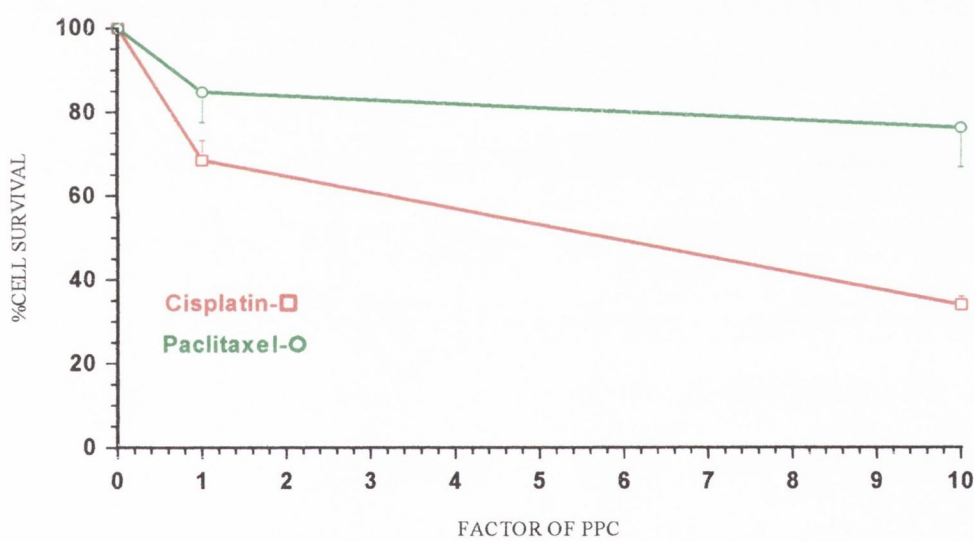


Fig. 3.26 Effect of Chemotherapy on a Stage IB Mucinous Adenocarcinoma of the Ovary

3.2.9 Effect of Chemotherapy on a Stage III Grade 2 Mucinous Adenocarcinoma of the Ovary (OV79)

The response of the mucinous adenocarcinoma can be seen in Fig. 3.27. Mean survival rates varied from 34.5% for topotecan to 84.3% for carboplatin at the PPC. The tumour responded to all the drugs at 10 x PPC. The IC50s for the individual drugs were: Cisplatin (3.6 x PPC), Paclitaxel (3.91 x PPC), Topotecan (0.79 x PPC), Cisplatin & Paclitaxel (>1 x PPC) and Carboplatin (4.61 x PPC).

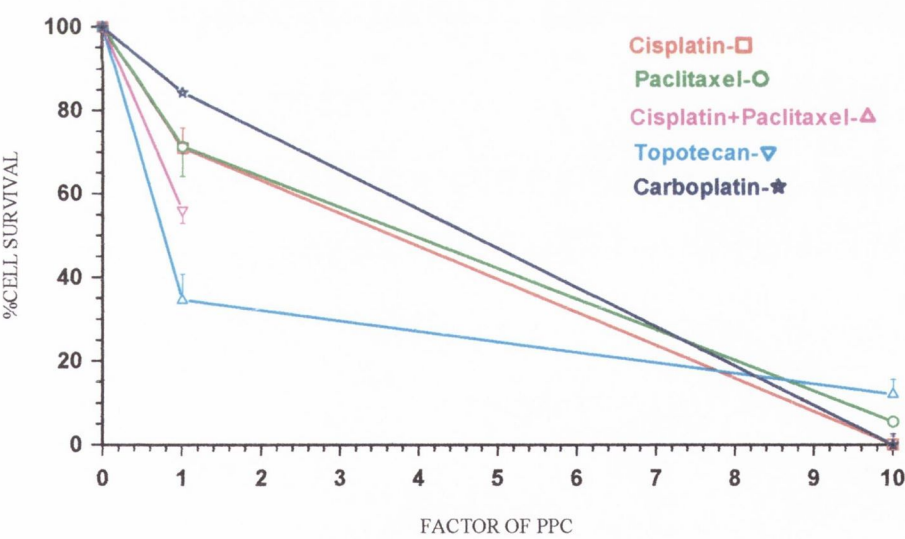


Fig. 3.27 Effect of Chemotherapy on a Stage III Grade 2 Mucinous Adenocarcinoma of the Ovary

3.2.10 Effect of Chemotherapy on a Stage III Grade 3 Mucinous Adenocarcinoma of the Ovary (OV80)

The effects of 6 drugs (paclitaxel, cisplatin, etoposide, 4-hydroxycyclophosphamide, doxorubicin and fluorouracil) on this tumour are shown in Fig. 3.28. The tumour responded well to fluorouracil with a survival rate of 8% and to doxorubicin with a 10% survival rate at the PPC. It displayed a borderline response to cisplatin at 48% and was quite resistant to paclitaxel, etoposide and 4-hydroxycyclophosphamide. It was sensitive to all the drugs except 4-hydroxycyclophosphamide at 10 x PPC. The IC50 for the drugs were Doxorubicin (0.55 x PPC), Fluorouracil (0.55 x PPC), 4-Hydroxycyclophosphamide (>10 x PPC), Paclitaxel (4.35 x PPC), Etoposide (4.0 x PPC) and Cisplatin (1 x PPC).

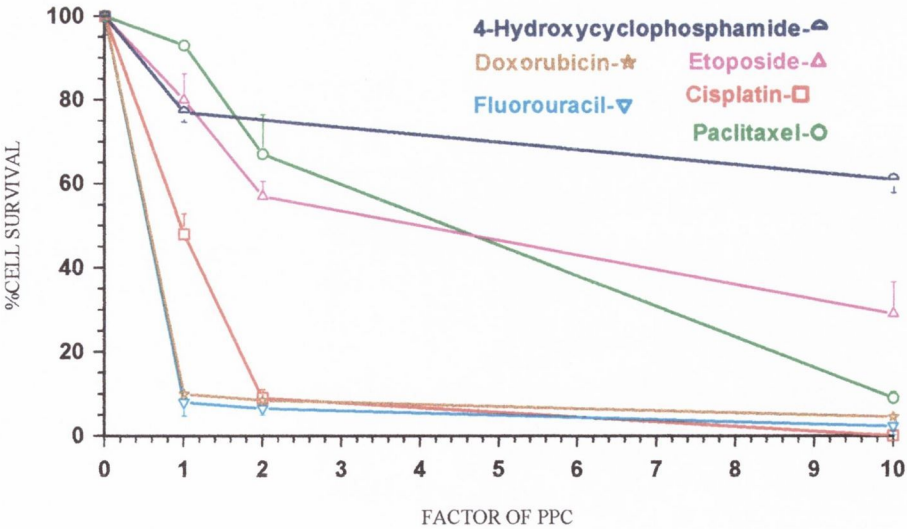


Fig. 3.28 Effect of Chemotherapy on a Stage III Grade 3 Mucinous Adenocarcinoma of the Ovary

3.2.11 Effect of Chemotherapy on a Stage IV Grade 3 Mucinous Adenocarcinoma of the Ovary (OV81)

The response of the mucinous adenocarcinoma can be seen in Fig. 3.29. Mean survival rates varied from 17.6% for doxorubicin to complete resistance to paclitaxel at the PPC. The tumour remained resistant to paclitaxel at 10 x PPC. The IC50s for the individual drugs were: Cisplatin (0.84 x PPC), Paclitaxel (>10 x PPC), Topotecan (3 x PPC), Cisplatin & Paclitaxel (>1 x PPC) and Doxorubicin (0.6 x PPC).

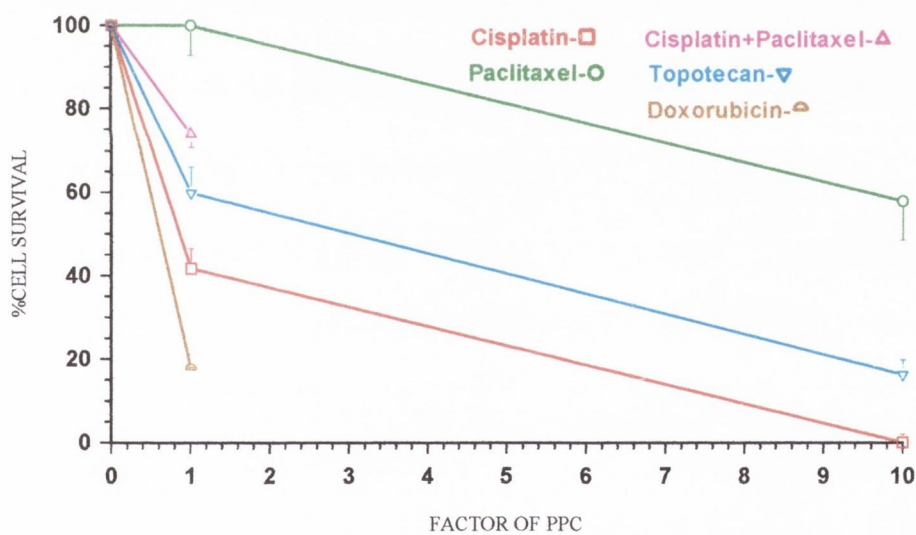


Fig. 3.29 Effect of Chemotherapy on a Stage IV Grade 3 Mucinous Adenocarcinoma of the Ovary

3.2.12 Effect of Chemotherapy on a Stage IV Grade 3 Adenocarcinoma of the Peritoneum (OV82)

Fig. 3.30 shows the response of the tumour to various cytotoxic agents. Mean survival rates varied from 6.2% for doxorubicin to 53.3% for paclitaxel at the PPC. The tumour responded to all the drugs at 10 x PPC. The IC50s for the individual drugs were: Cisplatin (0.79 x PPC), Paclitaxel (1.58 x PPC), Topotecan (0.78 x PPC), Cisplatin & Paclitaxel (0.92 x PPC) and Doxorubicin (0.55 x PPC).

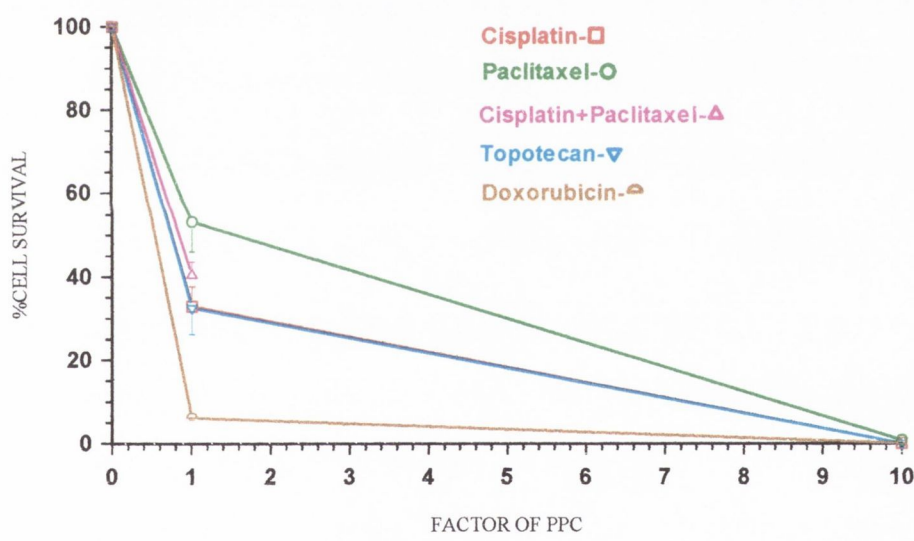


Fig. 3.30 Effect of Chemotherapy on a Stage IV Grade 3 Adenocarcinoma of the Peritoneum

3.2.13 Effect of Chemotherapy on a Stage I Grade 3 Endometrioid Adenocarcinoma of the Ovary (OV83)

This tumour responded well to single agent cisplatin, topotecan and cisplatin / paclitaxel combination at the PPC (Fig. 3.31). It was resistant to single agent

paclitaxel at this concentration and at 10 x PPC. The IC50s for the drugs were: Cisplatin (0.58 x PPC), Paclitaxel (>10 x PPC), Cisplatin / Paclitaxel (0.84 x PPC) and Topotecan (0.9x PPC).

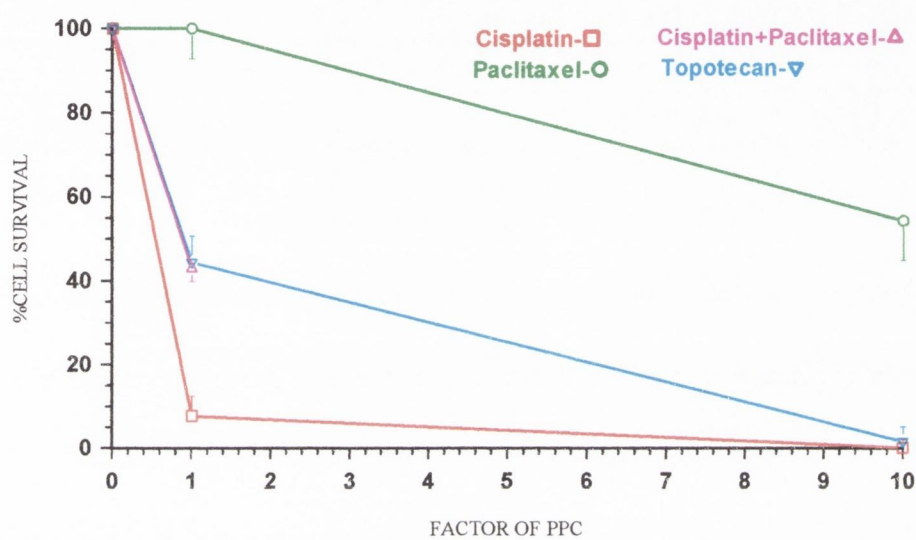


Fig. 3.31 Effect of Chemotherapy on a Stage I Grade 3 Endometrioid Adenocarcinoma of Ovary

3.2.14 Effect of Chemotherapy on a Stage II Grade 1 Endometrioid Adenocarcinoma of the Ovary (OV84)

This tumour was only examined at the PPC (Fig. 3.32). The tumour was resistant to all the drugs tested at this concentration. Mean survival rates varied from 52% for single agent paclitaxel to 92% for both carboplatin and topotecan. The IC50 was >1 x PPC for all the drugs.

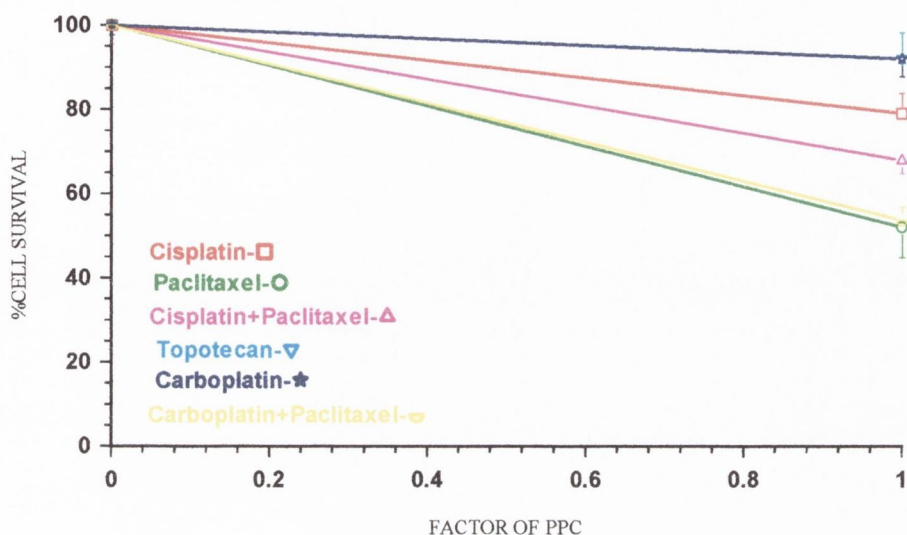


Fig. 3.32 Effect of Chemotherapy on a Stage II Grade 1 Endometrioid Adenocarcinoma of Ovary

3.2.15 Effect of Chemotherapy on a Stage II Transitional Cell Carcinoma of the Ovary (OV85)

This tumour shown in Fig. 3.33 responded to the single agent platinum drugs, topotecan and the cisplatin / paclitaxel combination at the PPC. Mean survival rates varied from 27.1% to 40.1%. It did not respond well to paclitaxel (71.9%) or to the carboplatin / paclitaxel combination (63.7%). The IC50s were: Cisplatin (0.7 x PPC), Paclitaxel (3.8 x PPC), Cisplatin / Paclitaxel (0.82 x PPC), Carboplatin (0.72 x PPC), Carboplatin / Paclitaxel (>1 x PPC) and Topotecan (0.79x PPC).

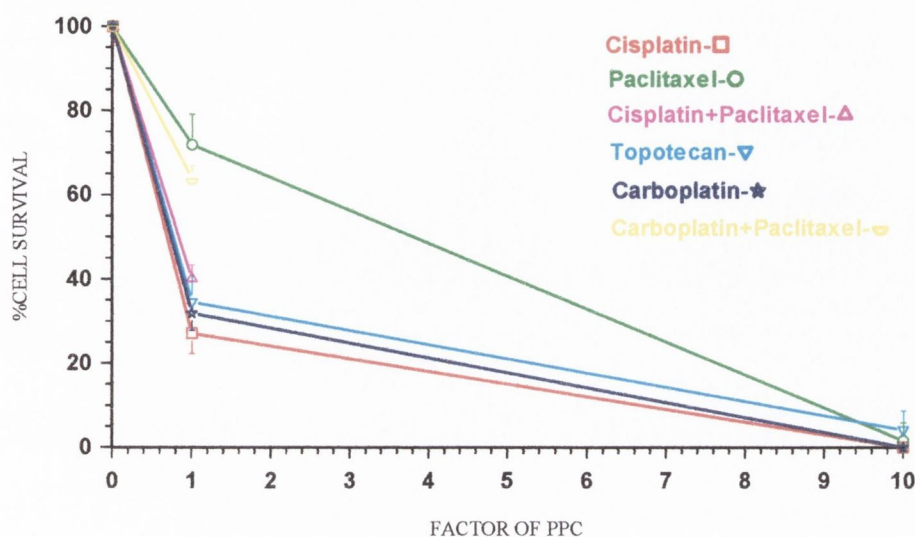


Fig. 3.33 Effect of Chemotherapy on a Stage II Transitional cell carcinoma of Ovary

3.2.16 Effect of Chemotherapy on 5 Borderline Mucinous Tumours of the Ovary (OV90-OV94)

Cisplatin: (Fig. 3.34) Five borderline mucinous tumours were examined for their response to chemotherapy. Mean survival rates varied from 9.9% to 82% at the PPC with three of the tumours being sensitive to the drug and two being resistant. One tumour remained resistant at 10 x PPC while total cell kill was observed with the other four. The IC₅₀ in this category of tumours varied from 0.52 to >10 x PPC.

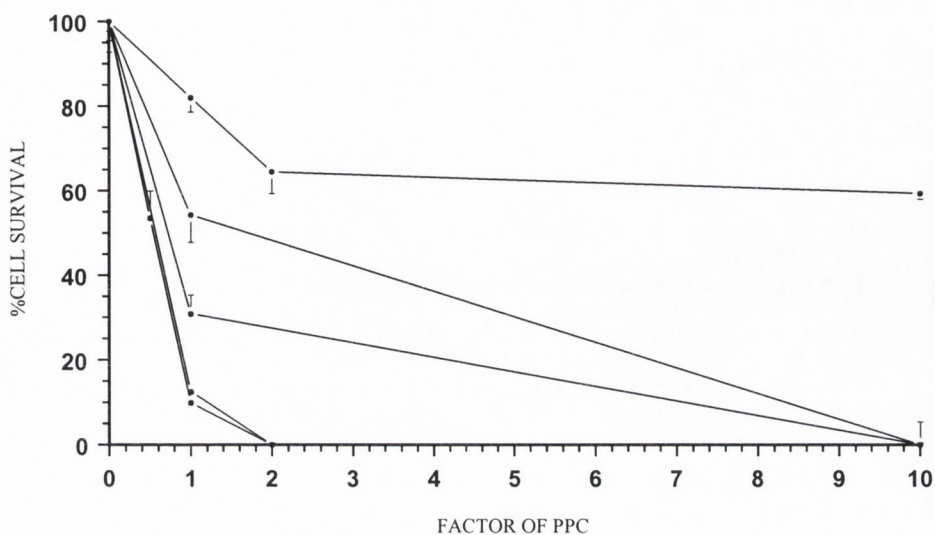


Fig. 3.34 Effect of Cisplatin on Mucinous Borderline Carcinomas of the Ovary

Paclitaxel: (Fig. 3.35) One of the tumours was sensitive to paclitaxel at the PPC and the other three were resistant with survival rates from 67.8% to 91.4%. One of these remained resistant at 10 x PPC. The IC₅₀ ranged from 0.8 x PPC to >10 x PPC.

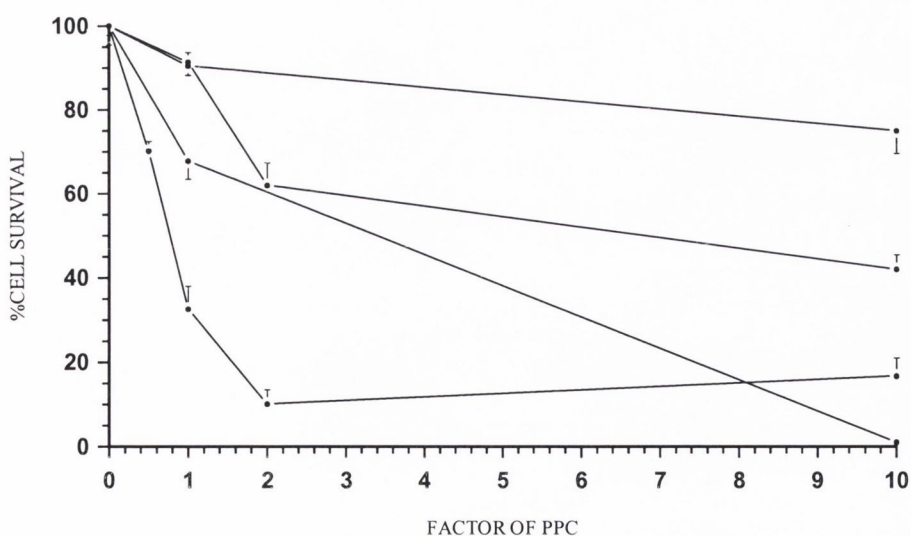


Fig. 3.35 Effect of Paclitaxel on Borderline Mucinous Tumours of the Ovary

Cisplatin and Paclitaxel: (Fig. 3.36) Survival rates of 12.5%, 24.2% 55.9% and 86.3% were observed when these carcinomas were tested at the PPC of the cisplatin / paclitaxel combination. The IC50 varied from 0.58 x PPC to greater than 1 x PPC.

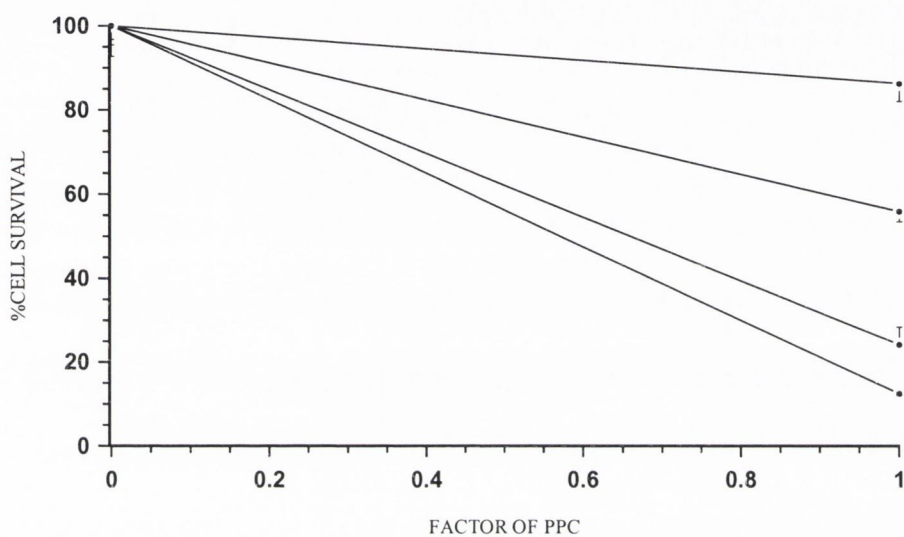


Fig. 3.36 Effect of Cisplatin & Paclitaxel on Borderline Mucinous Tumours of the Ovary

Topotecan: (Fig. 3.37) Three of the tumours tested with topotecan were found to be sensitive at the PPC with response rates varying from 5.6% to 25%. One of the tumours was resistant with a survival value of 56.3%. At 10 x PPC total cell kill was observed for one tumour and rates of 1.6%, 2% and 19.1% were observed for the other three tumours. IC50 values ranged from 0.30 to 2.6 x PPC.

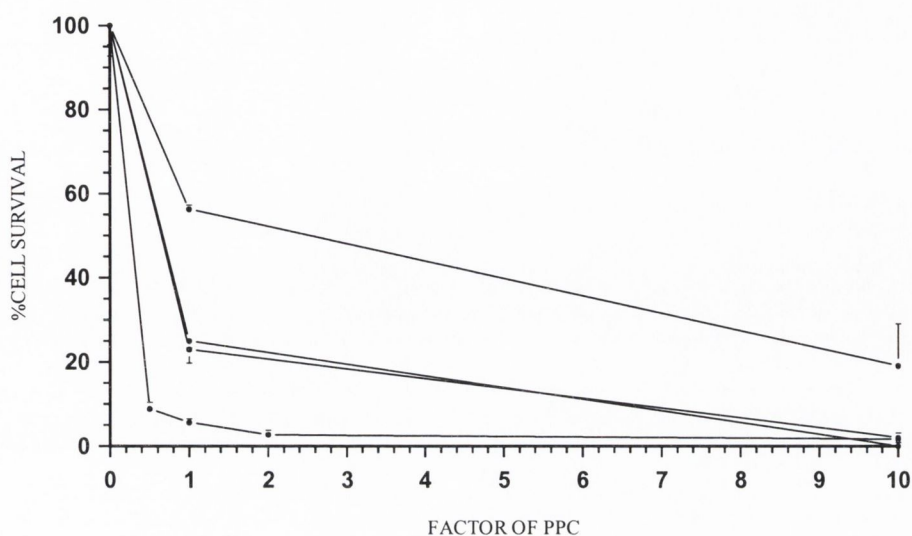


Fig. 3.37 Effect of Topotecan on Borderline Mucinous Tumours of the Ovary

3.2.17 Effect of Chemotherapy on 4 Borderline Serous Tumours of the Ovary (OV95-OV98)

Cisplatin: (Fig. 3.38) Four serous borderline tumours were examined for their response to chemotherapy. Mean survival rates varied from 7.7% to 70.3% at the PPC with three of the tumours being sensitive to the drug and one being resistant. All the tumours were sensitive at 10 x PPC. The IC₅₀ in this category of tumour varied from 0.55 to 1.5 x PPC.

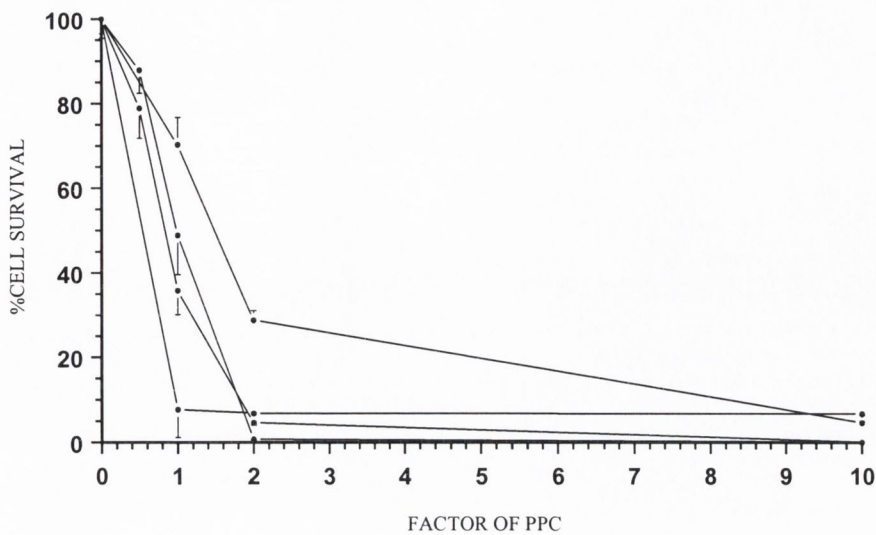


Fig. 3.38 Effect of Cisplatin on Borderline Serous Tumours of the Ovary

Paclitaxel: (Fig. 3.39) Three of the tumours were examined with paclitaxel and all displayed complete resistance at the PPC. At 10 x PPC survival rates of 14.5%, 44% and 66.6% were observed. The IC50 ranged from 5.65 x PPC to >10 x PPC.

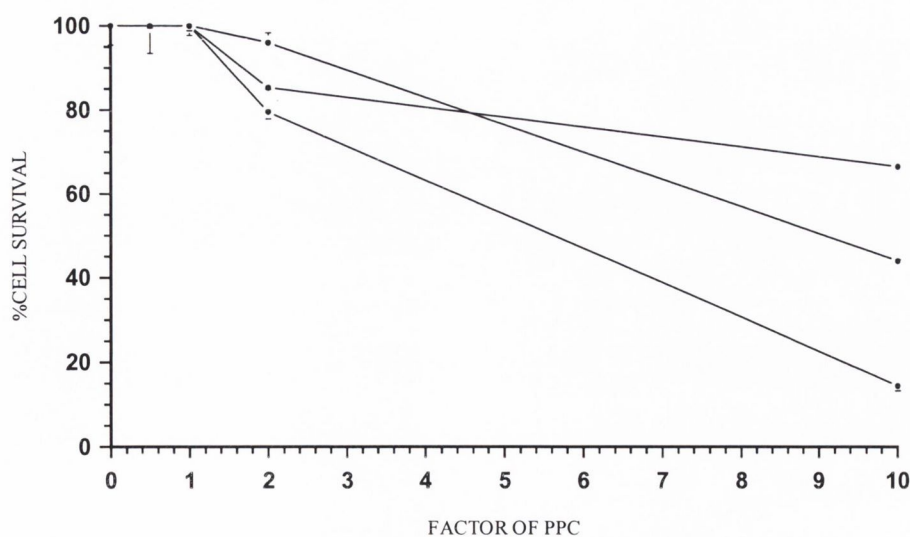


Fig. 3.39 Effect of Paclitaxel on Borderline Serous Tumours of the Ovary

Cisplatin and Paclitaxel: (Fig. 3.40) None of the tumours were sensitive at the PPC of the cisplatin / paclitaxel combination. Response rates of 58.2%, 61.4% and 84.7% were observed at this concentration. The IC50 was greater than 1 x PPC.

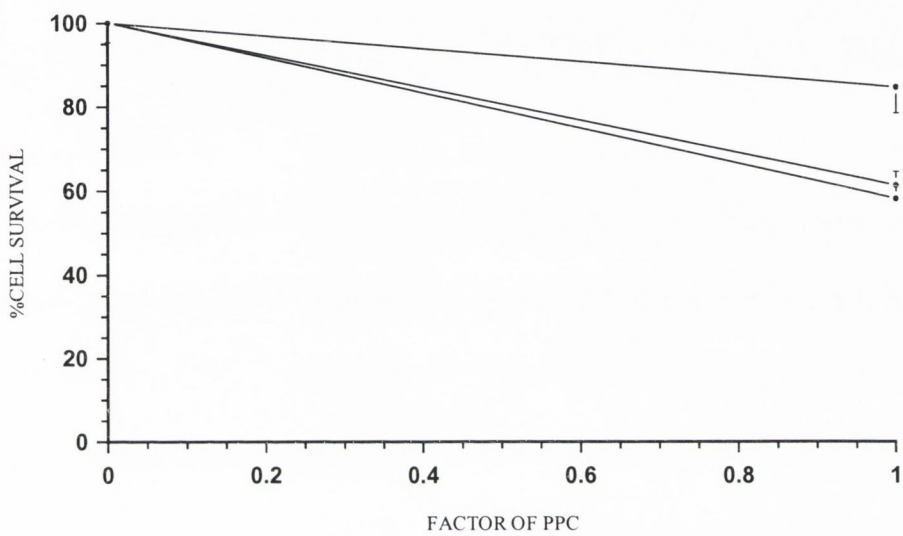


Fig. 3.40 Effect of Cisplatin & Paclitaxel on Borderline Serous Tumours of the Ovary

Topotecan: (Fig. 3.41) Two of the tumours tested with topotecan were found to be sensitive at the PPC with response rates of 45.6% and 47%. The other was resistant with a survival rate of 59.7%. At 10 x PPC rates of 3.1%, 9.04% and 23.2% were observed. IC50 values ranged from 0.90 to 1.75 x PPC.

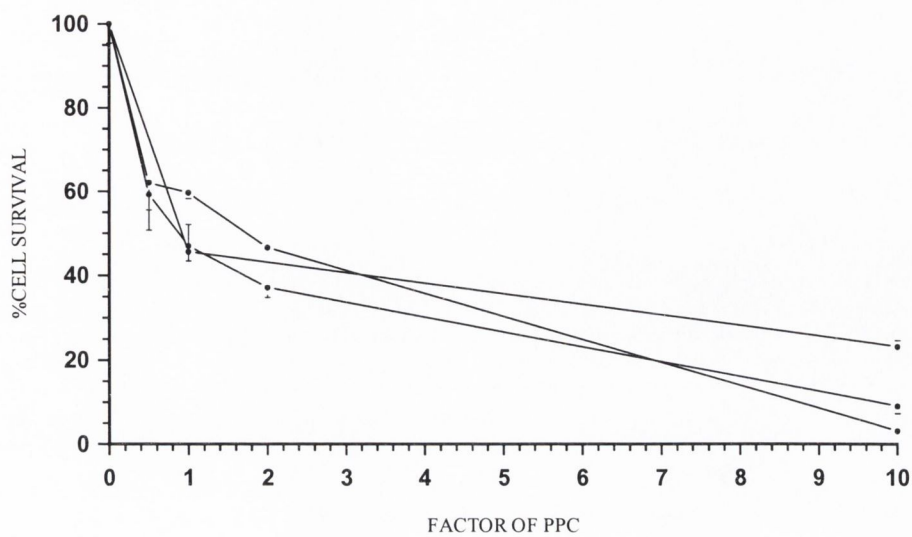


Fig. 3.41 Effect of Topotecan on Borderline Serous Tumours of the Ovary

CHAPTER 4

HETEROGENEITY IN THE RESPONSE OF CERVICAL AND ENDOMETRIAL TUMOURS TO CYTOTOXIC DRUGS

4.1 Culture of Cervical and Endometrial Specimens

Chemosensitivity testing was carried out on 30 cervical specimens and 25 endometrial specimens. The details of these specimens are shown in Tables 4.1 and 4.2.

Table 4.1 Cervical specimens with *in vitro* results

<u>Tumour Type</u>	<u>Stage</u>	<u>Grade</u>	<u>Number</u>
Squamous Cell Carcinoma	I	2	7
Squamous Cell Carcinoma	I	3	7
Squamous Cell Carcinoma	II	2	4
Squamous Cell Carcinoma	II	3	4
Squamous Cell Carcinoma	IV	3	1
Serous Papillary Adenocarcinoma	I	2	1
Serous Papillary Adenocarcinoma	II	2	1
Adenosquamous Carcinoma	I	2	1
Adenosquamous Carcinoma	I	3	1
Adenosquamous Carcinoma	II	3	1
Adenosquamous Carcinoma	III	3	1
Recurrent Squamous Carcinoma	IB	3	1

Table 4.2 Endometrial specimens with *in vitro* results

Tumour Type	Stage	Grade	Number
Endometrioid Adenocarcinoma	I	1	1
Endometrioid Adenocarcinoma	I	2	4
Endometrioid Adenocarcinoma	III	2	3
Adenosquamous Carcinoma	III	3	4
Serous Papillary Adenocarcinoma	I	1	1
Serous Papillary Adenocarcinoma	I	2	2
Serous Papillary Adenocarcinoma	II	3	1
Serous Papillary Adenocarcinoma	III	3	1
Serous Papillary Adenocarcinoma	IV	2	1
Serous Papillary Adenocarcinoma	IV	3	1
Recurrent Stromal Cell Sarcoma	ND	2	1
Stromal Cell Sarcoma	ND	ND	1
Adenosarcoma	ND	3	1
Leiomyosarcoma	IV	3	1
Leiomyosarcoma	ND	ND	1
Mixed Mullerian Tumour	III	4	1

Abbreviations: ND (Not Documented)

One hundred percent evaluability was achieved in culturing the endometrial and cervical specimens. Some of the cervical explants showed overgrowth of fibroblasts as seen in Fig. 4.0(a) and these flasks were discarded. An example of endometrial cell growth can be seen in Fig. 4.0(b)

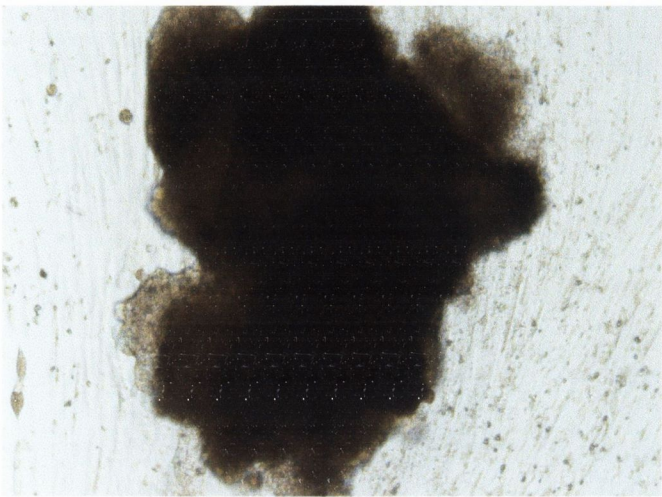


Figure 4.0 (a) Cervical culture showing fibroblast growth

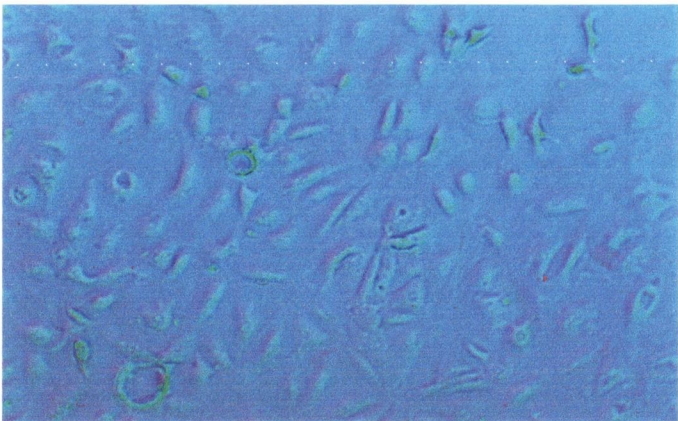


Figure 4.0 (b) Endometrial culture *in vitro*

4.2 *In Vitro* Effect of Chemotherapy on Cervical Cancers

4.2.1 Effect of Chemotherapy on 7 Stage I Grade 2 Cervical Squamous Carcinomas (CX1-CX7)

Cisplatin: (Fig. 4.1) Survival rates at the PPC varied from complete resistance to 9.1% survival for the seven tumours examined. One tumour was resistant at 10 x PPC with a survival rate of 65%. A concentration of 0.5 x PPC gave greater than 50% cell kill for one tumour. The IC50 in this category of tumours varied from 0.40 to >10 x PPC.

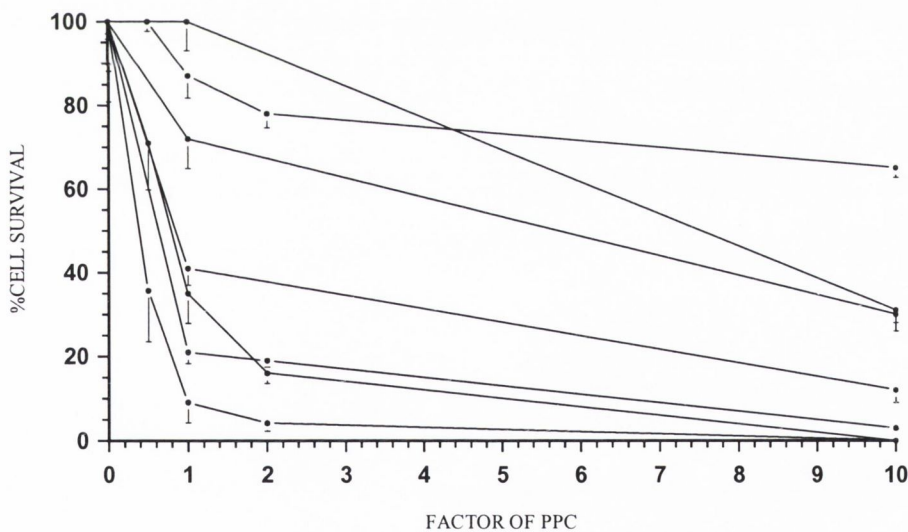


Fig. 4.1 Effect of Cisplatin on Stage I Grade 2 Cervical Squamous Carcinomas

Paclitaxel: (Fig. 4.2) Three of the tumours were sensitive to paclitaxel at the PPC and at 0.5 x PPC two of these were found to respond to the drug. The other tumours were resistant at the PPC and one of these just gave a borderline response at 10 x PPC. The IC50 ranged from 0.35 x PPC to 10 x PPC.

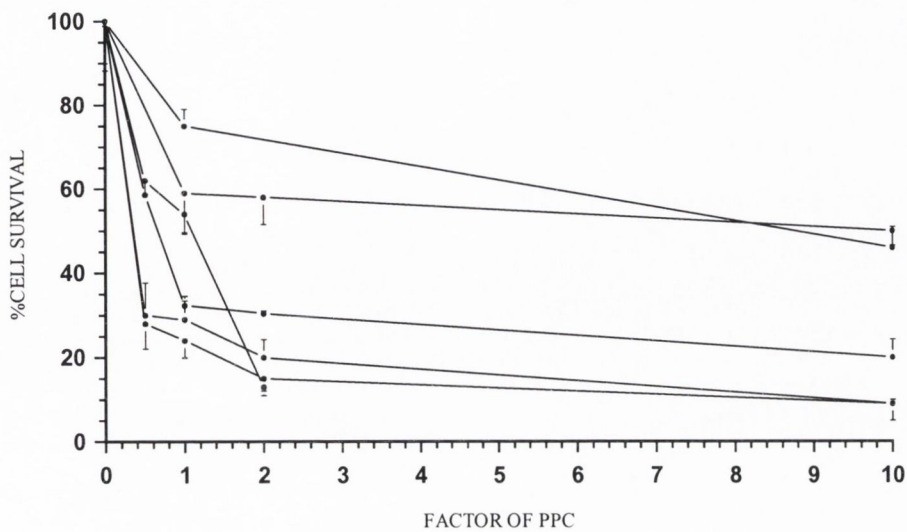


Fig. 4.2 Effect of Paclitaxel on Stage I Grade 2 Cervical Squamous Carcinomas

Cisplatin and Paclitaxel: (Fig. 4.3) Survival rates between 27.4% and 78% were observed when these carcinomas were tested at the PPC of the cisplatin / paclitaxel combination. Three of the tumours were sensitive to the drugs. The IC50 varied from 0.68 x PPC to greater than 1 x PPC.

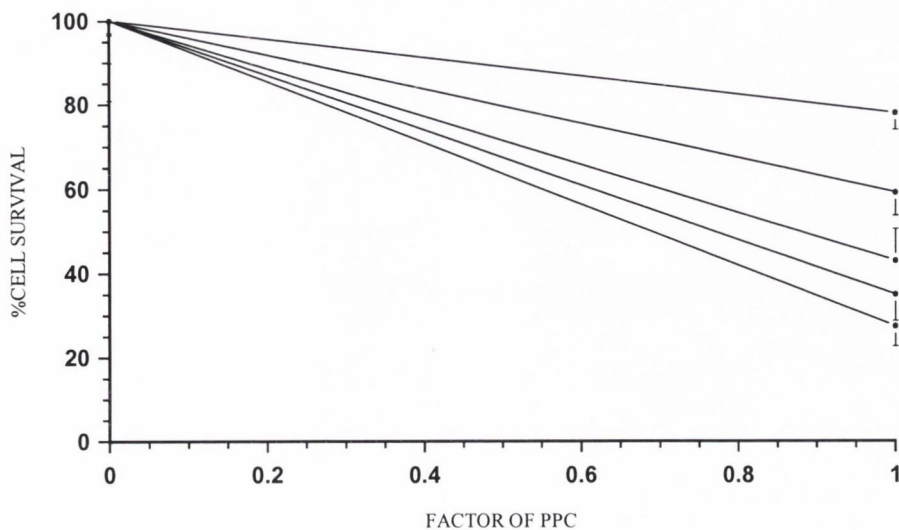


Fig. 4.3 Effect of Cisplatin & Paclitaxel on Stage I Grade 2 Cervical Squamous Carcinomas

Fluorouracil: (Fig. 4.4) Three of the four tumours tested with this drug were sensitive at the PPC. All of the tumours responded at 10 x PPC. IC50 values ranged from 0.49 to 1.9 x PPC.

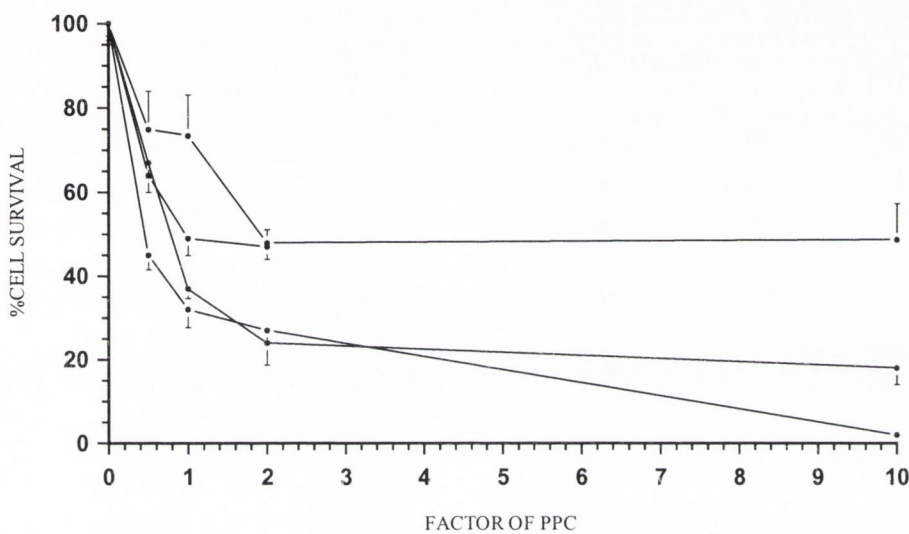


Fig. 4.4 Effect of Fluorouracil on Stage I Grade 2 Cervical Squamous Carcinomas

4.2.2 Effect of Chemotherapy on 7 Stage I Grade 3 Cervical Squamous Carcinomas (CX8-CX14)

Cisplatin: (Fig. 4.5) Mean survival rates varied from 19% to 100% at the PPC with two of the tumours being sensitive to the drug and five being resistant. Only four of the tumours were sensitive at 10 x PPC. The IC50 in this category of tumours varied from 0.40 to >10 x PPC.

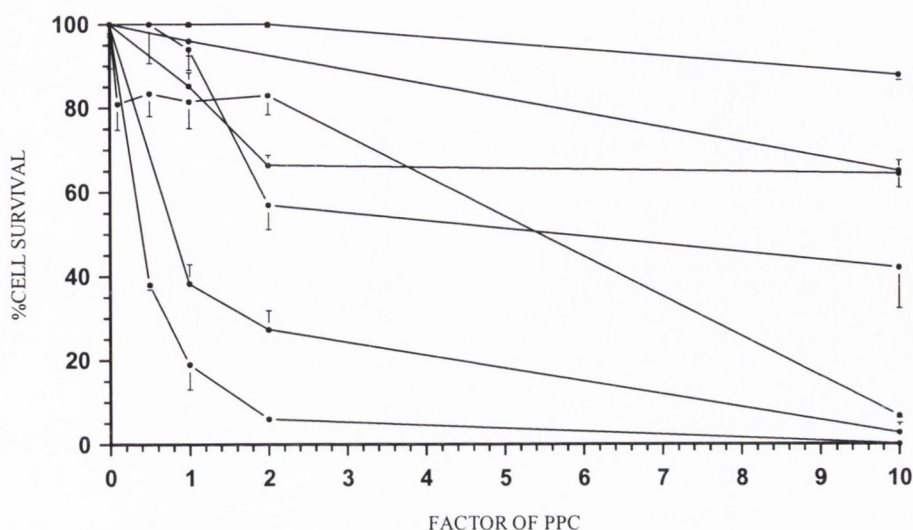


Fig. 4.5 Effect of Cisplatin on Stage I Grade 3 Cervical Squamous Carcinomas

Paclitaxel: (Fig. 4.6) Five of the tumours were examined with paclitaxel and two were sensitive at the PPC. At 10 x PPC four of the tumours responded and one was resistant with a survival rate of 85%. The IC50 ranged from 0.39 x PPC to >10 x PPC.

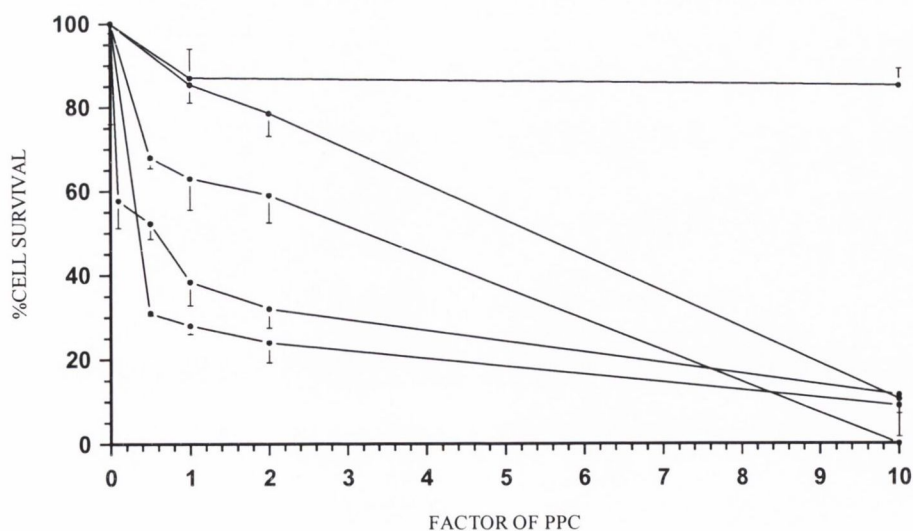


Fig. 4.6 Effect of Paclitaxel on Stage I Grade 3 Cervical Squamous Carcinomas

Cisplatin and Paclitaxel: (Fig. 4.7) Three tumours were examined at the PPC of the cisplatin / paclitaxel combination and only one responded. Response rates of 75% and 69.8% were seen with the other two. The IC50 varied from 0.58 x PPC to greater than 1 x PPC.

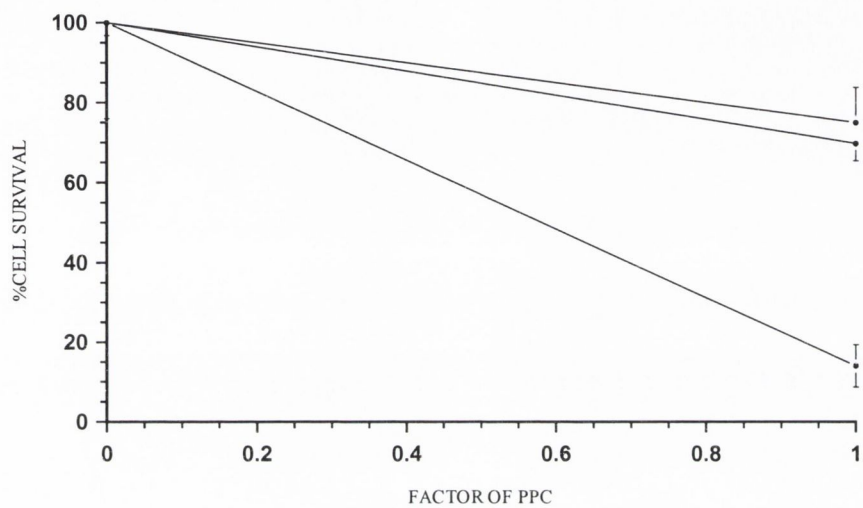


Fig. 4.7 Effect of Cisplatin & Paclitaxel on Stage I Grade 3 Cervical Squamous Carcinomas

Topotecan: (Fig. 4.8) One tumour was tested with topotecan and was sensitive at the PPC with a survival rate of 37.5%. The IC50 value was 0.90 x PPC.

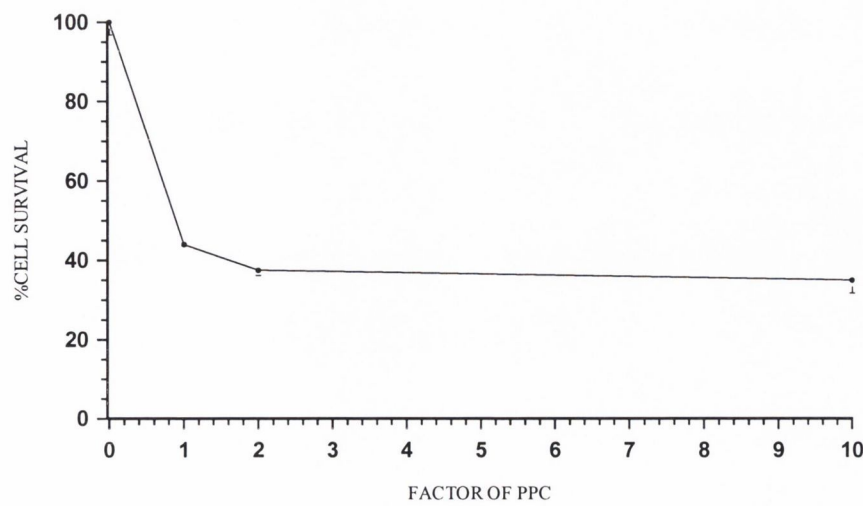


Fig. 4.8 Effect of Topotecan on Stage I Grade 3 Cervical Squamous Carcinomas

4.2.3 Effect of Chemotherapy on 4 Stage II Grade 2 Cervical Squamous Carcinomas (CX15-CX18)

Cisplatin: (Fig. 4.9) Mean survival rates varied from 37.1% to complete resistance at the PPC with only one of the four tumours being sensitive. Two of the tumours were sensitive at 10 x PPC. The IC50 in this category of tumours varied from 0.70 to >10 x PPC.

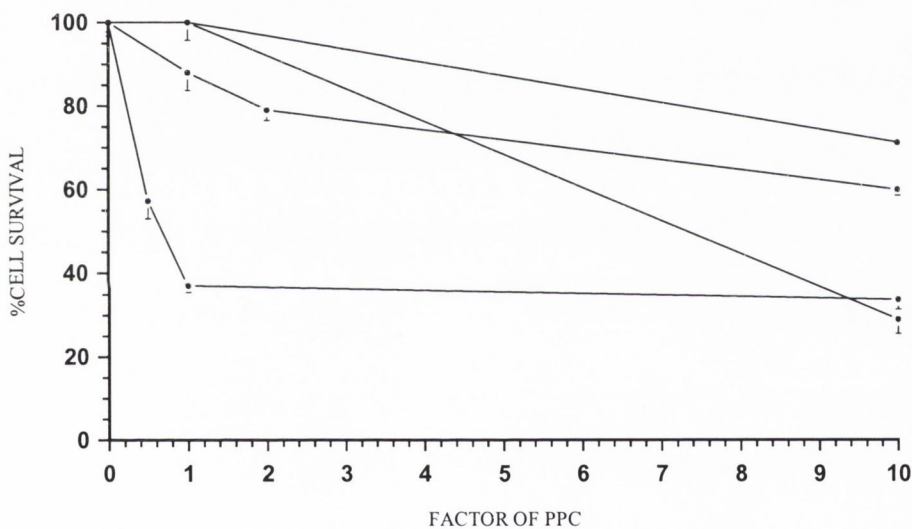


Fig. 4.9 Effect of Cisplatin on Stage II Grade 2 Cervical Squamous Carcinomas

Paclitaxel: (Fig. 4.10) Two of the tumours were examined with paclitaxel and both were sensitive at the PPC. At 10 x PPC one of the tumours responded with a 77% cell kill being observed but the other tumour remained completely resistant. The IC50 ranged from 4.3 x PPC to >10 x PPC.

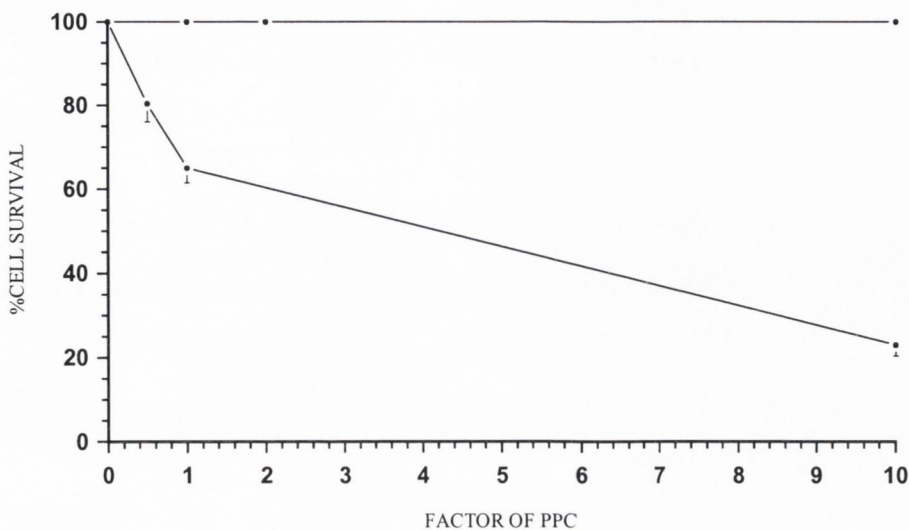


Fig. 4.10 Effect of Paclitaxel on Stage II Grade 2 Cervical Squamous Carcinomas

4-Hydroxycyclophosphamide: (Fig. 4.11) Two tumours were examined with the active metabolite of cyclophosphamide, 4-hydroxycyclophosphamide, and one responded at the PPC with a survival rate of 41%. At 10 x PPC this tumour did not display any additional cytotoxicity and the other tumour showed a 65.5% survival. The IC50 varied from 0.82 x PPC to greater than 10 x PPC.

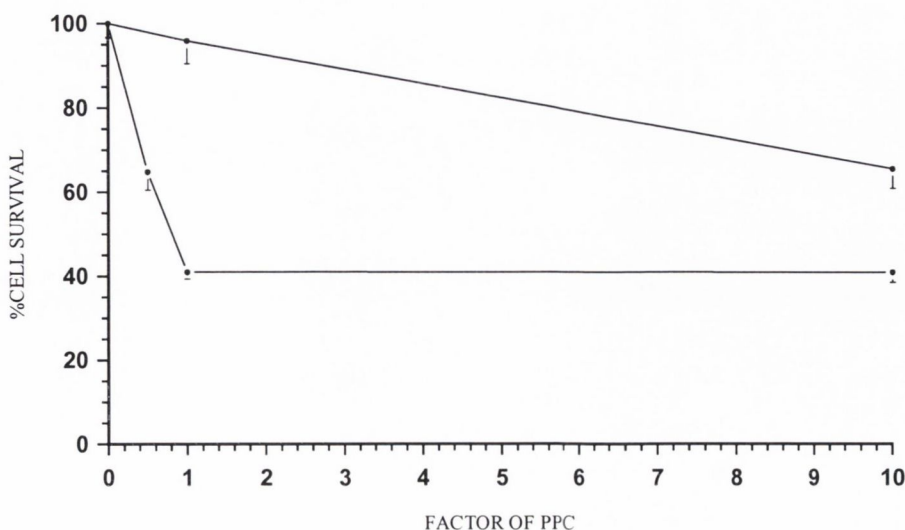


Fig. 4.11 Effect of 4-Hydroxycyclophosphamide on Stage II Grade 2 Cervical Squamous Carcinomas

4.2.4 Effect of Chemotherapy on 4 Stage II Grade 3 Cervical Squamous Carcinomas (CX19-CX22)

Cisplatin: (Fig. 4.12) Mean survival rates varied from 32% to 78.7% at the PPC with one of four tumours being sensitive to the drug. Three of the tumours were sensitive at 10 x PPC. The IC50 in this category of tumours varied from 0.75 to >10 x PPC.

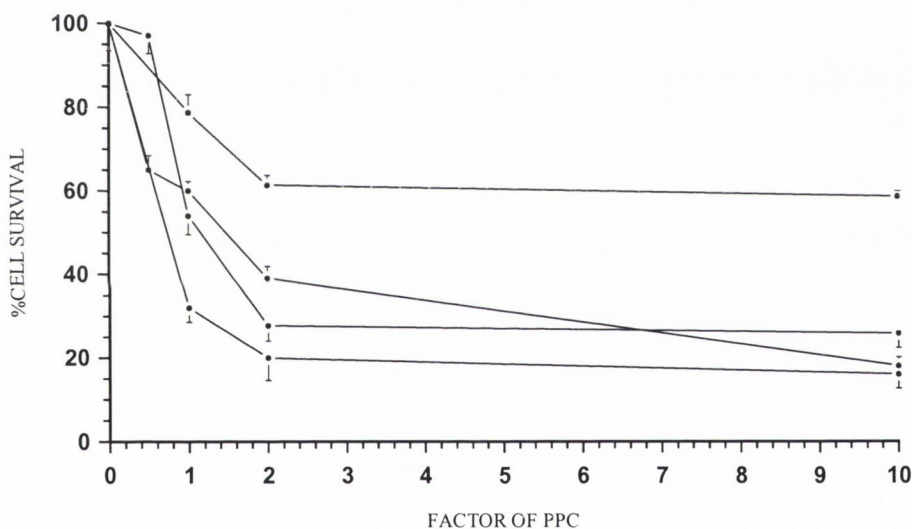


Fig. 4.12 Effect of Cisplatin on Stage II Grade 3 Cervical Squamous Carcinomas

Doxorubicin: (Fig. 4.13) Response rates varied from 37% survival to 87% at the PPC of doxorubicin. One of the tumours was resistant and remained resistant at 10 x PPC. The IC50 ranged from 0.5 x PPC to >10 x PPC.

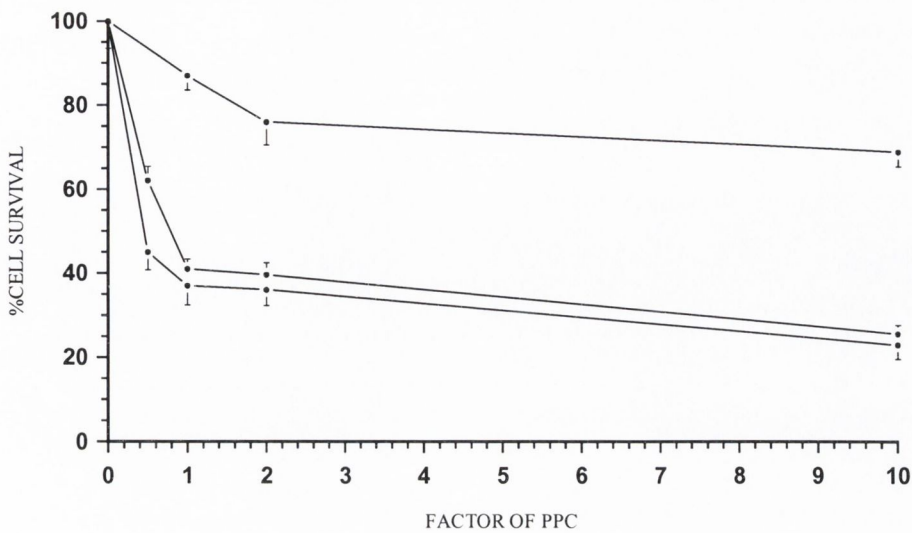


Fig. 4.13 Effect of Doxorubicin on Stage II Grade 3 Cervical Squamous Carcinomas

Cisplatin and Doxorubicin: (Fig. 4.14) Two of the tumours were sensitive at the PPC of the cisplatin / doxorubicin combination with response rates of 37% and 39.7%. The other tumour displayed a 60% cell survival. The IC50 varied from 0.78 x PPC to greater than 1 x PPC.

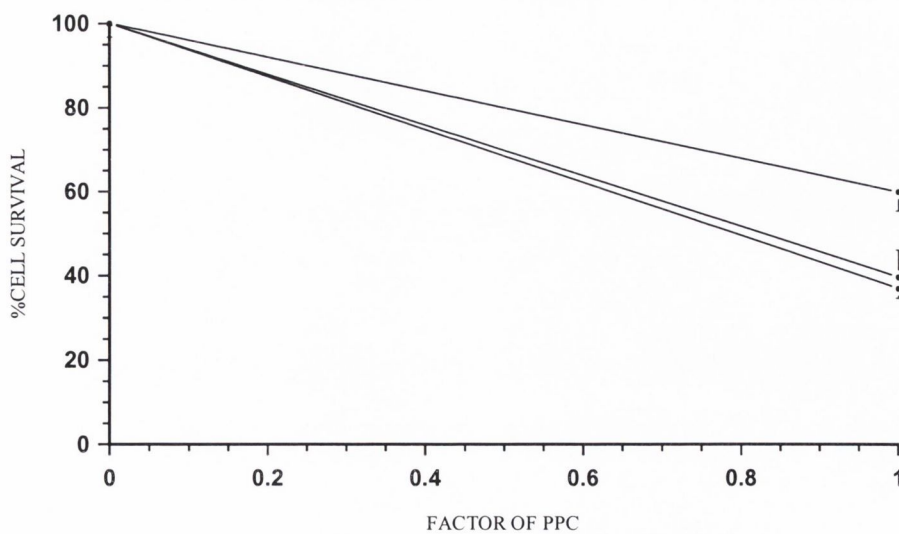


Fig. 4.14 Effect of Cisplatin & Doxorubicin on Stage II Grade 3 Cervical Squamous Carcinomas

4.2.5 Effect of Chemotherapy on a Stage IV Grade 3 Cervical Squamous Carcinoma (CX23)

The effects of 4 drugs (cisplatin, paclitaxel, bleomycin and fluorouracil) on this tumour are shown in Fig. 4.15. The tumour responded well to cisplatin with a survival rate of 7% and to paclitaxel with a rate of 40% survival at the PPC. The tumour was resistant to bleomycin and fluorouracil. It was sensitive to all the drugs except fluorouracil at 10 x PPC. The IC50 for the drugs were: Cisplatin (0.39 x PPC), Paclitaxel (0.8 x PPC), Fluorouracil (8.4 x PPC) and Bleomycin (>10 x PPC).

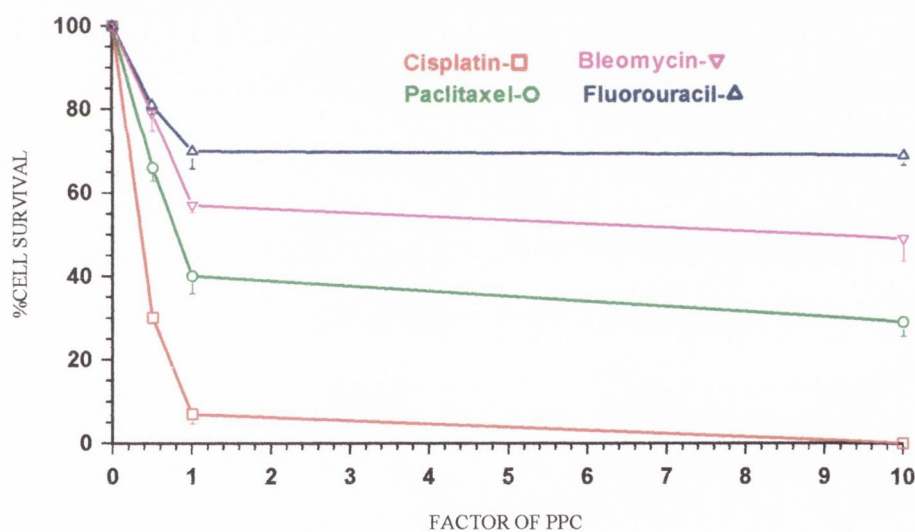


Fig. 4.15 Effect of Chemotherapy on a Stage IV Grade 3 Cervical Squamous Carcinoma

4.2.6 Effect of Chemotherapy on a Stage I Grade 2 Cervical Adenocarcinoma (CX24)

The effects of 4 drugs (cisplatin, paclitaxel, topotecan and the cisplatin/paclitaxel combination) on this tumour are shown in Fig. 4.16. The tumour responded well to all the drugs at the PPC and at 10 x PPC total cell kill was observed. At 0.5 x PPC the tumour was sensitive to the four drugs tested. The IC50 for the drugs were Cisplatin (0.45 x PPC), Paclitaxel (0.5 x PPC), Cisplatin / Paclitaxel (0.5 x PPC) and Topotecan (0.3 x PPC).

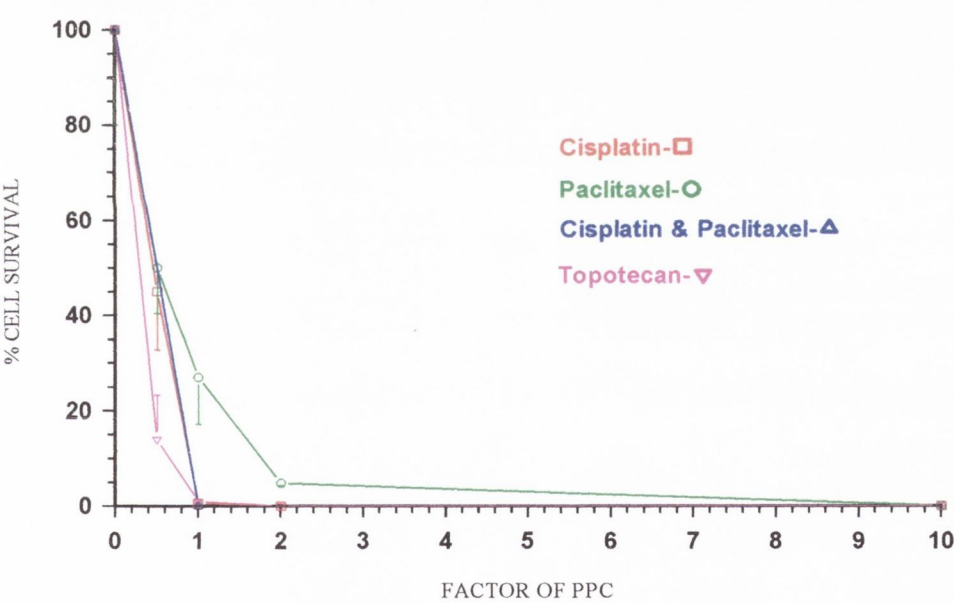


Fig. 4.16 Effect of Chemotherapy on a Stage I Grade 2 Cervical Adenocarcinoma

4.2.7 Effect of Chemotherapy on a Stage II Grade 2 Cervical Adenocarcinoma (CX25)

The effects of 3 drugs (cisplatin, doxorubicin and the cisplatin/doxorubicin combination) on this tumour are shown in Fig. 4.17. The tumour responded to the

drugs at the PPC. The response seen with the single agent drugs was a borderline response and the tumour responded better to the combination of cisplatin / doxorubicin. The IC50 for the drugs are Cisplatin (0.95 x PPC), Doxorubicin (1.0 x PPC), Cisplatin / Doxorubicin (0.55 x PPC).

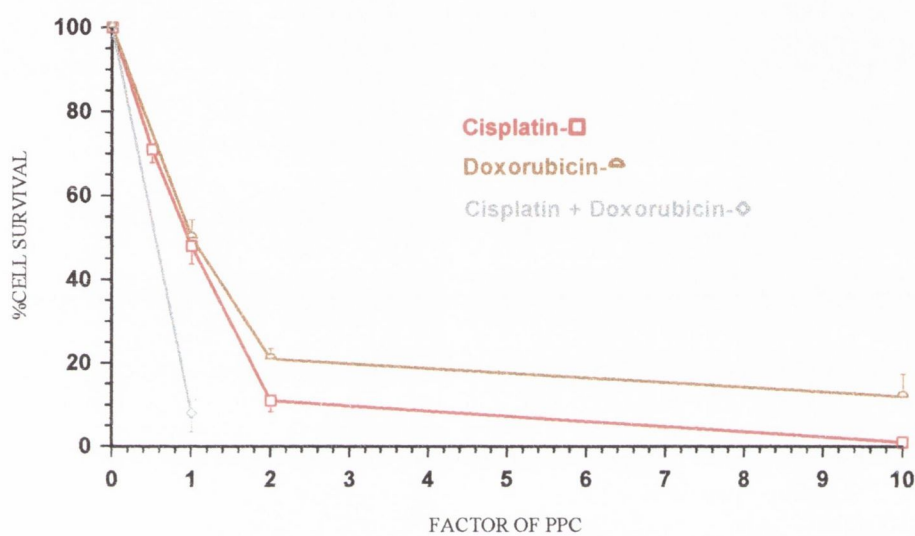


Fig. 4.17 Effect of Chemotherapy on a Stage II Grade 2 Adenocarcinoma of Cervix

4.2.8 Effect of Cisplatin on Adenosquamous Carcinomas of the Cervix (CX26-CX29)

Four different stage tumours were examined with cisplatin and the responses are shown in Fig. 4.18. The Stage I Grade 2 tumour was the only one that was sensitive at the PPC. At 10 x PPC all the tumours responded to the drug. The IC50 of cisplatin for each of the tumours was Stage I Grade 2 (0.7 x PPC), Stage I Grade 3 (1.65 x PPC), Stage II Grade 3 (1.8 x PPC) and Stage III Grade 3 (3.8 x PPC).

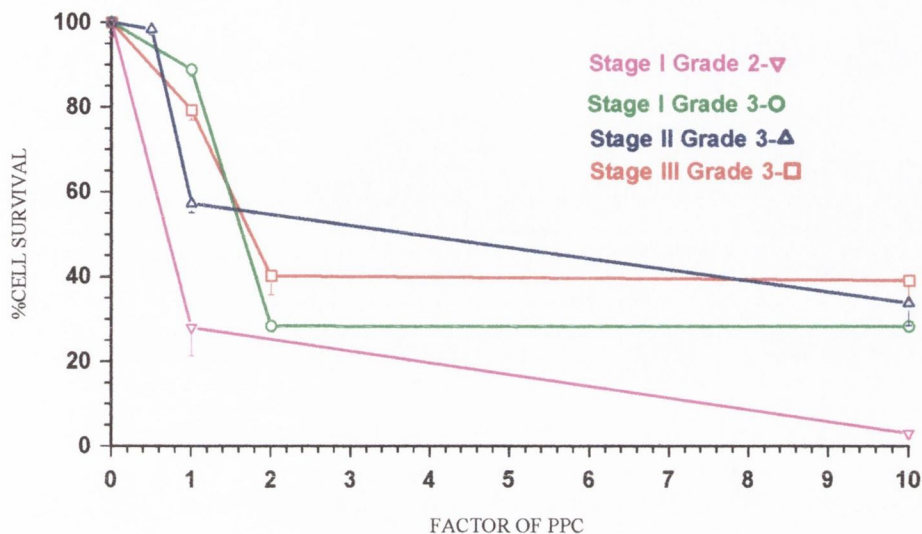


Fig. 4.18 Effect of Cisplatin on Adenosquamous Carcinomas of Cervix

4.2.9 Effect of Chemotherapy on a Grade 3 Recurrent Squamous Carcinoma of the Cervix (CX30)

The effects of 4 drugs (cisplatin, paclitaxel, topotecan and the cisplatin/paclitaxel combination) on this tumour are shown in Fig. 4.19. The tumour responded well to cisplatin, cisplatin / paclitaxel combination and to topotecan at the PPC but not to paclitaxel. Response rates varied from 11.1% cell survival for cisplatin to 94.1% survival for paclitaxel. At 10 x PPC the tumour was sensitive to all the drugs. The IC50 for the drugs were Cisplatin (0.60 x PPC), Paclitaxel (5.95 x PPC), Cisplatin / Paclitaxel (0.65 x PPC) and Topotecan (0.70 x PPC).

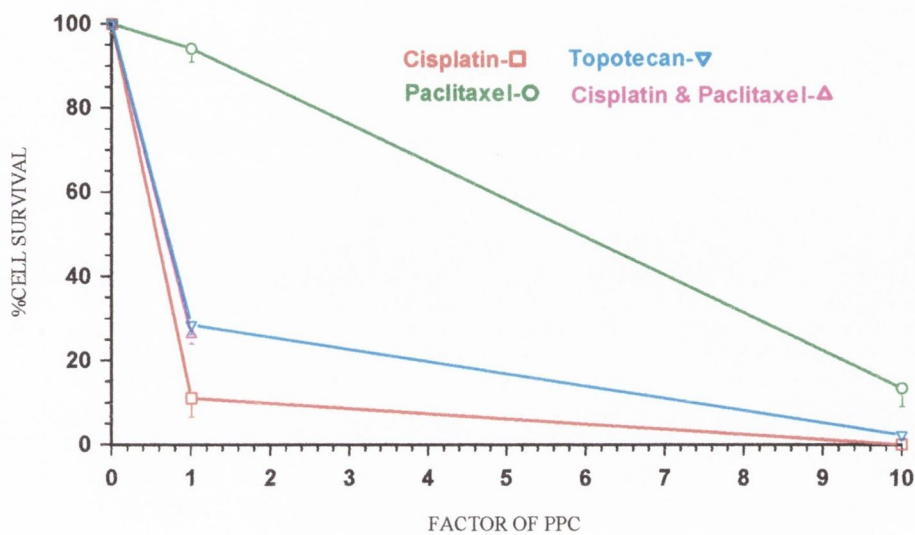


Fig. 4.19 Effect of Chemotherapy on a Grade 3 Recurrent Squamous Carcinoma of Cervix

4.3 *In Vitro* Effect of Chemotherapy on Endometrial Cancers

4.3.1 Effect of Chemotherapy on a Stage I Grade 1 Endometrioid Adenocarcinoma of the Endometrium (EN1)

This tumour was examined with the platinum drugs and with paclitaxel and the platinum / paclitaxel combinations at the PPC. The response is shown in Fig. 4.20. This tumour was resistant to all the drugs tested at this concentration. The IC50s are >1 x PPC.

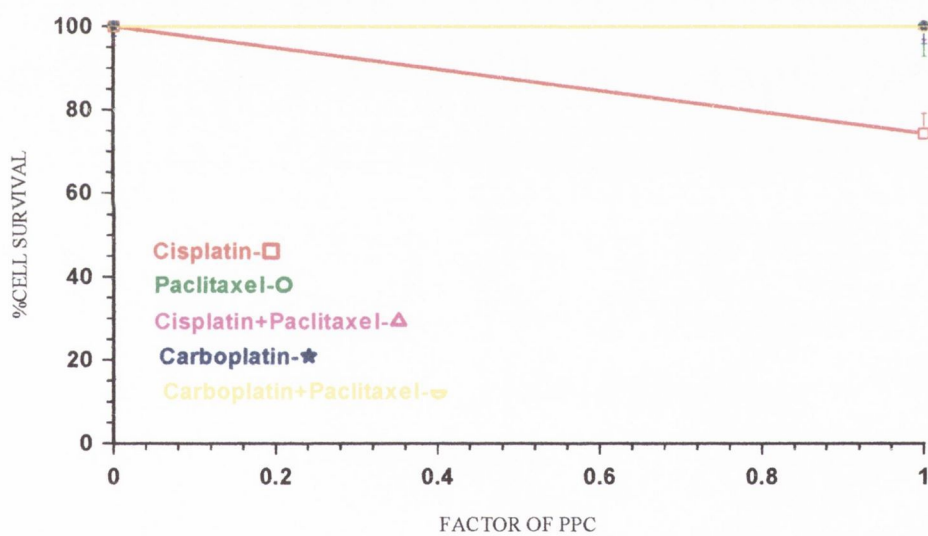


Fig. 4.20 Effect of Chemotherapy on a Stage I Grade 1 Endometrioid Adenocarcinoma of Endometrium

4.3.2 Effect of Chemotherapy on 4 Stage I Grade 2 Endometrioid Adenocarcinomas of the Endometrium (EN2-EN5)

Cisplatin: (Fig. 4.21) Mean survival rates at the PPC varied from complete resistance to 27% survival for the four tumours examined. All the tumours responded at 10 x PPC. The IC50 in this category of tumours varied from 0.70 to 7.45 x PPC.

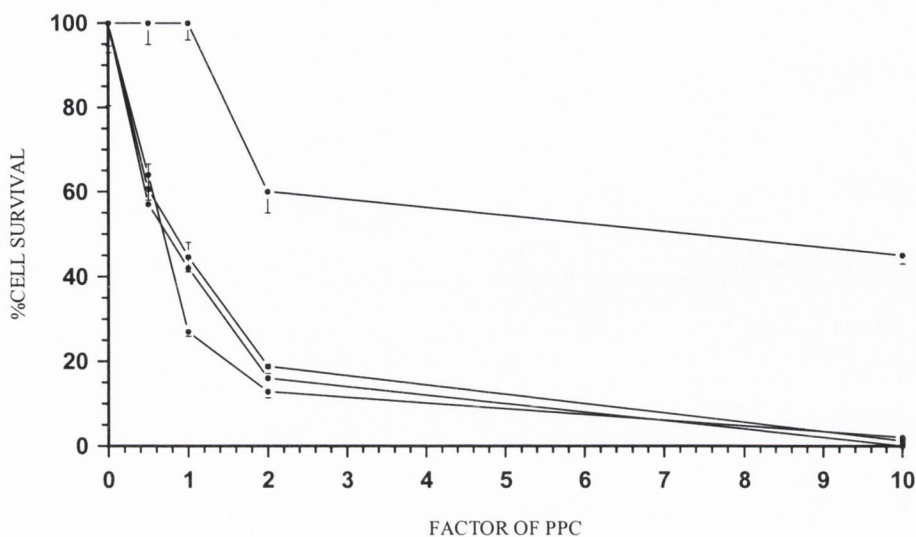


Fig. 4.21 Effect of Cisplatin on Stage I Grade 2 Endometrioid Adenocarcinomas

Paclitaxel: (Fig. 4.22) One of the tumours examined with paclitaxel was sensitive at the PPC and the other three were resistant with survival rates varying from 75% to 96.5%. At 10 x PPC all four tumours responded to paclitaxel. The IC50 ranged from 0.8 x PPC to 4.9 x PPC.

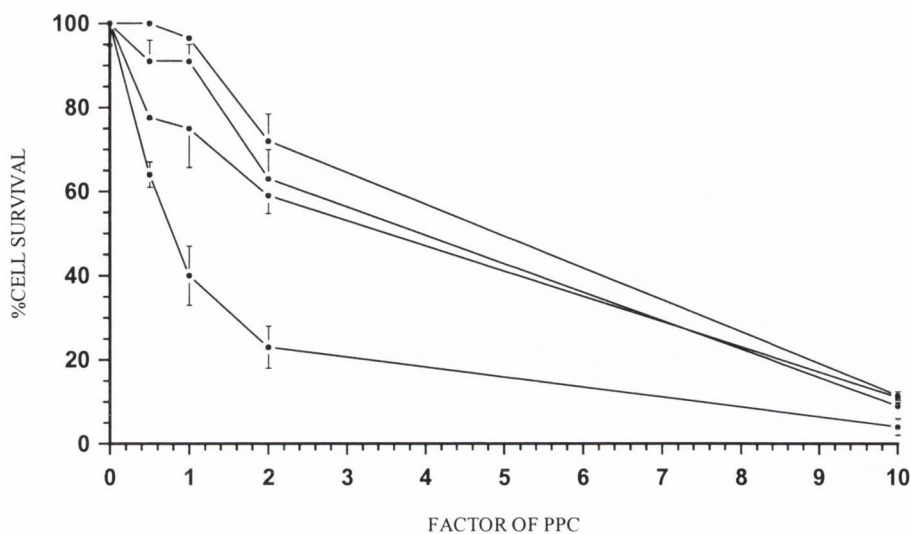


Fig. 4.22 Effect of Paclitaxel on Stage I Grade 2 Endometrioid Adenocarcinomas

Cisplatin and Paclitaxel: (Fig. 4.23) Three tumours were examined at the PPC of the cisplatin / paclitaxel combination and only one responded with a 27% survival rate. Response rates of 59.3% and 57% were seen with the other two. The IC50 varied from 0.68 x PPC to greater than 1 x PPC.

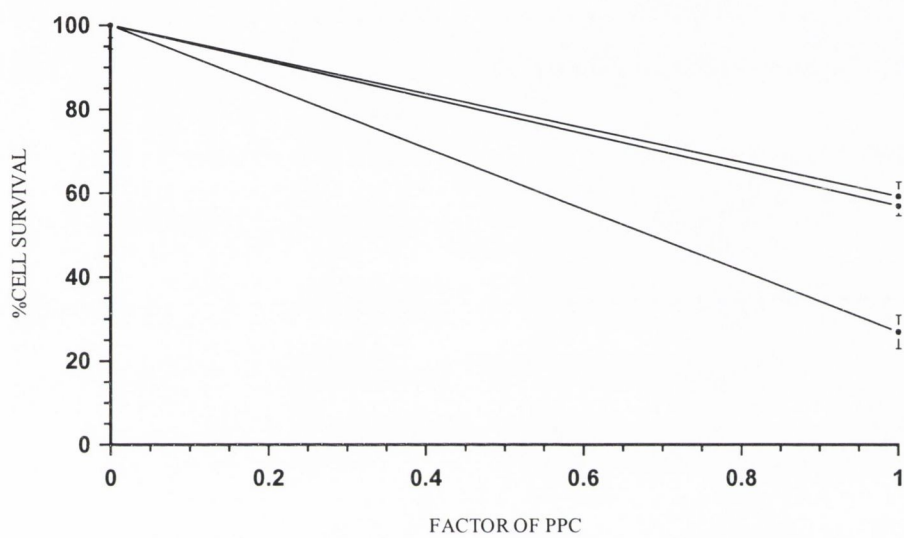


Fig. 4.23 Effect of Cisplatin & Paclitaxel on Stage I Grade 2 Endometrioid Adenocarcinomas

Topotecan: (Fig. 4.24) Two tumours were tested with topotecan and both were sensitive at the PPC with survival rates of 36% and 28.4%. The IC50 value ranged from 0.49 x PPC to 0.55 x PPC.

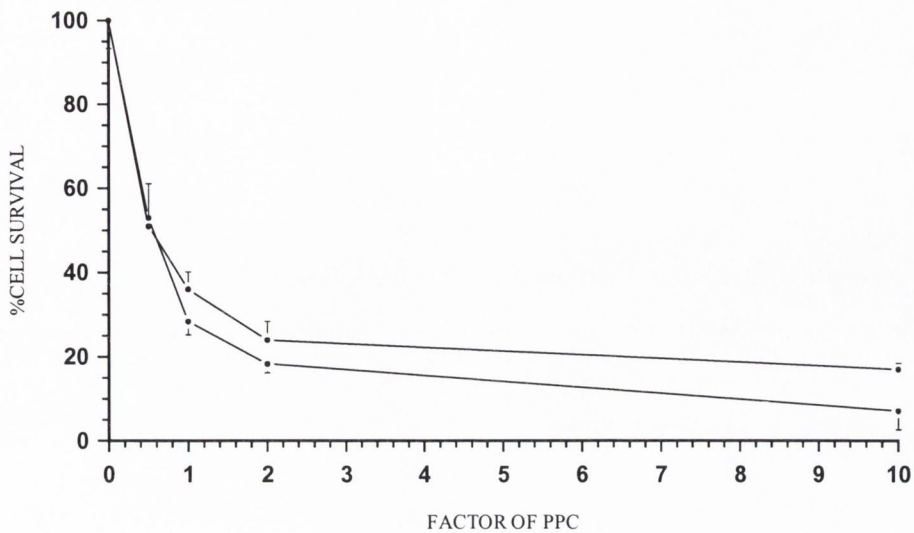


Fig. 4.24 Effect of Topotecan on Stage I Grade 2 Endometrioid Adenocarcinomas

4.3.3 Effect of Chemotherapy on 3 Stage III Grade 2 Endometrioid Adenocarcinomas of the Endometrium (EN6-EN8)

Cisplatin: (Fig. 4.25) Mean survival rates at the PPC varied from 13.4% survival to 85% survival for the three tumours examined. One tumour remained resistant at 10 x PPC with a survival rate of 78%. The IC50 in this category of tumours varied from 0.39 to > 10 x PPC.

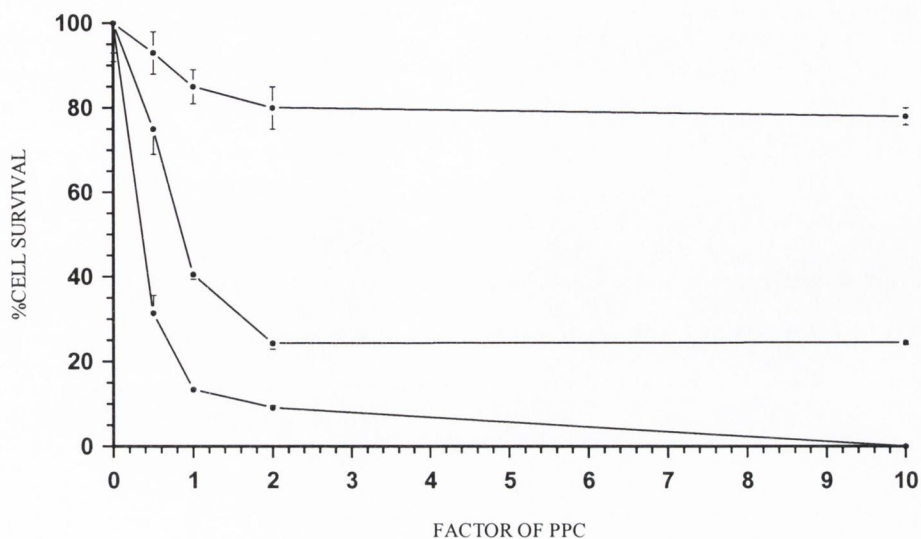


Fig. 4.25 Effect of Cisplatin on Stage III Grade 2 Endometrial Adenocarcinomas

Paclitaxel: (Fig. 4.26) One of the three tumours examined with paclitaxel was sensitive at the PPC and the other two were resistant with survival rates of 58% to 76.5%. At 10 x PPC two of the tumours were sensitive and the other had a survival rate of 57%. The IC₅₀ ranged from 0.8 x PPC to > 10 x PPC.

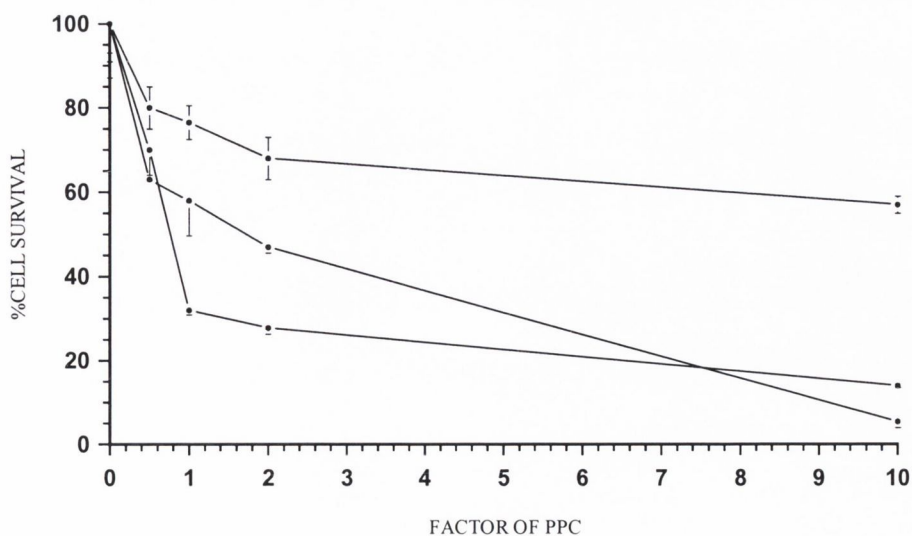


Fig. 4.26 Effect of Paclitaxel on Stage III Grade 2 Endometrial Adenocarcinomas

Cisplatin and Paclitaxel: (Fig. 4.27) Three tumours were examined at the PPC of the cisplatin / paclitaxel combination and only one responded with a 21.4% survival rate. Response rates of 53% and 73.5% were seen with the other two. The IC50 varied from 0.64 x PPC to greater than 1 x PPC.

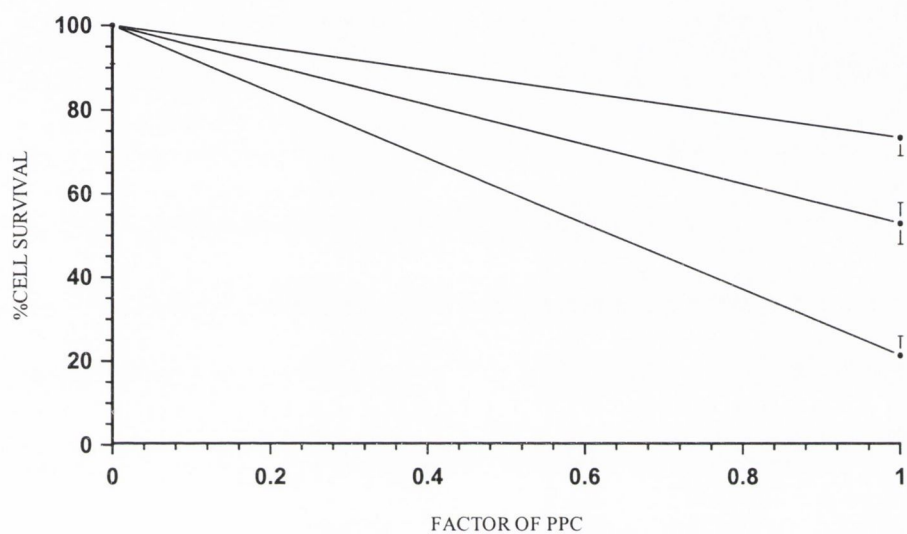


Fig. 4.27 Effect of Cisplatin & Paclitaxel on Stage III Grade 2 Endometrial Adenocarcinomas

Topotecan: (Fig. 4.28) Two of the tumours were sensitive at the PPC with survival rates of 29.9% and 32.1%. The other tumour was resistant even at 10 x PPC. The IC50 value ranged from 0.45 x PPC to > 10 x PPC.

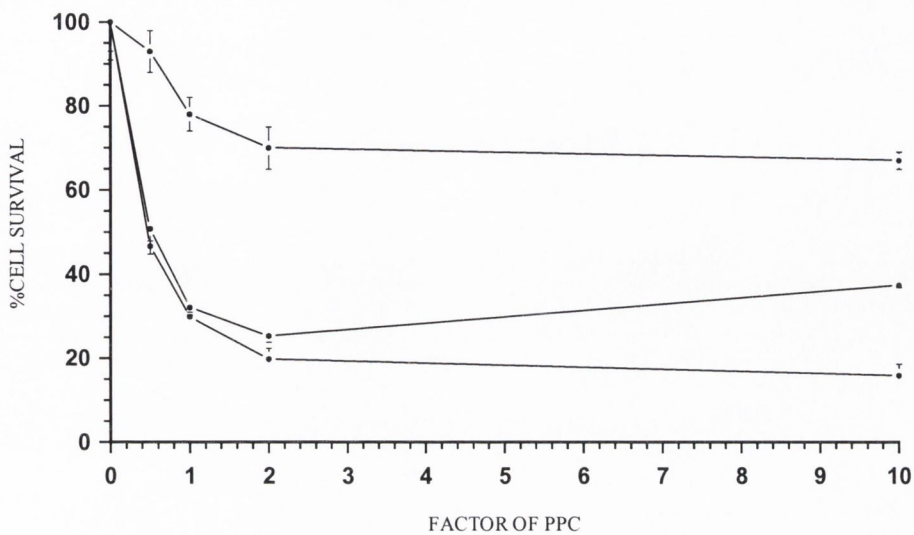


Fig. 4.28 Effect of Topotecan on Stage III Grade 2 Endometrial Adenocarcinomas

4.3.4 Effect of Chemotherapy on 4 Stage III Grade 3 Endometrial Adenosquamous carcinomas (EN9-EN12)

Cisplatin: (Fig. 4.29) Mean survival rates at the PPC varied from 7% survival to 72% survival, with two tumours being sensitive and two resistant. All the tumours responded at 10 x PPC. The IC50 in this category of tumours varied from 0.48 to 1.75 x PPC.

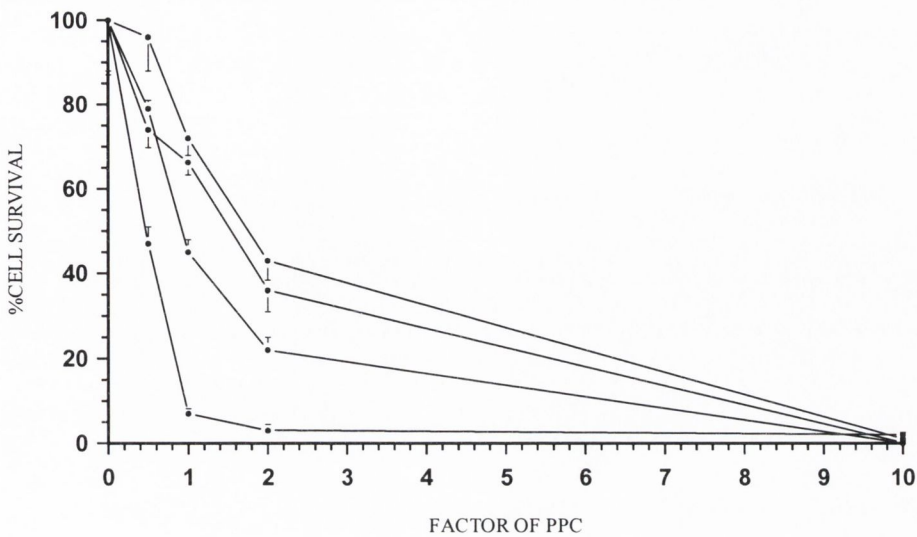


Fig. 4.29 Effect of Cisplatin on Stage III Grade 3 Endometrial Adenosquamous carcinomas

Paclitaxel: (Fig. 4.30) Two of the tumours were sensitive and two were resistant at the PPC. At 10 x PPC the two resistant tumours remained resistant. The IC50 ranged from 0.6 x PPC to > 10 x PPC.

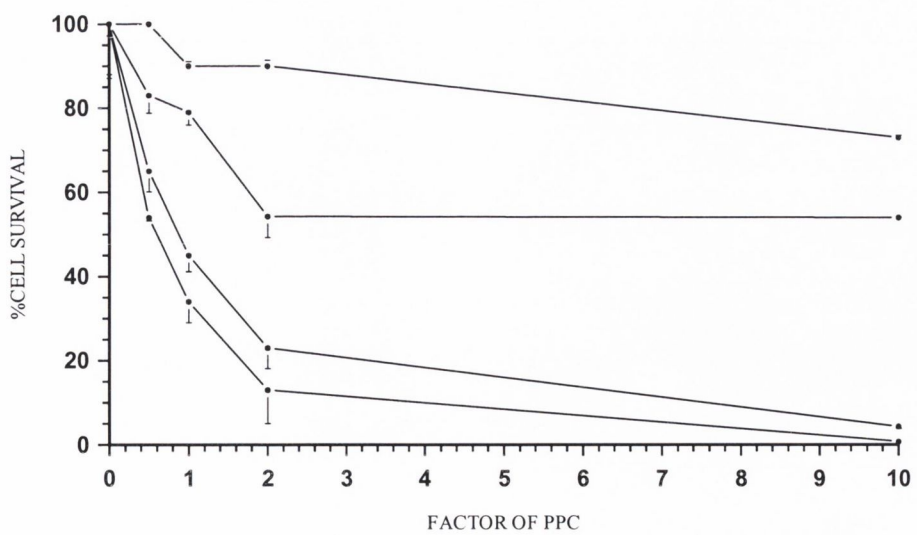


Fig. 4.30 Effect of Paclitaxel on Stage III Grade 3 Endometrial Adenosquamous Carcinomas

Cisplatin and Paclitaxel: (Fig. 4.31) A similar pattern was seen with the combination treatment, two responders and two non-responders. Survival rates ranged from 30% to 61%. The IC50 varied from 0.72 x PPC to greater than 1 x PPC.

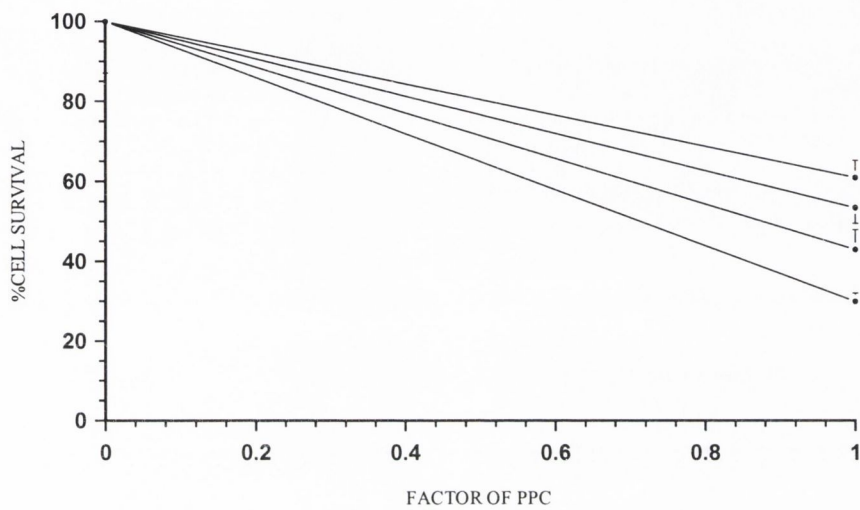


Fig. 4.31 Effect of Cisplatin & Paclitaxel on Stage III Grade 3 Endometrial Adenosquamous Carcinomas

Topotecan: (Fig. 4.32) Three of the tumours responded well to topotecan but one was quite resistant showing a 90% cell survival at the PPC and this tumour was still resistant at 10 x PPC. The IC50 value ranged from 0.4 x PPC to > 10 x PPC.

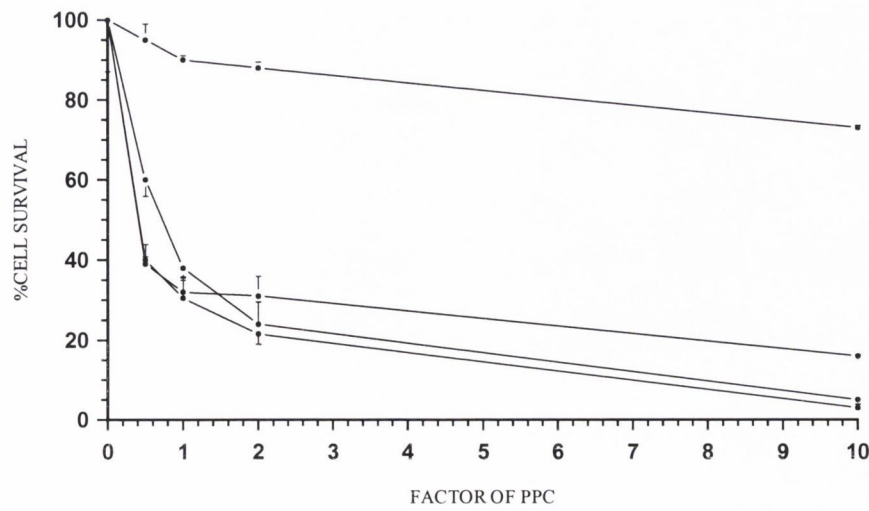


Fig. 4.32 Effect of Topotecan on Stage III Grade 3 Endometrial Adenosquamous Carcinomas

4.3.5 Effect of Chemotherapy on a Stage I Grade 1 Endometrial Adenocarcinoma (EN13)

The effects of 4 drugs (cisplatin, paclitaxel, topotecan and the cisplatin/paclitaxel combination) on this tumour are shown in Fig. 4.33. The tumour was sensitive to cisplatin / paclitaxel combination and to topotecan at the PPC but not to single agent cisplatin or single agent paclitaxel. Response rates varied from 30.6% cell survival for topotecan to 70.5% survival for paclitaxel. At 10 x PPC the tumour was sensitive to all the drugs. The IC50 for the drugs were Cisplatin (1.31 x PPC), Paclitaxel (2.65 x PPC), Cisplatin / Paclitaxel (0.95 x PPC) and Topotecan (0.7 x PPC).

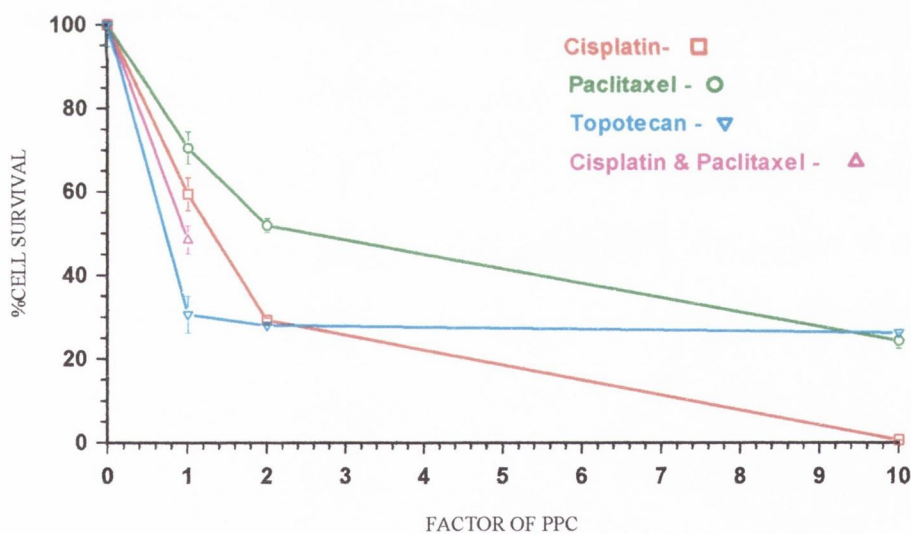


Fig. 4.33 Effect of Chemotherapy on a Stage I Grade 1 Endometrial Adenocarcinoma

4.3.6 Effect of Chemotherapy on 2 Stage I Grade 2 Endometrial Adenocarcinomas (EN14, EN15)

Cisplatin: (Fig. 4.34) Both tumours displayed a good response to cisplatin at the PPC with survival rates of 12.3% and 15.9% and total cell kill was observed in both at 10 x PPC. The IC50 ranged from 0.59 x PPC to 6 x PPC.

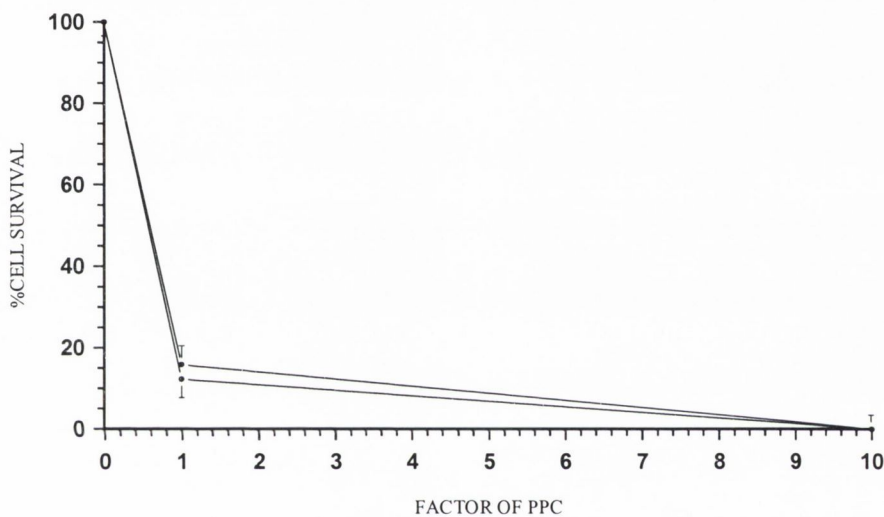


Fig. 4.34 Effect of Cisplatin on Stage I Grade 2 Endometrial Adenocarcinomas

Paclitaxel: (Fig. 4.35) None of the tumours responded well to paclitaxel at the PPC but a good response was seen at 10 x PPC. The IC50 varied from 3.6 x PPC to 4.05 x PPC.

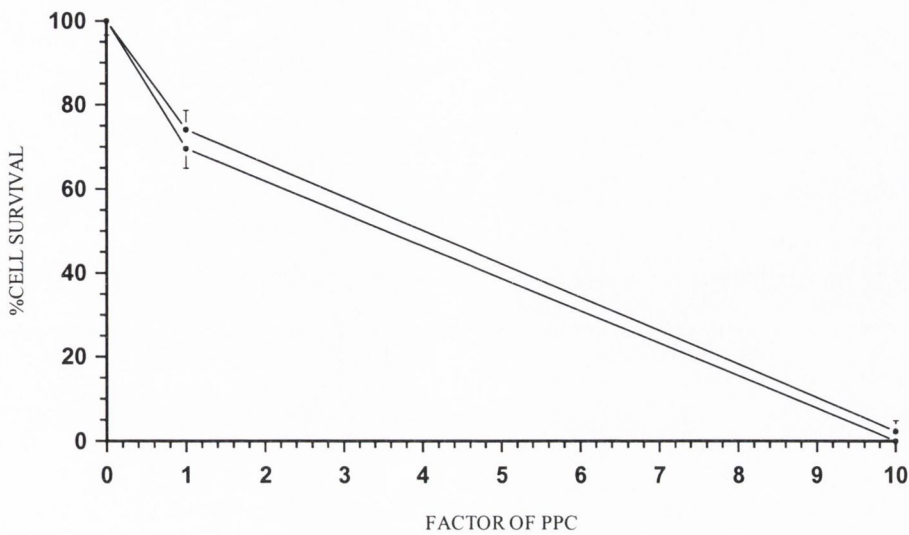


Fig. 4.35 Effect of Paclitaxel on Stage I Grade 2 Endometrial Adenocarcinomas

Cisplatin and Paclitaxel: (Fig. 4.36) One of the tumours displayed a response of 45.2% to the cisplatin / paclitaxel combination but the other tumour was quite resistant at the PPC with a survival rate of 92.7%. The IC₅₀ was 0.92 x PPC for one and >1 x PPC for the other tumour.

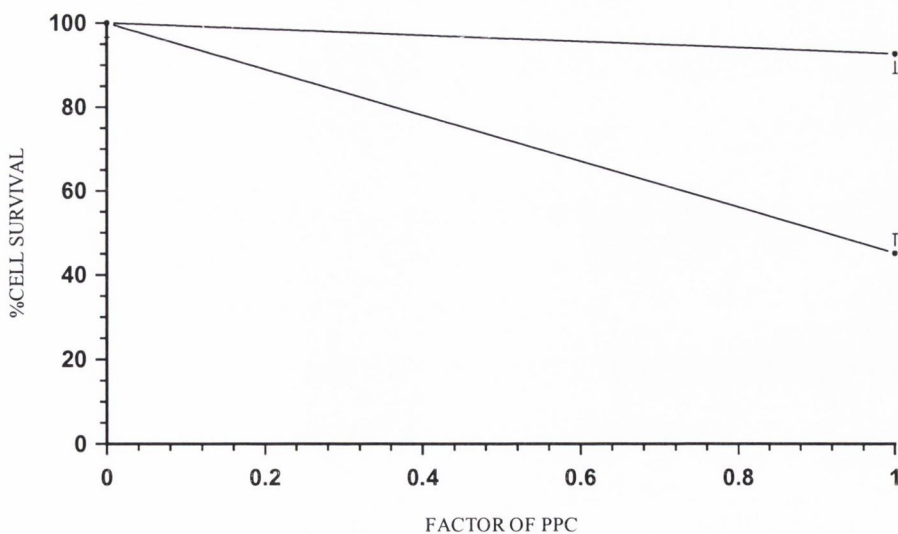


Fig. 4.36 Effect of Cisplatin & Paclitaxel on Stage I Grade 2 Endometrial Adenocarcinomas

Topotecan: (Fig. 4.37) One of the tumours responded well to topotecan at the PPC with a survival rate of 16.4%. The other tumour was extremely resistant at this concentration and also at 10 x PPC. The IC₅₀ for the sensitive tumour was 0.6 x PPC and for the resistant greater than 10 x PPC.

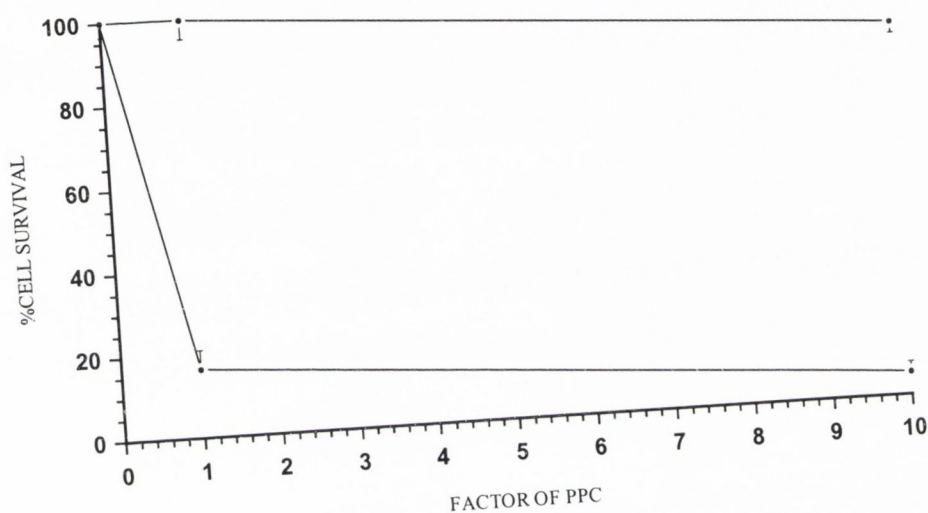


Fig. 4.37 Effect of Topotecan on Stage I Grade 2 Endometrial Adenocarcinomas

4.3.7 Effect of Chemotherapy on a Stage II Grade 3 Endometrial Adenocarcinoma (EN16)

The response of this tumour to the platinum compounds can be seen in Fig. 4.38. The tumour was resistant to both cisplatin and carboplatin, even at 20 x PPC. This tumour was examined early in the study so 20 x PPC was also tested. The IC₅₀ for the drugs were Cisplatin (>16 x PPC) and Carboplatin (>20 x PPC).

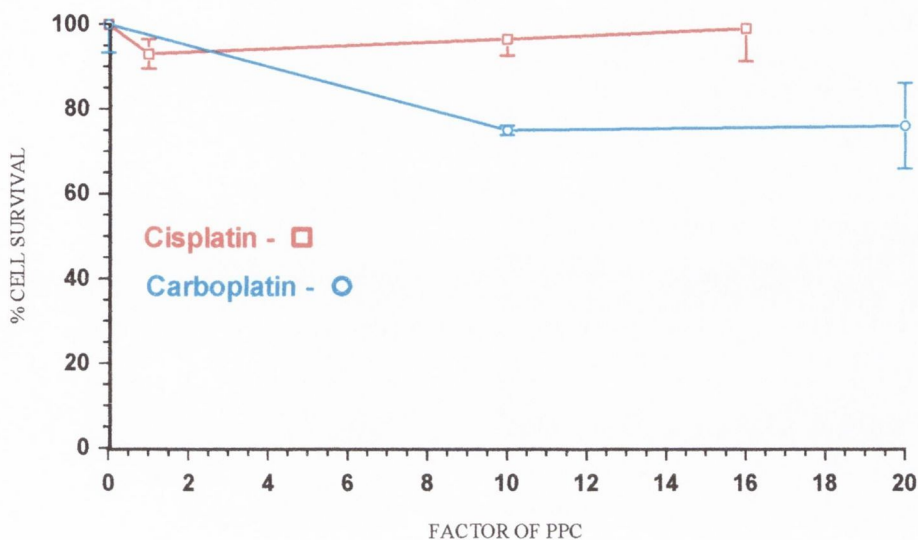


Fig. 4.38 Effect of Chemotherapy on a Stage II Grade 3 Endometrial Adenocarcinoma

4.3.8 Effect of Chemotherapy on a Stage III Grade 3 Endometrial Adenocarcinoma (EN17)

The response of this tumour is shown in Fig. 4.39. It was only sensitive to single agent carboplatin. It displayed a borderline response to cisplatin. Mean survival rates at the PPC varied from 39.2% to 100%. The tumour was sensitive to all the drugs except topotecan at 10 x PPC. The IC₅₀ for the drugs was: Cisplatin (1.4 x PPC), Paclitaxel (6.1 x PPC), Cisplatin / Paclitaxel (>1 x PPC), Carboplatin (0.8 x PPC), Carboplatin / Paclitaxel (>1 x PPC) and Topotecan (8.1 x PPC).

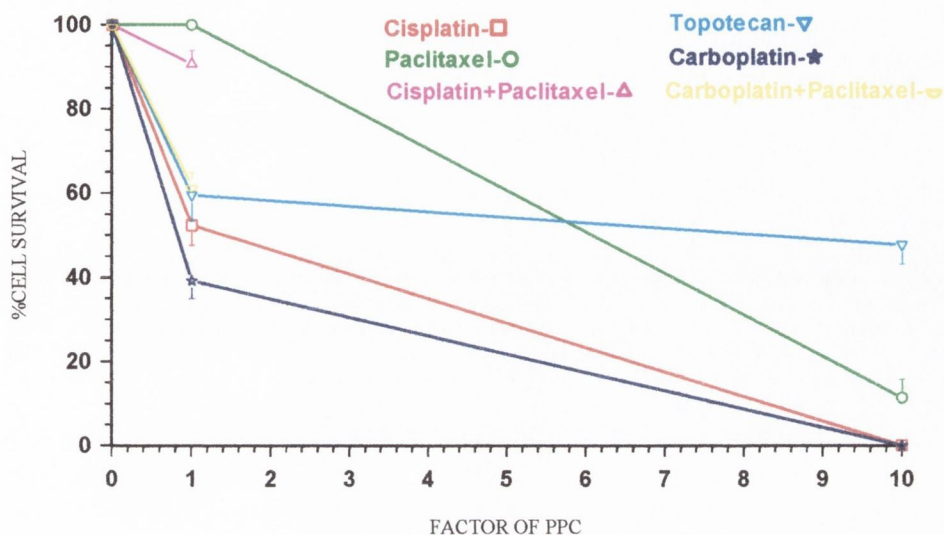


Fig. 4.39 Effect of Chemotherapy on a Stage III Grade 3 Endometrial Adenocarcinoma

4.3.9 Effect of Cisplatin on a Stage IV Grade 2 Endometrial Adenocarcinoma (EN18)

This tumour was resistant to cisplatin even at 10 x PPC with a survival rate of 82.1%, displayed in Fig. 4.40. The IC50 was > 10 x PPC.

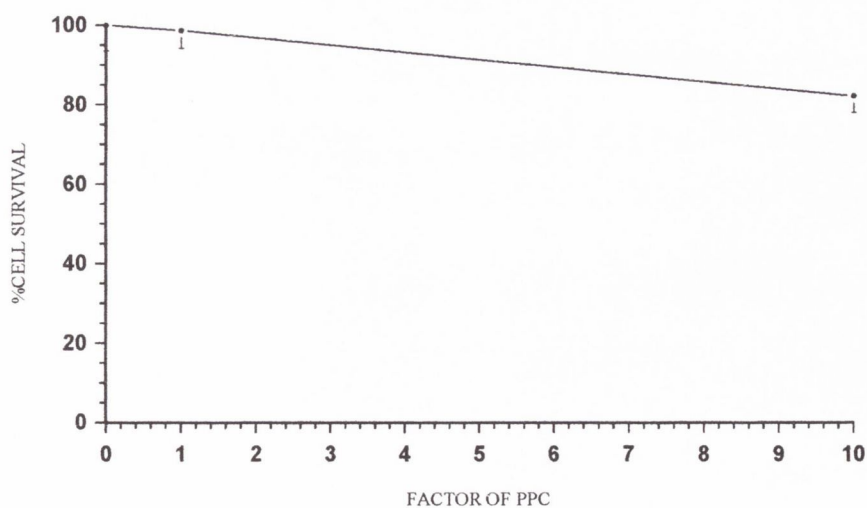


Fig. 4.40 Effect of Cisplatin on a Stage IV Grade 2 Endometrial Adenocarcinoma

4.3.10 Effect of Chemotherapy on a Stage IV Grade 3 Endometrial Adenocarcinoma (EN19)

The effects of cytotoxic drugs on this tumour are shown in Fig. 4.41. The tumour was sensitive to cisplatin and to topotecan at the PPC but not to single agent paclitaxel or to the combination of cisplatin / paclitaxel. Response rates varied from 7.3% cell survival for topotecan to 100% survival for paclitaxel. At 10 x PPC the tumour was resistant to paclitaxel and total cell kill was observed with cisplatin. The IC50 for the drugs were Cisplatin (0.5 x PPC), Paclitaxel (>10 x PPC), Cisplatin / Paclitaxel (> 1.0 x PPC) and Topotecan (0.55 x PPC).

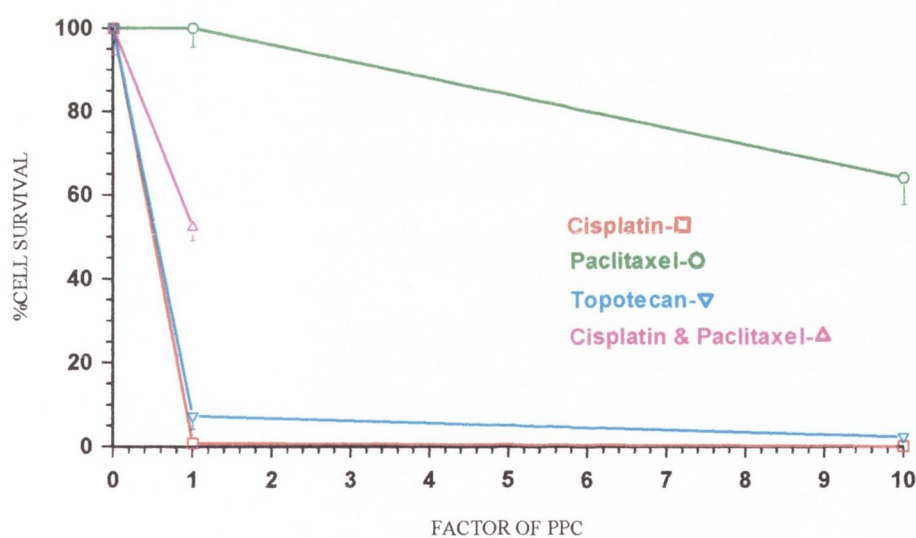


Fig. 4.41 Effect of Chemotherapy on a Stage IV Grade 3 Endometrial Adenocarcinoma

4.3.11 Effect of Chemotherapy on a Recurrent Endometrial Stromal Sarcoma (EN20)

The first biopsy from this patient was found to be resistant to cisplatin (Fig. 4.42), with a survival response of 78% at 10 x PPC. Two years later a second biopsy was taken from this patient (Fig. 4.43), which was still resistant to cisplatin but sensitive to paclitaxel at the PPC. Paclitaxel was not used on the first biopsy, as it was not part of routine treatment at the time. IC50 values were >10 x PPC for cisplatin and 0.84 x PPC for paclitaxel.

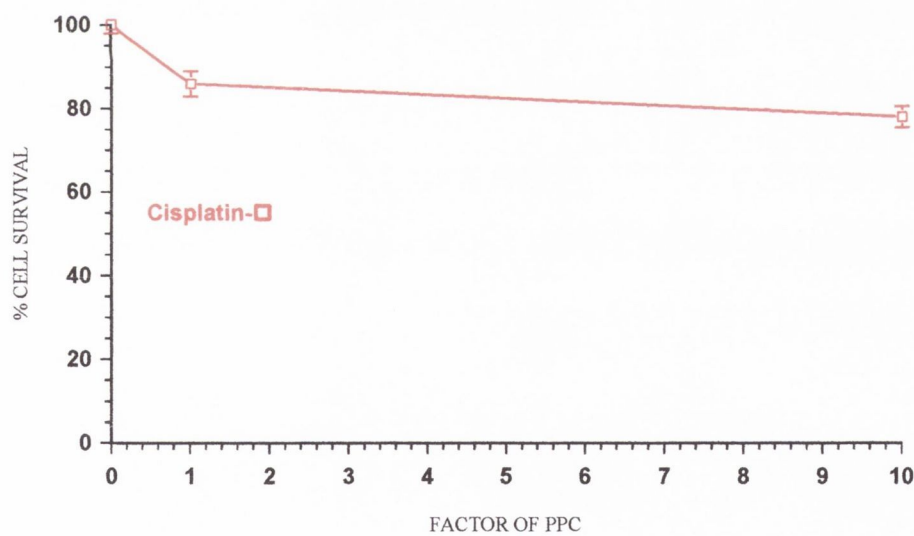


Fig. 4.42 Effect of Cisplatin on an Endometrial Stromal Sarcoma (1st Biopsy)

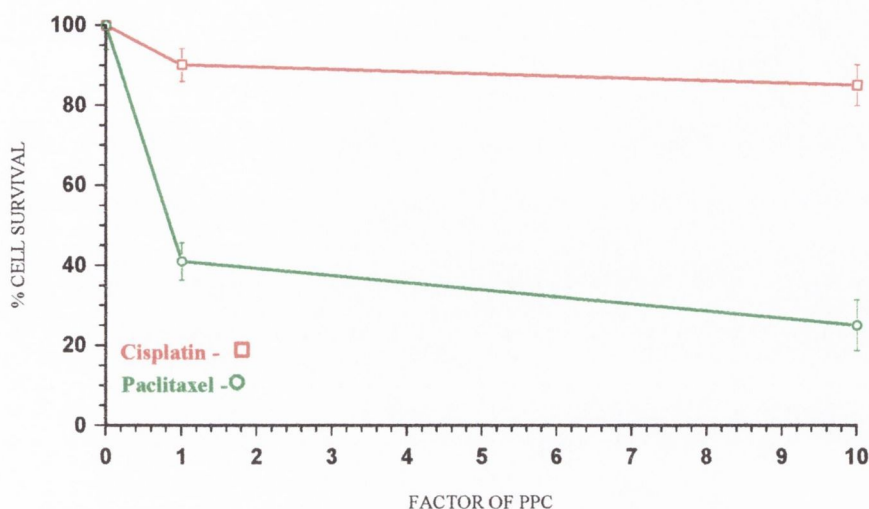


Fig. 4.43 Effect of Chemotherapy on a Recurrent Endometrial Stromal Sarcoma (2nd Biopsy)

4.3.12 Effect of Chemotherapy on an Endometrial Stromal Sarcoma (EN21)

This tumour was resistant to all the drugs at the PPC as seen in Fig. 4.44 with survival rates varying from 55% for paclitaxel to 79% for the cisplatin / paclitaxel combination. The IC₅₀ for the drugs were Cisplatin (1.5 x PPC), Paclitaxel (1.22 x PPC), Cisplatin / Paclitaxel (> 1.0 x PPC) and Topotecan (3.5 x PPC).

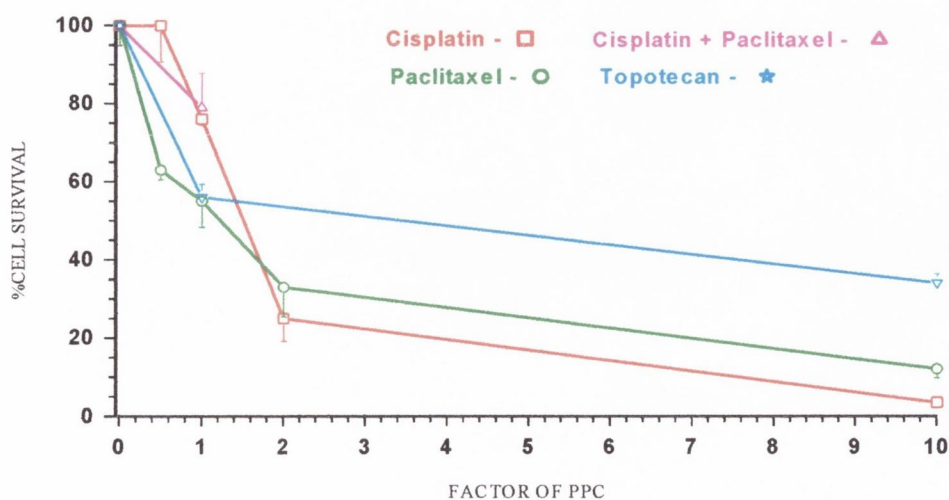


Fig. 4.44 Effect of Chemotherapy on an Endometrial Stromal Sarcoma

4.3.13 Effect of Cisplatin on an Endometrial Adenosarcoma (EN22)

The response of this tumour to cisplatin is displayed in Fig. 4.45. This tumour was resistant to cisplatin even at 10 x PPC with a survival rate of 56.9%. The IC50 was > 10 x PPC.

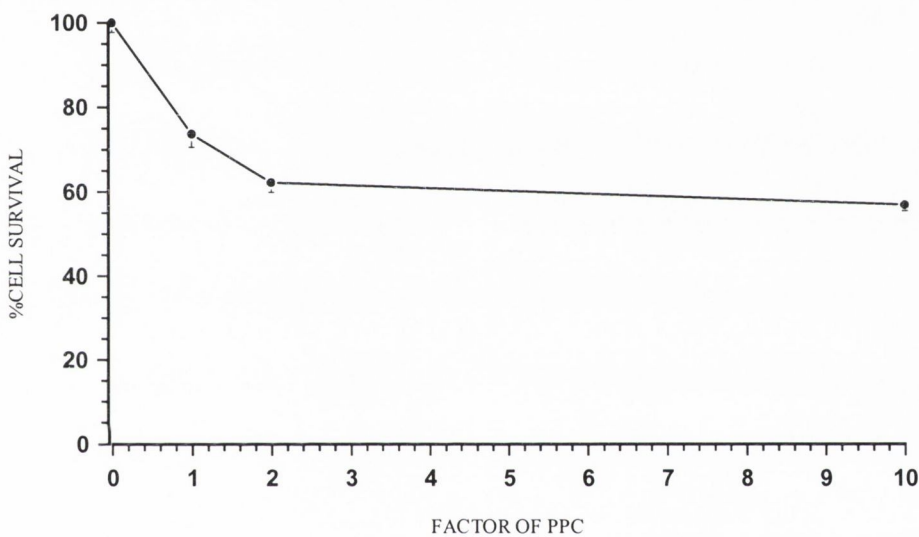


Fig. 4.45 Effect of Cisplatin on an Endometrial Adenosarcoma

4.3.14 Effect of Chemotherapy on Recurrent Endometrial Leiomyosarcomas (EN23, EN24)

Cisplatin: (Fig. 4.46) Both tumours were sensitive to cisplatin at the PPC with survival rates of 10.1% and 44%. Total cell kill was observed with one of the tumours at 10 x PPC and 2% survival with the other. The IC50 was 0.55 x PPC for one tumour and 0.9 x PPC for the other.

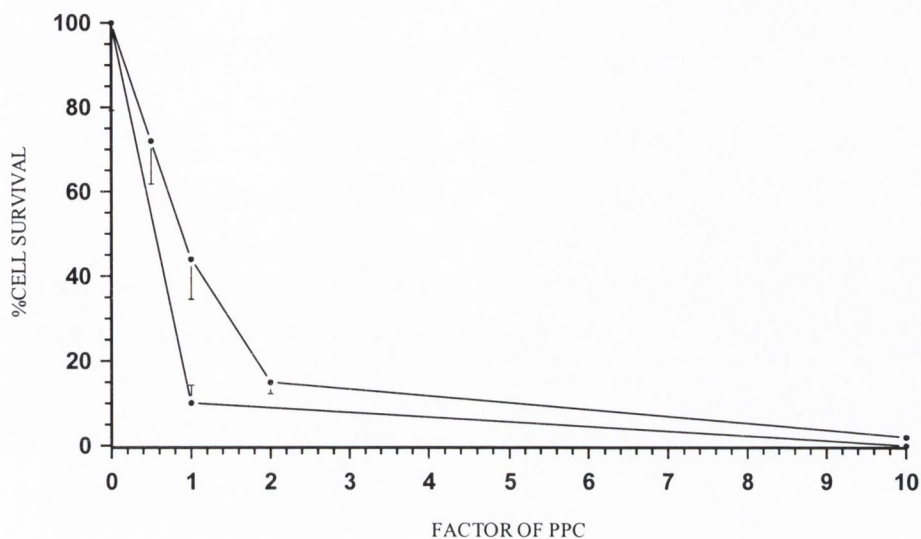


Fig. 4.46 Effect of Cisplatin on Recurrent Endometrial Leiomyosarcomas

Paclitaxel: (Fig. 4.47) Both tumours were resistant to paclitaxel at the PPC with survival rates of 62% and 80.1%. The two tumours were sensitive at 10 x PPC. The IC50 was 2.35 x PPC for one tumour and 5.5 x PPC for the other.

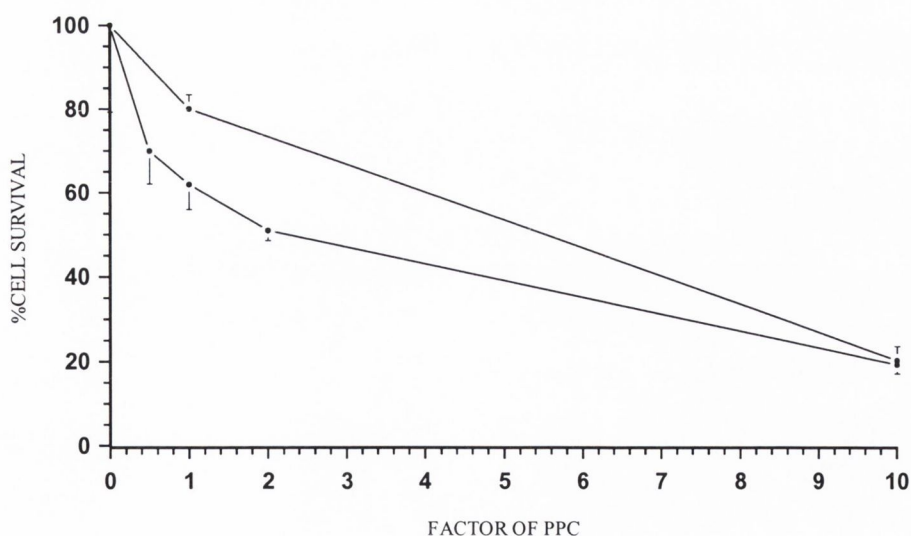


Fig. 4.47 Effect of Paclitaxel on Recurrent Endometrial Leiomyosarcomas

Cisplatin and Paclitaxel: (Fig. 4.48) One tumour gave a borderline response and the other was sensitive at the PPC. The IC50 was 0.72 x PPC for one tumour and >1 x PPC for the other.

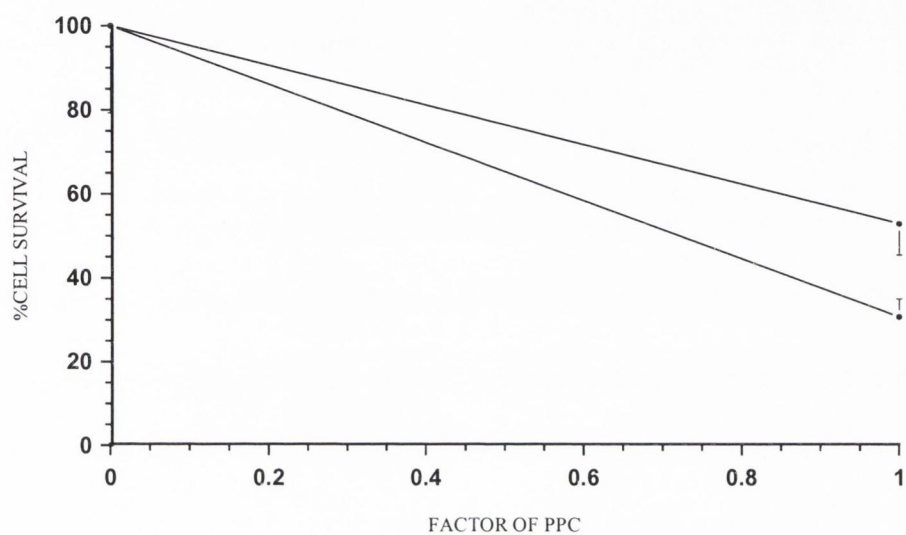


Fig. 4.48 Effect of Cisplatin & Paclitaxel on Recurrent Endometrial Leiomyosarcomas

4.3.15 Effect of Chemotherapy on a Mixed Mullerian Tumour of the Endometrium (EN25)

The response of this tumour is shown in Fig. 4.49. It was only examined at the PPC and was sensitive to all the drugs tested. Complete cell kill was observed with paclitaxel and a mean survival rate of 4.14% was observed with the cisplatin / paclitaxel combination. A survival rate of 40.8% was seen with single agent cisplatin. IC50 values were: Cisplatin (0.84 x PPC), Paclitaxel (0.5 x PPC) and Cisplatin / Paclitaxel (0.54 x PPC).

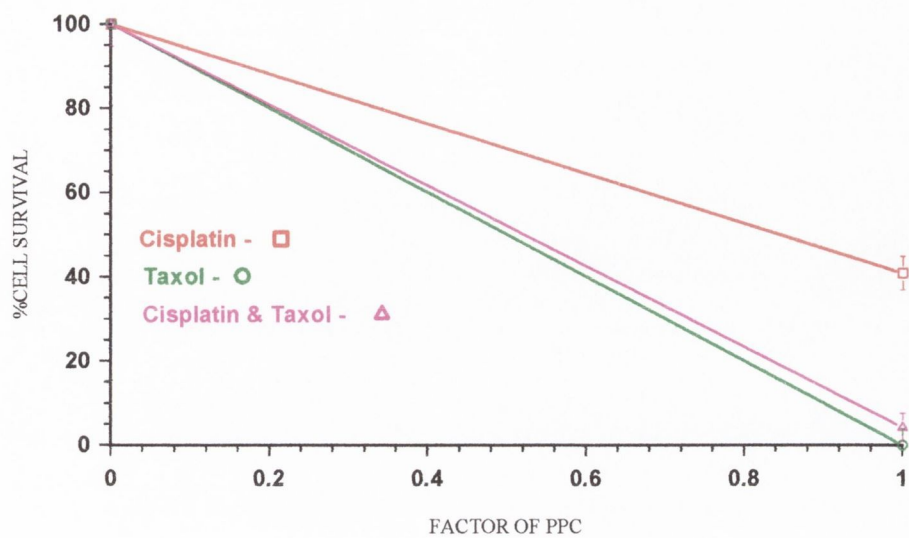


Fig. 4.49 Effect of Chemotherapy on a Stage III Mixed Mullerian Tumour of Endometrium

CHAPTER 5

***IN-VITRO-IN VIVO* CORRELATIONS**

5.1 *In Vitro In Vivo* Correlations

The relationship between drug action on a tumour *in vitro* and the patient's clinical response to the same drug was evaluated. In addition to determining the response to chemotherapy progression free survival (PFS) and overall survival (OS) were examined in these patients to determine if the assay system could be of prognostic value. Fifty-four percent of the ovarian cancer patients presented with a recurrence within 1 year of surgery and an additional 20% within 3 years. Retrospective correlations between *in vitro* chemosensitivity / resistance and clinical response were available in 76 cases. Correlations were made for 64 of 87 ovarian cancer patients. The remaining 23 ovarian specimens consisted of 11 with no *in vitro* results, 4 who were lost to follow-up, 7 patients who had died prior to receiving chemotherapy and 1 patient who did not receive chemotherapy. However, the information that was available on these patients was included in the survival analysis. Nine endometrial cancer patients and 3 cervical cancer patients received chemotherapy and their clinical response was included in the retrospective correlations. Details of *in vitro-in vivo* correlations are given in Table 5.1. The response to the chemotherapy regimen the patient received and the corresponding *in vitro* response to this regimen at the PPC is shown. The patients included in the retrospective correlations included 25 who showed no evidence of disease at the 12-month follow-up stage. Exceptions to the sensitive definition occurred in 5 patients. Ov36 and ov39 had received neoadjuvant chemotherapy, which significantly reduced the size of their tumours. Ov19 and ov24 demonstrated a very good initial response to the chemotherapy but recurred at 10 and 11 months respectively. Ov33 was displaying a good response to chemotherapy but died from another cause.

Forty of the patients included in the retrospective analysis developed recurrences within 12 months of operation. Exceptions to the resistant definition included a recurrent patient who had previous chemotherapy and was undergoing repeat surgery, ov66. Other exceptions included patients sent to the hospice for palliative care where the only follow-up was date of death, ov38, ov42, ov72, and ov74. Ov46 had to change her chemotherapy regimen due to allergies and this was taken into consideration when determining her *in vivo* response.

Statistical analysis is shown in Table 5.2. In 37 cases the tumour was found to be resistant *in vitro* and in 36 of these cases the patient presented with a recurrence, had evidence of active disease or died from the disease suggesting 97.2% prediction accuracy for resistance. The mean PFS for this group was 8.79 months. In 39 instances the tumour was sensitive to a drug *in vitro*. Twenty-nine of these patients were found to be sensitive *in vivo* with a mean PFS of 20.96 months, suggesting 74.4% prediction accuracy for sensitivity. The sensitivity²⁸⁶ $[(\text{true positives})/(\text{true positives} + \text{false negatives})]$ of the assay was 0.97. Thus, the probability of a positive *in vitro* test for any patient whose tumour responded clinically was 97%. The specificity $[(\text{true negative})/(\text{true negative} + \text{false positives})]$ was 0.78. The probability of a negative *in vitro* test for a patient who failed to respond clinically was, therefore, 78%. The association between *in vitro* and *in vivo* results for all correlations, as measured by chi-square, was highly significant ($p < 0.0001$).

Table 5.1 *In vitro* and *in vivo* results and patient outcome

Abbreviations: PFS (Progression free survival in months), Prog (Progressed), OS (Overall survival in months), Prev chemo (Previous chemotherapy), U (Unknown), LTF (lost to follow-up), NC (Not cultured), NE (Not evaluable), ND (Not documented)

Number	Specimen	Grade	Stage	Age	PFS	Prog	OS	Status	Prev chemo	<i>In vivo</i>	<i>In vitro</i>
Ovarian Carcinomas											
OV1	Serous papillary adenocarcinoma	1	I	29	4.00	Yes	78.00	Alive	No	Resistant	NC
OV2	Serous papillary adenocarcinoma	1	I	53	16.00	No	16.00	Alive	No	Sensitive	Sensitive
OV3	Serous papillary adenocarcinoma	2	I	43	31.00	No	31.00	Alive	No	Sensitive	Sensitive
OV4	Serous papillary adenocarcinoma	2	I	49	24.00	No	24.00	Alive	No	No chemo	Sensitive
OV5	Serous papillary adenocarcinoma	3	II	40	32.00	No	32.00	Alive	No	Sensitive	NC
OV6	Serous papillary adenocarcinoma	2	III	65	17.00	No	17.00	Alive	No	Sensitive	Sensitive
OV7	Serous papillary adenocarcinoma	2	III	61	U	U	U	LTF	No	LTF	Sensitive

OV8	Serous papillary adenocarcinoma	2	III	59	14.00	Yes	18.00	Alive	Yes	Sensitive	Sensitive
OV9	Serous papillary adenocarcinoma	2	III	55	1.00	Yes	1.00	RIP	Yes	Resistant	Resistant
OV10	Serous papillary adenocarcinoma	2	III	31	U	U	U	LTF	No	LTF	Sensitive
OV11	Serous papillary adenocarcinoma	2	III	63	57.00	No	57.00	Alive	No	Sensitive	Sensitive
OV12	Serous papillary adenocarcinoma	2	III	77	12.00	Yes	12.00	Alive	No	Resistant	Resistant
OV13	Serous papillary adenocarcinoma	2	III	70	4.00	Yes	20.00	RIP	No	Resistant	Resistant
OV14	Serous papillary adenocarcinoma	2	III	53	21.00	No	21.00	Alive	No	Sensitive	Sensitive
OV15	Serous papillary adenocarcinoma	2	III	43	13.00	No	U	LTF	No	Sensitive	Sensitive
OV16	Serous papillary adenocarcinoma	2	III	62	26.00	No	26.00	Alive	No	Sensitive	Sensitive
OV17	Serous papillary adenocarcinoma	2	III	78	8.00	Yes	8.00	RIP	No	Resistant	Sensitive
OV18	Serous papillary adenocarcinoma	2	III	45	10.00	Yes	U	LTF	No	Resistant	Sensitive
OV19	Serous papillary adenocarcinoma	2	III	46	10.00	Yes	11.50	RIP	No	Sensitive	Sensitive
OV20	Serous papillary adenocarcinoma	2	III	57	31.00	No	31.00	Alive	No	Sensitive	Sensitive
OV21	Serous papillary adenocarcinoma	2	III	70	.25	Yes	.25	RIP	No	RIP	Resistant
OV22	Serous papillary adenocarcinoma	2	III	60	13.00	Yes	24.00	Alive	Yes	Sensitive	Sensitive
OV23	Serous papillary adenocarcinoma	2	III	54	12.00	Yes	32.00	RIP	No	Resistant	Resistant
OV24	Serous papillary adenocarcinoma	2	III	60	11.00	Yes	16.00	Alive	No	Sensitive	Sensitive

OV25	Serous papillary adenocarcinoma	2	III	32	5.00	Yes	5.00	RIP	Yes	Resistant	Sensitive
OV26	Serous papillary adenocarcinoma	2	III	74	14.00	Yes	14.00	Alive	No	Sensitive	Sensitive
OV27	Serous papillary adenocarcinoma	2	III	70	9.00	Yes	24.00	RIP	No	Resistant	Sensitive
OV28	Serous papillary adenocarcinoma	2	III	58	5.00	Yes	U	LTF	No	Resistant	Resistant
OV29	Serous papillary adenocarcinoma	2	III	76	27.00	Yes	27.00	RIP	No	Sensitive	NC
OV30	Serous papillary adenocarcinoma	2	III	65	12.00	Yes	12.00	RIP	No	Resistant	NC
OV31	Serous papillary adenocarcinoma	3	III	43	5.00	Yes	8.00	RIP	No	Resistant	Resistant
OV31A	Serous papillary adenocarcinoma	3	III	43	3.00	Yes	3.00	RIP	Yes	Resistant	Resistant
OV32	Serous papillary adenocarcinoma	3	III	57	6.00	Yes	24.00	Alive	No	Resistant	Resistant
OV33	Serous papillary adenocarcinoma	3	III	64	9.00	Yes	9.00	RIP	No	Sensitive	Sensitive
OV34	Serous papillary adenocarcinoma	3	III	43	7.00	Yes	17.00	RIP	Yes	Resistant	Resistant
OV35	Serous papillary adenocarcinoma	3	III	76	18.00	No	18.00	Alive	No	Sensitive	Sensitive
OV36	Serous papillary adenocarcinoma	3	III	70	5.00	Yes	5.00	RIP	Yes	Sensitive	Sensitive
OV37	Serous papillary adenocarcinoma	3	III	66	16.00	No	16.00	Alive	No	Sensitive	Sensitive
OV38	Serous papillary adenocarcinoma	3	III	65	18.00	Yes	41.00	RIP	No	Resistant	Resistant
OV39	Serous papillary adenocarcinoma	3	III	66	U	U	U	LTF	Yes	Sensitive	Sensitive

OV40	Serous papillary adenocarcinoma	3	III	84	7.00	Yes	7.00	RIP	No	RIP	Sensitive
OV41	Serous papillary adenocarcinoma	3	III	76	32.00	Yes	46.00	Alive	No	Sensitive	Sensitive
OV42	Serous papillary adenocarcinoma	3	III	64	14.50	Yes	14.50	RIP	No	Resistant	Resistant
OV43	Serous papillary adenocarcinoma	3	III	36	9.00	Yes	34.00	Alive	No	Resistant	Resistant
OV44	Serous papillary adenocarcinoma	3	III	54	17.00	Yes	U	LTF	No	Sensitive	Sensitive
OV45	Serous papillary adenocarcinoma	3	III	48	13.00	Yes	24.00	Alive	No	Sensitive	Sensitive
OV46	Serous papillary adenocarcinoma	3	III	48	15.00	Yes	31.00	Alive	No	Resistant	Resistant
OV47	Serous papillary adenocarcinoma	3	III	55	17.00	Yes	30.00	Alive	No	Sensitive	Sensitive
OV48	Serous papillary adenocarcinoma	3	III	61	76.00	No	76.00	Alive	No	Sensitive	NE
OV49	Serous papillary adenocarcinoma	3	III	59	11.00	Yes	25.00	RIP	No	Resistant	NC
OV50	Serous papillary adenocarcinoma	3	III	73	6.00	Yes	13.00	RIP	No	Resistant	NC
OV51	Serous papillary adenocarcinoma	2	IV	61	7.00	Yes	7.00	RIP	No	Resistant	Sensitive
OV52	Serous papillary adenocarcinoma	2	IV	50	1.00	Yes	1.00	RIP	No	RIP	Sensitive
OV53	Serous papillary adenocarcinoma	2	IV	45	4.00	Yes	19.00	RIP	No	Resistant	Resistant
OV54	Serous papillary adenocarcinoma	2	IV	60	11.00	Yes	23.00	RIP	No	Resistant	Sensitive
OV55	Serous papillary adenocarcinoma	2	IV	58	35.00	Yes	47.00	RIP	No	Sensitive	Sensitive

OV56	Serous papillary adenocarcinoma	2	IV	39	U	U	U	LTF	No	LTF	Resistant
OV57	Serous papillary adenocarcinoma	2	IV	78	.20	Yes	.20	RIP	No	RIP	Resistant
OV58	Serous papillary adenocarcinoma	2	IV	29	9.00	Yes	22.00	Alive	Yes	Resistant	Resistant
OV59	Serous papillary adenocarcinoma	2	IV	57	12.00	Yes	15.00	Alive	No	Resistant	Resistant
OV60	Serous papillary adenocarcinoma	2	IV	43	24.00	Yes	50.00	Alive	No	Resistant	NC
OV61	Serous papillary adenocarcinoma	3	IV	47	6.00	Yes	20.00	RIP	No	Resistant	NC
OV62	Serous papillary adenocarcinoma	3	IV	49	10.00	Yes	25.00	Alive	No	Resistant	Resistant
OV63	Serous papillary adenocarcinoma	3	IV	47	7.00	Yes	13.00	RIP	No	Resistant	Resistant
OV64	Serous papillary adenocarcinoma	3	IV	60	1.00	Yes	1.00	RIP	No	RIP	Sensitive
OV65	Serous papillary adenocarcinoma	3	IV	74	75.00	No	75.00	Alive	No	Sensitive	Resistant
OV66	Serous papillary adenocarcinoma	3	IV	62	19.50	Yes	19.50	RIP	Yes	Resistant	Resistant
OV67	Serous papillary adenocarcinoma	3	IV	50	1.00	Yes	1.00	RIP	No	RIP	Sensitive
OV68	Serous papillary adenocarcinoma	3	IV	54	3.00	Yes	U	LTF	No	Resistant	Sensitive
OV69	Serous papillary adenocarcinoma	3	IV	58	6.00	Yes	12.00	RIP	No	Resistant	NC
OV70	Clear cell carcinoma	3	ND	71	3.00	Yes	U	LTF	Yes	Resistant	Resistant

OV71	Clear cell carcinoma	3	I	62	39.00	No	39.00	Alive	No	Sensitive	Sensitive
OV72	Clear cell carcinoma	3	II	69	14.00	Yes	33.00	RIP	No	Resistant	Resistant
OV73	Clear cell carcinoma	3	II	71	3.00	Yes	3.00	RIP	Yes	Resistant	Sensitive
OV74	Clear cell carcinoma	3	III	64	14.50	Yes	14.50	RIP	No	Resistant	Resistant
OV75	Clear cell carcinoma	3	III	85	.40	Yes	.40	RIP	No	RIP	Sensitive
OV76	Clear cell carcinoma	3	III	58	6.00	Yes	6.00	RIP	No	Resistant	Resistant
OV77	Clear cell carcinoma	ND	IV	47	6.00	Yes	8.00	RIP	No	Resistant	Resistant
OV78	Mucinous adenocarcinoma	ND	I	52	5.00	Yes	29.00	Alive	No	Resistant	Resistant
OV79	Mucinous adenocarcinoma	2	III	47	12.00	Yes	13.00	Alive	No	Resistant	Resistant
OV80	Mucinous adenocarcinoma	3	III	41	3.00	Yes	3.00	RIP	Yes	Resistant	Resistant
OV81	Mucinous adenocarcinoma	3	IV	49	12.00	Yes	U	LTF	No	Resistant	Resistant
OV82	Peritoneal adenocarcinoma	3	IV	71	19.00	No	19.00	Alive	No	Sensitive	Sensitive
OV83	Endometrioid adenocarcinoma	3	I	44	17.00	No	17.00	Alive	No	Sensitive	Sensitive
OV84	Endometrioid adenocarcinoma	1	II	66	U	U	U	LTF	No	LTF	Resistant

OV85	Transitional cell carcinoma	ND	II	52	14.00	No	14.00	Alive	No	Sensitive	Sensitive
OV86	Adenosquamous carcinoma	2	III	40	13.00	Yes	13.00	RIP	No	Resistant	NE
Endometrial Carcinomas											
EN3	Endometrioid adenocarcinoma	2	I	42	42.00	No	42.00	Alive	No	Sensitive	Sensitive
EN9	Adenosquamous carcinoma	3	III	77	8.00	Yes	LTF	LTF	No	Resistant	Resistant
EN11	Adenosquamous carcinoma	3	III	91	12.00	Yes	12.00	RIP	No	Resistant	Sensitive
EN12	Adenosquamous carcinoma	3	III	42	10.00	Yes	LTF	LTF	No	Resistant	Resistant
EN18	Serous papillary adenocarcinoma	2	IV	53	5.00	Yes	5.00	RIP	No	Resistant	Resistant
EN19	Serous papillary adenocarcinoma	3	IV	57	20.00	No	20.00	Alive	No	Sensitive	Sensitive
EN20	Stromal sarcoma	2	ND	50	9.00	Yes	11.00	RIP	Yes	Resistant	Resistant
EN22	Adenosarcoma	3	ND	65	6.00	Yes	U	LTF	No	Resistant	Resistant
EN24	Leiomyosarcoma	ND	ND	39	12.00	Yes	16.00	RIP	Yes	Resistant	Resistant

Cervical Carcinomas

CX15	Squamous carcinoma	2	II	70	6.00	Yes	6.00	RIP	No	Resistant	Resistant
CX16	Squamous carcinoma	2	II	75	12.00	Yes	28.00	RIP	No	Resistant	Resistant
CX22	Squamous carcinoma	3	II	42	11.00	Yes	31.00	RIP	U	Resistant	Sensitive

Table 5.2 *In vitro-In vivo* correlations

Sensitive <i>In vitro</i>	Sensitive <i>In vitro</i>	Resistant <i>In vitro</i>	Resistant <i>In vitro</i>
Sensitive <i>In vivo</i>	Resistant <i>In vivo</i>	Sensitive <i>In vivo</i>	Resistant <i>In vivo</i>
29	10	1	36
p<0.0001 Chi-Square			

Tumour details and *in vitro* and *in vivo* response were examined for the ovarian cancers in Kaplan-Meier curves to determine their influence on PFS and OS. Analysis was confined to the malignant ovarian samples, as chemotherapy was not relevant in the benign/normal group and numbers were too few in the cervical and endometrial groups. The grade 1 tumours were excluded from evaluation, as only 2 existed. Cumulative (Cum) survival was graphed against progression free survival in months. Tables 5.3 and 5.4 summarise the median survival times in months and their significance. The 50th percentile refers to the survival time in months when 50% of the patients had an event. In some cases this value was not reached so the 25th percentile was quoted.

Table 5.3 Log rank and quartiles for progression free survival

		25 th percentile	50 th percentile	75 th percentile	p
Grade	2	27	12	7	.7013
	3	18	10	6	
Stage	Early	-	-	5	.0141
	Late	17	11	6	
Age	<55	17	10	5	.488
	>55	27	12	6	
Previous	Yes	9	5	3	.0023
	No	27	12	6	
<i>In vivo</i>	Sensitive		35	14	.0000
	Resistant	12	7	5	
<i>In vitro</i>	Sensitive	35	14	7	.0009
	Resistant	12	7	4	

Table 5.4 Log rank and quartiles for overall survival

		25 th percentile	50 th percentile	75 th percentile	p
Grade	2	47	27	11.5	.7514
	3		20	8	
Stage	Early	-	-	33	.0231
	Late	47	20	8	
Age	<55		32	11.5	.5011
	>55	47	24	9	
Previous	Yes	19.5	5	3	.0017
	No		32	12	
<i>In vivo</i>	Sensitive			47	.0001
	Resistant	33	19	8	
<i>In vitro</i>	Sensitive		47	7	.2461
	Resistant	41	19.5	8	

5.2 Tumour Grade and survival

The present group of specimens contained only 2 grade 1 tumours so these were excluded from survival analyses. Seventy-nine percent of the grade 2 tumours progressed with a median PFS of 12 months and 80% of the grade 3 tumours with a median PFS of 10 months (Figure 5.1). Median OS was 27 months for the grade 2 tumours and 20 months for the grade 3 tumours. Fifty-three percent of the grade 2 and 60% of the grade 3 tumours had died at time of follow up (Figure 5.2). This was not significant.

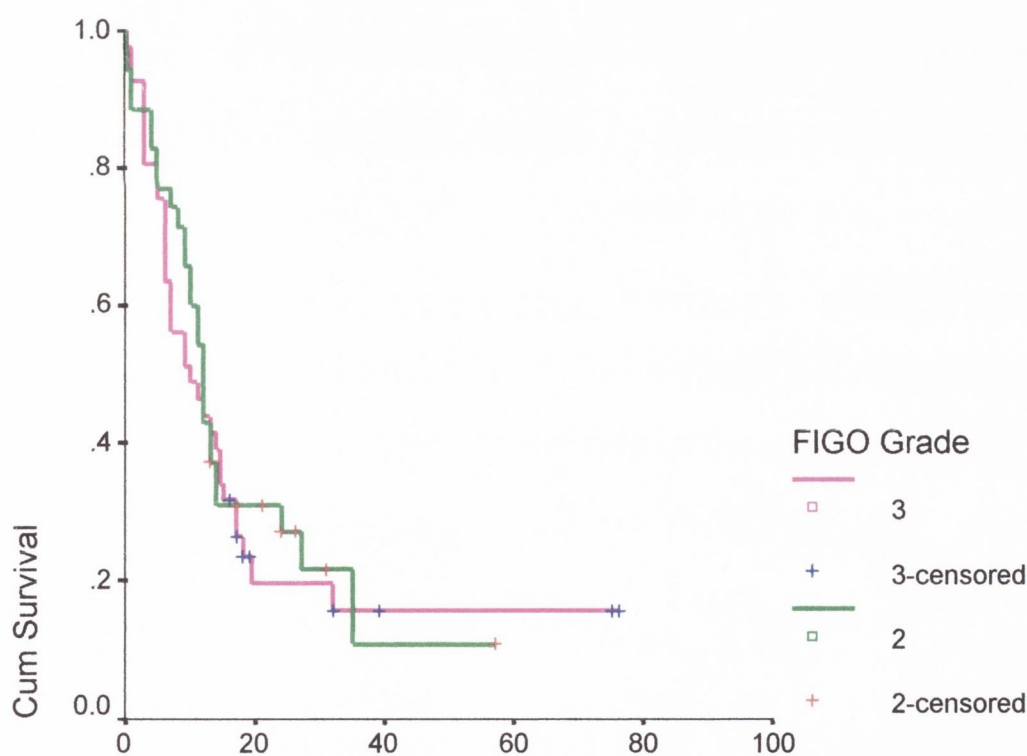


Fig. 5.1 PFS in relation to tumour grade

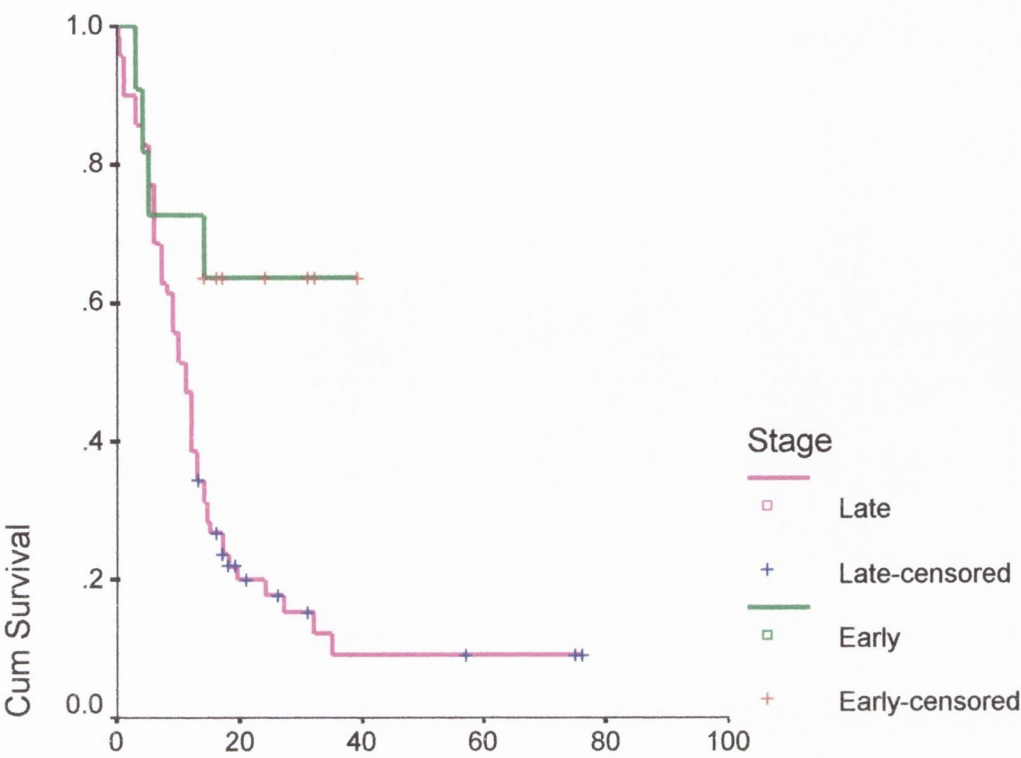


Fig. 5.3 PFS in relation to tumour stage

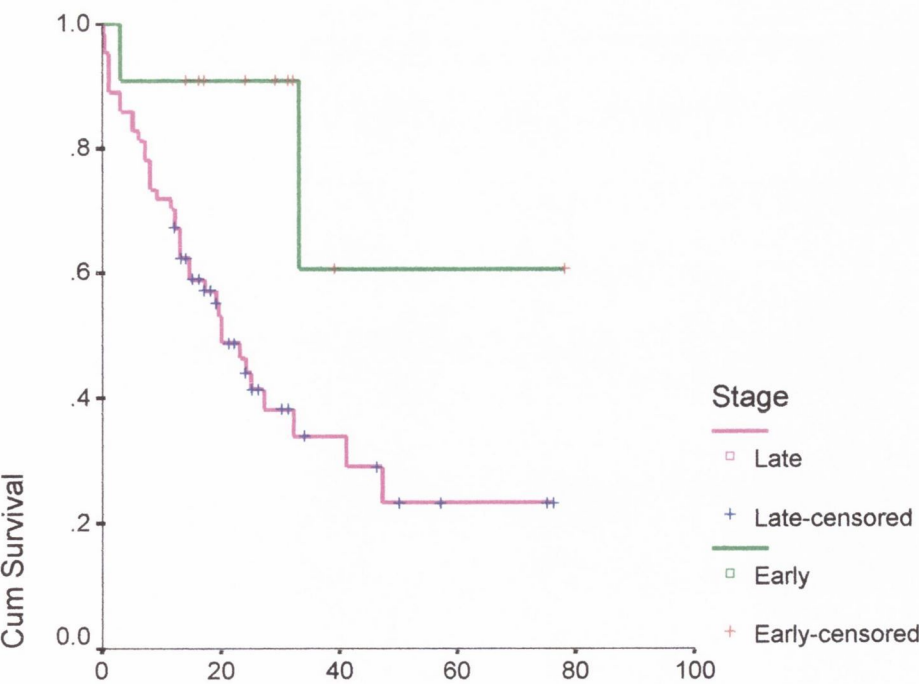


Fig. 5.4 OS in relation to tumour stage

5.4 Previous Chemotherapy and survival

Median PFS for patients who had previous chemotherapy was 5 months and for those receiving chemotherapy for the first time median survival was 15 months. Survival plots for these are shown in Figures 5.5 and 5.6. Sixty-percent of patients receiving first line chemotherapy presented with a recurrence or progression of disease and all the patients who had received previous chemotherapy had disease progression or a recurrence ($p<0.0001$). Overall survival was examined in this group with 73% of the patients who had received chemotherapy previously having died at time of follow up compared to 39% in the group that had not received chemotherapy ($p<0.001$). The median was not reached so the 75th percentile was 3 months in the previously treated group compared to 14.5 months in the group with no previous chemotherapy.

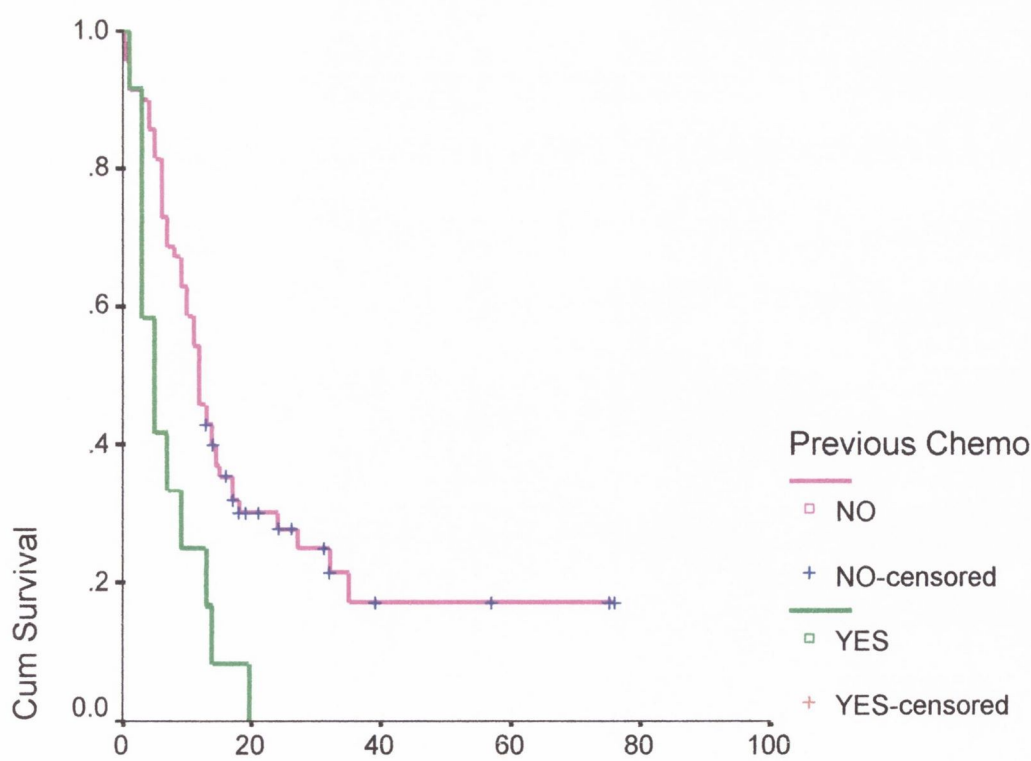


Fig. 5.5 PFS in relation to previous chemotherapy

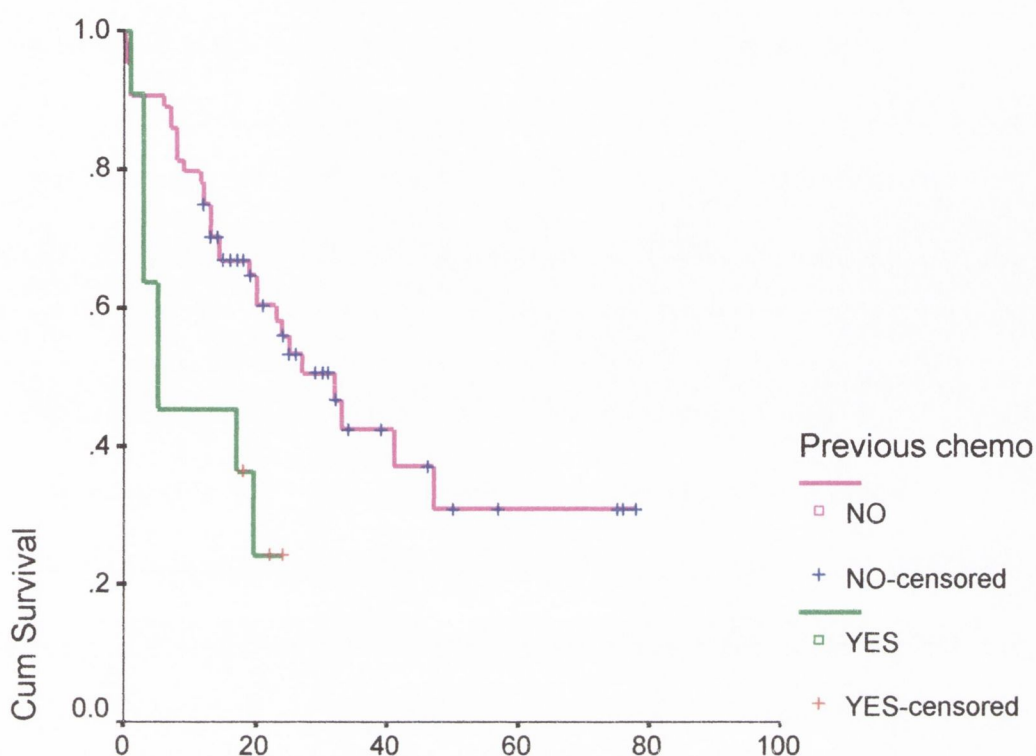


Fig. 5.6 OS in relation to previous chemotherapy

5.5 *In vitro* response to chemotherapy and survival

PFS was examined in relation to sensitivity or resistance to chemotherapy *in vitro* (Fig. 5.7). Median PFS for the sensitive group was 14 months and 7 months for the resistant group. Ninety-seven percent of the resistant group had presented with disease progression or a recurrence compared to 64% of the sensitive group ($p < 0.001$). Overall survival is shown in Figure 5.8 but this was not significant. Sixty-four percent of the resistant tumours had died at time of follow up with a median OS of 19.5 months. The sensitive group had a median OS of 47 months and 43% of these had died at time of follow up.

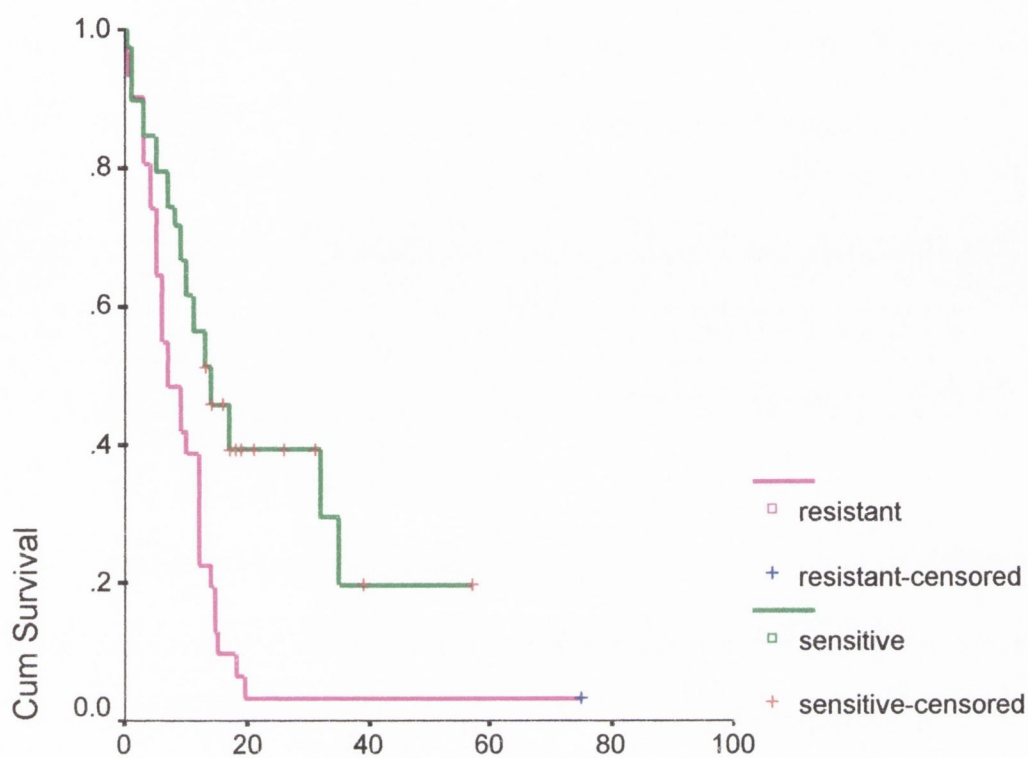


Fig. 5.7 PFS in relation to in vitro response to chemotherapy

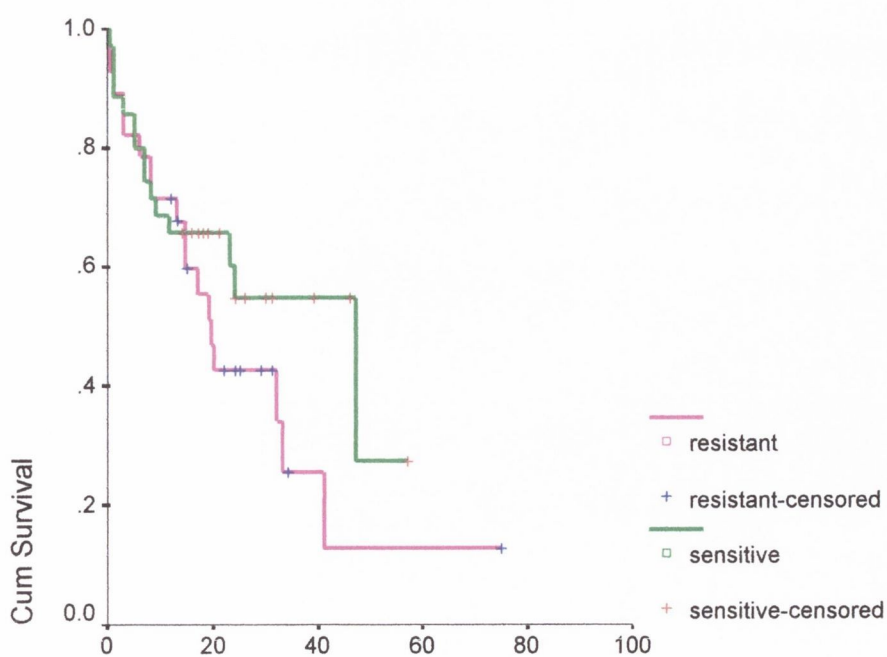


Fig. 5.8 OS in relation to in vitro chemotherapy response

5.6 *In vivo* response to chemotherapy and survival

Patient’s charts were followed up to evaluate how they responded to chemotherapy. PFS in relation to *in vivo* response is displayed in Figure 5.9. Median PFS for the group of patients found to be sensitive *in vivo* was 35 months and 7 months for the resistant group. Forty-three percent of the sensitive group had presented with a recurrence or disease progression compared to 100% in the resistant group ($p<0.0001$). Overall survival is shown in Figure 5.10. Seventy-two percent of the resistant group had died at time of follow up with a median OS of 19 months. Eighteen percent of the sensitive group had died at time of follow up. The median was not reached for this group but the 75th percentile was 47 months for this group compared to a 75th percentile of 8 months in the resistant group ($p<0.001$).

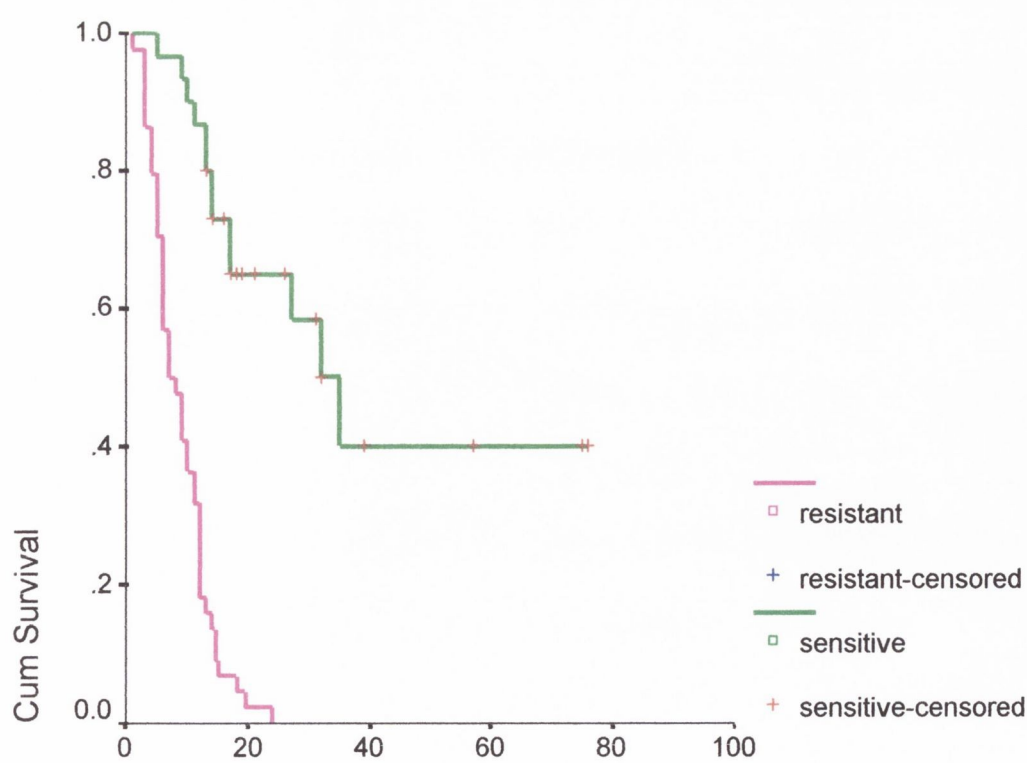


Fig. 5.9 PFS in relation to in vivo chemotherapy response

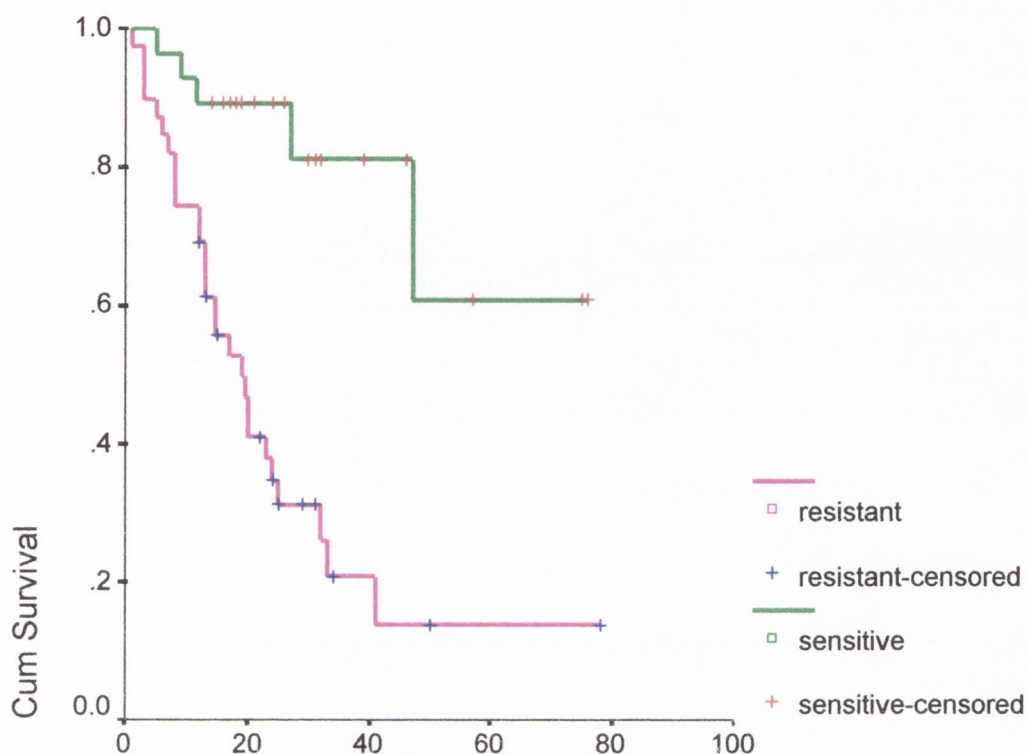


Fig. 5.10 OS in relation to in vivo chemotherapy response

5.7 Patient Age and survival

Patient's age was categorised at the mean into under 55's and over 55's. Median PFS in the under 55 group was 10 months and 12 months in the over 55 group (Figure 5.11). Approximately the same percentage of events had occurred in each group. No significant observation was made here. Similarly when examining OS (Figure 5.12) no significant observation was made. Forty-seven percent of the under 55 group had died at time of follow up compared to 58% of the over 55 group. Median OS in the under 55 group was 32 months and 24 months in the over 55 group. Age was also treated as a continuous variable but no significant observations were observed with survival. Patient's age was also examined using a one-way ANOVA to determine if it

had any influence on the other categories examined. It was examined against tumour grade, stage, previous chemotherapy, *in vitro* response and *in vivo* response. A significant relationship was observed between age and *in vivo* response only ($p<0.05$).

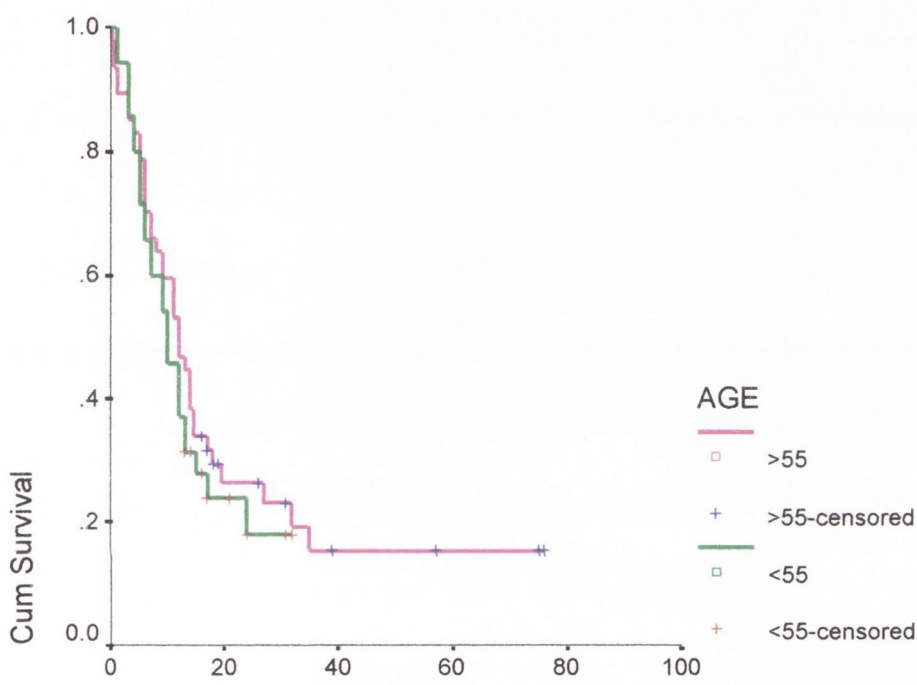


Fig. 5.11 PFS in relation to age category

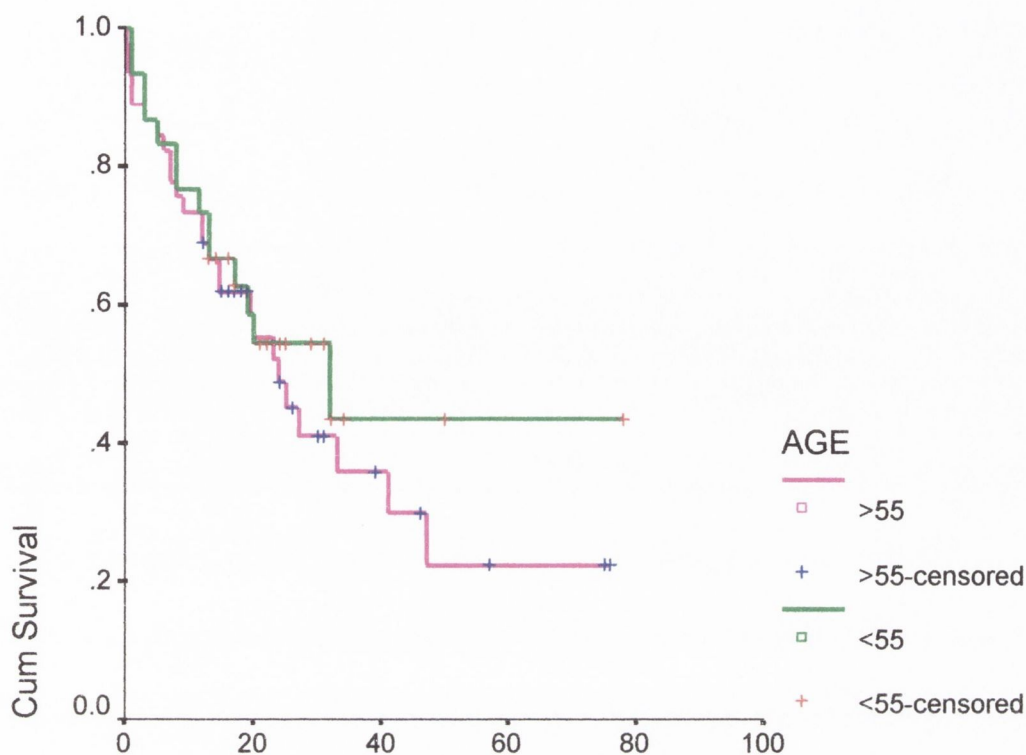


Fig. 5.12 OS in relation to age category

5.8 Multivariate Analysis

The variables that were significant in univariate analyses were then analysed in a multivariate analysis using a Cox proportional hazards regression model. Using corrected progression free survival as endpoint, previous chemotherapy, *in vitro* response and *in vivo* response remained significant in multivariate analysis. For overall survival previous chemotherapy and *in vivo* response remained significant. Cox hazard ratios and confidence intervals are shown in Tables 5.5 and 5.6.

Table 5.5 Cox regression analysis for PFS

Variable	P-value	Hazard Ratio	95% CI
<i>In vivo</i> chemo response	0.000	0.078	0.029-0.209
<i>In vitro</i> chemo response	0.038	2.353	1.049-5.277
Previous chemotherapy	0.034	2.155	1.059-4.385

Table 5.6 Cox regression analysis for OS

Variable	P-value	Hazard Ratio	95% CI
<i>In vivo</i> chemo response	0.001	0.156	0.071- 0.488
Previous chemotherapy	0.005	3.349	1.437- 7.805

CHAPTER 6

POTENTIAL PROGNOSTIC MARKERS AND PREDICTORS OF RESPONSE TO CHEMOTHERAPY IN OVARIAN CANCER

6.1 CD31 Results

A summary of the 79 specimens stained for CD31 is shown in Table 6.1.

Table 6.1 Specimens stained for CD31, VEGF and MDR

Tumour Type	Stage	Grade	Number
Serous Papillary Adenocarcinoma	IC	1	1
Serous Papillary Adenocarcinoma	IC	2	1
Serous Papillary Adenocarcinoma	II	3	1
Serous Papillary Adenocarcinoma	III	2	18
Serous Papillary Adenocarcinoma	III	3	17
Serous Papillary Adenocarcinoma	IV	2	6
Serous Papillary Adenocarcinoma	IV	3	8
Clear Cell Adenocarcinoma	ND	3	1
Clear Cell Adenocarcinoma	IIIC	3	2
Clear Cell Adenocarcinoma	IV	ND	1
Mucinous Adenocarcinoma	III	3	1
Mucinous Adenocarcinoma	IV	3	1
Primary Peritoneal Adenocarcinoma	IV	3	1
Adenosquamous	III	2	1
Borderline Mucinous	I	N/A	2
Borderline Serous	I	N/A	2
Benign	N/A	N/A	10
Normal	N/A	N/A	5

Abbreviations: ND (Not Documented), N/A (Not Applicable)

Positive control slides showed staining, an example can be seen in Fig. 6.1. Negative control reagent slides were clear of staining as displayed in Figure 6.2. Sections stained with CD31 were scored as 0, 1, and 2. In some of the more vascular malignant tumours CD31 appeared to be localised to the endothelial cell boundary, giving the vessels a striped appearance Fig 6.3. Specimen details, CD31 scores and follow up details are shown in Table 6.2.

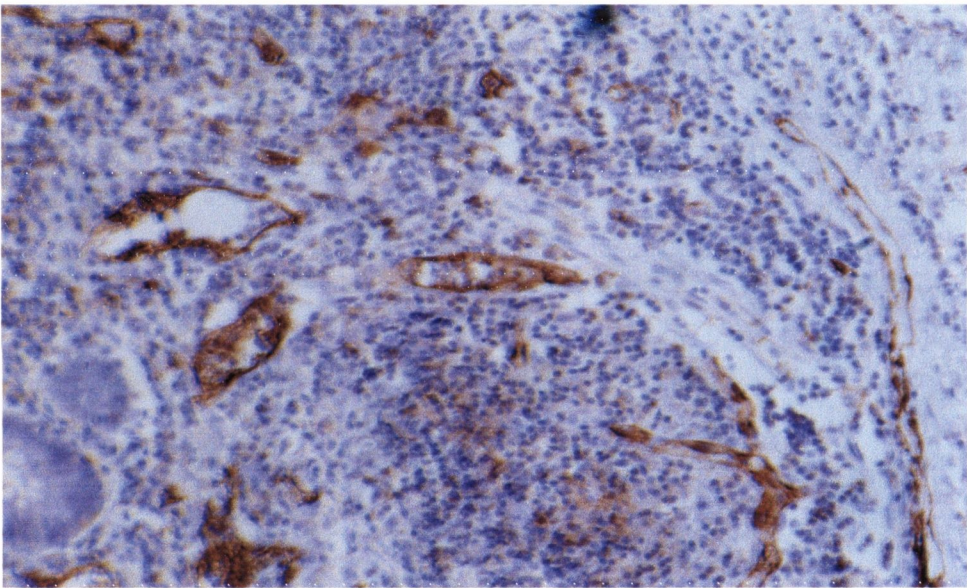


Figure 6.1 Positive Control for CD31 Expression

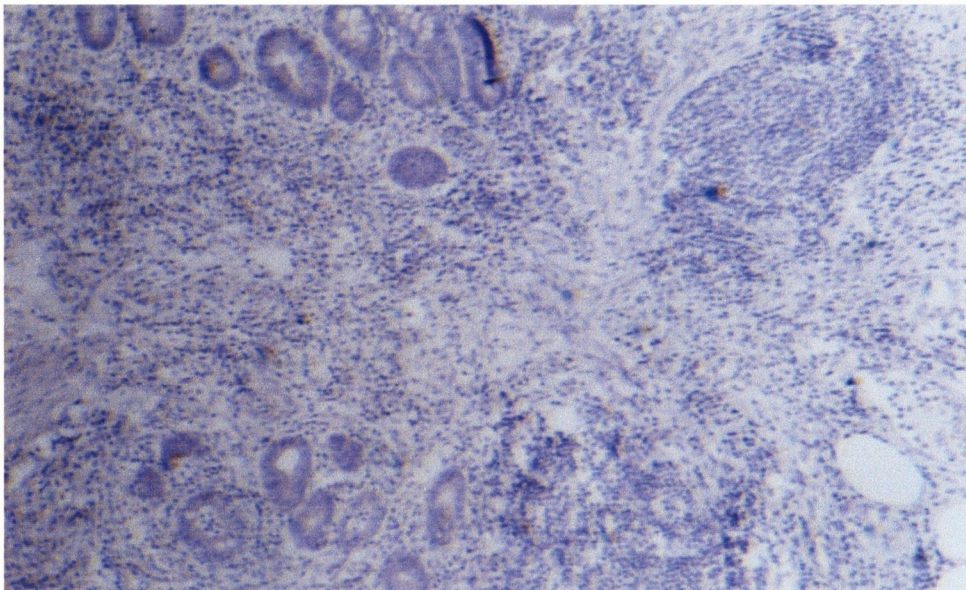


Figure 6.2 Negative Control for CD31 Staining

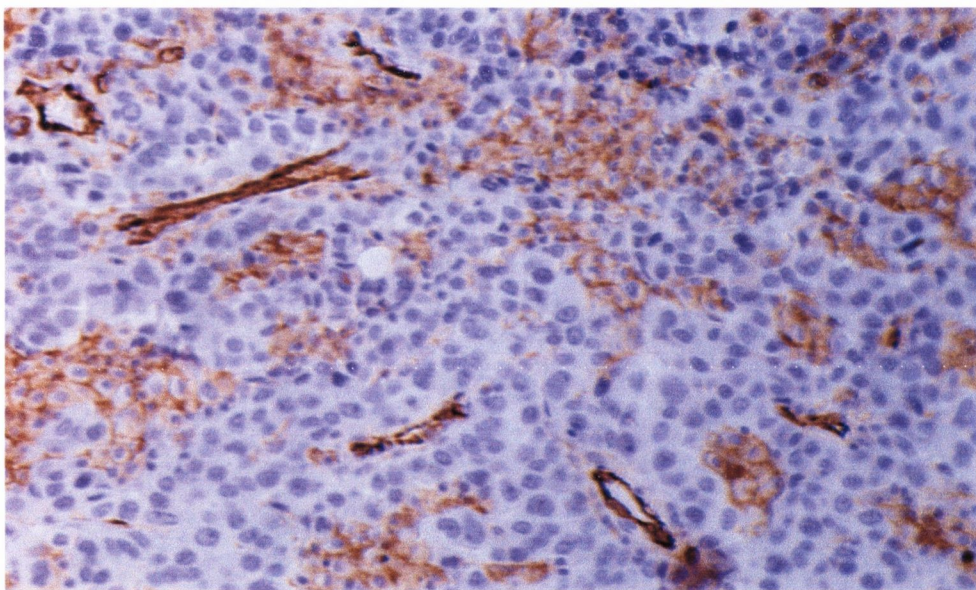


Figure 6.3 CD31 Staining in an Ovarian Adenocarcinoma

Table 6.2 Staining scores and follow-up

Abbreviations: G (Grade), PFS (Progression free survival), Prog (Progressed), OS (Overall survival), Prev (Previous chemotherapy), U (Unknown), NC (Not cultured), NE (Not evaluable), N/A (Non applicable), ND (Not documented), LTF (lost to follow-up).

No	Pathology	G	Stage	Age	PFS	Prog	OS	Status	Prev	MDR	VEGF	CD31	<i>In vivo</i>	<i>In vitro</i>
OV1	Serous Papillary Adenocarcinoma	1	I	29	4	Yes	78	Alive	No	1	1	0	Resistant	NC
OV3	Serous Papillary Adenocarcinoma	2	I	43	31	No	31	Alive	No	1	1	2	Sensitive	Sensitive
OV5	Serous Papillary Adenocarcinoma	3	II	40	32	No	32	Alive	No	2	0	1	Sensitive	NC
OV7	Serous Papillary Adenocarcinoma	2	III	61	U	LTF	U	LTF	No	1	1	1	Sensitive	Sensitive
OV8	Serous Papillary Adenocarcinoma	2	III	59	14	Yes	18	Alive	Yes	1	1	1	Sensitive	Sensitive
OV9	Serous Papillary Adenocarcinoma	2	III	55	1	Yes	1	RIP	Yes	2	2	2	Resistant	Resistant
OV11	Serous Papillary Adenocarcinoma	2	III	63	57	No	57	Alive	No	0	0	0	Sensitive	Sensitive

OV13	Serous Papillary Adenocarcinoma	2	III	70	4	Yes	20	RIP	No	1	0	2	Resistant	Resistant
OV14	Serous Papillary Adenocarcinoma	2	III	53	21	No	21	Alive	No	0	0	2	Sensitive	Sensitive
OV15	Serous Papillary Adenocarcinoma	2	III	43	13	No	U	LTF	No	1	1	1	Sensitive	Sensitive
OV16	Serous Papillary Adenocarcinoma	2	III	62	26	No	26	Alive	No	0	0	0	Sensitive	Sensitive
OV17	Serous Papillary Adenocarcinoma	2	III	78	8	Yes	8	RIP	No	1	2	0	Resistant	Sensitive
OV18	Serous Papillary Adenocarcinoma	2	III	45	10	Yes	U	LTF	No	2	0	1	Resistant	Sensitive
OV19	Serous Papillary Adenocarcinoma	2	III	46	10	Yes	12	RIP	No	0	0	1	Sensitive	Sensitive
OV20	Serous Papillary Adenocarcinoma	2	III	57	31	No	31	Alive	No	1	0	1	Sensitive	Sensitive
OV21	Serous Papillary Adenocarcinoma	2	III	70	0.25	Yes	0.3	RIP	No	1	0	1	RIP	Resistant
OV22	Serous Papillary Adenocarcinoma	2	III	60	13	Yes	24	Alive	Yes	0	0	0	Sensitive	Sensitive
OV25	Serous Papillary Adenocarcinoma	2	III	32	5	Yes	5	RIP	Yes	0	1	0	Resistant	Sensitive
OV28	Serous Papillary Adenocarcinoma	2	III	58	5	Yes	U	LTF	No	1	2	2	Resistant	Resistant
OV29	Serous Papillary Adenocarcinoma	2	III	76	27	Yes	27	RIP	No	1	0	0	Sensitive	NC
OV30	Serous Papillary Adenocarcinoma	2	III	65	12	Yes	12	RIP	No	0	0	0	Resistant	NC

OV31	Serous Papillary Adenocarcinoma	3	III	43	3	Yes	3	RIP	Yes	2	2	2	Resistant	Resistant
OV31A	Serous Papillary Adenocarcinoma	3	III	43	5	Yes	8	RIP	No	2	1	2	Resistant	Resistant
OV32	Serous Papillary Adenocarcinoma	3	III	57	6	Yes	24	Alive	No	0	0	0	Resistant	Resistant
OV33	Serous Papillary Adenocarcinoma	3	III	64	9	Yes	9	RIP	No	1	1	1	Sensitive	Sensitive
OV34	Serous Papillary Adenocarcinoma	3	III	43	7	Yes	17	RIP	Yes	0	0	1	Resistant	Resistant
OV35	Serous Papillary Adenocarcinoma	3	III	76	18	No	18	Alive	No	0	0	1	Sensitive	Sensitive
OV39	Serous Papillary Adenocarcinoma	3	III	66	U	U	U	LTF	Yes	2	1	1	Sensitive	Sensitive
OV40	Serous Papillary Adenocarcinoma	3	III	84	7	Yes	7	RIP	No	0	2	2	RIP	Sensitive
OV42	Serous Papillary Adenocarcinoma	3	III	64	14.5	Yes	15	RIP	No	0	0	1	Resistant	Resistant
OV43	Serous Papillary Adenocarcinoma	3	III	36	9	Yes	34	Alive	No	2	0	2	Resistant	Resistant
OV44	Serous Papillary Adenocarcinoma	3	III	54	17	Yes	U	LTF	No	2	0	2	Sensitive	Sensitive
OV45	Serous Papillary Adenocarcinoma	3	III	48	13	Yes	24	Alive	No	1	0	1	Sensitive	Sensitive
OV46	Serous Papillary Adenocarcinoma	3	III	48	15	Yes	31	Alive	No	0	0	1	Resistant	Resistant

OV47	Serous Papillary Adenocarcinoma	3	III	55	17	Yes	30	Alive	No	1	2	2	Sensitive	Sensitive
OV48	Serous Papillary Adenocarcinoma	3	III	61	76	No	76	Alive	No	2	0	0	Sensitive	NE
OV49	Serous Papillary Adenocarcinoma	3	III	59	11	Yes	25	RIP	No	2	0	0	Resistant	NC
OV50	Serous Papillary Adenocarcinoma	3	III	73	6	Yes	13	RIP	No	2	2	2	Resistant	NC
OV51	Serous Papillary Adenocarcinoma	2	IV	61	7	Yes	7	RIP	No	1	1	1	Resistant	Sensitive
OV52	Serous Papillary Adenocarcinoma	2	IV	50	1	Yes	1	RIP	No	0	1	2	RIP	Sensitive
OV54	Serous Papillary Adenocarcinoma	2	IV	60	11	Yes	23	RIP	No	2	1	2	Resistant	Sensitive
OV55	Serous Papillary Adenocarcinoma	2	IV	58	35	Yes	47	RIP	No	2	0	1	Sensitive	Sensitive
OV58	Serous Papillary Adenocarcinoma	2	IV	29	9	Yes	22	Alive	Yes	0	0	1	Resistant	Resistant
OV60	Serous Papillary Adenocarcinoma	2	IV	43	24	Yes	50	Alive	No	2	0	1	Resistant	NC
OV61	Serous Papillary Adenocarcinoma	3	IV	47	6	Yes	20	RIP	No	1	0	0	Resistant	NC
OV62	Serous Papillary Adenocarcinoma	3	IV	49	10	Yes	25	Alive	No	1	0	0	Resistant	Resistant

OV63	Serous Papillary Adenocarcinoma	3	IV	47	7	Yes	13	RIP	No	2	1	1	Resistant	Resistant
OV64	Serous Papillary Adenocarcinoma	3	IV	60	1	Yes	1	RIP	No	0	2	2	RIP	Sensitive
OV65	Serous Papillary Adenocarcinoma	3	IV	74	75	No	75	Alive	No	2	0	0	Sensitive	Resistant
OV66	Serous Papillary Adenocarcinoma	3	IV	62	19.5	Yes	20	RIP	Yes	2	0	2	Resistant	Resistant
OV68	Serous Papillary Adenocarcinoma	3	IV	54	3	Yes	U	LTF	No	2	1	1	Resistant	Sensitive
OV69	Serous Papillary Adenocarcinoma	3	IV	58	6	Yes	12	RIP	No	1	1	2	Resistant	NC
OV70	Clear cell Adenocarcinoma	3	ND	71	3	Yes	U	LTF	Yes	0	0	0	Resistant	Resistant
OV75	Clear cell Adenocarcinoma	3	III	85	0.4	Yes	0.4	RIP	No	1	1	1	RIP	Sensitive
OV76	Clear cell Adenocarcinoma	3	III	58	6	Yes	6	RIP	No	2	0	0	Resistant	Resistant
OV77	Clear cell Adenocarcinoma	ND	IV	47	6	Yes	8	RIP	No	1	2	2	Resistant	Resistant
OV80	Mucinous Adenocarcinoma	3	III	41	3	Yes	3	RIP	Yes	1	2	2	Resistant	Resistant
OV81	Mucinous Adenocarcinoma	3	IV	49	12	No	U	LTF	No	2	0	2	Resistant	Resistant

OV82	Primary peritoneal	3	IV	71	19	No	19	Alive	No	0	1	2	Sensitive	Sensitive
OV86	Adenosquamous carcinoma	2	III	40	13	Yes	13	RIP	No	0	0	0	Sensitive	NE
OV91	Borderline mucinous	N/A	I	68	74	No	74	Alive	No	1	0	0		
OV92	Borderline mucinous	N/A	I	50	34	No	34	Alive	No	1	1	2		
OV96	Borderline serous	N/A	I	47	31	No	31	Alive	No	1	0	1		
OV98	Borderline serous	N/A	I	75	33	No	33	Alive	No	0	1	0		
OV99	Benign	N/A	N/A	45	34	No	34	Alive	No	1	1	1		
OV100	Benign	N/A	N/A	43	51	Yes	78	Alive	No	0	0	0		
OV101	Benign	N/A	N/A	64	26	No	26	Alive	No	1	0	0		
OV102	Benign	N/A	N/A	71	79	No	79	Alive	No	0	1	1		

OV103	Benign	N/A	N/A	67	27	No	27	Alive	No	0	0	0
OV104	Benign	N/A	N/A	45	79	No	79	Alive	No	0	0	1
OV105	Benign	N/A	N/A	52	31	No	31	Alive	No	0	0	0
OV106	Benign	N/A	N/A	76	28	No	28	Alive	No	1	0	0
OV107	Benign	N/A	N/A	50	26	No	26	Alive	No	0	1	0
OV108	Benign	N/A	N/A	36	27	No	27	Alive	No	1	0	0
OV109	Normal	N/A	N/A	45	69	No	69	Alive	No	0	0	1
OV110	Normal	N/A	N/A	75	78	No	78	Alive	No	1	0	1
OV111	Normal	N/A	N/A	46	79	No	79	Alive	No	1	0	0
OV112	Normal	N/A	N/A	71	79	No	79	Alive	No	1	0	0
OV113	Normal	N/A	N/A	45	70	No	79	Alive	No	0	0	0

6.1.1 CD31 in tumour types

Fifty-two of the tumours examined for CD31 were serous papillary adenocarcinomas of various stages and grades. Four clear cell carcinomas, 2 mucinous adenocarcinomas, one adenosquamous, 1 primary peritoneal, four borderline tumours, ten benign tumours and five normal ovaries were also examined. Apart from fourteen specimens all the serous papillary specimens displayed some degree of staining with the CD31 antibody. One of the clear cell carcinomas showed a score of 2, 1 a score of 1 and the others displayed no staining. The mucinous tumours displayed a high degree of staining, as did the primary peritoneal. The adenosquamous tumour showed no staining. Of the four borderline tumours one of the mucinous showed a high degree of staining with a score of two, one of the serous tumours a score of one and the other two showed no staining. Of the 15 benign and normal specimens, five showed a small degree of staining and the remaining showed no staining. Significantly more staining was observed in the malignant group than in the borderline or benign/normal group ($p < 0.01$).

6.1.2 CD31 and tumour grade

CD31 and its relationship to tumour grade was examined in 59 of the tumour specimens. The specimens that displayed no staining of CD31 consisted of 1 well, 8 moderately and 8 poorly differentiated tumours. The low staining group consisted of 11 moderately and 11 poorly differentiated tumours. Seven moderately and 13 poorly differentiated tumours displayed a high degree of staining for CD31. No significant association was observed between degree of staining and tumour grade.

6.1.3 CD31 in early and late stage tumours

Twenty-two of the specimens examined showed a high degree of CD31 staining with a score of 2. Of these 8 were stage IV tumours, 12 were stage III tumours, and 2 stage I tumours. Twenty-three specimens showed a small degree of staining, 6 of these were stage IV tumours, 15 stage III tumours, a stage II tumour and 1 stage I borderline tumour. The specimens that displayed no staining consisted of 3 stage I tumours, 12 stage III and 3 stage IV tumours. No significant correlation was observed between the degree of CD31 staining and FIGO stage.

6.1.4 CD31 and patient outcome

PFS and OS were examined for specimens stained with CD31. The PFS time for the patients in the various staining groups is displayed in Table 6.2. Kaplan-Meier curves were constructed for PFS and patients that were alive and free from disease were censored. OS entries were censored for patients still alive at time of follow up. In some cases it was not possible to calculate the median survival so the 75th percentile is quoted but in a few cases where all patients were event free there is no percentile calculated. Median PFS, 25th and 75th percentiles and log rank values are displayed in Table 6.3. Median OS, 25th and 75th percentiles and log rank values are displayed in Table 6.4. Eighty-two percent of the high staining group had tumour progression as compared to 62% in the low staining group and 48% in the no staining group. Patient samples that had a score of 2 had a median PFS of 6 months. Median PFS time in the group that displayed a low degree of staining was 14.5 months and 51 months in the no staining group ($p<0.01$) (Figure 6.4). OS was also examined in each group and at

time of follow-up 69% of high staining group had died, 39% of low staining and 29% of the no staining group. Corrected 75th percentile OS in the no staining group was 25 months. In the low staining group the 75th percentile was 13 months and in the high staining group 3 months ($p<0.01$) (Figure 6.5).

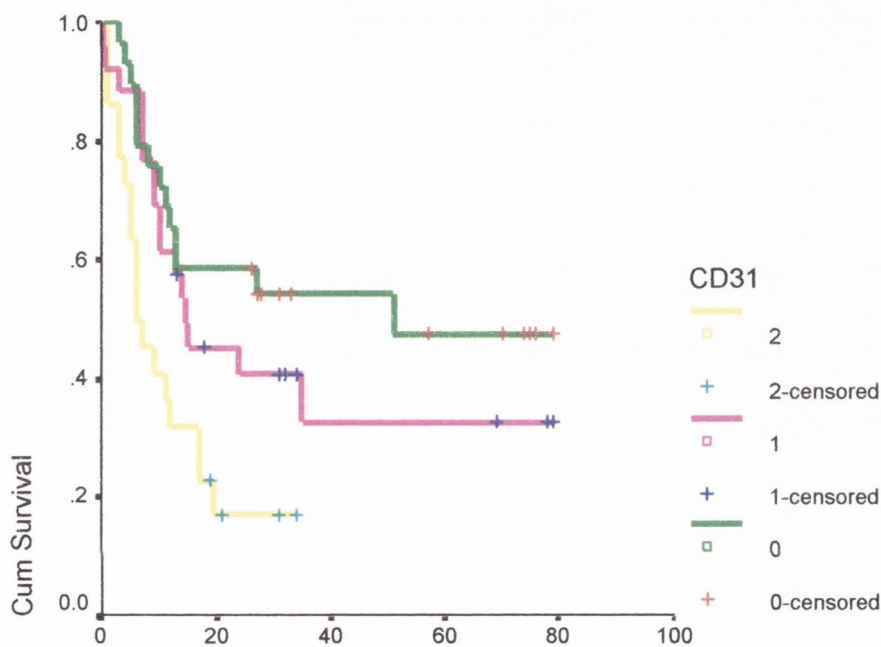


Figure 6.4 PFS in relation to CD31 expression

Table 6.3 Log rank and quartiles for progression free survival

		25 th percentile	50 th percentile	75 th percentile	p
Pathology	Malignant	19.5	10	6	<0.0001
	Benign	-	-	-	
Grade	1	4	4	4	0.14
	2	27	12	7	
	3	17	9	6	
Stage	Early	-	-	-	0.035
	Late	17	10	6	
Age	<55		15	6	0.85
	>55		14	6	
Previous	Yes	13	5	3	<0.0001
	No		17	7	
MDR	0		15	7	0.29
	1		14	6	
	2	24	11	6	
VEGF	0		27	10	<0.0001
	1		9	5	
	2	7	5	3	
CD31	0		51	10	0.0056
	1		14.5	9	
	2	17	6	4	
<i>In vivo</i>	Sensitive		35	14	<0.0001
	Resistant	11	7	5	
<i>In vitro</i>	Sensitive	35	13	7	0.009
	Resistant	10	6	4	

Table 6.4 Log rank and quartiles for overall survival

		25 th percentile	50 th percentile	75 th percentile	p
Pathology	Malignant		20	8	<0.0001
	Benign	-	-	-	
Grade	1				0.56
	2		23	8	
	3		19.5	8	
Stage	Early	-	-	-	0.003
	Late		19.5	8	
Age	<55			13	0.28
	>55		27	12	
Previous	Yes		17	3	0.02
	No			13	
MDR	0			13	0.25
	1			9	
	2		23	8	
VEGF	0			25	<0.0001
	1		23	8	
	2	8	7	3	
CD31	0			25	0.004
	1			13	
	2		13	3	
<i>In vivo</i>	Sensitive			47	0.0006
	Resistant	25	13	8	
<i>In vitro</i>	Sensitive		47	7	0.37
	Resistant		14.5	6	

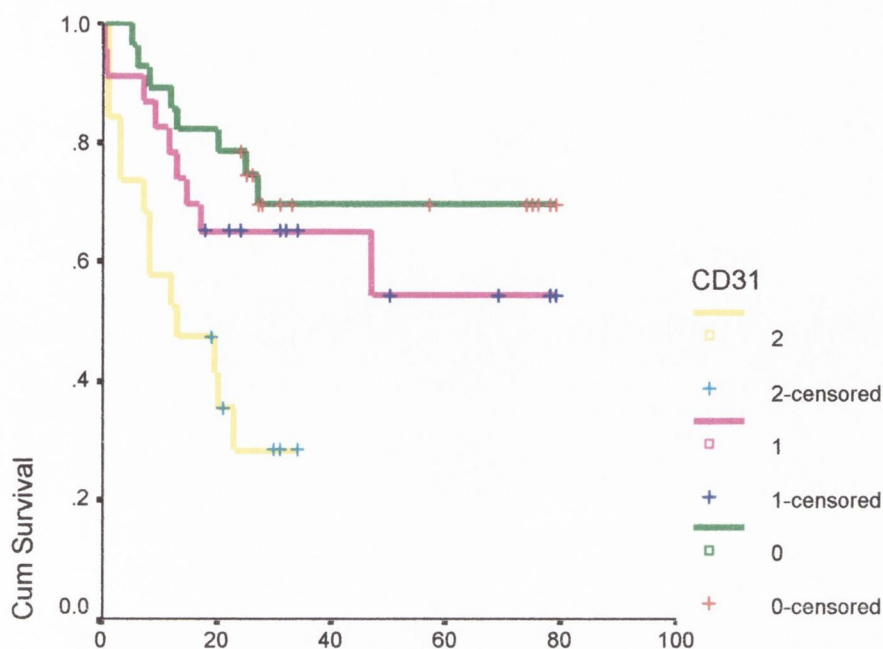


Figure 6.5 OS in relation to CD31 expression

6.1.5 CD31 and *in vitro* chemotherapy response

Forty-nine of the specimens that were stained were correlated with *in vitro* chemotherapy response. Those that displayed no staining included 5 specimens, which were sensitive *in vitro* and 5 that were resistant. Twenty specimens displayed a low degree of staining, 14 of these were sensitive *in vitro* and 6 resistant. Nineteen specimens displayed a high degree of staining, 9 of these were sensitive and 10 resistant. No significant association was observed between *in vitro* response and degree of staining.

6.1.6 CD31 and *in vivo* chemotherapy response

Sixty of the cases that were stained were followed up *in vivo* for their response to chemotherapy. Seventeen specimens displayed no staining and of these 6 were sensitive *in vivo* and 11 were resistant. Twenty-two cases showed a low degree of staining and 11 of these were sensitive, 9 resistant and two died before they could receive treatment. Of the 21 specimens that displayed a high degree of staining 5 were sensitive, 13 resistant and 3 died before receiving treatment. No significant correlation was observed between CD31 staining and *in vivo* chemotherapy response.

6.1.7 CD31 and Patient Age

Median age was calculated in the 3 staining groups. The median age in the no staining group was 60 years (range 29-78), 51 years in the low staining (range 29-85) and 54.5 in the high staining group (range 36-84). No significant relationship was observed between patient age and degree of staining. Patient's age was also categorised, according to the mean into under 55's and over 55's but no significant relationship was observed with degree of staining in these categories.

6.2 VEGF Results

Seventy-nine specimens in total were examined using the VEGF antibody; a summary of these can be seen in Table 6.1. Staining scores, patient details and follow up are shown in Table 6.2.

Positive control slides showed staining, an example can be seen in Fig. 6.6. Negative VEGF slide is shown in Fig. 6.7 and was clear of staining. An example of a VEGF stained ovarian section is shown in Fig. 6.8 showing cytoplasmic staining. Sections stained with VEGF were scored as 0, 1, and 2.

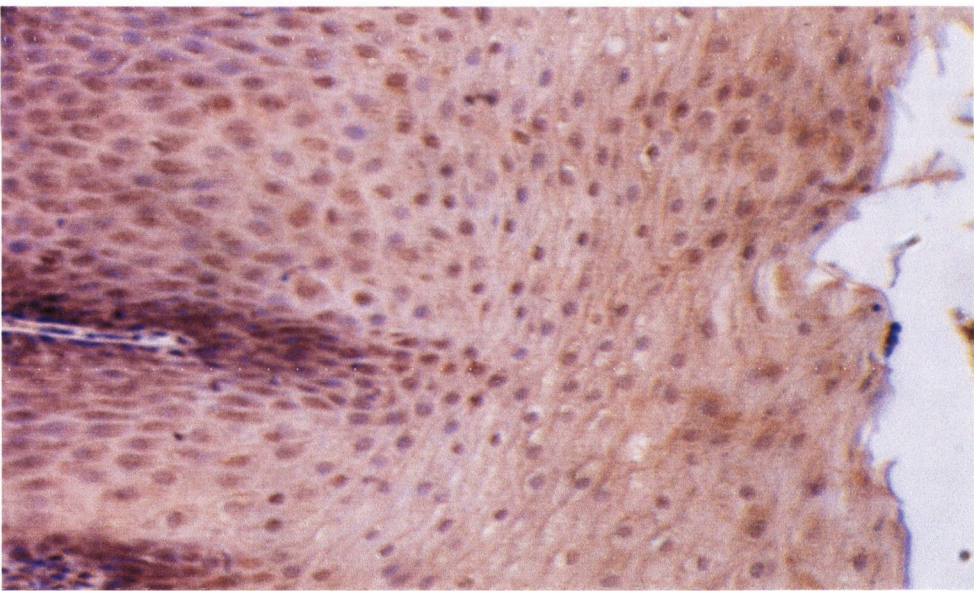


Figure 6.6 Positive Control for VEGF Staining

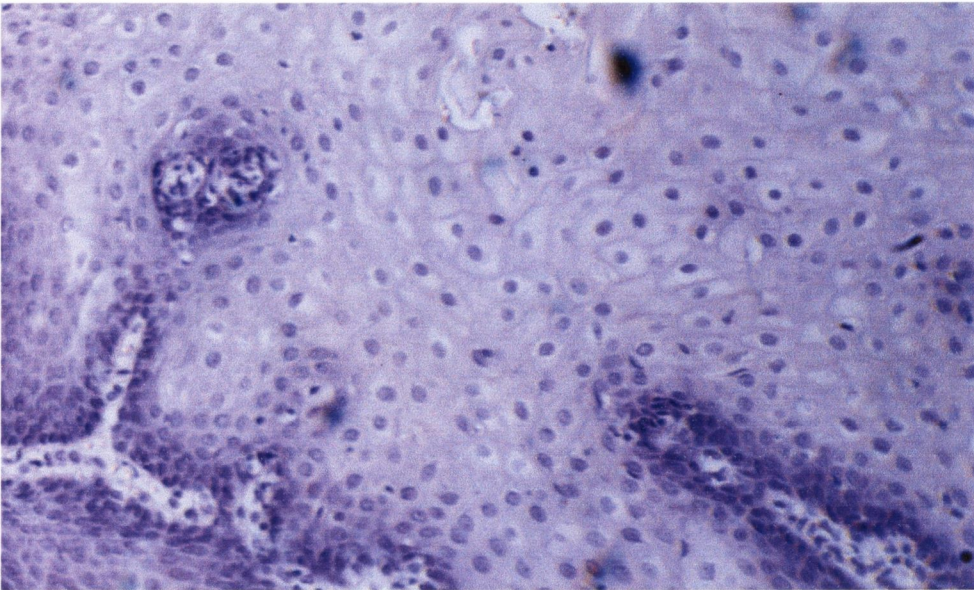


Figure 6.7 Negative Control for VEGF Staining

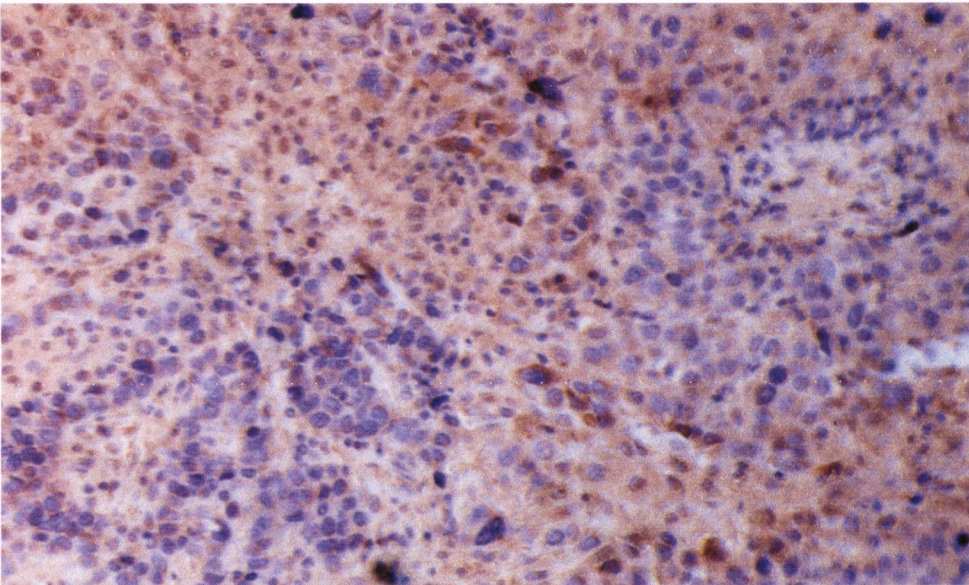


Figure 6.8 VEGF Staining in an Ovarian Adenocarcinoma

6.2.1 VEGF in specimen pathology types

Fifty-two of the tumours examined for VEGF were serous papillary adenocarcinomas of various stages and grades. Four clear cell carcinomas, 2 mucinous adenocarcinomas, 1 primary peritoneal tumour, 1 adenosquamous, 4 borderline tumours, 10 benign tumours and 5 normal ovaries were also examined. Twenty-three of the 52 serous papillary adenocarcinomas displayed various degrees of staining with the VEGF antibody and 29 showed no staining. One of the clear cell carcinomas showed a score of 2, 1 a score of 1 and the other two displayed no staining. One of the mucinous tumours showed a high degree of staining while the other showed no staining. The primary peritoneal tumour showed a low degree of staining. The adenosquamous tumour showed no staining. Of the 4 borderline tumours one of the mucinous and one of the serous tumours showed a low degree of staining and the other two showed no staining. Of the 15 benign and normal specimens 3 of the benign specimens displayed a low degree of staining and the rest of the specimens showed no staining. No association was observed between degree of staining and pathology but the high staining group consisted of just malignant samples.

6.2.2 VEGF and tumour grade

VEGF and its relationship to tumour grade was examined in 59 of the tumour specimens. The specimens that displayed no staining of VEGF consisted of 15 moderately and 18 poorly differentiated tumours. The low staining group consisted of 1 well, 8 moderately and 8 poorly differentiated tumours. Three moderately and 6

poorly differentiated tumours displayed a high degree of staining for VEGF. This relationship was not significant.

6.2.3 VEGF in early and late stage tumours

Ten of the specimens examined showed a high degree of VEGF staining with a score of 2. Of these 2 were stage IV tumours and 8 were stage III tumours. Nineteen specimens showed a low degree of staining, 7 of these were stage IV tumours, 8 stage III tumours and 4 stage I tumours. The specimens that displayed no staining included a stage I tumour, 1 stage II tumour, 23 stage III tumours and 8 stage IV tumours. No significant correlation was observed between VEGF staining and tumour stage.

6.2.4 VEGF and patient outcome

The PFS time for the patients in the various staining groups is displayed in Table 6.2. Censored Quartiles and log rank values for PFS and OS are displayed in Tables 6.3 and 6.4 respectively. The entire high staining group presented with tumour progression compared to 60% of the low staining and 55% of the no staining group. Patient samples that had a score of 0 had a median PFS of 27 months. Median PFS time in the group that displayed a low degree of staining was 9 months and in the group with a high degree of staining the median was 5 months. A significant relationship was observed between VEGF staining and PFS. As the degree of staining increased PFS decreased ($p<0.0001$). The Kaplan-Meier curve for VEGF and PFS is shown in Figure 6.9. Eighty-nine percent of the high staining group had died at time of follow-up compared to 50% in the low staining group and 30% in the no staining

group. Corrected median OS for the group of specimens with no staining was not calculable but the 75th percentile was 25 months. The group that displayed a low degree of staining had a median OS of 23 months and for the high staining group the median OS was 7 months. There was a significant relationship between the degree of staining and OS as displayed in Figure 6.10 ($p<0.0001$).

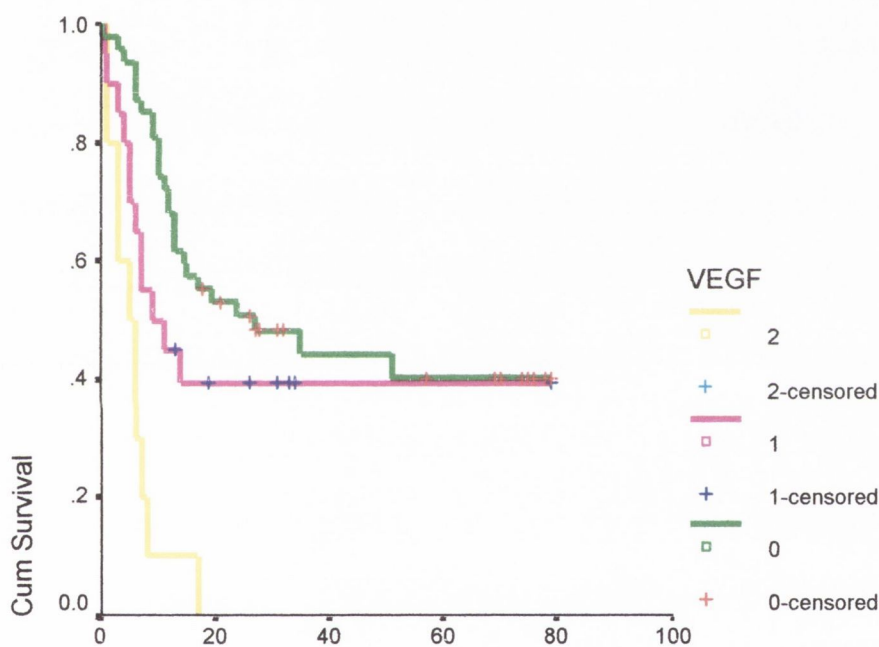


Figure 6.9 PFS in relation to VEGF expression

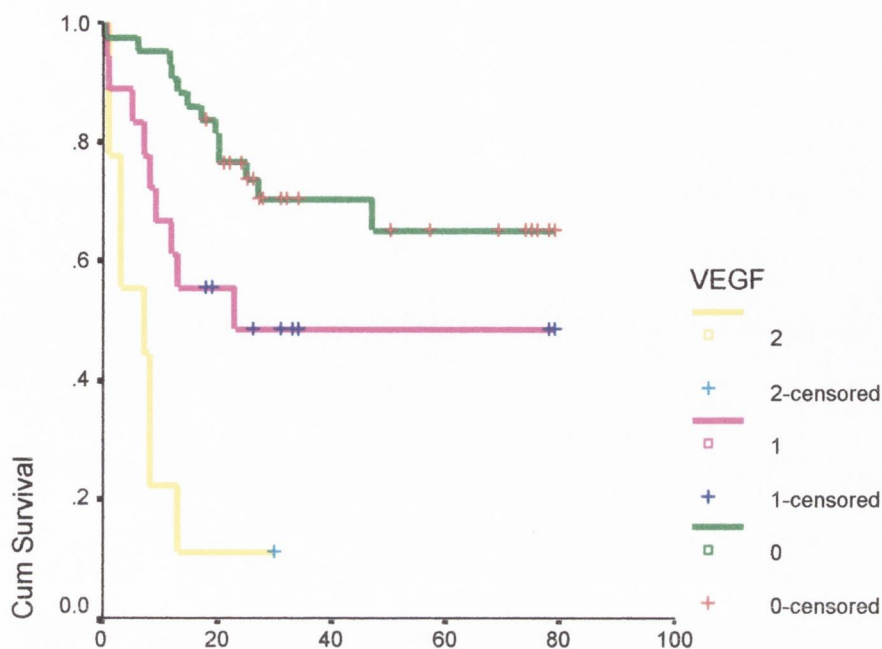


Figure 6.10 OS in relation to VEGF

6.2.5 VEGF and *in vitro* chemotherapy response

Forty-nine of the specimens that were stained for VEGF were correlated with *in vitro* chemotherapy response. Those that displayed no staining included 11 specimens, which were sensitive *in vitro* and 14, which were resistant. Fifteen showed a low degree of staining with 13 of these being sensitive and 2 resistant. Nine specimens displayed a high degree of staining, 4 of these were sensitive and 5 resistant. No significant correlation was observed between VEGF staining and *in vitro* chemotherapy response.

6.2.6 VEGF and *in vivo* chemotherapy response

Sixty of the cases that were stained were followed up *in vivo* for their response to chemotherapy. Thirty-three specimens displayed no staining and of these 14 were sensitive *in vivo*, 18 resistant and 1 died before receiving treatment. Seventeen cases showed a low degree of staining and 7 of these were sensitive and 8 resistant and two died before they could receive treatment. Of the specimens that displayed a high degree of staining 1 was sensitive, 7 resistant and 2 died before receiving treatment. No significant correlation was observed between VEGF staining and *in vivo* chemotherapy response.

6.2.7 VEGF and Patient Age

Median age was calculated in the 3 staining groups. The median age in the no staining group was 57 years (range 29-76), 56 years in the low staining (range 29-85) and 56.5 in the high staining group (range 41-84). No significant relationship was observed between patient age and degree of staining. The degree of staining was also examined in under 55's and over 55's but no significant association was found.

6.3 MDR Results

Seventy-nine specimens were examined using the MDR antibody; a summary of the specimens is shown in Table 6.1. Staining scores and follow-up are shown in Table 6.2. Positive control slides showed staining, an example can be seen in Fig. 6.11. A negative control slide is displayed in Figure 6.12 and an example of an ovarian specimen stained with MDR is shown in Figure 6.13. Sections stained with MDR were scored as 0, 1, and 2. Cell membrane staining was observed and cytoplasmic staining.

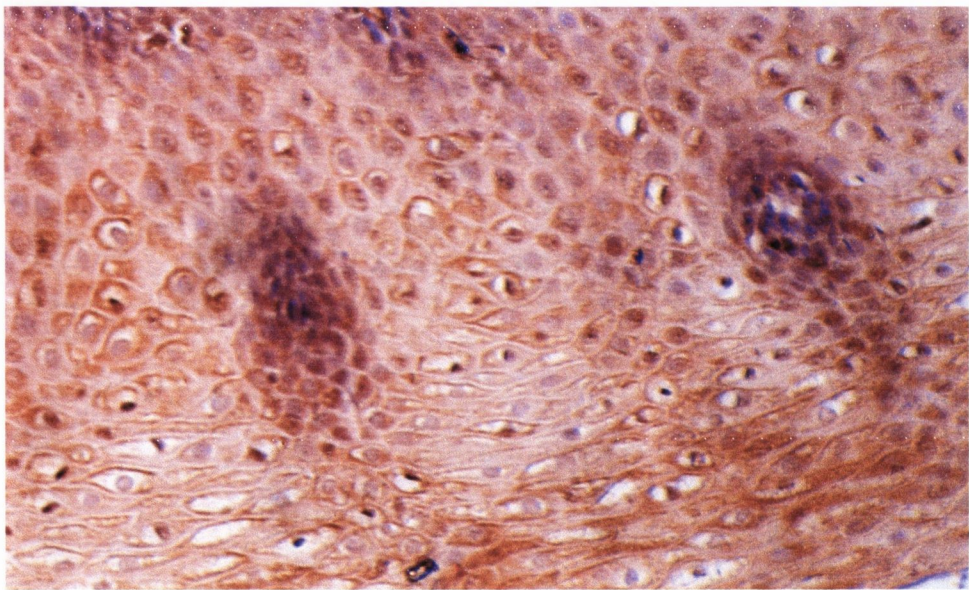


Figure 6.11 Positive Control for MDR Staining

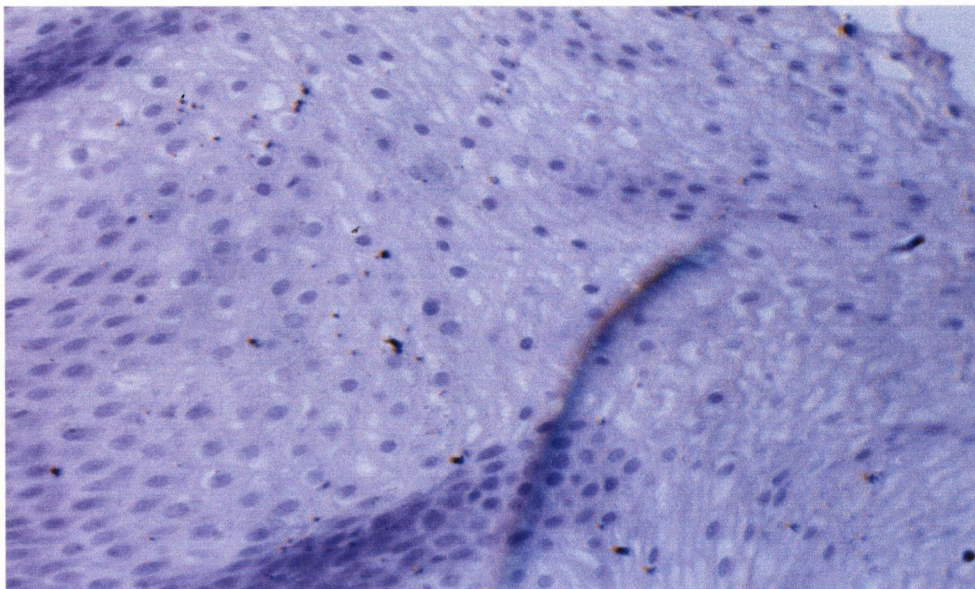


Figure 6.12 Negative Control for MDR Staining

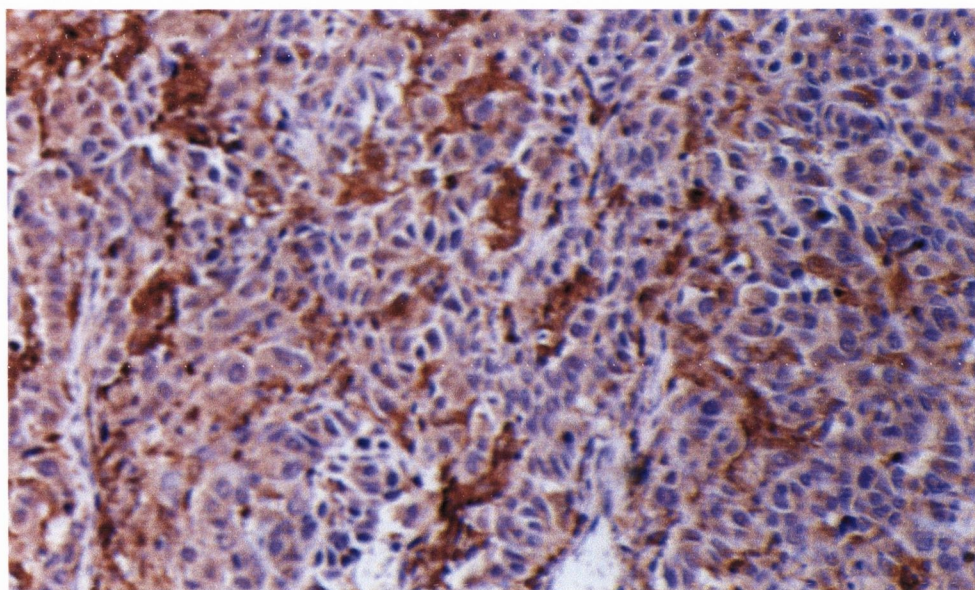


Figure 6.13 MDR Staining in an Ovarian Adenocarcinoma

6.3.1 MDR in specimen pathology types

Fifty-two of the tumours examined for MDR were serous papillary adenocarcinomas of various stages and grades. Four clear cell carcinomas, 2 mucinous adenocarcinomas, 1 primary peritoneal tumour, 1 adenosquamous, 4 borderline tumours, 10 benign tumours and 5 normal ovaries were also examined. Thirty-six of the 52 serous papillary adenocarcinomas displayed various degrees of staining with the MDR antibody and 16 showed no staining. Three of the clear cell carcinomas displayed staining, 1 a high degree of staining, 2 low degree staining and 1 showed no staining. Both of the mucinous tumours stained with the MDR antibody, 1 a high degree of staining and 1 low. The primary peritoneal tumour and the adenosquamous tumour showed no staining. Of the four borderline tumours the mucinous showed a low degree of staining, one of the serous tumours also had a score of 1 and the other displayed no staining. Of the 15 benign and normal specimens 4 of the benign and three normal specimens displayed a low degree of staining and the rest of the specimens showed no staining. Significantly more staining was observed in the malignant group than in the normal/benign group ($p<0.05$).

6.3.2 MDR and tumour grade

The relationship between MDR and tumour grade was examined in 59 of the tumour specimens. The specimens that displayed no staining of MDR consisted of 10 moderately and 9 poorly differentiated tumours. The low staining group consisted of 1 well, 11 moderately and 8 poorly differentiated tumours. Five moderately and 15

poorly differentiated tumours displayed a high degree of staining for MDR. This relationship was not significant.

6.3.3 MDR in early and late stage tumours

Twenty of the specimens examined showed a high degree of MDR staining with a score of 2. Of these 8 were stage IV tumours, 11 were stage III tumours and 1 stage II tumour. Twenty-four specimens showed a small degree of staining, 5 of these were stage IV tumours, 14 stage III tumours and 5 stage I tumours. The specimens that displayed no staining included 14 stage III tumours, 4 stage IV tumours and a stage I borderline serous tumour. No significant correlation was observed between FIGO stage and degree of MDR staining.

6.3.4 MDR and patient outcome

The PFS time for the patients in the various staining groups is displayed in Table 6.2. Quartiles and log rank values for censored patients for PFS and OS are shown in Tables 6.3 and 6.4. Patient samples that had a score of 2 had a median PFS of 11 months. Those with a low degree of staining had a median PFS of 14 months and in the group with no staining the median was 15 months. The Kaplan-Meier curve for PFS in relation to MDR expression is displayed in Figure 6.14 but this was not significant. Eighty-four percent of the high staining group had disease progression compared to 57% in the low staining and 56% in the no staining group. Median OS for the specimens that displayed no staining was 23 months. The 75th percentiles were calculated for the low and no staining groups with values of 9 and 13 months

respectively as compared to 8 months in the high staining group. 67% of the high staining group had died of disease at time of follow-up, 39% of the low staining group and 35% of the no staining group. There was a trend towards decreased survival and increased staining (Figure 6.15) but this was not significant.

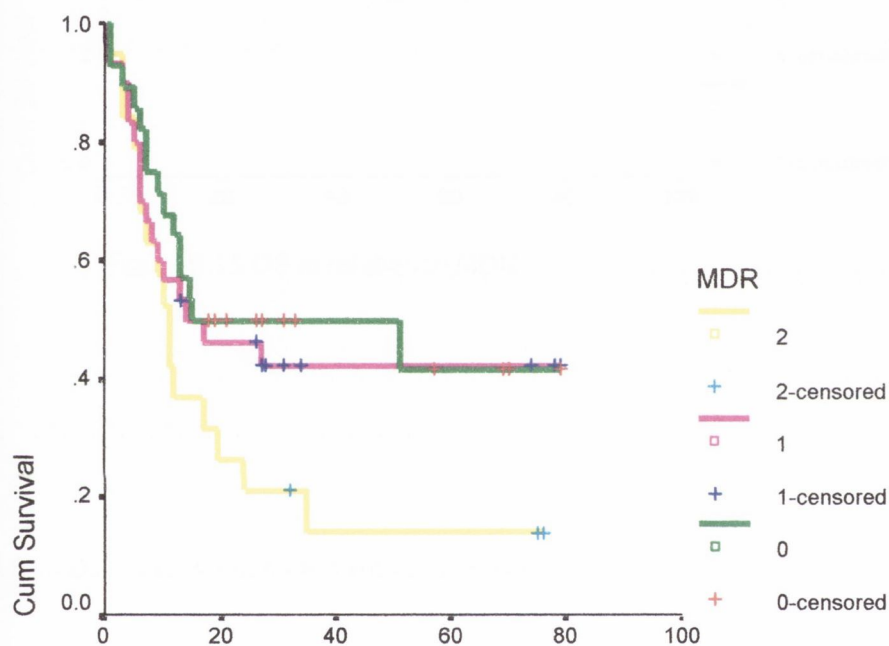


Figure 6.14 PFS in relation to MDR expression

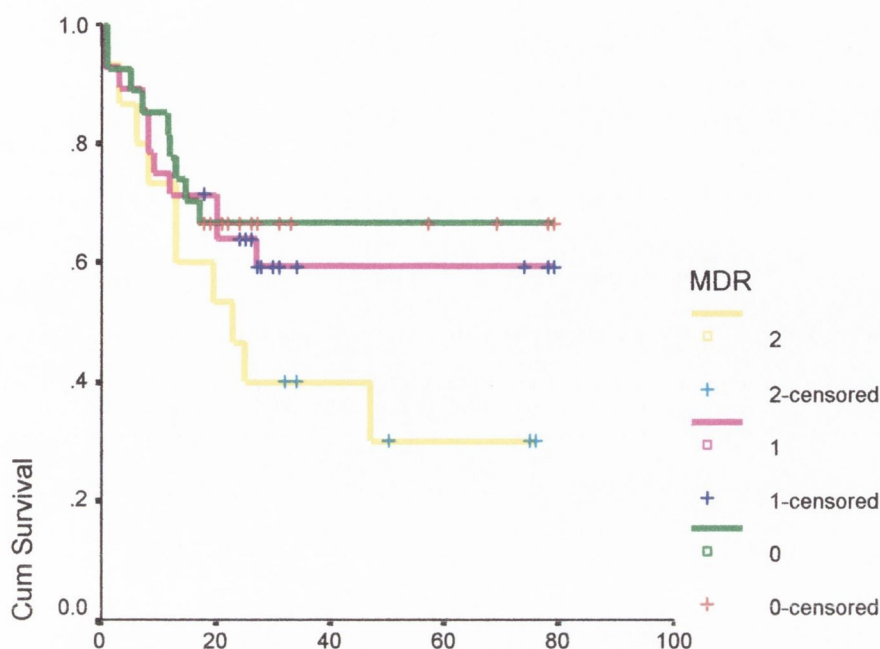


Figure 6.15 OS in relation to MDR

6.3.5 MDR and *in vitro* chemotherapy response

Forty-nine of the specimens that were stained for MDR were available for correlation with *in vitro* chemotherapy response. Ten of the stained specimens were either not cultured due to mistakenly placing in formalin or not evaluable due to lack of viable cells. Those that displayed no staining included 11 specimens that were sensitive *in vitro* and 6 resistant. Seventeen specimens displayed a low degree of staining, 11 of which were sensitive *in vitro* and 6 resistant. Fifteen specimens displayed a high degree of staining, 6 of these were sensitive and 9 resistant. The association between *in vitro* response and degree of MDR was not significant.

6.3.6 MDR and *in vivo* chemotherapy response

Sixty of the cases that were stained were followed up *in vivo* for their response to chemotherapy. Nineteen specimens displayed no staining and of these 7 were sensitive *in vivo*, 9 resistant and 3 died before receiving treatment. Twenty-one cases showed a low degree of staining and 9 of these were sensitive, 10 resistant and 2 died before receiving treatment. Of the specimens that displayed a high degree of staining 6 were sensitive and 14 resistant *in vivo*. This relationship was not significant.

6.3.7 MDR and Patient Age

Median age was calculated in the 3 staining groups. The median age in the no staining group was 55 years (range 29-84), 58 years in the low staining (range 29-85) and 54.5 in the high staining group (range 36-74). No significant relationship was observed between patient age and degree of staining. No association was found between degree of staining and the under 55 and over 55 age groups.

6.4 Correlation between CD31, VEGF and MDR

The resulting staining scores for specimens examined with all three antibodies are displayed in Table 6.2. A significant association was observed between VEGF and CD31 ($p < 0.0001$). No significant relationships were observed between VEGF and MDR or between CD31 and MDR.

6.5 Tumour Details and Patient Outcome

Other tumour details were examined in Kaplan-Meier curves to identify if they had any effect on PFS or OS. Only specimens that had been stained were examined here, as our objective was to determine if any of the antibodies could act as a prognostic indicator or a predictor of response to chemotherapy.

6.5.1 Pathology

PFS and OS were examined in the malignant and benign/normal group. Progression or recurrence of tumour was observed in 81% of the malignant group and one patient in the benign group presented at a later stage with a malignant tumour ($p<0.0001$). Median PFS was calculated only for the malignant group and this was 10 months. Fifty-nine percent of patients in the malignant group had died at time of follow up compared to no events in the benign group ($p<0.0001$). Median OS was 20 months in the malignant group.

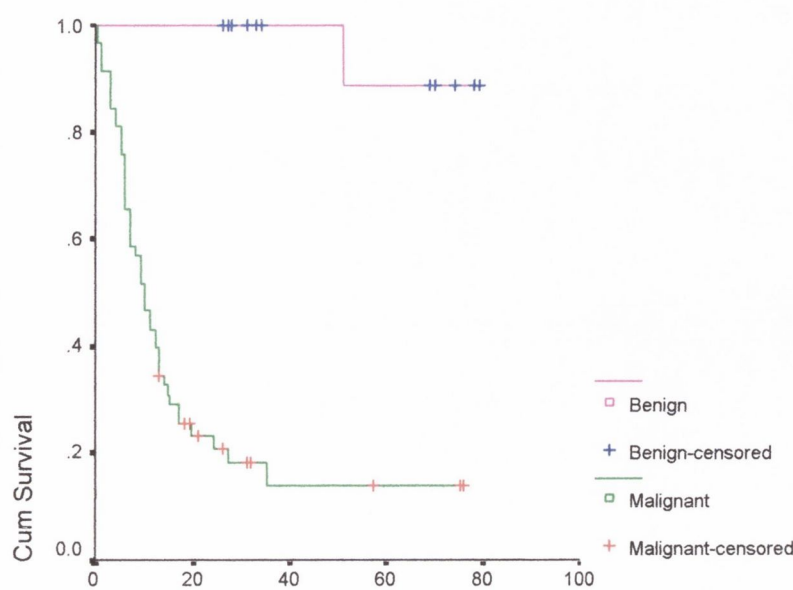


Figure 6.16 PFS in relation to Pathology

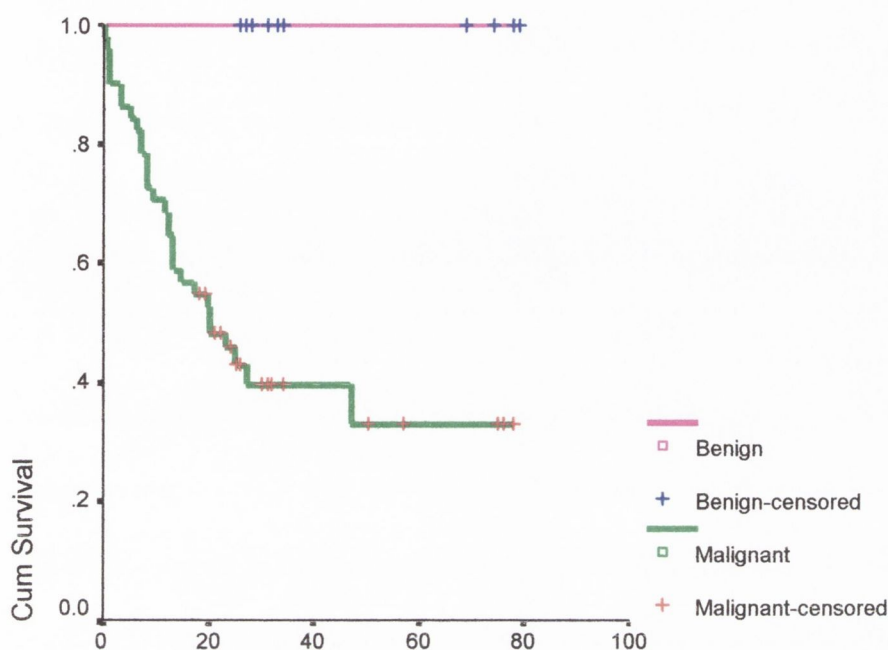


Fig. 6.17 OS in relation to pathology

6.5.2 Tumour Grade

Figure 6.18 displays the PFS for the 3 tumour grades and Figure 6.19 displays the OS for the FIGO grade tumours. The median PFS for the tumour grades were 4 months for the grade 1 tumours, 12 months for the grade 2 tumours and 9 months for the grade 3 tumours. Seventy-six percent of the grade 2 tumours had progressed and 84% of the grade 3 tumours. The median OS was calculated for the grade 2 and 3 tumours with survival values of 23 months and 19.5 months respectively. Fifty-nine percent of the grade 2 and 3 tumours had died at time of follow up. No significant relationship was observed between tumour grade and progression free or OS.

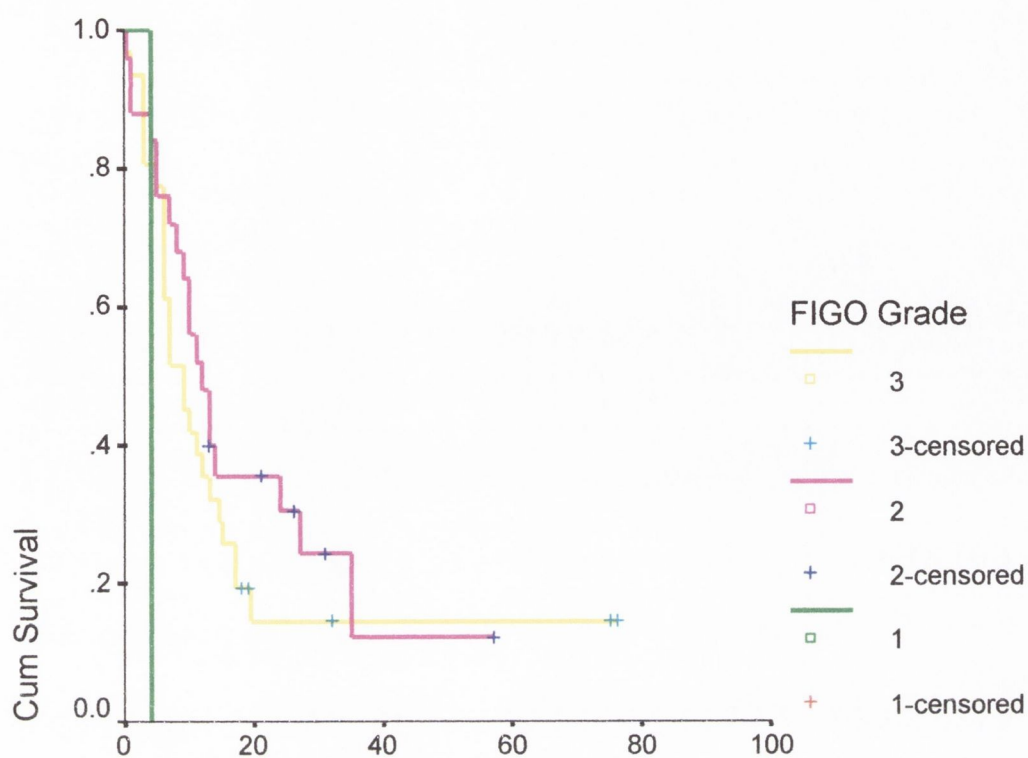


Fig. 6.18 PFS in relation to tumour grade

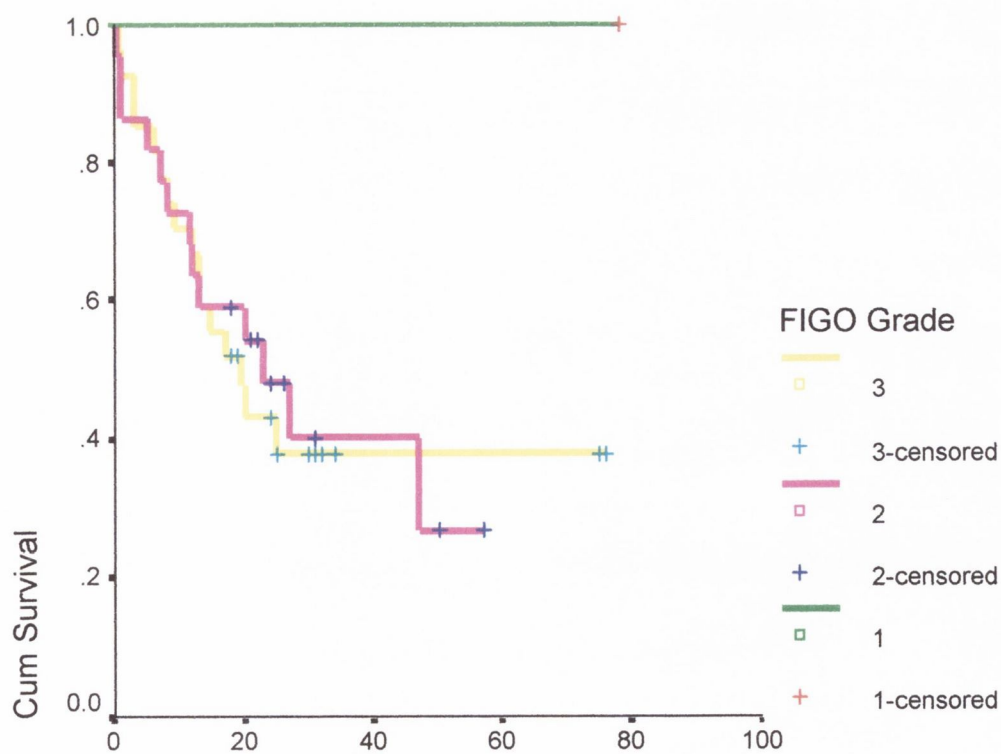


Fig. 6.19 OS in relation to tumour grade

6.5.3 Tumour Stage

PFS in relation to tumour stage is graphed in Figure 6.20 and OS in Figure 6.21. Median PFS for the late stage tumours was 10 months but was not calculable for the early stage tumours as most of these were censored. Eighty-one percent of the late stage tumours had progressed or recurred as compared to 14% in the early stage tumours. Late stage tumours had a significantly lower PFS ($p<0.01$). The median OS was 19.5 months for the late stage tumours and again no value for the early stage tumours. None of the patients in the early stage tumours had died at time of follow up, the median follow up was 33 months but 62.5% of the late stage tumours had. OS was significantly lower for the late stage tumours ($p<0.01$).

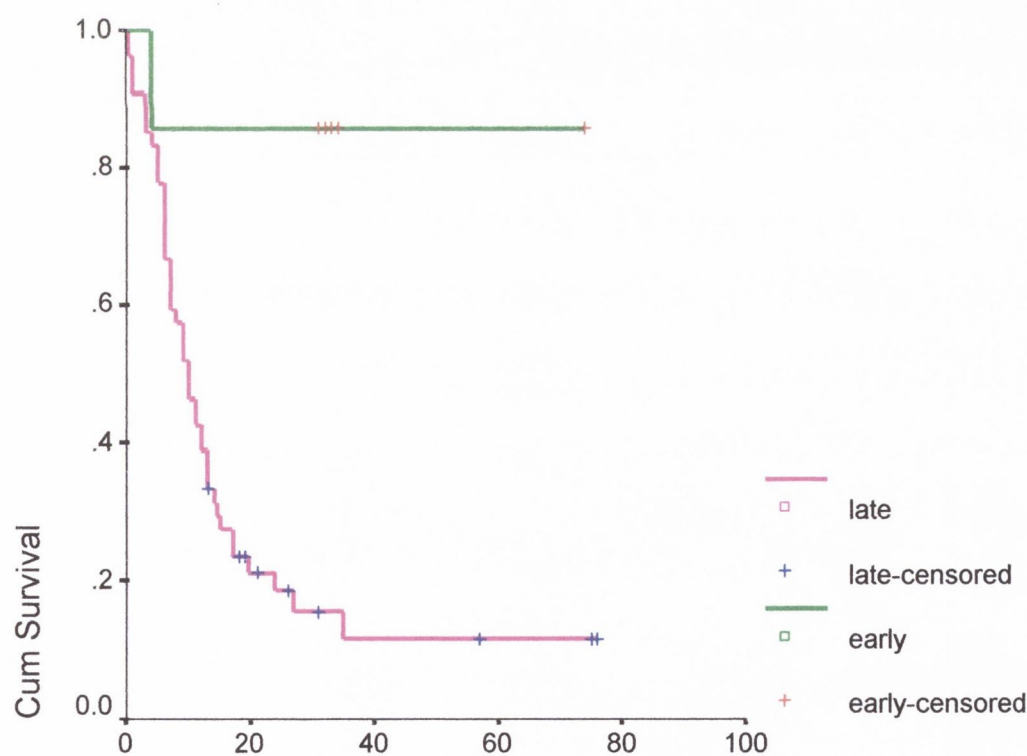


Fig. 6.20 PFS in relation to tumour stage

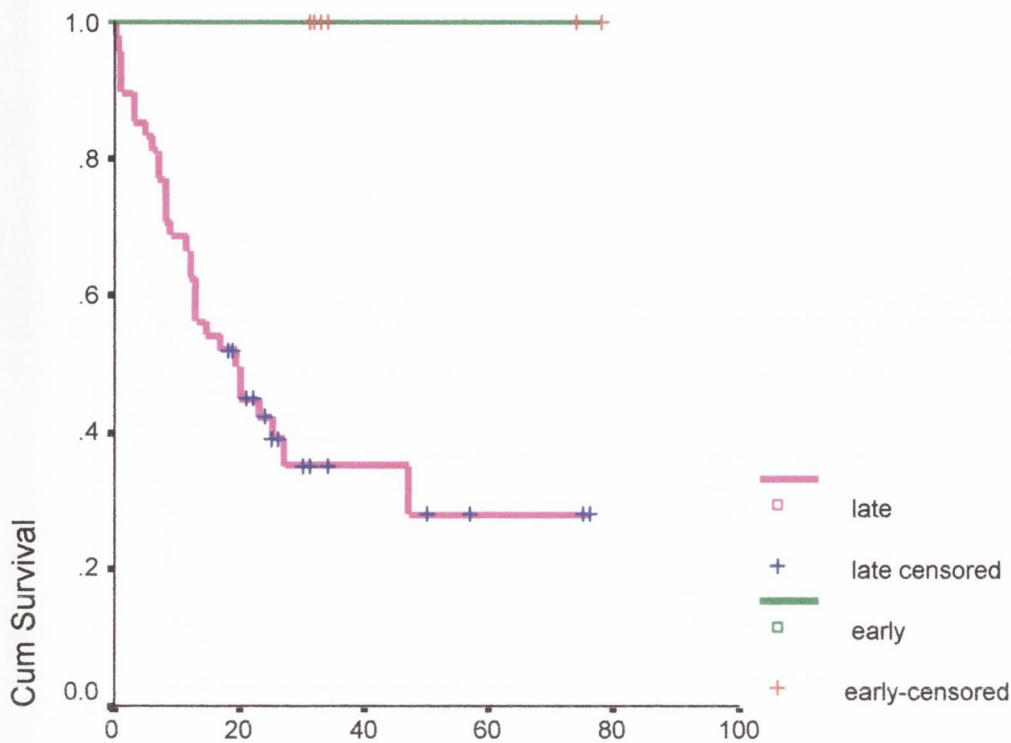


Fig. 6.21 OS in relation to FIGO Stage

6.5.4 Previous Chemotherapy

PFS and OS were examined for patients who had previous chemotherapy and for previously untreated patients. The Kaplan-Meier curve for PFS in these patients is displayed in Figure 6.22. All of the patients who had previous chemotherapy presented with a recurrence or disease progression and 57% of the patients who received chemotherapy for the first time had a recurrence. Median PFS in the group that had previous chemotherapy was 5 months and in the group that had no previous treatment the median was 17 months ($p < 0.0001$). OS was graphed for these patients in Figure 6.23. The group of patients who had previous chemotherapy had a 75th percentile OS of 3 months and 67% of these patients had died of disease. For the other group the 75th percentile was 13 months and 39% had died of disease ($p < 0.05$).

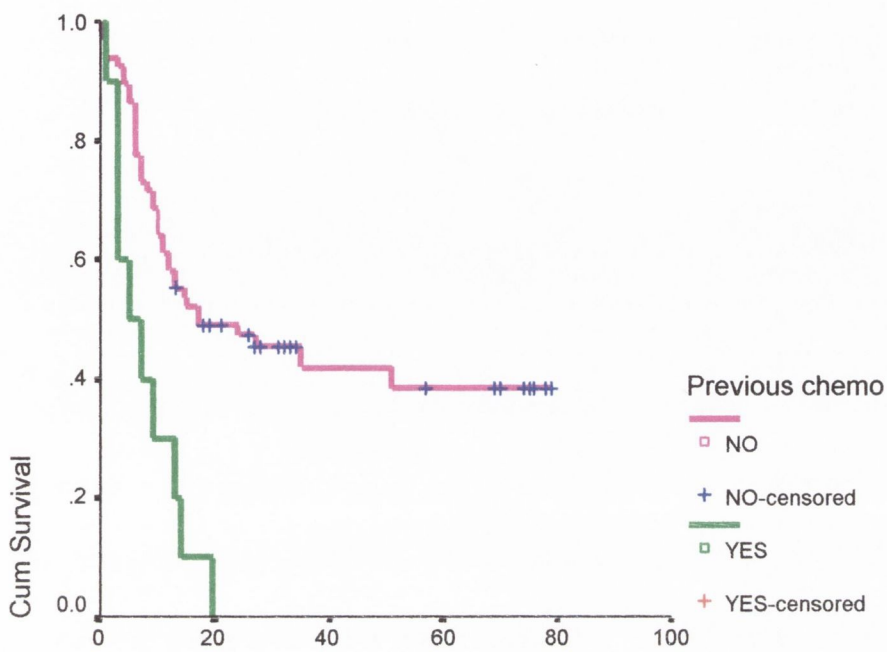


Fig. 6.22 PFS in relation to previous chemotherapy

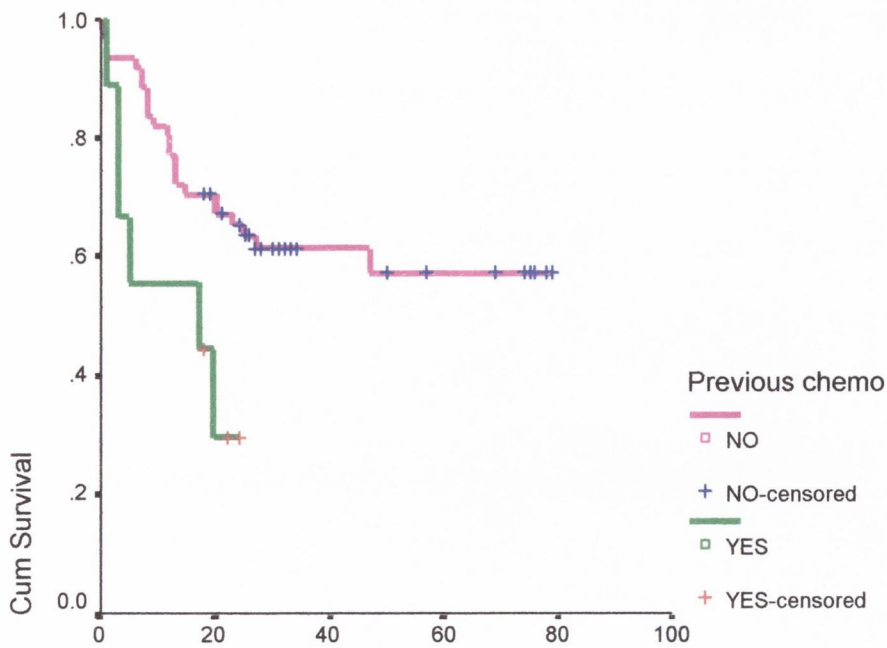


Fig. 6.23 OS in relation to previous chemotherapy

6.5.5 *In vitro* response to chemotherapy

PFS was examined in relation to sensitivity or resistance to chemotherapy *in vitro* (Figure 6.24). Median PFS for the sensitive group was 13 months and 6 months for the resistant group. Disease progression or recurrence occurred in 95.2% of the resistant group compared to 69% of the sensitive group, $p < 0.01$. OS for this group is shown in Figure 6.25 but this was not significant. Median OS was 47 months for the sensitive group and 14.5 months for the resistant group. Sixty-seven percent of the resistant group had died of disease at time of follow-up and 50% of the sensitive group.

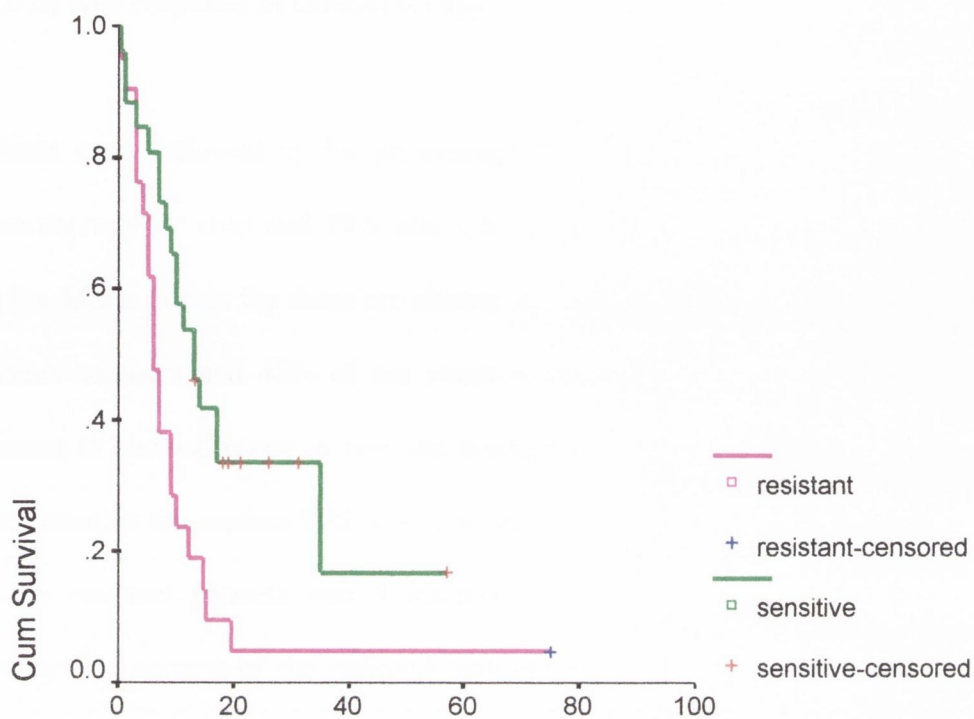


Fig. 6.24 PFS in relation to in vitro chemotherapy response

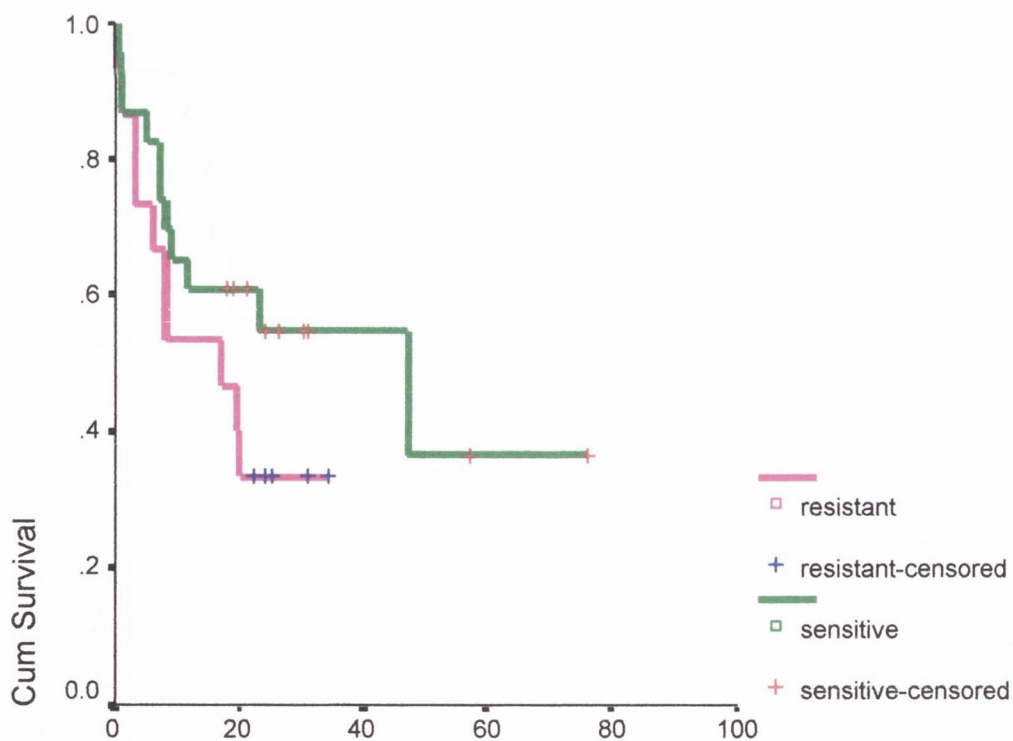


Fig. 6.25 OS in relation to in vitro response to chemotherapy

6.5.6 *In vivo* response to chemotherapy

Patients were followed up for an average of 2.5 years to assess their response to chemotherapy *in vivo* and PFS and OS were determined for these patients. The Kaplan-Meier curves for these are shown in Figures 6.26 and 6.27 respectively. The resistant tumours and 45% of the sensitive tumours recurred. For those that were resistant to chemotherapy *in vivo* the median PFS was 7 months and for those that were sensitive the median PFS was 35 months ($p<0.0001$). The 75th percentile for OS for the resistant patients was 8 months and 47 months for the sensitive group. Seventy-five percent of the resistant tumours had died of disease and 22.2% of the sensitive tumours ($p<0.001$).

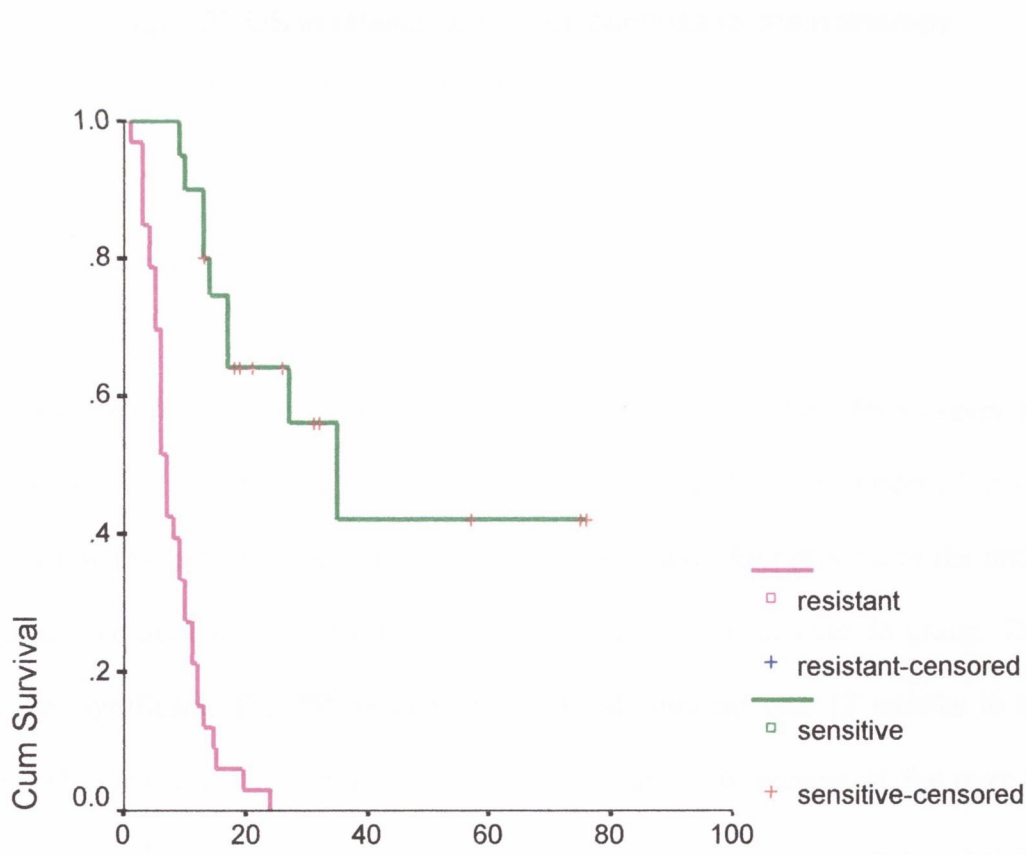


Fig. 6.26 PFS in relation to *in vivo* response to chemotherapy

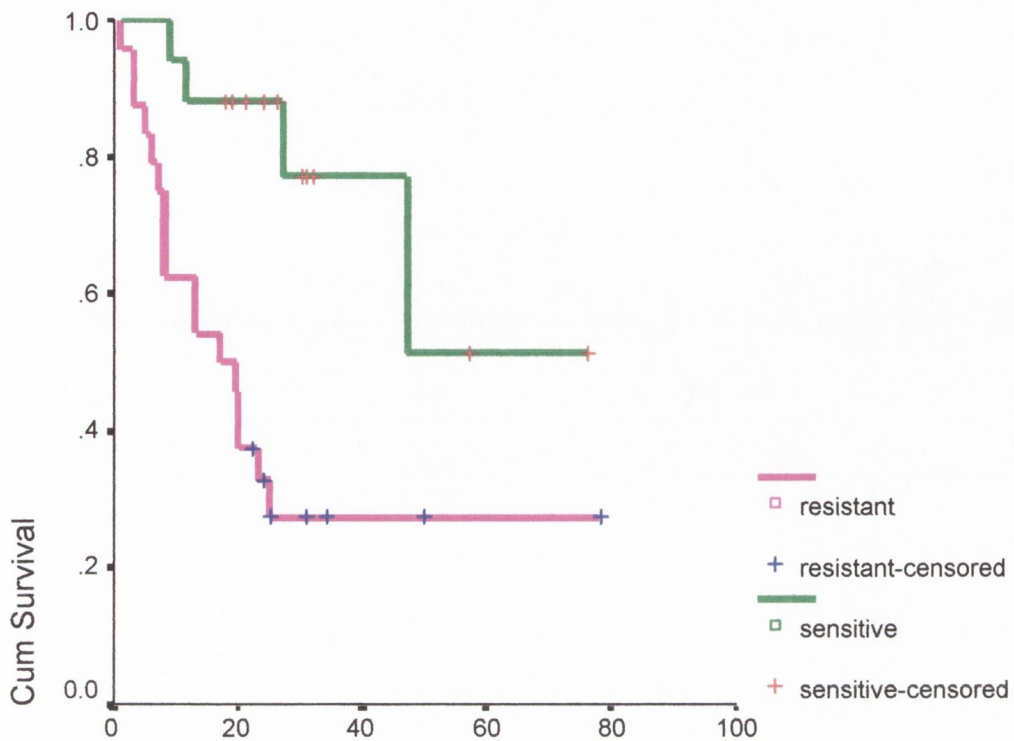


Fig. 6.27 OS in relation to in vivo response to chemotherapy

6.5.7 Patient Age

Patient's age was categorised into under 55 and over 55. Kaplan-Meier curves for PFS and OS are shown in Figures 6.28 and 6.29. Median PFS in the under 55 group was 15 months and 14 months in the over 55 group. Sixty-four percent of the under 55 group had demonstrated disease progression and 61% of the over 55 group. This was not significant. The 75th percentile for overall survival was 13 months in the under 55 group and 12 months in the over 55 group. Fifty percent of the over 55 group had died at time of follow-up and 35% of the under 55 group. No significant relationship was observed with age.

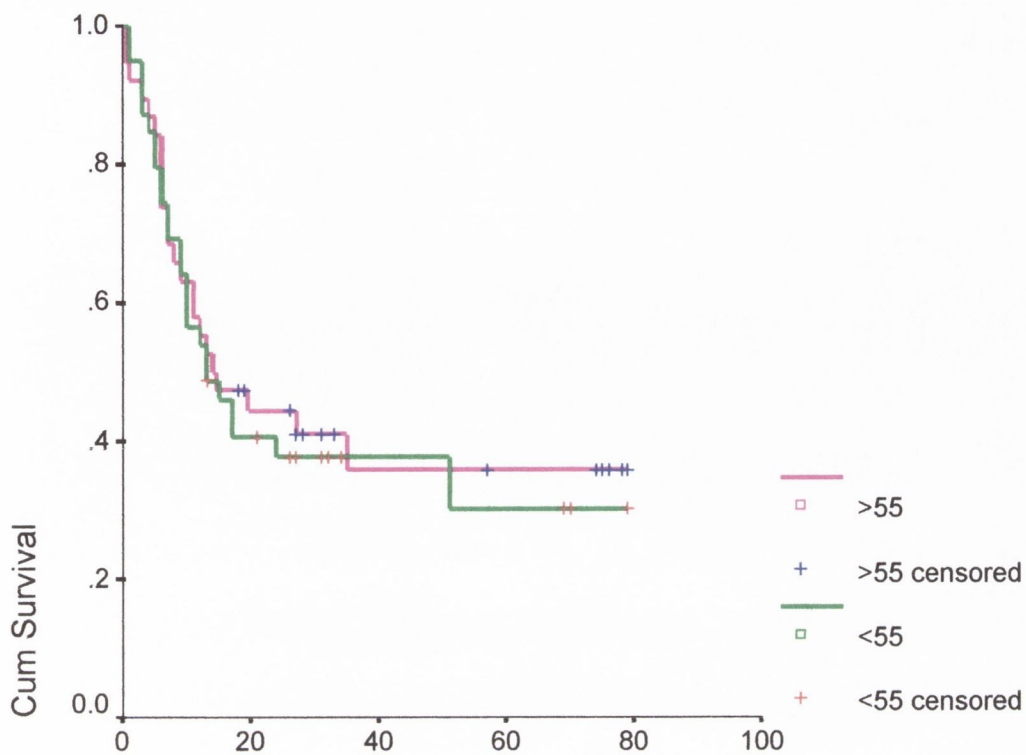


Fig. 6.28 PFS in relation to patient age

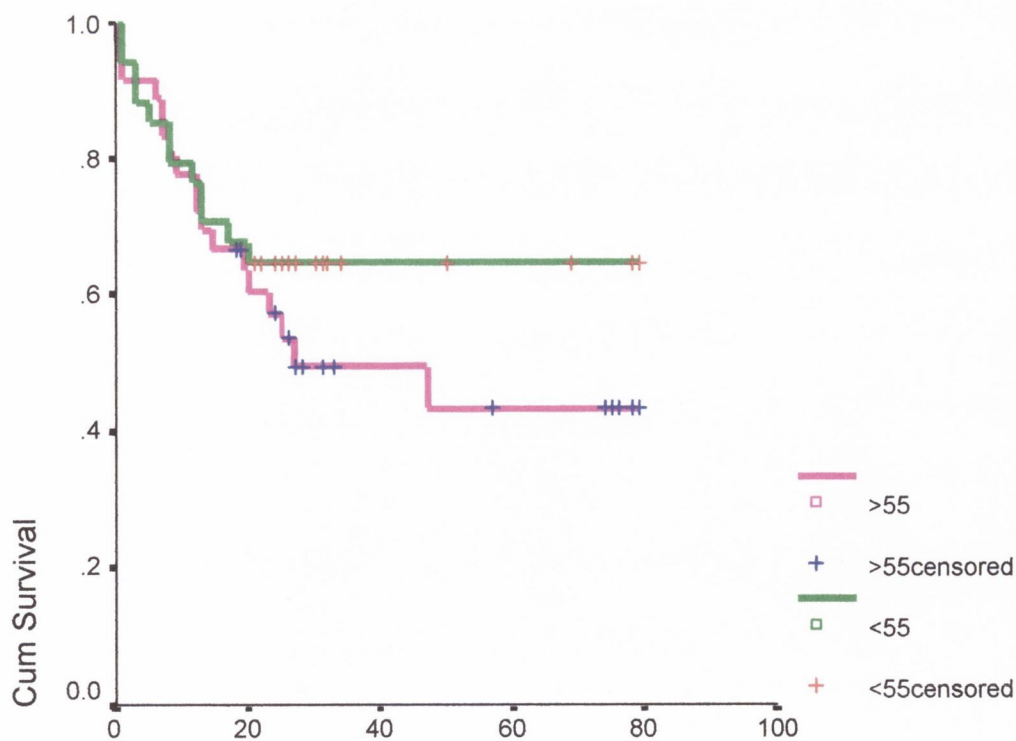


Fig 6.29 OS in relation to patient age

6.6 Multivariate Analysis

Variables that were significant in univariate analysis (log rank-Tables 6.3 and 6.4) were examined in a Cox proportional hazards regression model. Using corrected progression free survival as endpoint only VEGF staining and response to chemotherapy *in vivo* remained significant. In overall survival VEGF staining and response to chemotherapy *in vivo* were also significant, as was previous chemotherapy. Cox hazard ratios and confidence intervals are displayed in Tables 6.5 and 6.6.

Table 6.5 Cox regression analysis for PFS

Variable	P-value	Hazard Ratio	95% CI
<i>In vivo</i> chemo response	<0.0001	0.095	0.036-0.251
VEGF Staining	0.023	0.183	0.066-0.502

Table 6.6 Cox regression analysis for OS

Variable	P-value	Hazard Ratio	95% CI
<i>In vivo</i> chemo response	0.001	0.158	0.051- 0.489
VEGF Staining	<0.0001	0.12	0.038-0.375
Previous chemotherapy	0.04	2.978	1.053- 8.421

CHAPTER 7

DISCUSSION

7.1 Heterogeneity in response to chemotherapy in ovarian cancers

The Mothersill explant culture technique proved to be highly successful in culturing ovarian tumours. Sufficient growth was achieved for most specimens after 7-10 days in culture. A drug incubation time of 3 days was selected, as this exposure time was sufficient to produce cytotoxicity and short enough to minimise the variable effects of cell proliferation and cell death over the assay period. The MTS assay was then carried out to determine the %cell kill caused by the cytotoxic drugs. In the present study the evaluability rate of the assay system for culturing ovarian specimens was 94.4%.

The proportion of tumours that can be evaluated successfully with an *in vitro* drug response system is related to several factors. The tumour must be viable to enable it to grow *in vitro* and careful evaluation and selection by the surgeon of viable tumour tissue is essential. Delays in placing the tumour in transport media and bringing it to the lab can lead to necrosis, thereby decreasing the amount of cells capable of proliferation. The variations in proliferative potential *in vitro* as a function of histological type can also influence evaluability. Careful attention to aseptic technique is essential to prevent microbial overgrowth²⁸⁷.

An evaluability rate greater than 80% is desirable. Other assay techniques had variable evaluability rates when examining ovarian cancers. The HTCA method had evaluability rates from 40%-70%^{159 160 161 162} which would not be favourable in a clinical setting. The thymidine assay had evaluability rates between 75% and 92%²⁸⁸ and the FCA had rates of between 75% and 95%²¹⁰. The ATP assay has also been

used to examine ovarian cancers and has yielded evaluability rates around 90%²⁰⁰. The present assay system combines a culture technique and a cytotoxicity assay to improve evaluability and to mimic the *in vivo* situation more accurately. The explant technique has the advantage of retaining the properties of tissue organisation and heterogeneity that are present *in vivo*¹⁹⁰. A limitation of this assay was the yield of cells achieved for some specimens and a decision had to be made about which drugs to be tested. The MTT assay was used to assess chemosensitivity in ovarian cancers and had an evaluability rate of 75%²⁰³ in one study and 90% in a more recent study²⁸⁹. One problem encountered with the MTT assay is in the use of DMSO to solubilise the formazan crystals. DMSO increases the absorbance values and intra-experimental variation. When using DMSO it is necessary to remove the medium from the plate first and it is difficult to avoid removing cells when doing this²⁹⁰. The MTS assay is a modified version of the MTT assay, which obviates many of the problems previously encountered with MTT^{186 187}. MTS can be used to examine both proliferating and non-proliferating cells, which is an advantage over the thymidine assay. Since the MTS assay examines resting tumour cells, which may be less sensitive to drugs than proliferating cells it may have a potential role in examining chemoresistance. The MTS assay offers the advantages of speed, simplicity, low cost, non-radioactivity and it is possible to test multiple drugs at several concentrations.

Chemosensitivity / resistance testing can be carried out on fresh tumour tissue before treatment for ovarian cancer is administered. The turnaround time for the procedure should make results available in a clinically relevant time frame. The present assay system has that advantage in that a result can be obtained within 10-13 days, before the patient would normally receive chemotherapy.

Tumours of similar stage and grade were examined for their response to cytotoxic drugs. Each tumour proved to be a highly individual dynamic system; even tumours that are classified under the same histotype are highly individualistic. The present study showed that tumours of similar stage and grade had a variable response to the same concentration of cytotoxic drug. Similar results were observed in chemosensitivity in cell lines confirming heterogeneity of the *in vitro* drug response in morphologically similar neoplasms²¹⁰. Sevin *et al.*²¹¹ showed heterogeneity of drug response in fresh human ovarian carcinomas. The present study investigated ovarian tumours of similar grade and stage using a different assay system to the Sevin study.

The most prevalent subtype of ovarian cancer in the present study was serous papillary adenocarcinoma and as it is difficult to diagnose this disease at an early stage most of the tumours were late stage tumours. Three early stage tumours were examined *in vitro* and 56 late stage serous papillary adenocarcinomas. This allowed the examination of the heterogeneity in response among tumours of the same clinical stage and FIGO grade.

Seventy percent of the stage III grade 2 tumours responded to cisplatin at the PPC as seen in Figure 3.6. The assay system showed that some tumours responded at the lower concentration of 0.5 x PPC (Fig.3.6 (a)) suggesting that full dose chemotherapy was unnecessary. All of the tumours in this group responded at 10 x PPC. The tumours in the other categories; Stage III G3 (Fig. 3.10), Stage IV G2 (Fig. 3.14) and Stage IV G3 (Fig. 3.18) displayed a mixed response to cisplatin with some showing resistance at 10 x PPC (Fig. 3.10) and in others total cell kill was observed.

Drug resistance in ovarian cancer is a serious clinical problem. Drug resistance tests could obviate administering chemotherapy when the tumour is resistant to specific agents. The assay allows the identification of ineffective drugs, thus reducing unnecessary patient exposure to toxic agents. An example of extreme drug resistance to paclitaxel is shown in Figure 3.7 where 100% survival was observed at 10 x PPC. This would suggest that this patient was unlikely to benefit from this drug. A similar resistance pattern was seen with one of the tumours in the Stage III, G3 category (Fig. 3.11). Resistance patterns at 10 x PPC were also observed with cisplatin in the other serous papillary adenocarcinoma groups (Figs. 3.10, 3.14, 3.18,) and against the cisplatin / paclitaxel combination (Figs. 3.8, 3.12, 3.16). Host elements may operate against accurate *in vitro* predictions of clinical response but if cancer cells survive high exposures of drugs in the laboratory, host factors are unlikely to render cells responsive *in vivo*²⁹¹.

The results of the present *in vitro* study also showed that with some tumours combination therapy was more effective but for others single agent therapy was equally effective. About 80% of the tumours responded to the combination of cisplatin and paclitaxel, which would probably be the treatment selected by the oncologist for these patients. Paclitaxel in combination with a platinum drug is the standard treatment for epithelial ovarian cancer^{71 74}. The use of single agent therapy may be as effective and less toxic to the patient but it would not compensate for acquired resistance, which is the reason combination therapy is used in ovarian cancer.

Not all of the tumours were examined with topotecan, as it was available at a later stage in the study. This drug is recommended in patients with a recurrence following

platinum therapy³². Over 75% of the tumours examined with this drug were sensitive at the PPC.

Clear cell carcinomas of the ovary have been regarded as chemoresistant in contrast to ovarian surface epithelial carcinomas with response rates of less than 5% to platinum based therapy²⁹². The present study consisted of 8 clear cell carcinomas, 3 of which were sensitive *in vitro* to cisplatin and 4, which were sensitive to the platinum/paclitaxel combination (Fig. 3.24). All of the tumours displayed sensitivity to topotecan. Patients with this tumour type have a poor prognosis and the results support the investigation of chemotherapeutic agents *in vitro* to assess how each tumour would respond.

Mucinous tumours fall into the same group as clear cell carcinomas as regards chemoresistance. Four mucinous tumours were examined but they varied in stage and grade. Two displayed only a partial response to cisplatin (Figs 3.28 + 3.29). Patients with this tumour type would normally receive the standard chemotherapy regimen of platinum/paclitaxel. Of interest was the fact that two of the late stage tumours responded to doxorubicin. There may be a role for this type of chemosensitivity testing for mucinous adenocarcinomas.

Tumours that would be regarded as chemosensitive would include the serous group as discussed above but also the endometrioid and transitional cell carcinomas. Two endometrioid tumours were examined; one was sensitive to chemotherapy and the other resistant. The transitional cell tumour was sensitive. Greater numbers of these

tumour types would be needed to investigate this further but unfortunately the incidence of these tumours was low in the present study.

Borderline ovarian tumours are characterised by epithelial proliferation in the absence of stromal invasion and have a very favourable prognosis. Surgical principles for the management of these tumours are identical to those in the management of invasive disease. Adjuvant chemotherapy is recommended for patients with invasive implants. The borderline tumours were examined *in vitro* in the event that invasive implants were present. Two thirds of the borderline tumours responded well to single agent platinum but overall the borderline mucinous tumours responded better to chemotherapy than the borderline serous tumours.

After surgery, chemotherapy is the treatment of choice for patients with advanced ovarian cancer. Approximately one-third of tumours do not respond to initial chemotherapy and advanced-stage ovarian cancer patients experience relapse rates of 70% to 80% after achieving complete clinical response to platinum- and paclitaxel-based chemotherapy regimens. The ability to predict the response of individual ovarian tumours to chemotherapy would allow selection of patients likely to respond and avoid administration to those resistant. Similar tumours show a heterogeneous response *in vitro* when exposed to various chemotherapy regimens. This strongly suggests the need to characterise a tumour chemosensitivity / resistance profile in each patient before deciding chemotherapy. A prospective study is required to compare the results of chemotherapy based on chemosensitivity and resistance using the present assay system and chemotherapy, as currently used based on large clinical trials.

7.2 Heterogeneity in response to chemotherapy in cervical and endometrial cancers

Cervical and endometrial tumours of similar stage and grade displayed heterogeneity in their response to chemotherapy. Numbers were smaller in this group than in the ovarian cancer group. Whereas sufficient tumour tissue can be obtained from ovarian cancer patients due to the nature of this disease, cervical specimens are more difficult to obtain, as the pathologist needs the entire specimen to assess the margins of the tumour. Also the incidence of cervical cancer and endometrial cancer in the hospitals supplying tissue in the present study was lower than ovarian cancer.

An evaluability of 100% was achieved for culturing the cervical and endometrial specimens. The advantages of the assay system have been discussed above. Cervical and endometrial specimens demonstrated a similar pattern of heterogeneity in their response to cytotoxic drugs as the ovarian cancers. Sevin *et al.*²¹¹ showed heterogeneity of drug response in fresh human ovarian carcinomas but little work has been carried out on cervical and endometrial specimens.

The effect of cisplatin on the Stage I, G2 squamous carcinomas are shown in Fig. 4.1 with responses at the PPC varying from complete resistance to 9.1% survival. In one of the tumours a concentration of 0.5 x PPC was sufficient to give greater than 50% cell kill. The response rates using paclitaxel varied from 75% survival to 24% survival at the PPC (Fig 4.2). Five of the tumours were examined using the cisplatin / paclitaxel combination only at the PPC (Fig. 4.3) and three responded well (27.4% survival to 43% survival) and two specimens were resistant. Two of the tumours that

were examined using fluorouracil (Fig. 4.4) were sensitive but the remaining two only gave a borderline response and one of these was examined at 10 x PPC but no additional cytotoxicity was observed at this concentration. Similar patterns were observed in the other groups of cervical specimens.

It was observed that some of the cervical specimens responded well to the cisplatin and paclitaxel combination, which may encourage the use of paclitaxel in the treatment of cervical cancers. In the United States of America paclitaxel and cisplatin have been shown to be highly active in advanced and recurrent squamous cell carcinoma of the cervix and the Gynecologic Oncology Group (GOG) are conducting a phase III randomised study comparing the combination of paclitaxel and cisplatin with cisplatin alone¹⁰³. The response of some of the early stage tumours to platinum/paclitaxel may encourage further investigations to be carried out into this treatment for cervical cancer. The adenocarcinomas examined responded well to chemotherapy as shown in Figs 4.16 and 4.17. The adenosquamous cancers were collected at an early stage in the present study and were only examined with cisplatin; the early stage tumours responded better to this drug. The recurrent tumour also showed a good response to platinum. This patient had recurred after 29 years of initial diagnosis so was going to receive additional radiotherapy.

Previous studies examining chemosensitivity are limited which is probably due to the fact that most cervical cancer patients receive postoperative radiotherapy. One study using a clonogenic assay was only 36% evaluable¹⁹¹ and another study which was 82% evaluable only contained 6 patients¹⁹².

Responses of the endometrial tumours to chemotherapy varied with some showing a good response at the PPC and others being quite resistant. Again studies are few and numbers small, examining chemosensitivity in endometrial cancers. The clonogenic assay had a 35% evaluability rate¹⁹¹ and the ATP assay a 68% evaluability¹⁹⁸. The MTT assay has been used to establish the cytotoxicity of a new compound irinotecan and compared this with cisplatin in 49 specimens of ovarian and endometrial cancers, heterogeneity in response was observed in the 22 endometrial samples.²⁰⁴

In the present study some of the endometrial cancers displayed almost complete resistance to some of the drugs and others again showed responses at 0.5 x PPC. This may suggest a role for these drugs in the treatment of endometrial cancers and also a cost saving if lower doses of the drugs were used. However, prospective clinical trials would have to be carried out to determine if a significant increase in survival could be obtained by treating the patients with chemotherapy as opposed to radiotherapy. Results have just been presented from GOG trial 122²⁹³ demonstrating a significant survival advantage for advanced-stage endometrial cancer patients treated with platinum-doxorubicin as opposed to radiotherapy.

An interesting case was observed of a recurrent stromal sarcoma (Fig.4.43). The first biopsy obtained in the present study was resistant to cisplatin; a drug this patient had received but she had developed a recurrence. This patient did not receive any chemotherapy at this stage as she had a very good recovery post-op. However, her tumour recurred two years later and again it was resistant to cisplatin. Paclitaxel was available at this stage and her tumour was found to be sensitive to this agent.

Unfortunately this patient died due to post-op complications so it was not possible to evaluate this further.

The role of chemosensitivity testing in cervical and endometrial cancer may change as trials examining the effectiveness of chemotherapy in these diseases are carried out. The present assay system proved to be 100% evaluable in determining the response of these tumours to chemotherapy.

7.3 *In vitro- in vivo* correlations

The correlation between *in vitro* response to chemotherapeutic drugs and *in vivo* response for mainly ovarian cancer patients was highly significant for the 76 patients ($p < 0.0001$). In 7 advanced stages of ovarian carcinoma the patient died before they could receive any adjuvant therapy and no correlation was possible and four patients were lost to follow-up. The patients with cervical and endometrial cancer usually received postoperative radiation and correlations between clinical outcome to chemotherapy and the *in vitro* chemosensitivity / resistance results could only be carried out for 12 patients in this group. The results of the *in vitro* assay were passed on to the physician in the event of a recurrence. While *in vitro-in vivo* correlations do not prove that chemosensitivity testing will ultimately benefit significant numbers of cancer patients, such correlations are a first step towards establishing the validity of any assay system.

Negative predictive accuracy relates to the reliability of the assay to identify ineffective agents that will fail to produce a clinical response and this assay system had a negative predictive accuracy of 97.2%.

Positive predictive accuracy is a measure of an assays reliability to identify agents that will cause clinical responses and this assay had a positive predictive accuracy of 74.4%. The lower rate of the positive predictive accuracy is due to the fact that 10 of the 39 patients testing as sensitive *in vitro* were resistant *in vivo*. This could be explained by rapid development of drug resistance under therapy.

In general negative predictive accuracies are higher than the positive predictive accuracies suggesting that these technologies are better at identifying ineffective agents. Previous studies²⁸⁸ examining chemosensitivity in ovarian cancers using some

of the endpoints described in chapter 1 have reported positive predictive accuracies in the order of 53% to 88% and negative predictive accuracies from 71% to 100%. The fact that these assays would vary in their positive and negative predictive reliability is not surprising when the concept of drug delivery *in vivo* is considered and has been an accepted theory for several years based on the differences between *in vitro* models and *in vivo* pharmacodynamics^{166 200 201}.

For an *in vitro* test to be incorporated into routine procedure a significant increase in patient survival would have to be observed for assay directed chemotherapy regimens. Progression free survival and overall survival were examined for the cohort of patients in the present study. This analysis was confined to the ovarian cancers (ov1 – ov86) as the majority of patients who received chemotherapy were in this group and numbers in the cervical and endometrial groups were small. Progression free survival was chosen as the most appropriate parameter of response as all the patients had different follow up times but OS was also considered.

Eighty percent of the ovarian specimens in the present study were serous papillary adenocarcinomas so no analysis was carried out on survival and tumour type. Tumour grade had no influence on progression free survival or overall survival. Tumour stage had a significant effect on PFS and OS. Fifty-nine percent of the late stage tumours had died at time of follow-up compared to 13% in the early stage group ($p<0.01$).

Patients who had received chemotherapy prior to inclusion in this study had a significantly lower PFS and OS compared to the group of patients who had not

received chemotherapy. There was a three-fold difference in PFS ($p<0.0001$) between the two groups and almost a 5-fold difference in OS ($p<0.001$). The patients who had previous chemotherapy had most likely developed acquired resistance to the chemotherapy drugs. A significant increase in PFS was observed when patients responded to their chemotherapy *in vivo*, which would be expected. Median PFS for patients whose tumours were sensitive *in vivo* was 5 times greater than those whose tumours were resistant ($p<0.0001$). A significant increase in OS was observed in patients who responded to chemotherapy *in vivo*. Five of 28 patients who had responded to chemotherapy *in vivo* had died at time of follow up compared to 28 patients out of a group of 39 who had been resistant *in vivo* ($p<0.001$).

While *in vitro* drug-response assays discriminate between clinically inactive agents, this does not necessarily translate to an accurate prediction of patient survival. Various trials have identified agents capable of causing short-term responses without translating clinical response into a survival benefit^{176 294 295}. In the present study patients with an *in vitro* sensitive assay result survived for twice as long without a recurrence or progression of disease as the patients with an *in vitro* resistant result and only 1 patient with an *in vitro* resistant assay displayed a clinical response to the drug ($p<0.001$). *In vitro* response remained significant in multivariate analysis ($p<0.05$). A trend was observed, albeit non significant towards decreased OS in the *in vitro* resistant group with 64% of these patients having died at time of follow up compared to 43% in the sensitive group. Patients whose tumours had been sensitive in the *in vitro* assay survived for more than twice as long as those whose tumours were resistant *in vitro*. Patients were not followed up for the same length of time, as this would require a longer study period, which may yield significant observations for

OS. The current results would suggest that drugs found to be resistant *in vitro* have a high probability of poor clinical response and survival.

The correlation between *in vitro* test results and *in vivo* patient response is greatly affected by the definition and method of clinical response assessment. In medical oncology a 50% or more reduction of tumour size for 1 month or more is sufficient for a partial response. In contrast the World Health Organisation²⁹⁶ recommends a minimum of 3 months observation time. Previous studies only examined patients for 1 month after chemotherapy to determine their response to the treatment^{167 196}. This follow up period is not sufficient to determine their true response. The present study monitored patients for at least a year but a longer follow up period may demonstrate a significant reduction in overall survival for those who had an *in vitro* resistant test result.

Three recent studies have been carried out examining if *in vitro* testing has an influence on survival^{200 289 297}. One study had an 89% evaluability rate using the ATP assay and correlations were carried out on 38 patients. Similar differences in PFS and OS were observed as in the present study. Correlations were only carried out on ovarian stage III patients and demonstrated a sensitivity of 95%, specificity of 44%, a positive predictive accuracy of 66% and a negative predictive accuracy of 89%. The study was carried out before paclitaxel was routinely used for the treatment of ovarian cancer but was incorporated for 14 of the later patients. The *in vitro* response in the study did influence PFS and OS in univariate analysis but it was not examined in multivariate analysis²⁰⁰. Similar OS values were observed as in the present study,

however, the results of the present study showed a longer median OS for the resistant group but this may be due to the use of paclitaxel.

A study using the MTT assay²⁸⁹ had a sensitivity of 81%, specificity of 67%, a positive predictive accuracy of 64% and a negative predictive accuracy of 83%. The evaluability of this assay was 90%. Again this study was carried out before paclitaxel became widely available.

The other study²⁹⁷ utilised the extreme drug resistance assay, which is a form of clonogenic assay. This study just examined resistance to platinum to determine if it had an influence on PFS and OS. They found that platinum resistant tumours had a significantly decreased PFS and OS in univariate and multivariate analysis. The negative predictive accuracy was 85% in this study.

Clearly the present assay system has a better sensitivity, specificity, negative and positive predictive accuracies and evaluability than other systems. An *in vitro* resistant result corresponded with a significant decrease in progression free survival. A trend was observed towards a decreased overall survival but a longer follow up period is necessary to accurately determine the influence of the assay on OS. As observed in other studies the *in vitro* assays are more accurate at identifying inactive drugs rather than identification of drugs that will be effective. The present assay did show an improvement in positive predictive accuracies compared to the other studies. *In vitro* assay systems can deliver the drug directly to the tumour cells *in vitro* but the human situation is a lot different. After injection, the drugs are subject to biotransformation and biodistribution. Individual differences in drug metabolism that may prevent an active drug from reaching the tumour *in vivo* cannot be modelled *in vitro* using the current assay systems. Angiogenesis may also influence how a tumour

will respond *in vivo* but it is not possible to account for this *in vitro*. This makes it difficult for the *in vitro* assay to predict *in vivo* sensitivity accurately. However, if a tumour is resistant *in vitro* it is unlikely to show efficacy *in vivo*.

In addition to improved survival in the patient there is also a question of cost. The avoidance of unnecessary treatment could reduce costs involved in treating patients.

7.4 Potential prognostic factors and predictors of response to chemotherapy in ovarian cancer

Angiogenesis Markers

CD31

The majority of women with advanced stage ovarian cancer die of progressive disease^{44 298}. It has been reported that MVD has some relation to metastases and predicts patient prognosis in several solid organ cancers including those arising in the breast, lung, prostate and head and neck²⁹⁹. These reports imply that angiogenesis is progressively stimulated during tumour progression^{223 300 301 302}. The present study attempted to establish the significance of angiogenesis in ovarian cancer.

Significantly more staining was observed in the malignant group than in the borderline or benign group. The malignant group in the present study consisted mainly of serous papillary adenocarcinomas so it was not possible to distinguish between malignant tumour types. One study did observe higher MVD in early stage mucinous tumours relative to serous and benign²²¹. The two late stage mucinous tumours in the present study displayed a high degree of staining but further studies with a larger series of specimens would be necessary to validate this observation. Hollingsworth *et al.*²⁴² in a study of 43 patients found that none of the angiogenesis variables were associated with tumour type.

In the present study of 59 cases of ovarian cancers no correlation was detected between CD31 expression and histological differentiation; this is in agreement with Hollingsworth *et al.*²⁴² who found no association between tumour grade and angiogenesis.

No correlation existed between CD31 and FIGO stage but the number of early stage tumours were few as most ovarian cancers are diagnosed at a late stage. This is in contrast to a study by Darai *et al.*²³⁹ who found a significant relationship between CD31 and histological type, stage and tumour differentiation in a group of 60 patients. Another group found MVD did not differ between early and late stage serous tumours but they did find higher MVD in early stage mucinous tumours²²¹. This would contradict the putative association with poor outcome, as this group would have a good prognosis. It may be that mucinous and serous tumours are characterised by different angiogenic pathways or that MVD is only relevant in late stage tumours. Numbers of mucinous tumours were too low in our study to allow any statistical evaluation of this.

No association was observed between staining and patient age in the present study, which is in agreement with Darai *et al.*²³⁹.

The association between MVD and survival has been investigated in many tumour types yielding conflicting reports²⁹⁹. A statistically significant reduction in progression free and overall survival was found with increasing degree of CD31 staining in the present study, which is in agreement with Darai *et al.*²³⁹. Also Hollingsworth *et al.*²⁴² concluded that increased intratumour microvessel density is associated with decreased overall and disease-free survival in ovarian cancer but only disease-free survival remained significant in multivariate analysis. The present results

are similar to Obermair *et al.*³⁰³ who found that microvessel density was a significant prognostic indicator in univariate analysis but this failed to attain statistical significance in multivariate analysis. Other studies, however, have failed to repeat these observations, one study on 60 ovarian cancer patients found that microvessel density did not provide any prognostic information in these tumours²⁴³. This group, however, used the anti factor VIII-related monoclonal antibody which has been reported to be less sensitive than CD31^{221 222 223}. Studies^{304 305 306} examining tumours of different origins failed to find the significance of angiogenesis but none of these studies used CD31 to evaluate angiogenesis.

Increased vascularity may suggest improved tumour oxygenation and drug delivery and thereby improved response to chemotherapy. However, this remains contradictory in many cases^{241 242 248 307}. In the present study no correlation existed between the degree of CD31 staining and *in vitro* or *in vivo* response, however, a large percentage of the resistant tumours were in the high staining groups. Gasparini *et al.*²⁴¹ examined the relationship between the degree of vascularisation and response to platinum-based combined induction chemotherapy in patients with FIGO stage III-IV ovarian disease. There was a significant association with the degree of staining and lack of response. Due to the small number of patients with a pathological complete response they were unable to examine the association of the probability of complete response with degree of vascularisation. A study carried out on vascular density as a predictor of chemotherapy response in breast cancer observed a trend between degree of staining and lack of response but it did not reach significance³⁰⁸, which was similar to the findings in the present study. In a study of non-small cell lung cancers²⁴⁸ microvessel density was significantly reduced in resistant tumours

when compared with sensitive tumours. This was explained by the fact that tumour vasculature is often inadequate for the tumour mass, solid tumours have subpopulations of cells with very low oxygen and nutrient levels which may limit the effectiveness of therapy.

If patients present with highly angiogenic primary tumours and these are the patients most likely to develop distant recurrences, then it might follow that this group of patients are most likely to benefit from adjuvant therapy. However, in contrast to this hypothesis, some studies demonstrate that angiogenic tumours have a more aggressive phenotype and do not benefit from therapy as anticipated. This seems to be the pattern observed in the present study. Tumours of low angiogenic index may be more likely to benefit from adjuvant therapy but they may be the subgroup of patients that do not need chemotherapy. Patients with highly angiogenic tumours should therefore be considered for trials that include the use of anti-angiogenic strategies.

Angiogenesis is just one step in the multistep process of metastasis. Tumour cells must also proliferate, penetrate host tissues and vessels, survive within the vasculature, escape the host's immune system and then begin growth at a metastatic site. The findings in the present study are in agreement with the majority of studies suggesting MVD is a prognostic marker in ovarian cancer but no definite conclusions can be drawn at present on the real clinical usefulness of this approach. Before adopting MVD as a prognostic marker in the routine clinical setting appropriate prospective trials are needed to validate the results observed in retrospective studies.

VEGF

Tumour angiogenesis is believed to be mediated by soluble factors that are released by tumour cells, such as VEGF. The expression of VEGF was assessed in 79 patients and compared with histopathological parameters that are useful in predicting prognosis and disease free survival. The correlation between VEGF immunostaining and *in vitro* and *in vivo* response to chemotherapy was also evaluated.

Previous immunohistochemical studies^{234 235 236} have demonstrated mean VEGF expression was significantly greater in malignant neoplasms than seen in borderline, benign or normal samples, suggesting a strong correlation between VEGF-induced angiogenesis and aggressiveness of ovarian tumours. In the present study more staining was observed in the series of malignant specimens than in the benign/normal group but this did not reach significance, despite the fact that the high degree of staining was only observed in the malignant specimens. Similarly, Abu-Jawdeh *et al.*²³⁶ by using in situ hybridisation, observed strong expression of VEGF mRNA in neoplastic cells in all serous ovarian carcinomas evaluated, whereas serous borderline tumours had variable VEGF mRNA expression, and no benign tumours had strong expression, suggesting an important role for VEGF in the angiogenesis associated with ovarian malignancy.

In the present cohort of patients no significant association was observed between VEGF immunostaining and architectural grade, which is in agreement with a study by Fujimoto *et al.*²³¹ but two thirds of the present group that showed a high degree of staining were poorly differentiated tumours and the remaining third were moderately differentiated. This is in contrast to two other studies^{246 309} who found that VEGF was

correlated to tumour grade. In a study of small cell lung carcinoma no interrelationship was found between VEGF expression and tumour grade²⁴⁸.

No significant relationship was observed between VEGF immunostaining and FIGO stage; this is in agreement with two other studies on ovarian cancers^{231 243}. Volm *et al.*²⁴⁸ found no significant relationship between VEGF and tumour stage in non-small cell lung carcinomas. In contrast to these findings other investigators did find a significant relationship between VEGF staining and FIGO stage^{246 309 310}. The specimens in the present study that did show a high degree of staining, however, were all late stage tumours.

In the present study patients whose disease recurred or progressed or who died from disease displayed significantly higher VEGF staining. Median corrected disease free survival varied from 5 months in the group with the highest degree of staining to 27 months in the group that showed no staining. This result is in agreement with other studies reporting a correlation between induced neoangiogenesis and tumour progression and / or metastases^{237 238 243}. Paley *et al.*²³⁷ suggested that VEGF expression by neoplastic cells was of independent prognostic value in patients with early stage ovarian cancers and patients with VEGF-rich tumours had a shorter disease-free survival. The sample size of borderline and early stage tumours was too small to determine if VEGF had any prognostic significance in the present study. Yamamoto *et al.*²³⁸ found that ovarian cancer patients with strong VEGF immunoreactivity showed poorer survival rates than those with weak or no immunostaining but the prognostic significance was related to its correlation with FIGO stage and it was not an independent prognostic indicator. The present results

demonstrate that VEGF is an independent prognostic indicator in disease free survival and overall survival.

There is increasing evidence that angiogenesis has a critical role in the spread and progression of ovarian carcinoma. Animal studies have shown that carcinoma of the ovary metastasises to the peritoneal membranes as tiny avascular seeds, which do not grow beyond a limited size until after neovascularisation¹¹. However, the pathogenesis of the angiogenic event is not well defined in neoplastic lesions. Many cytokines act in concert with VEGF to induce neovascularisation³¹¹, and it is probable that a precise, coordinated regulation of contributing components is necessary.

Seventy percent of the specimens displaying a high degree of VEGF staining were resistant *in vivo* and 50% were resistant *in vitro* but no significant relationship was observed between *in vitro* or *in vivo* chemotherapy response and VEGF immunostaining. Studies analysing the relationship between angiogenesis and chemotherapy response are limited. A study by Volm *et al.*²⁴⁸ showed that expression of VEGF was significantly lower in resistant non-small cell lung carcinomas than in sensitive tumours. This study only looked at doxorubicin resistance *in vitro* and was explained by the oxygenation of the neoplastic tissue. It has been shown that hypoxia can induce resistance to a number of antineoplastic agents including doxorubicin³¹²³¹³. Also reduced vascularisation may up-regulate resistance-related proteins and may represent an important factor to the poor response of tumours to chemotherapy and radiation³¹⁴.

A study on gastric carcinomas²⁴⁷ showed that VEGF positive cases showed a significantly higher response to chemotherapy than VEGF negative cases. This

relationship was explained by the drug delivery through angiogenesis and vascular permeability.

These two studies were in contrast with a study on breast carcinomas. Foekens *et al.*²⁵⁰ found high tumour levels to be associated with a poor rate of response and a short progression free survival and post relapse overall survival, on chemotherapy given for advanced oestrogen receptor negative breast cancer. This was explained by the fact that VEGF could induce endothelial cell proliferation and indirectly contribute to the drug resistant phenotype of a tumour via the expression of drug-resistance-associated proteins such as glutathione S-transferase- π ³¹⁵. The present study found no significant relationship between VEGF and MDR.

The mechanisms responsible for tumour angiogenesis are complex, and whether secretion of vascular endothelial growth factor induces tumour growth or tumour cells secrete vascular endothelial growth factor during tumour growth is still unknown.

A significant correlation between degree of CD31 staining and VEGF staining was demonstrated in the present study, which is in agreement with a study by Nakanishi *et al.*²⁴³. They found a significant association between MVD and VEGF expression in that the MVD of VEGF-rich tumours was significantly higher than that of VEGF-poor tumours suggesting VEGF has a crucial role in angiogenesis of ovarian cancer. Shen *et al.*²⁴⁶ observed no relationship between VEGF and MVD but the antibody used for detection of MVD was FVIII-related antigen, which has been shown to be less sensitive than CD31^{221 222 223}. Hartenbach *et al.*²⁴⁴ found no correlation between

microvessel density and VEGF expression but this may be explained by the small sample size and they also used the antibody to FVIII-related antigen.

Angiogenesis might be induced differently depending on the organ involved and the histological type. The present cohort of specimens contained mainly serous papillary adenocarcinomas of the ovary so it was not possible to determine if this was true. Angiogenesis seems to be necessary for cancer cell growth and to allow cancers to increase in volume, but other mechanisms may play a crucial role in tumour aggressiveness. The present study indicates that VEGF is important as a prognostic indicator in progression free and overall survival in ovarian cancers.

Angiogenesis inhibitors have shown promising results in preclinical animal models but have been disappointing when extrapolated to the clinical setting³¹⁶. Much effort has been put into the identification of endogenous angiogenic inhibitors and activators and less is known about how these factors work together to form vessels at any given stage of tumour development. Few studies assessing antiangiogenic agents have demonstrated improvement in survival. With single-agent therapy unlikely to provide clinical benefits in a large population of patients, there are many unanswered questions. It is still unclear who benefits from therapy, the mechanisms of the toxicities, the best combinations that work or even why they may work. Despite the setbacks results just released using an angiogenesis inhibitor bevacizumab have demonstrated a survival advantage in colorectal cancer patients³¹⁷ but it was not effective in other cancers.

MDR in Ovarian Cancers

Nearly half of all patients with cancer have tumours that are intrinsically resistant to chemotherapy. A large number of the remaining patients develop or acquire resistance during the course of their chemotherapy despite being responsive initially. Drug resistance is estimated to cause over 90% of all cancer deaths³¹⁸. The mechanisms responsible for intrinsic and acquired drug resistance in ovarian cancer are not clearly understood. P-gp, which is encoded by the MDR gene, has been extensively studied in cell lines and human tumour samples³¹⁹. Conflicting evidence on the frequency and prognostic significance of P-gp expression in ovarian carcinomas can be found in various studies^{273 274}. The discrepancies in reports may be due to the different sensitivity of the detection techniques used. The present study used pressure cooker antigen retrieval, which was highly efficient at antigen unmasking and had been recommended by the hospital pathology laboratory.

Significantly more staining was observed in the ovarian malignant group compared to the normal/benign group in the present study. No relationship was observed between degree of staining and histological type as the majority of specimens in this group comprised of serous papillary adenocarcinomas. This was in agreement with another study on ovarian cancers where no association was found between MDR1 and histology but this group of tumours also consisted of mainly serous carcinomas²⁷⁵.

No significant association was observed between MDR staining and tumour grade although the group that displayed a high degree of staining consisted of moderately

and poorly differentiated tumours. Similar observations were made by Kavallaris *et al.*²⁷⁵ in a study of epithelial ovarian cancers.

Of the tumours with a high score of staining only one was a stage II and the remaining were late stage tumours, however, no significant relationship was found between degree of staining and FIGO stage of the ovarian tumours. This may have been due to the small number of early stage tumours in our group as most ovarian cancers, which are diagnosed, are at least stage III tumours. This is in agreement with the study on epithelial ovarian cancers²⁷⁵.

Increased MDR staining was associated with a decrease in progression free survival in the present group of ovarian specimens but this relationship did not reach significance. Studies examining MDR and survival in ovarian cancers have reported conflicting results. Kavallaris *et al.*²⁷⁵ found that patients with MDR1 positive tumours had a significantly shorter event free survival time than patients with MDR1 negative tumours suggesting that even low-level expression of the MDR1 gene may offer a selective growth advantage to these tumours. This analysis was carried out on previously untreated tumours. In contrast to this Bourhis *et al.*²⁷³ stated that MDR1 overexpression does not occur in untreated ovarian cancers, favouring the hypothesis that MDR1 is associated with acquired chemoresistance. The opinion that MDR1 is only associated with acquired resistance is contradicted by other studies who demonstrate its overexpression in tumours known to be resistant to chemotherapy such as kidney, liver, colon and adrenal gland²⁶². The overexpression of MDR in the kidney and liver is possibly associated with its role for excretion and detoxification of a wide variety of substances but its role in the adrenal gland is not fully understood.

Holzmayer *et al.*²⁷⁴ demonstrated that MDR1 expression in ovarian carcinomas is not limited to pre-treated tumours, which is similar to what was observed in the present study as overexpression of MDR was detected in 24% of the untreated tumours. Another study examining MDR1 mRNA expression did not observe amplification of the gene in recurrent ovarian cancers suggesting that overexpression of this gene is not a common pathway for the development of chemoresistance in ovarian cancers³²⁰.

One study showed no relation between progression free survival and P-pg staining in untreated ovarian carcinomas²⁷⁹. Another study on ovarian cancers demonstrated that MDR was expressed in over 30 late stage cancers and its expression correlated with survival³²¹. A recent study demonstrated that P-glycoprotein was an independent predictor of both overall and progression free survival²⁷⁶. The significance of MDR on progression free and overall survival was not evident in the present study.

The relationship between MDR staining and *in vitro* chemotherapy response was not significant in the present study although the highest number of resistant tumours were in the high staining group. Kamazawa *et al.*³²² examined mRNA expression of MDR1 as a predictor of paclitaxel-based chemotherapy and found expression of the gene to be higher in nonresponders. This was a small study of 27 patients and mRNA expression does not necessarily correlate with protein overexpression.

No significant association was found between the degree of MDR staining and *in vivo* chemotherapy response in 55 of the ovarian cancers examined in the present study. Seventy percent of the specimens that displayed a high degree of staining were resistant *in vivo*. This trend was in agreement with a study by Baekelandt *et al.*²⁷⁶ who

found P-glycoprotein negative patients responded significantly better to chemotherapy. In multivariate analysis P-gp was an independent predictor of both overall and progression free survival. This study examined the response to cisplatin and epirubicin. Investigators examining MDR and paclitaxel response in cell lines and a small number of patients reported significantly higher expression of MDR for non responders than for responders³²².

Mechanisms responsible for drug resistance in ovarian cancer appear to be variable and further studies are required to elucidate the role MDR associated genes play in this carcinoma. It is likely that MDR plays a role in the resistance to chemotherapy and could act as a prognostic factor in some ovarian cancers but as yet it is not possible to determine which tumours. Perhaps molecular profiling of tumours will answer this in the future.

Reversal of multidrug resistance has been the target of clinical trials in various malignancies³²³. Results have been disappointing and the major limitation of most of these agents has been that they reverse MDR at concentrations that result in unacceptable toxicity³²⁴. The quest for suitable MDR reversal agents continues.

Tumour Details and Survival

Other tumour parameters were examined in the present subgroup of specimens that were stained to determine if they had a prognostic role in ovarian cancer. As expected malignant tumours had a decreased progression free and overall survival compared to benign tumours. Tumour grade had no influence on PFS or OS but the sample size in the well-differentiated tumours was small. Moderately and poorly differentiated tumours are a more frequent observation in ovarian cancers. Tumour stage had a significant effect on PFS and OS. Significantly shorter PFS and OS were observed with patients who had been treated with chemotherapy previously. A significant correlation was observed between *in vitro* response to chemotherapy and PFS but not with OS. As expected a significant relationship was observed between *in vivo* response to chemotherapy and PFS and OS. There were some exceptions to this with some patients surviving for quite some time with progressive disease. No association with survival and patient age was observed in the group of patients examined. All the significant variables in the univariate analysis were examined in multivariate analysis. For progression free survival, response to chemotherapy *in vivo* remained an independent prognostic indicator, as did VEGF staining. For overall survival *in vivo* response to chemotherapy and whether or not the patient had previous chemotherapy remained significant. VEGF remained significant in overall survival also. VEGF expression was shown to be an independent prognostic variable and its potential value in the treatment of ovarian cancer needs to be determined in a prospective trial.

CHAPTER 8

CONCLUSIONS AND RECOMMENDATIONS FOR FURTHER RESEARCH

8.1 Conclusions

The explant culture technique enables sufficient growth of gynaecological tumours within 7-10 days for chemosensitivity / resistance testing. The MTS assay can therefore be completed in the postoperative period and identify ineffective agents prior to commencement of chemotherapy. The MTS assay is simple, rapid, flexible nonradioactive and measures changes in cell viability. The new reagent MTS obviates many of the problems previously encountered with MTT. The MTS endpoint used in conjunction with the explant technique has the advantage of retaining the properties of tissue organisation and heterogeneity that are present *in vivo*. Evaluability, reproducibility, number of cells required, as well as time and cost are of significant importance in a chemosensitivity / resistance assay. The assay system proved to be 96.5% evaluable in culturing the ovarian, cervical and endometrial specimens. The need to individualise chemotherapy is demonstrated by the variable response of tumours of the same clinical stage and histological grade.

In vitro-in vivo correlations are of major importance in establishing the validity of any assay system and the correlation between *in vitro* and *in vivo* response was highly significant ($p < 0.0001$). The fundamental criteria for evaluation of *in vitro* drug-response tests are its correlation with patient survival and improved quality of life. Other beneficial effects would be reduction of toxicity and reduction of costs. *In vitro* response remained an independent prognostic indicator in multivariate analysis for progression free survival but not for overall survival.

In vitro chemosensitivity / resistance assays are only as useful as the drugs available, but with increasing diversity of cytotoxic agents, individualisation of chemotherapy should be biologically justified. Randomised trials are urgently required to compare empirical choice of drugs with assay-guided therapy to determine patient benefit and cost effectiveness. Medical oncologists are likely to be resistant to using the results from such studies as the previous expectations of chemosensitivity testing were unfulfilled by the clonogenic assays. Methods have improved and *in vitro* testing has evolved from assays that had a 50% success rate and a 2-4 weeks turnaround time to current assays, which can predict survival in ovarian cancer patients.

Increased staining with CD31 was associated with decreased PFS and OS in univariate analysis but when other factors were taken into account in multivariate analysis CD31 was not significant. Microvessel density provided no indication as to patient response.

Quantifying VEGF has proven to be a valuable independent prognostic indicator in PFS and OS but fails to provide an adequate measure for resolving the response to chemotherapy drugs in ovarian cancer. The literature on chemotherapy response and angiogenesis is limited and contradictory. Seventy percent of the specimens in the present study that displayed a high degree of VEGF staining were resistant *in vivo* suggesting a possible role for VEGF in chemotherapy response but other angiogenic factors may be involved. A significant correlation was detected between CD31 and VEGF suggesting VEGF plays a role in angiogenesis.

MDR1 expression was detected in previously untreated ovarian tumours. A nonsignificant trend was observed between increased MDR1 staining and decreased survival. A correlation between the degree of staining and the response to

chemotherapy could not be confirmed in the present study although 70% of the specimens that displayed a high degree of staining were resistant *in vivo*. No association was found between MDR1 and CD31 or VEGF.

MDR1, CD31 and VEGF do play a role in some ovarian malignancies but other factors are likely to be involved. None of these candidate markers were accurate in predicting the response to chemotherapy. Perhaps molecular profiling of tumours in the future will determine which factors will be important for determining the response of the various tumours to chemotherapy. VEGF and CD31 were useful in predicting the PFS and OS of the patients. The mechanism by which VEGF influences survival is not understood. A longer study period with a larger group of patients may determine this.

8.2 Recommendations for future research

Based on the results of this retrospective study a prospective controlled clinical trial using the explant technique in combination with the MTS assay should be carried out to compare the results of chemotherapy based on chemosensitivity and resistance testing and currently used chemotherapy. Such a trial has commenced using the ATP assay³²⁵ in Europe but recruitment has been slow.

The role of other angiogenic factors such as platelet-derived growth factor or basic fibroblast growth factor in vascularisation should be investigated with a view to the elucidation of angiogenesis of ovarian cancers. The examination of a larger study group of early stage tumours and of other histological types may provide additional answers on the role of angiogenesis in ovarian cancer. VEGF expression was shown to be an independent prognostic variable and its potential value in the treatment of ovarian cancer needs to be determined in a prospective trial.

The role of other multidrug resistance mechanisms such as lung resistance protein or multidrug resistance protein need to be investigated in ovarian cancer as it is likely that more than one mechanism is involved in this very complex cancer.

The molecular profiling of tumours may assist in the future treatment of gynaecological cancers.

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