Evaluation of the Mevalonate Pathway as a Target for the Treatment of Ovarian Cancer

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Abstract

Ovarian cancer is the 5th leading cause of cancer-related death. The disease responds initially to treatment which is most often surgical cytoreduction followed by chemotherapy. The primary response rates to chemotherapy are approximately 80%. Unfortunately, most patients relapse and eventually tumours become refractory to frontline therapy. The lack of widely effective therapies at this points leads to a low 5-year survival. Therefore, new therapeutic agents or treatment strategies are required.

It has been reported previously that gain of function mutations of p53 which upregulate the mevalonate pathway in breast cancer. *TP53* is commonly altered in high grade serous ovarian cancer which might suggest that the mevalonate pathway may also be deregulated in ovarian cancer. The result reported in this thesis indicate that p53 upregulate the expression of key enzymes of the mevalonate pathway in ovarian cancer cell lines. In particular, it was found that 3-Hydroxy-3-Methylglutaryl-CoA Reductase (HMGCR), the rate limiting enzymes of the pathway, geranylgeranyl transferase-I (GGTI), GGTII and farnesyltransferase are upregulated in number of ovarian cancer cell lines. These observations suggest that pharmaceutical inhibition of the mevalonate pathway may be a promising therapeutic approach.

Pitavastatin, a member of statin family of HMGCR inhibitors, has been found to have significant activity against ovarian cancer cells and induce regression of ovarian cancer xenografts in mice in previously published result from our laboratory. Although repurposing statins for use in oncology is an attractive strategy, there are legitimate concerns about the potential for the drug to cause myopathy. Therefore, other pharmacological agents which inhibit the mevalonate pathway were evaluated to test the hypothesis that dual inhibition of the mevalonate pathway would synergistically cause ovarian cancer cell death.

Bisphosphonates, such as zolendronic acid, are inhibitors of farnesyl diphosphate synthase. Zolendronic acid, and to lesser extends risedronate, potentiated the activity of pitavastatin in several assays assessing the growth and viability of ovarian cancer cells. In contrast, the geranylgeranyl transferase I inhibitor, GGTI-2133, antagonised the activity of pitavastatin. Similarly, knockdown of either GGTI-β or GGTII-β by RNAi failed to potentiate the activity of pitavastatin. However, combined knockdown of both geranylgeranyl transferases potentiated the activity of pitavastatin.

To identify further drugs which could interact synergistically with pitavastatin, a library of 100 off-patent drugs was screened in combination with pitavastatin in cell growth assays. Several compounds were identified which potentiated the activity of pitavastatin and/or had notable activity as single agents. The most striking hit from this screen was prednisolone, a synthetic glucocorticoid. Subsequent studies confirmed the synergistic interaction between prednisolone and pitavastatin in several cell growth and viability assays. To evaluate the mechanism underlying this synergistic interaction, publically-available expression data were interrogated to identify mevalonate pathway enzymes whose expression was regulated by prednisolone. The effect of these candidate genes was then tested in ovarian cancer cells and levels of HMGCR, farnesyl diphosphate synthase and geranylgeranyl transferase II were found to be reduced.

These data suggest that drug combinations inhibiting multiple points in the mevalonate pathway may increase the therapeutic window for pitavastatin and offer a potential treatment for ovarian cancer.

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Abbreviation

ACTH	Adrenocorticotropic hormone
ATM	Ataxia-telangiectasia mutated
ATR	Ataxia telangiectasia and RAD3-related
BER	Base excision repair
Cdks	Cyclin dependent kinases
CRH	Corticotrophin-releasing hormone
DRC	Dose response curve
ECACC	European collection of authenticated cell cultures
ERCC1	Excision repair cross-complementing group 1
FDPS	Farnesyl diphosphate synthase
FOH	Farnesol
FTase	Farnesyl transferase
GAPs	GTPase activating proteins
GDIs	Guanine nucleotide dissociation inhibitors
GDP	Guanosine diphosphate
GEFs	Guanine nucleotide exchange factors
GGOH	Geranylgeraniol
GGPPS	Geranylgeranyl pyrophosphate synthase
GGTII-β	Geranylgeranyl Transferase-II beta subunit
GGTI-β	Geranylgeranyl Transferase-I beta subunit
GR	Glucocorticoid receptor
GTP	Guanosine-5-triphosphate
HIDS	Hyperimmunoglobulinaemia D syndrome

HLH	Helix–loop–helix domain
HMGCR	3-Hydroxy-3-Methylglutaryl-coa Reductase
HMGCS	HMG-CoA synthase
HPA	Hypothalamic–pituitary–adrenal
HR	Homologous recombination
Insig	Insulin-induced gene
MAU	Mevalonic aciduria
MMR	Mismatch repair
MP	Mevalonate pathway
NBPs	Nitrogen bisphosphonates
NER	Nucleotide excision repair
OC	Ovarian cancer
PARP	Poly ADP ribose polymerase
REP1	Rab escort protein 1
SCAP	SREBP cleavage-activating protein
SRB	Sulforhodamine B
SRE	Sterol response element
SREBP	Sterol regulatory element binding protein

Publications

1- Abed MN, **Abdullah M**, Richardson A (2016) Antagonism of Bcl-XL is necessary for synergy between carboplatin and BH3 mimetics in ovarian cancer cells. J Ovarian Res 9:25.

2- de Wolf E, **Abdullah M**, Jones SM, et al (2017) Dietary geranylgeraniol can limit the activity of pitavastatin as a potential treatment for drug-resistant ovarian cancer. Sci Rep 7:5410.

3- Uche FI, Abed M, **Abdullah M**, et al (2017) O9 Isolation, identification and anti-cancer activity of minor alkaloids from Triclisia subcordata Oliv. Biochem Pharmacol 139:112.

4- **Abdullah M**, Abed M, Richardson A (2017) Inhibition of the mevalonate pathway augments the activity of pitavastatin against ovarian cancer cells. Sci Rep. 7:8090

5- **Abdullah, M**. Khanim, F. Richardson, A, Prednisolone augments the cytotoxic activity of pitavastatin against ovarian cancer cells by down-regulating mevalonate pathway enzymes. **Manuscript in preparation.**

Poster presentations

1. **Abdullah M**, Richardson A (2016) Bisphosphonates potentiate the activity of pitavastatin against ovarian cancer cells. Eur J Cancer 61:S190–S191.
2. **Abdullah M**, Khanim F, Richarson A (2016) Pitavastatin and prednisolone, novel drug combination, display significant synergy in ovarian cancer cell lines. Eur J Cancer 69:S150.
3. Robinson E, **Abdullah M**, Jones S, et al (2016) Preclinical evaluation of pitavastatin as a treatment for chemotherapy-resistant ovarian cancer. Eur J Cancer 69:S150.
4. Robinson E, **Abdullah M**, Jones S, et al (2016) Pitavastatin is a potential treatment for drug-resistant ovarian cancer. Eur J Cancer 61:S192.

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Chapter One

Introduction

1. Introduction

1.1. Ovarian cancer

Ovarian cancer (OC) is a group of molecularly and aetiologically distinct diseases that share the same anatomical location. It usually presents as complex cystic mass in the pelvis. OC consists of abnormal cells found in the ovary, that have the capability to spread or invade other tissues of the body and share other hallmarks of cancers (Figure 1-1). The disease tends to be diagnosed at an advanced stage, with limited prospects for successful treatment and usually poor overall survival. The incidence rates of OC are projected to rise by 15% in the UK between 2014 and 2035, to 32 cases per 100,000 females by 2035 (Figure 1-2) (Bast, Hennessy and Mills, 2009; Smittenaar *et al.*, 2016; Cancer research UK, 2017). OC is a relatively uncommon but fatal disorder and considered as the leading cause of death among gynaecological cancers (Jessmon *et al.*, 2017). Although the majority of patients respond initially to the treatment (Ledermann *et al.*, 2012), relapse after an initial response to treatment can occur and the development of resistance leads to failure of chemotherapy (Ling *et al.*, 2005). The prognosis of OC patients had not changed significantly since three decades ago despite significant improvements in the understanding of the biology of the malignancy (Vaughan *et al.*, 2011).

The majority of OC patients, approximately 75%, are detected at an advanced stage due to the obscure nature of the symptoms and the lack of the precise detection method (Bharwani, Reznek and Rockall, 2011; Wright, Bohlke and Edelson, 2016). The lack of early diagnosis impairs patients' prognosis and leads to a poor overall 5-year survival rate of around 40%. More specifically, the survival rate varies significantly from 85% for early stage disease to 25% in the advanced stages (Colombo *et al.*, 2006; Lu *et al.*, 2016). Consequently, it could be argued that early diagnosis and resistance to chemotherapy are the key obstacles for

successful treatment. This review introduces the present status of our understanding of OC pathology and treatment. It also offers insights into the potential of the mevalonate pathway (MP) as a promising target for therapy.

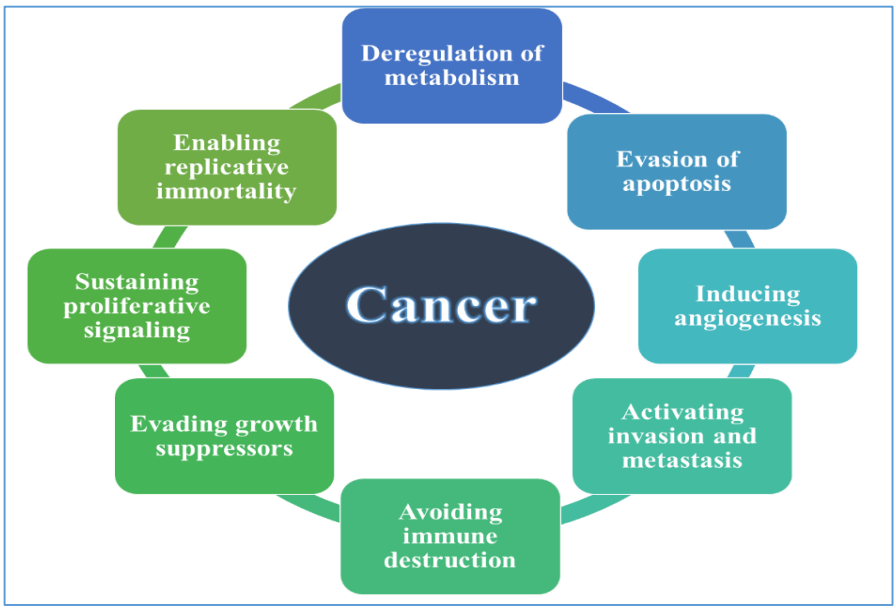


Figure 1-1 The Hallmarks of Cancer reproduced from (Hanahan and Weinberg, 2011)

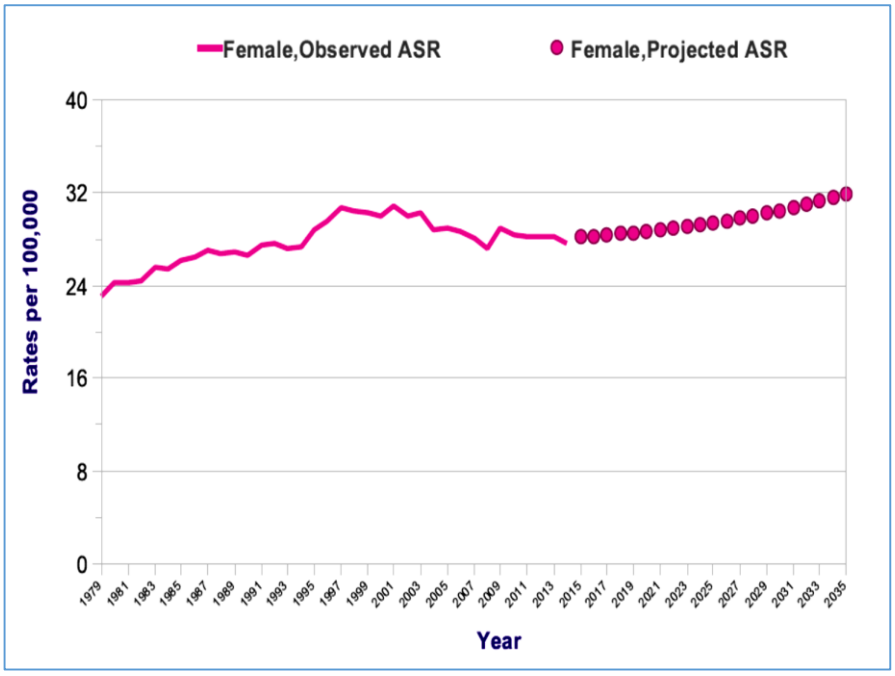


Figure 1-2 Observed and Projected Age-standardised Incidence Rates, Females, UK (Smittenaar *et al.*, 2016)**.**

1.1.1. Epidemiology of ovarian cancer

Ovarian cancer is one of the most devastating diseases worldwide (Scarlett *et al.*, 2012). It is the 5th and 7th leading cause of cancer-related mortality and is responsible for the death of approximately 4272, 14000 and 151000 patients per annum, in UK, USA and worldwide, respectively (Siegel, Naishadham and Jemal, 2013; Siegel, Miller and Jemal, 2015, 2016; Berek *et al.*, 2017; Coburn *et al.*, 2017; Kroeger and Drapkin, 2017). In the UK, OC is the 5th most common cancer and the annual mortality equates to more than half of total newly diagnosed cases (7011 patients) each year (Figure 1-3) (Rooth, 2013; Ovarian cancer research UK, 2014). Many risk factors have been recognised which can increase the prospect of developing OC.

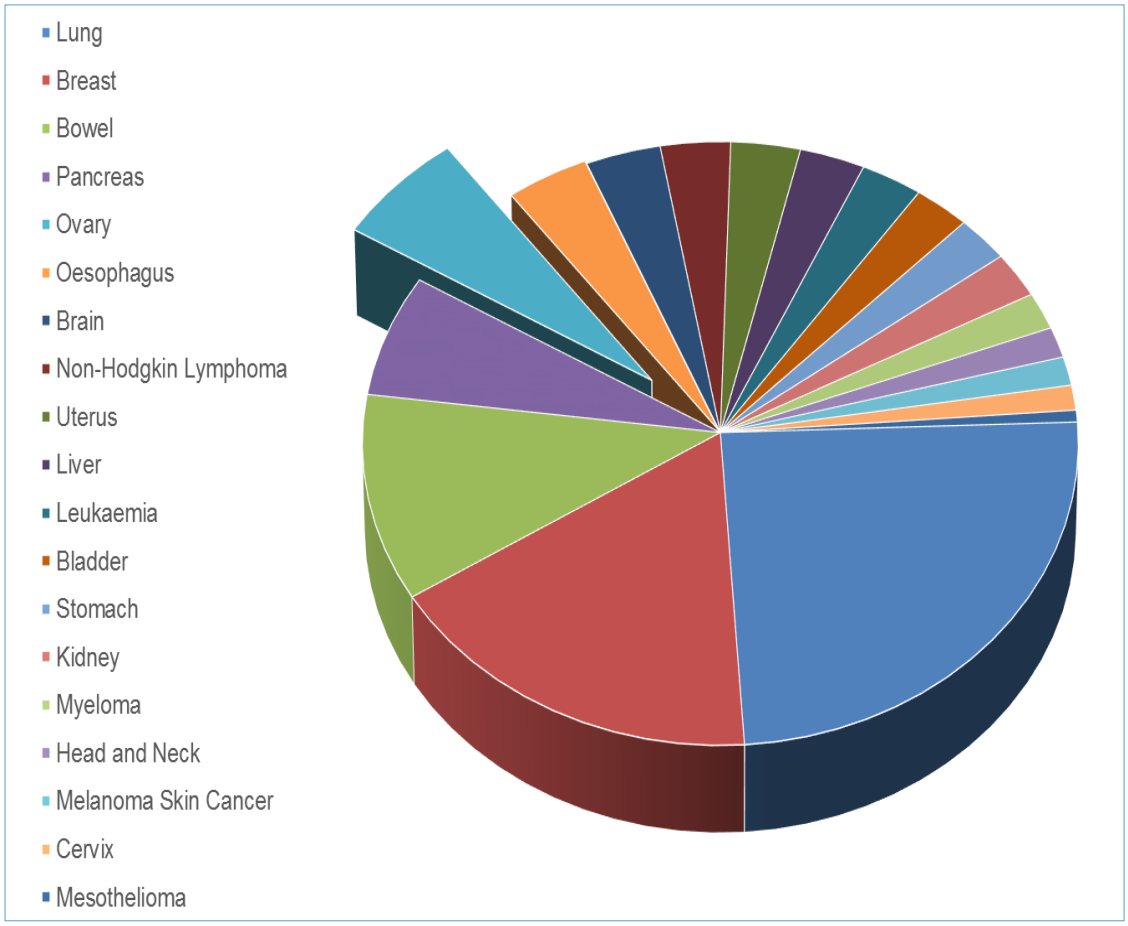


Figure 1-3: Common Causes of Cancer Deaths in female, UK (Ovarian cancer research UK, 2014).

1.1.1.1. Age

Age has a significant impact not only on the incidence and prevalence of OC but also on survival rate and prognosis. OC is primarily a disease of old age (Chan *et al.*, 2006). The crude and age-standardized annual incidences are 20.9 and 16.2 per 100,000 females in UK, respectively. The incidence increases from approximately 5 per 100,000 females in the age group 30-34 to approximately 15 per 100,000 females in age group 45-49. After menopause, the prevalence of OC represents more than 80% of all diagnosed cases and the incidence increases abruptly, reaching a plateau at 69 per 100,000 for female age group 80-84 (Figure 1-4) (Maas *et al.*, 2005; Ovarian cancer research UK, 2014).

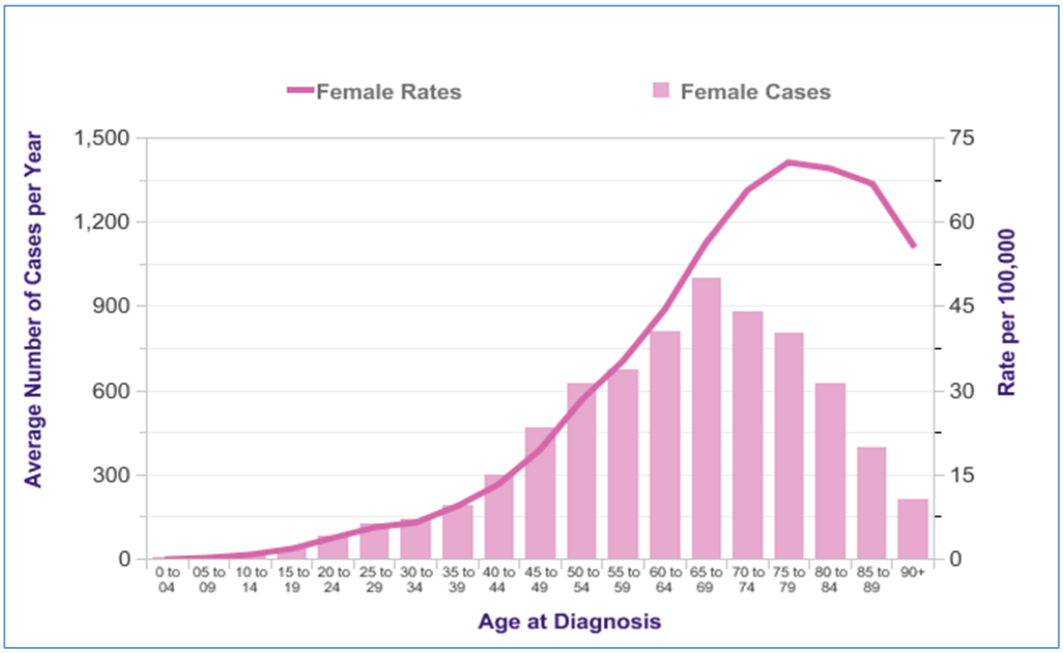


Figure 1-4: Average number of new cases every year and age-specific incidence rates of ovarian cancer in UK, 2012-2014 (Cancer research UK, 2017)**.**

Survival rate of OC patients is inversely influenced by the age. The five-year survival rate declines from around 85% for age group 15-39 to less than 30% in the age group over 70 year (Figure 1-5). In addition, age seems to be prognostic factor for OC treatment. Younger women with the disease exhibit better prognosis in comparison to old age women (Maas *et*

al., 2005; Ng, Low and Ilancheran, 2012). The accumulation of somatic mutations during lifetime is likely to contribute to the development of OC (Rooth, 2013). For instance, *TP53*, *BRCA1*, *NF1* and *CHK2* mutations was more frequently detected in older women with serous OC (Encinas *et al.*, 2015). In addition, there is a dramatic increase in the loss of heterozygosity of chromosome 17, an event strongly linked to OC, with age reaching 80% in the 60-69 years' group. Therefore, age is considered the most significant risk factor of cancer (Pieretti and Turker, 1997).

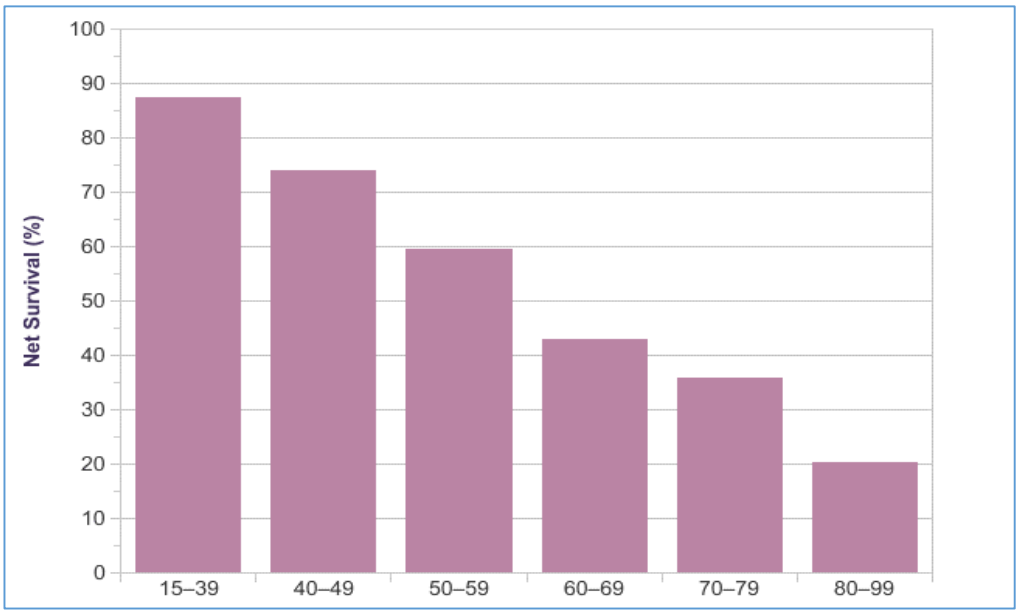


Figure 1-5: Five-year survival rates of ovarian cancer by age. England 2009-2013 (Cancer Research UK, 2017).

1.1.1.2. Geographical factors

There is a considerable variation in the incidence and mortality of OC according to geographical location (Figure 1-6). Generally, the incidence is high in Europe and USA (developed countries; 9.9 per 100,000) in comparison to Africa and Asia (less developing country; 5 per 100,000) (Hennessy, Coleman and Markman, 2009; Bharwani, Reznek and Rockall, 2011). In Europe, the incidence varies greatly in different regions and these disparities might achieve 40%, with peak incidence noted in Eastern and Northern Europe,

but lower incidence in Southern and Western Europe. For example, Lithuania, Latvia and Bulgaria had the highest incidence rate (approximately 19 per 100,000). In contrast, Portugal and Cyprus were had the lowest rate (7 per 100,000) (Cancer research UK, 2017). In addition, the incidence of OC in United States and the United Kingdom is 3–7 times greater than in Japan (Berek *et al.*, 2017). The incidence is generally lower in countries with Hispanic, Asian and African women (Asia, Africa, Mexico) than predominantly Caucasian population countries (Europe, Canada). The reduced life expectancy in less developed countries may explain the low incidence, because OC is more prevalent in older women. In addition, race, social habits, life style and environmental factors might influence the national incidences variation (Chornokur *et al.*, 2013; Lowe *et al.*, 2013; Coburn *et al.*, 2017).

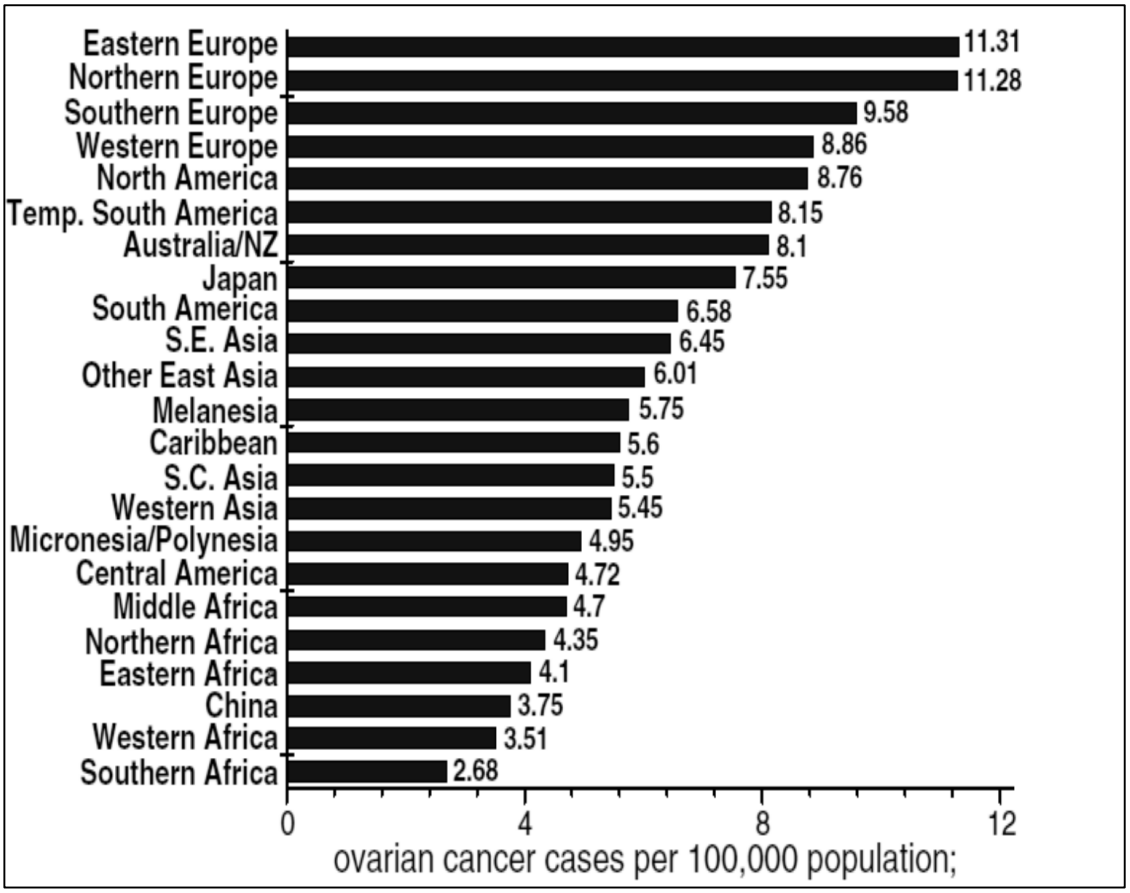


Figure 1-6: Incidence of ovarian cancer in different region worldwide (Reproduced with permission from (Chornokur *et al.*, 2013)**).** NZ: New Zealand; S.E.: South-Eastern; S.C.: South-Central**.**

1.1.1.3. Reproductive factors

Several reproductive factors have a potential effect on the risk of OC. Multi-parity and the use of oral contraceptive pills for 5 years or more are established protective factors (Tworoger *et al.*, 2007; Beral *et al.*, 2008; Tsilidis *et al.*, 2011). Epidemiological studies show that the relative risk of OC is inversely related to the number of children and to duration of use of oral contraceptive pills (Table 1-1). In addition, some studies illustrate that the risk decreased even in case of incomplete pregnancies. The evidence for breast feeding having a protective power are conflicting but feeding for more than 18 months has been shown to decrease the risk of OC by up to 30% (Jordan *et al.*, 2010, 2012; Pasalich *et al.*, 2013; Ferris *et al.*, 2014). Recently, a meta-analysis emphasised the protective role of tubal ligation against OC (Cibula *et al.*, 2011).

Table 1-1 Relative risk of ovarian cancer by parity and duration of oral contraceptive use (Cancer research UK, 2017).

Relative risk for OC by parity	**Number of children**	**Relative risk**
	3+	1
	2	1.21
	1	1.60
	0	2.12
Relative risk for OC by duration of oral contraceptive use (mean)	**Oral contraceptive use**	**Relative risk**
	Never	1.0
	Less than 1 year (0.4 years)	1.0
	1-4 years (2.4 years)	0.78
	5-9 years (6.8 years)	0.64
	10-14 years (11.16 years)	0.56
	15 years or more (18.3 years)	0.42

In contrast to the protective factors, infertility and null-parity are assumed to raise the risk of OC by increasing the rate of exposure to ovulation. However, there are controversial data about in-vitro fertilization; some authors state that there is no association while other show there is increased risk of OC in women undergoing in-vitro fertilization (Le *et al.*, 2012; Stewart *et al.*, 2013). Endometriosis, which is the growth of endometrium tissue in other parts of the body, is also linked with an elevated risk of OC (Stewart *et al.*, 2013; Wang *et al.*, 2013). Progesterone, which is raised during pregnancy or supplied by oral contraceptives, might have a role in clearing the transformed epithelial cells from the ovarian surface (Smith and Xu, 2008). In summary it appears that factors associated with a reduced the frequency of ovulation might have a protective effect against OC.

1.1.1.4. Genetic factors

Inherited and somatic mutations are associated with an elevated risk of OC (Bast, Hennessy and Mills, 2009). Women with a family history (i.e. sister, daughter, and mother) of OC have a three-fold increased risk of developing OC (Schorge *et al.*, 2010). Inherited mutations in genes such as *BRCA1* and *BRCA2* and DNA mismatch repair are reported to influence the risk of OC (Levy-Lahad and Friedman, 2007).

BRCA1 or *BRCA2* germline mutation account for 90% of inherited OC (Schorge *et al.*, 2010). However, only 10-15% of women with mutation in *BRCA* genes develop OC (Hennessy, Coleman and Markman, 2009). The lifetime risk of OC is 2% among UK women (Sasieni *et al.*, 2011; Cancer research UK, 2017) but the cumulative risk is amplified to 30-60% in woman with *BRCA1* and 15-30% in woman with *BRCA2* mutations and a family history of breast or OC (Gayther and Pharoah, 2010). Furthermore, woman whose family suffer from Lynch II syndrome, a hereditary nonpolyposis colorectal malignancy, have a 7% chance to develop OC (Bast, Hennessy and Mills, 2009). Genetic and non-genetic factors

within families might be the cause of familial aggregation of cancer. However, twin studies had suggested the importance of inherited mutation (Gayther and Pharoah, 2010).

On the other hand, somatic mutations also contribute to the development of OC. The most common somatic mutations noted in epithelial OC include *TP53*, a tumour suppressor gene, and K-Ras (11%) and B-Raf (0.5%), signalling molecules. *TP53* mutation is observed in 50-80% of advanced OC. p53 controls the function of many genes involved in DNA repair, cell cycle arrest, programmed cell death and differentiation of damaged cells (Despierre *et al.*, 2010; Bell *et al.*, 2011).

Other factors that might contribute to OC risk are summarized below (Table 1-2). The key documented factors influencing OC risk, such as age, oral contraceptive use and parity, offer limited potential for alteration to reduce the incidence of OC. Therefore, factors such as hormone replacement therapy and breast feeding might of particular importance (Banks, 2001).

Table 1-2 Summary of some factors influence ovarian cancer risk

Factors	Risk of OC	Mechanism	References
Hormone replacement therapy	Long term use increase the risk of OC	Oestrogen might stimulate proliferation of ovarian cells	(Beral *et al.*, 2007; Zhou *et al.*, 2008)
Smoking	Increase the risk of mucinous OC	Unknown mechanism	(Jordan *et al.*, 2006; Tworoger *et al.*, 2008)
Physical activity	Decrease the risk of OC	Reducing oestrogens level, and decreasing the occurrence of ovulation, and subsequently exposure to progesterone in the luteal phase of the cycle	(Chiaffarino *et al.*, 2007)
BMI	Increase the risk of OC	Adiposity provoke the production of endogenous sex steroid hormones	(Olsen *et al.*, 2007)
NSAID	No association, or decrease the risk have been reported	possibly through the inhibition of prostaglandin synthesis, which in turn involved in promoting cell proliferation and inhibiting apoptosis	(Pinheiro *et al.*, 2009; Murphy *et al.*, 2012)
Hysterectomy	Decrease the risk of OC	Several factors have been postulated such as reducing growth factors, blood supply, anovulation and blocking of access of carcinogens to ovary	(Chiaffarino *et al.*, 2005)
Talcum powder and asbestos	Increase the risk of OC	Damage to epithelial cells which might induce inflammation	(Schorge *et al.*, 2010; Camargo *et al.*, 2011; Lowe *et al.*, 2013)

1.1.2. Classification of ovarian cancer

Ovarian cancer is a heterogeneous disease (Domcke *et al.*, 2013; Kroeger and Drapkin, 2017). The World Health Organization classified OC based on histogenesis of the normal ovary, indicating the tissue the tumours appear to be derived from such as epithelial tumour, germ cell tumour, sex cord-stromal tumour (Kaku *et al.*, 2003). It has been proposed that 90% of primary malignant OC arise from either the epithelial surface of the ovary or the inclusion cysts or the mullerian system (Cho and Shih, 2009). However, there is a growing body of evidence which supports the theory that the primary site of origin of OC may not be the epithelial cells of the ovaries. Ovarian epithelial cancers share common characteristics that are similar to the cells lining the fallopian tube, endometrium and endocervix, and which have an embryological site of origin, the mullerian ducts, that is distinct from that of the ovary (Dubeau, 2008). This has raised the possibility that OC may originate in the fallopian tube (George, Garcia and Slomovitz, 2016).

Epithelial OC is categorised into serous (60%), mucinous (12-15%), endometrioid (20-25%) and clear cell tumour (4-12%). The majority of serous ovarian carcinoma (90%) are high grade serous OC. Other less frequent histological type includes Brenner, mixed epithelial type, and undifferentiated carcinomas (Wang *et al.*, 2005; Kroeger and Drapkin, 2017). Each subdivision might be further divided according to their behaviour into benign, border line (Low malignant potential) and malignant tumour (Mok *et al.*, 2007); or depending on the morphological characteristics of the tumour into papillary, glandular and solid tumour (Soslow, 2008).

1.1.3. Staging of ovarian cancer

In the absence of metastasis, histological conformation and accurate staging of OC is vital for determination of a prognosis and treatment strategy. It is usually done during surgery. The International Federation of Gynaecological Oncology (FIGO) developed a system for classification of OC. The system classifies OC into four major stages. Each stage is divided into sub-stages that reflect certain clinical, pathological, or biological prognostic factors (Table 1-3) (Colombo *et al.*, 2006; Decruze and Kirwan, 2006). The stages reflect dissemination of the disease from the ovaries into the pelvis, peritoneal cavity and then the rest of the body.

1.1.4. Signs, symptoms and diagnosis

Ovarian cancer has been described as a silent killer because the symptoms do not commence until the late stage of the disease (Goff *et al.*, 2007). The clinical presentation associated with OC might be not specific, vague, and usually have a common characteristic with other abdominal and gastrointestinal disorder (Goff *et al.*, 2007; Lanceley *et al.*, 2011). The majority of the patients are diagnosed at an advanced stage. Despite this, the OC patients might have discomfort for several months before diagnosis (Goff *et al.*, 2004), but most of the patients symptoms go unreported and unrecognised by the primary clinical care. Furthermore, half of the women with OC are not referred to gynaecological cancer clinics (Vine *et al.*, 2003). Doctors and patients must be alert to any symptoms to prevent any delay in diagnosis (Goff, 2012). The most frequently encountered symptoms with OC are listed below (Table 1-4).

Table 1-3 FIGO staging of ovarian cancer stage (Berek, et al., 2012)

Stages	Description
I	Tumour confined to ovaries
IA	Growth limited to one ovary; no ascites. No tumour on the external surface; capsule intact
IB	Growth limited to both ovaries; no ascites. No tumour on the external surfaces; capsules intact
IC	Tumour either Stage IA or IB, but with tumour on surface of one or both ovaries, or with capsule ruptured, or with ascites, or with positive peritoneal washings
II	Tumour comprising 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	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
III	Tumour involving one or both ovaries with histologically confirmed peritoneal implants outside the pelvis and/or positive regional lymph 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 histologic 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 regional lymph nodes
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 IVa.

Table 1-4 Ovarian cancer symptoms and their frequency (Goff, et al., 2000)

Symptom	Frequency (% of patients)
Increased abdominal size	61
Abdominal Bloating	57
Fatigue	47
Abdominal pain	36
Indigestion	31
Urinary frequency	27
Pelvic pain	26
Constipation	25
Urinary incontinence	24
Back pain	23
Pain with intercourse	17
Unable to eat normally	16
Palpable mass	14
Vaginal bleeding	13
Weight loss	11
Nausea	9
Bleeding with intercourse	3
Diarrhoea	1
Deep venous thrombosis	1
None	5

The confirmation of OC diagnosis is only made by histological analysis of a specimen during surgery. However, the urgent referral of suspected cases of OC to the diagnostic centre is required. In 1990, Jacobs, et al. established the risk of malignancy index (RMI), which combines the result of three independently associated variables to predict the likelihood of ovarian malignancy (CA125 blood level, ultrasound examination and menopausal status) to overcome the limitation of the ultrasound and CA125 alone. This also facilitates the referral

of the suspected patients to gynaecological oncologist (Jacobs *et al.*, 1990; Cancer research UK, 2017).

Carbohydrate antigen 125 (CA125) is a tumour marker for screening, diagnosis, and therapeutic monitoring in OC (Felder *et al.*, 2014). It was first discovered by Bast, Knapp, and colleagues (Bast *et al.*, 1981) in 1981 and is also known as MUC16. CA125 is a 22,000 amino acids protein encoded by *MUC16* gene and it is a cell surface transmembrane glycoprotein which can be released extracellularly by proteolytic cleavage into body fluids such as blood and ascites (Bafna, Kaur and Batra, 2010). In addition, It is expressed by epithelial cells in endocervix, endometrium and fallopian tube to protect the luminal surface from physical stress (Hung *et al.*, 2013). Elevated levels of CA125 were reported in 90% of women with advanced stage OC, but in only 50% of patient at early stage of OC (Gupta and Lis, 2009). Finally, utilizing CA125 in diagnosis of OC in the general population is complicated by its increase in other conditions such as endometriosis and ovarian cyst (Asher, Hammond and Duncan, 2010; Lutz *et al.*, 2011; Babic *et al.*, 2017).

Human Epididymis protein 4 (HE4) is new cancer specific biomarker which has a relatively high sensitivity to detect OC and its expression in non-gynaecological cancers had not been observed (Li *et al.*, 2009). The physiological role of HE4, which is encoded by the gene *WFDC2,* has not been identified. However, HE4 protein is upregulated in ovarian malignancies compared to benign OC and other type of carcinomas (Chung *et al.*, 2013; Steffensen *et al.*, 2016). Several studies have recognized HE4 level is upregulated in cases where CA125 level is not elevated, so HE4 can be used as complementary biomarker for OC (Montagnana *et al.*, 2009; Moore *et al.*, 2009; Steffensen *et al.*, 2011). Lastly, improved imaging techniques have been employed to diagnose cancerous tissue and angiogenesis at an early stages of the disease (Fleischer *et al.*, 2012).

1.1.5. Aetiology of ovarian cancer

There are several theories that explain the aetiology of OC. The earlier theories, incessant ovulation, gonadotropin and pelvic contamination theories, were based on the epidemiological data. Recently, the dualistic theory had connected the anatomical and molecular genetic with OC aetiology. The details anatomy and biology of the ovary in Figure 1-7

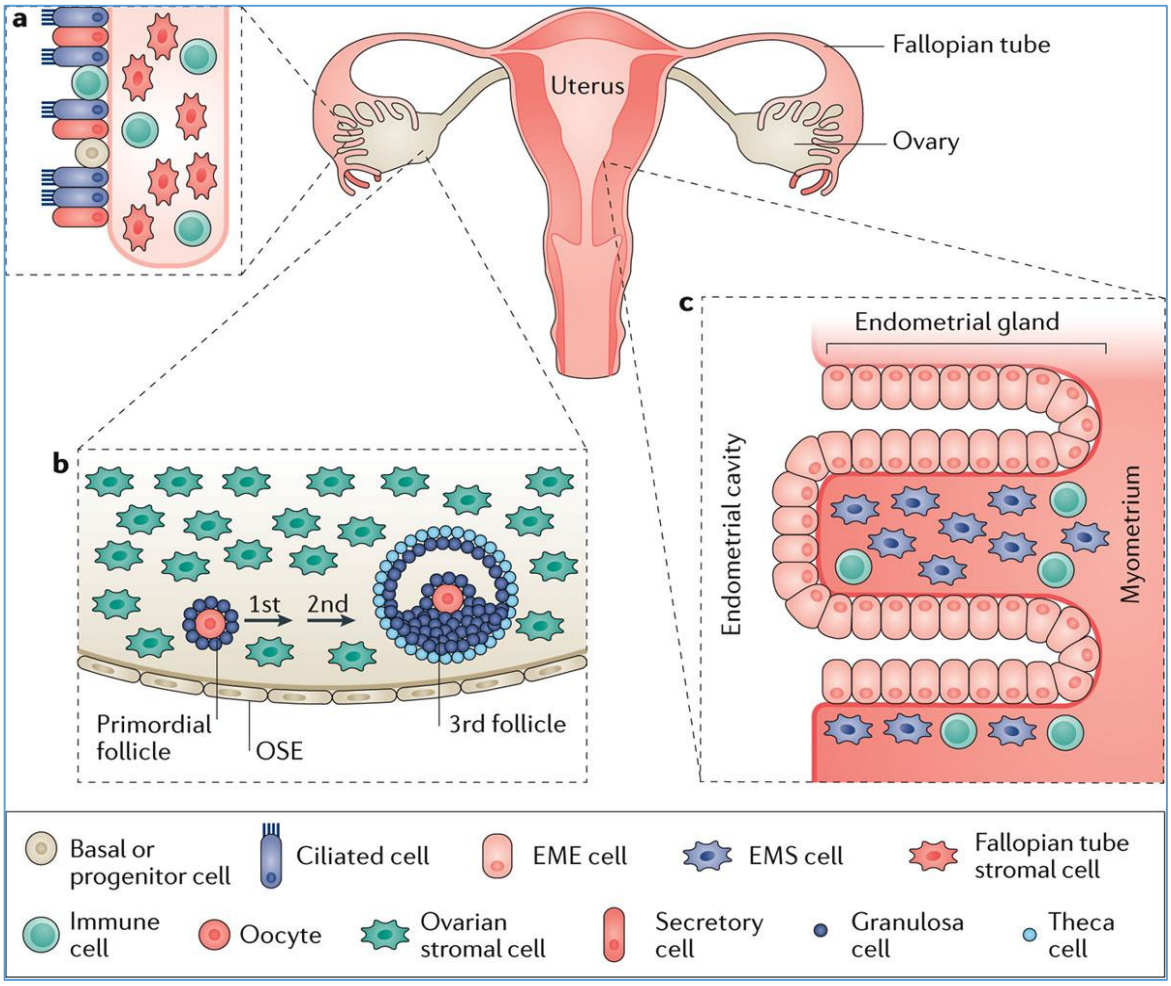


Figure 1-7 Anatomy and biology of the ovary, fallopian tube and uterus

(a) The fallopian tube is responsible for fluid synthesis and egg transportation which is supported by muscular contraction of the tube wall. The fallopian tube comprises two principal types of epithelial cell secretory cells and ciliated cells. The small basally-situated cells stand for recently described epithelial stem and/or progenitor cells. In addition, immune surveillance plays an important role through the presence of immune cells in the epithelium and stromal tissue.

(b) The main cells of the ovarian cortex are ovarian surface epithelium (OSE) (these cells are implicated in the repair of ovarian surface after ovulation), stromal cells and follicles at various stages of maturation, such as theca cells, granulosa cells and oocytes. Theca cells and granulosa cells synthesize androgens and oestrogens, respectively, and support the development of oocyte.
(c) The endometrium is composed of cells of the endometrial epithelium (EME) and endometrial stroma (EMS). These cells respond to systemic and paracrine signals and undergo cyclical changes during the menstrual cycle and support the nourishment of the embryo during fertilization and implantation. Reprinted with permission from (Karnezis *et al.*, 2017)**.**

1.1.5.1. Incessant ovulation theory

The incessant ovulation theory, which was proposed by Fathalla more than 40 years ago, linked the frequency of ovulation and the increased opportunity of developing ovarian malignancy (Fathalla, 1971). This theory is supported by the protective effects of the oral contraceptive pills (Fathalla, 2016). It was postulated that frequent injury to the ovarian epithelial cells, provoked through ovulation in nulliparous women, as a possible mechanism associated with development of OC. As consequence of repeated trauma, it was hypothesized that the cells required an increased rate of DNA synthesis and cell proliferation to repair the disruption of the epithelial cells. The increased rate of DNA synthesis might cause replication errors which trigger cells transformation into malignant or premalignant phenotype (Schüler *et al.*, 2013).

In addition, ovulation is accompanied by production of excessive amount of free radicals which might also contribute to DNA damage and hence be a causative factor of cancer (Murdoch, 2005). *In vitro,* it is found that frequent subculture of epithelial cells from the rat ovaries induces genetic mutation and malignant transformation (Testa *et al.*, 1994). However, a limitation to this theory is that the populations of the epithelial cells of the ovary that are affected by repeated repair would not be the same with each ovulation (Smith and Xu, 2003) and the use of the progesterone only contraceptive pills, that does not interrupt

ovulation, has the same potential in preventing OC as the combined oral contraceptive (Landen, Birrer and Sood, 2008).

1.1.5.2. Gonadotropin stimulation theory

The early evidence that led to the gonadotropin theory was the development of OC in rodents after oophorectomy caused by excessive gonadotropin stimulation (Schüler *et al.*, 2013). Gonadotropin hormones (LH and FSH), that are secreted from the pituitary gland, increase the oestrogenic stimulus of the surface epithelial cells of ovary to initiate the ovulation process. This stimulation might promote malignant cellular transformation by promoting production of oestrogen (Fleming *et al.*, 2006; Smith and Xu, 2008). After ovulation, the ruptured follicle becomes a corpus luteum which produces progesterone. Progesterone in turn, negatively inhibits gonadotropin levels that surge abruptly after menopause. The increment in gonadotropin levels in menopause women might contribute to the increased risk of OC, because it fosters the inflammatory milieu that could not induce ovulation. (Mertens-Walker, Baxter and Marsh, 2012). It has also been suggested that these hormones induce the evolution of OC rather than the causation (Landen, Birrer and Sood, 2008).

1.1.5.3. Pelvic contamination theory

The pelvic contamination theory hypothesised that inflammation induced by irritation of the peritoneum might be linked to the development of OC (Heintz, Hacker and Lagasse, 1985). Inflammation resulting from ovulation or exposure to xenobiotics such as asbestos and talc, might influence malignant transformation of ovarian epithelial cells (Mok *et al.*, 2007; Camargo *et al.*, 2011). Furthermore, endometriosis and pelvic inflammatory disease influence the risk of OC (Murdoch, 2005; Lowe *et al.*, 2013). On the other hand, tubal ligation and hysterectomy have been shown to reduce the risk through preventing exposure of the ovary to environmental factors (Chiaffarino *et al.*, 2005; Ness *et al.*, 2011).

1.1.5.4. Dualistic theory

The dualistic theory was proposed by Shih and Kurman (Shih and Kurman, 2004) and it is based on their idea that ovarian carcinoma might be divided into two distinctive broad categories (Figure 1-8) depending on analysis of the molecular pathogenesis of benign, borderline and malignant OC. Firstly, type I tumours have low proliferative activity, are less sensitive to chemotherapy and composed of low grade serous, endometrioid, mucinous, clear cells and Brenner tumours. Type I carcinomas have distinctive molecular pathogenesis such as K-Ras mutation for mucinous and serous tumours and B-Raf for serous tumour and β-catenin and *PTEN* mutation for endometrioid tumours. In contrast, type II tumours, such as high grade serous, undifferentiated carcinomas and mixed mesodermal malignancy, evolve rapidly, are relatively responsive to chemotherapy, lack conclusive precursor lesions and are characterized by frequent *TP53* mutation and *CCNE1* amplification (Fleming *et al.*, 2006; Landen, Birrer and Sood, 2008; Kurman and Shih, 2010; Jones and Drapkin, 2013; Tang *et al.*, 2017).

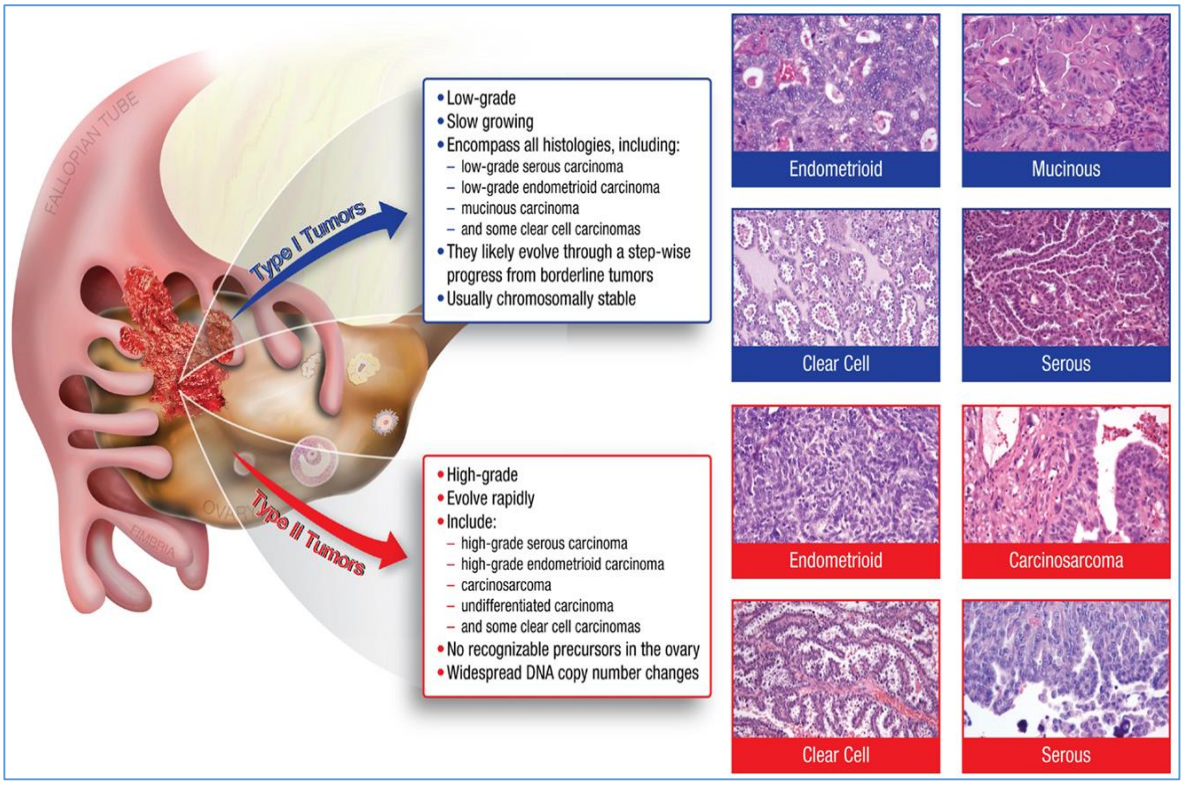


Figure 1-8 Classification of ovarian cancer according to dualistic theory (Jones and Drapkin, 2013)**.**

1.1.6. Treatment

In UK, a team of different disciplines work together to manage OC including a gynaecological oncologist, pathologist, radiologist and non-surgical oncologist and this has improved the patient survival (Guppy, Nathan and Rustin, 2005).

The management plan of OC is principally dependent on the stage of the disease at diagnosis and the patients associated co-morbidity. In the early stages of OC, surgery is considered as the cornerstone of the treatment plan and alongside tumour debulking, it also aims to confirm the diagnosis and inform optimal staging. Optimal staging includes palpation and careful inspection of the peritoneal surfaces with biopsies of suspected foci of cancers (Trimbos *et al.*, 2003). Also, the standard surgery management includes bilateral salpingo-oophorectomy, total hysterectomy, omentectomy, lymphadenectomy and removal of the all microscopic lesions (Lécuru *et al.*, 2017). In contrast to the early stage disease, adjuvant

chemotherapy is essential in advanced stages OC. However, some cases, such as wide spread disease and extensive ascites, neoadjuvant therapy is used prior to surgery (Guppy, Nathan and Rustin, 2005). The success of surgery is the most important prognostic factors in the management of advanced OC (Agarwal and Kaye, 2003). Although the prognosis for patients with early stage OC is better than those with advanced disease, 10-30% of the early stage OC patients relapse after surgery and require chemotherapy (Colombo *et al.*, 2006).

1.1.6.1. Chemotherapy

The chemotherapeutic schedules of OC treatment have been revised over the last 30 years. In the 1970, melphalan was replaced by combination therapy of cyclophosphamide and doxorubicin. Cisplatin was added to this combination or to doxorubicin alone in the 1980s. At the same time, it was established that carboplatin has approximately equivalent efficacy to cisplatin, but with a reduced toxicity profile. In the late 20th century, it was found that paclitaxel is effective in the treatment of relapsed platinum-refractory disease. Currently, paclitaxel with either carboplatin or cisplatin used as first line chemotherapy for treatment of OC. Generally, the drug combination is given every three weeks for 6 cycles (Agarwal and Kaye, 2003).

1.1.6.1.1. Platinum

Rosenberg discovered cisplatin during an investigation of the electrical field effect on bacterial growth. He found that a small amount of platinum reacted with ammonium chloride present in medium to inhibit bacterial cell division. In 1967, cisplatin was shown to have anticancer activity by inhibiting the development of sarcoma in mouse tumour model (Rosenberg, 1985). The clinical applications of cisplatin include testicular, bladder and OC. It is also used for osteogenic sarcoma, head and neck cancer, endometrial and cervical cancer and non-small cell lung cancer (Reedijk, 1987; Pabla and Dong, 2008; Ahmad, 2010).

Platinum's effect is mediated by aqueous hydrolysis of the drug inside the cell to form active species (Agarwal and Kaye, 2003). The cytotoxic effects of platinum complexes are mediated via the formation of DNA inter-strand cross links or intra-strand DNA cross links, mostly at N7 position of guanine and possible at N3 position of adenine and O6 position of cytosine, chelation of the O-atoms and N-atoms of guanine and lastly by cross linking of DNA to protein (Katzung, Masters and Trevor, 2012). Binding of platinum to DNA inhibits DNA replication and protein synthesis that eventually leads to cancer cell apoptosis (Ahmad, 2010). The frequently encountered adverse effects of cisplatin are nausea, vomiting, bone marrow toxicity, neurotoxicity and nephrotoxicity (Reedijk, 1987; Pabla and Dong, 2008).

Carboplatin has replaced cisplatin in many chemotherapeutic combination regimens. It has a similar pharmacological profile with the advantage of substantially less renal and gastrointestinal adverse effects. However, its main adverse effect remains bone marrow suppression. In addition, the third generation platinum analogue, oxaliplatin, has similar pharmacological activities as carboplatin and cisplatin, but with advantages of additive activities against the resistant tumours than the older generations of platinum (Katzung, Masters and Trevor, 2012).

1.1.6.1.2. Paclitaxel

Taxanes (paclitaxel and docetaxel) are either derived from natural sources or by semisynthetic processes. Paclitaxel, an ester alkaloid, was first derived from the Pacific yew (*Taxus brevifolia*) and the European yew (*Taxus baccata*). Docetaxel, a semisynthetic taxane, exhibits less neurotoxicity than paclitaxel. Both drugs act by interfering with the normal as well cancer cell mitosis through binding to the β-subunit of tubulin and consequently leads to the stabilization of the microtubules and cell cycle arrest (Harries and Gore, 2002). Furthermore, taxanes induce apoptosis and have anti-angiogenic properties

(Crown and O'Leary, 2000). Paclitaxel is effective in ovarian, advanced breast, non-small cell and small cell lung, head and neck, oesophageal, prostate, and bladder cancers. The adverse effects of taxane include hypersensitivity, neurotoxicity, bone marrow suppression, neutropenia and fluid retention (Katzung, Masters and Trevor, 2012).

1.1.6.1.3. Pegylated liposomal doxorubicin

Doxorubicin is a member of the anthracyclin antibiotics family that was isolated from *Streptomyces peucetius* var *caesius.* Four major mechanisms for the cytotoxic effect of doxorubicin have been proposed:(1) topoisomerase II inhibition; (2) blockade of the synthesis of DNA and RNA due to inter-chelation into DNA; (3) free radicals production via an enzyme-mediated reductive process; (4) changing the fluidity of cellular membrane and influencing ion transport (Katzung, Masters and Trevor, 2012).

Doxorubicin has also been encapsulated in liposomes with a layer of polyethylene glycol to produce pegylated liposomal doxorubicin (PLD). The pegylation process improves the pharmacokinetic and toxicological properties of the drug and aids in drug delivery to the tumour (Thigpen *et al.*, 2005; Shen *et al.*, 2010). PLD has been shown to be useful in treatment of relapsed OC (Oskay-Oezcelik *et al.*, 2008) as well as in combination with cyclophosphamide in platinum-sensitive cancers. The overall response for the combination was 31% and the overall survival was 8.2 months (Floquet *et al.*, 2014).

1.1.6.1.4. Topotecan

Topotecan is a semisynthetic analogue of camptothecin. It inhibits the enzyme topoisomerase I which participates in key processes during DNA replication, transcription and repair mechanism. The enzyme relieves torsional stress by cutting and re-ligating a single DNA strand during DNA synthesis. Topoisomerase I binds covalently with DNA to

from a complex, and this forms the site of topotecan action. Topotecan stabilizes the complex and inhibits DNA synthesis (Goff *et al.*, 2007; Lorusso *et al.*, 2010).

Topotecan is indicated as second line therapy for both platinum-sensitive and platinum resistant OC relapsed after platinum-based chemotherapy (Abushahin *et al.*, 2008; Morris *et al.*, 2008). Topotecan's toxicity profile presents less challenges to the oncologist in comparison to the doxorubicin and paclitaxel therapy. The adverse effects of topotecan are usually short lived, reversible and noncumulative. However, neutropenia, alopecia, leukopenia, stomatitis, thrombocytopenia and anaemia are noted in patients using topotecan therapy (Dunton *et al.*, 2002).

1.1.6.1.5. Melphalan

Melphalan, an alkylating agent which is phenylalanine derivative of nitrogen mustard and was initially synthesized in 1953 (Rothbarth, Vahrmeijer and Mulder, 2002). Melphalan exerts its cytotoxic effect by inhibiting DNA synthesis through the formation of the intra-strand and inter-strand cross link and also by causing DNA-protein crosslinks. Cell death is usually the result of transfer of melphalan alkyl group to the DNA (Katzung, Masters and Trevor, 2012). The major adverse effects of melphalan, especially at large doses, are bone marrow suppression including leukopenia and thrombocytopenia and secondary neoplasm has also been reported (Rothbarth, Vahrmeijer and Mulder, 2002). Although melphalan was first chemotherapeutic agent for the treatment of OC (McGuire and Markman, 2003), it was replaced by a combination of cyclophosphamide and doxorubicin (Agarwal and Kaye, 2003).

1.1.6.2. Molecularly-targeted therapy

Molecularly-targeted therapy might be defined as drugs that inhibit the cancerous phenotype of tumours by modulating the function of a specific protein or other cellular components.

These targets are critical for tumour development and progression and can act as "drivers" of carcinogenesis. Examples include c-Kit mutations in gastrointestinal stromal tumours, epidermal growth factor receptor mutations in non-small cell lung cancer, HER2 amplification in breast cancer, and the BCR-ABL translocation in chronic myelogenous leukaemia (Ellis and Hicklin, 2009; Huang *et al.*, 2014). Targeted therapy differs from traditional chemotherapy by acting selectively on cancer cells and less destructive to normal cells. However, the main obstacle to the success of these medications is heterogeneity of the cells in tumours and the development of drug resistance (Ellis and Hicklin, 2009).

1.1.6.2.1. Angiogenesis inhibitors

Angiogenesis is an essential factor for tumour growth and metastasis. Targeting the formation of new blood vessels is a promising strategy in breast, renal and OCs (Weis and Cheresh, 2011). The FDA had approved several inhibitors for cancer treatment such as the humanized antibody bevacizumab, which binds VEGF-A, the tyrosine kinase inhibitor sorafenib, which targets Raf and VEGF and PDGF receptors, and the tyrosine kinase inhibitor sunitinib, which targets VEGF and PDGF receptors (Chung, Lee and Ferrara, 2010). Bevacizumab is a monoclonal antibody directed against vascular endothelial growth factor. It displayed substantial single agent activity in OC. However, the 21 % response rate was low and the 6-month progression survival rate were less than 50% in platinum-sensitive relapsed OC (Burger *et al.*, 2007). Apart from the cost and resistance, hypertension, proteinuria, gastrointestinal perforation are the main limitation reported with angiogenesis inhibitors (Banerjee and Kaye, 2013).

A recent meta-analysis (Y. S. Wu *et al.*, 2017) of 5 clinical trials (Perren *et al.*, 2011; Aghajanian *et al.*, 2012; Burger *et al.*, 2012; Pujade-Lauraine *et al.*, 2014; Coleman *et al.*, 2015) indicated that bevacizumab combined with traditional chemotherapy significantly

prolongs the progression free survival and overall survival in OC patients. It had been suggested that these agents are relatively well tolerated and should be considered for further study as single agent or in combination with other cytotoxic agents (Banerjee, Bookman and Gore, 2011; Burger *et al.*, 2012). The European Medicines Agency approved the use of Bevacizumab as first line therapeutic agent in combination with carboplatin and paclitaxel for treating advanced stage OC (Sapiezynski *et al.*, 2016).

1.1.6.2.2. Poly(ADP)Ribose Polymerase (PARP) inhibitors

PARP enzymes, which was first described over 50 years ago by Mandel, are a family of proteins implicated in the regulation of several cellular processes such as DNA-repair, inflammation and cell fate. PARP-1, a nuclear enzyme, facilitates DNA repair (mild damage) or triggers cell death (severe damage) by binding to both single-stranded and double-stranded DNA breaks (Wiggans *et al.*, 2015; Meehan and Chen, 2016).

Therapy directed at DNA repair pathways in OC had gained considerable interest because the lifetime risk of OC in women with BRCA mutations is elevated by 39–44% (Banerjee, Bookman and Gore, 2011; Shi *et al.*, 2017). PARP inhibitors can kill cancer cells specifically by preventing the repair of single strand gaps which may be degenerate into double strand breaks in DNA if they encountered by a replication fork. In tumour cells which lack functional BRCA genes, replication forks collapse and chromatin breaks leading to loss of cell viability (Figure 1-9). However, normal cells which retain single wild type copy of the BRCA gene are spared (Farmer *et al.*, 2005). This concept is called synthetic lethality because neither PARP inhibitors nor BRCA mutation alone are lethal but the combination results in cell death.

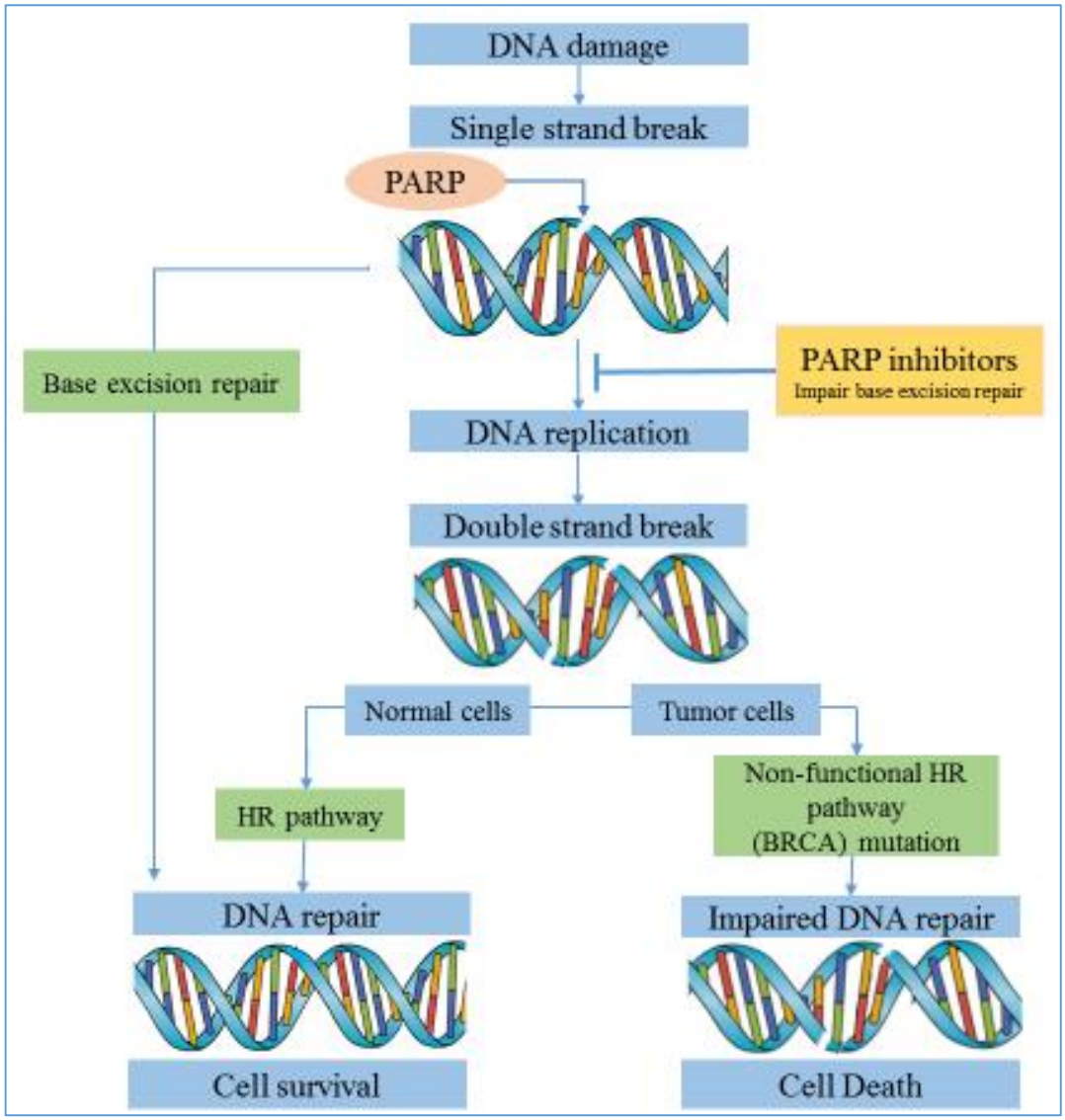


Figure 1-9 Role of PARP inhibitors in DNA repair and synthetic lethality.

PARP1 binding to single strand break results in activation of base excision repair. In the presence of PARP inhibitors and the lack of DNA repair, DNA replication generates a double strand breaks. In homologous recombination deficient cells, the lack of accurate repair of the double strand breaks persist resulting in cell death. Reproduced with permission from (Sonnenblick *et al.*, 2014).

1.1.6.2.3. Other signalling molecules

Several signalling mechanisms are aberrant in ovarian tumours which cause activation of oncogenic pathway participating in cell proliferation, angiogenesis, migration and survival. Many drugs are under development for targeting cellular changes implicated in malignant

transformation such as phosphoinositide 3-kinase/protein kinase B (PI3K/AKT), platelet-derived growth factor receptor (PDGFR), protein kinase C (PKC) and mitogen-activated protein kinase (MAPK). These agents include SF1126 and GSK690693 (PI3 kinase/AKT pathway inhibitors), dasatinib (Src inhibitor), sorafenib (Raf and VEGF receptor inhibitor), imatinib (PDGFR, c-kit inhibitor), and enzastaurin–LY-31765 (PKC inhibitor). Lastly, MORab-003 (Morphotek, Inc.), a monoclonal antibody against the folate receptor which is overexpressed in > 90% of OCs, is being evaluated in a phase II trial (Banerjee, Bookman and Gore, 2011).

Preclinical evidence suggests that epidermal growth factor receptor (EGFR) and human epidermal growth factor 2 receptor (HER2), tyrosine kinase receptors involved in cell proliferation and survival, are potential targets in OC. However, a clinical trial showed that the effectiveness of these agents were relatively limited (Teplinsky and Muggia, 2015).

1.1.7. Cytotoxic drug resistance in ovarian cancer

Resistance is main obstacle in management of cancer that leads to treatment failure and unsatisfactory overall patient survival (Holohan *et al.*, 2013). The response rate to the initial treatment of advanced OC is ordinarily high and is successful in 60-80% of patients. However, the survival rate has not improved in the last two decades and the 5-year survival rate remains as low as 40 % of the total patients (Ling *et al.*, 2005; Kigawa, 2013). The effort of incorporation of the newer cytotoxic agents (gemcitabine, liposomal doxorubicin and topotecan) to the conventional first line regimen (platinum-taxane) in GOG 182-ICON 5 study was unsuccessful in improving the overall survival and progression free survival of patients with advanced OC after optimal or suboptimal cytoreduction (Bookman *et al.*, 2009). Therefore, there is a pressing demand to develop new drugs or (re)sensitize cancer cells to existing chemotherapy (Witham *et al.*, 2007).

Several mechanisms of chemotherapy drug resistance in OC have been suggested, involving alterations in drug transport, changes in cellular detoxification, evading the induction of apoptosis and increased DNA repair activity (Figure 1-10) (Ling *et al.*, 2005; Kigawa, 2013). The potential involvement of multiple resistance mechanisms might contribute to a drug-resistant phenotype (Witham *et al.*, 2007).

1.1.7.1. Altered drug flux

One of the major mechanisms of drug resistance is the decrease in drug accumulation in cancer cells by increasing the drug efflux or reducing the uptake or a combination of both mechanisms (Tapia and Diaz-Padill, 2013). In 1976, *MDR1*, which encodes P-gp protein, the first member of ATP-binding cassette transporters (ABC) family, was identified (Callaghan, Luk and Bebawy, 2014) and represents a major advance in the understanding of drug resistance. Generally ABC proteins are efficient large transmembrane pumps involved in transport of metabolic products, nutrients, lipids, wide variety of drugs and chemical compounds against a concentration gradient (O'Connor, 2007).

At least eight ABC proteins are recognised in humans to have a role in resistance to cytotoxic agents as well as variety of other classes of drugs (Gottesman and Ambudkar, 2001) Specifically, P-gp overexpression is implicated not only in chemoresistance and failure of chemotherapy but it also associated with poor prognosis in patient with cancers such as OC, breast cancer, sarcoma and other malignancies (Ricciardelli *et al.*, 2013). P-gp has been reported to decrease intracellular accumulation of platinum-based compounds and other cytotoxic drugs and its expression has been shown to correlate with drug resistance. Therefore, it is considered as an attractive target to improve the clinical outcome of anticancer therapy (Tapia and Diaz-Padill, 2013; Kilari, Guancial and Kim, 2016).

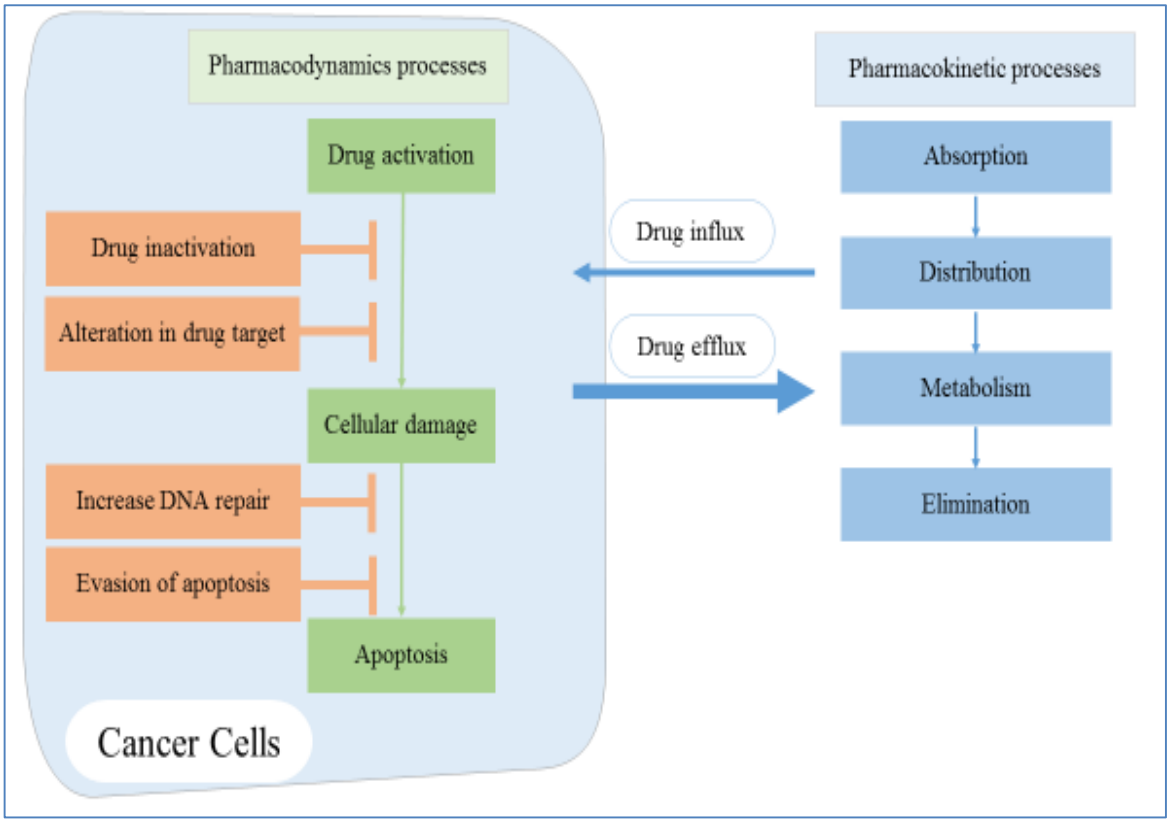


Figure 1-10 General principles of drug resistance.

Drug absorption, distribution, metabolism and elimination control the quantity of drug that reaches the tumour. The anticancer activity of a drug can be reduced by: poor drug influx or excessive efflux; drug inactivation or lack of activation; alterations in the drug target, such as mutation or changes in expression levels; activation of adaptive pro-survival responses; and a lack of cell death induction due to dysfunctional apoptosis. Reproduced with permission from (Holohan *et al.*, 2013).

Co-administration of P-gp inhibitors with chemotherapeutic drugs has given mixed results. P-gp inhibitors such as Valspodar, might elevate drug plasma concentrations beyond acceptable toxicity caused by pharmacokinetic drug interactions which reduce drug clearance and metabolism (Fracasso *et al.*, 2001). Yakirevich, et al., (Yakirevich *et al.*, 2006), studied consecutive sections from 60 patients of ovarian serous carcinoma and showed that P-gp is an independent prognostic factor and its expression directly correlated with the chemotherapeutic response and was inversely correlated with survival rate.

The entry of cisplatin and carboplatin into cancer cells is regulated by copper transporter-1 (CTR1). This protein is encoded by *CTR1* gene and deletion of this gene result in a reduction of the intracellular accumulation of cisplatin and ultimately triggers cisplatin resistance in various cell line including OC cell lines (Holzer, Manorek and Howell, 2006). In addition, ATP7A and ATP7B, copper exporters, have been reported to participate in resistance to cisplatin by retaining the drugs in intracellular compartment and blocking their interaction with DNA (Samimi *et al.*, 2004). Lastly, overexpression of ATP7A and ATP7B had been reported in OC and associated with increased resistance and lower survival rate (Samimi *et al.*, 2003).

1.1.7.2. Increase cellular detoxification

Glutathione (GSH) is a hydrophilic tripeptide composed of cysteine, glycine and glutamate. It is one of the most copious intracellular thiol molecules in cells and plays a crucial function in eliminating the toxicity of various cellular toxins include cisplatin and its analogues (Forman, Zhang and Rinna, 2009; Traverso *et al.*, 2013). Cysteine and methionine residues have the ability to inactivate the platinum-based drugs by binding to the sulphur atom in GSH (Jansen, Brouwer and Reedijk, 2002). This reaction is under the control of the glutathione S-transferase and the product is inactive and eliminated from cancer cells (Wang And and Guo, 2007). The role of GSH in acquired and intrinsic drug resistance might be explained by increased detoxification and elevated efflux of the cytotoxic drugs (Belotte *et al.*, 2014). It has been observed that several tumours with elevated level of GSH might be more resistant to chemotherapy (Traverso *et al.*, 2013). It has also been demonstrated that acquisition of the resistance to chemotherapy in cell lines derived from OC patients was associated with higher levels of GSH and glutathione-dependent enzymes (Lewis, Hayes and Wolf, 1988). Therefore, clinical development of a glutathione analog prodrug was initiated to reduce intracellular levels of GSH and overcome platinum inactivation. Canfosfamide

preferentially targets cancer cells that overexpress glutathione S-transferase. *In vitro* and *in vivo* antiproliferative activity of canfosfamide on cancer cells with glutathione S-transferase overexpression have been shown (Ramsay and Dilda, 2014). In addition, clinical trials demonstrated that canfosfamide was well tolerated and improve the clinical outcome when combined with standard chemotherapy in relapsed OC and non-small cell lung cancer (Sequist *et al.*, 2009; Vergote *et al.*, 2010).

1.1.7.3. Increased DNA repair activity

Chemotherapy drug resistance can be mediated by an increase in the capacity of DNA damage repair of the cancer cells (Housman *et al.*, 2014). The inter- and intra-strand DNA adducts caused by platinum compounds induce cell cycle arrest or apoptosis in cancer cells. The cell fate after cytotoxic therapy is dictated by the balance between DNA damage and DNA repair (Florea and Büsselberg, 2011). Several DNA repair mechanisms can be activated depending on type of lesion inflicted including nucleotide excision repair (NER), mismatch repair (MMR), homologous recombination repair (HR) and base excision repair (BER) (Torgovnick and Schumacher, 2015).

NER and MMR are among the well-recognized DNA repair pathways which appear to have a crucial function in mediating resistance to the treatment with platinum drug by participating in the detection and resolution of cisplatin induced DNA damage. Enhanced NER has been shown to be correlated with cisplatin resistance (Torgovnick and Schumacher, 2015). At least 20 protein involved in the process of NER, the excision of the damaged DNA requires dimerization of the excision repair cross-complementing group 1 (ERCC1) with xeroderma pigmentosum complementation group F (XPF). The dimerized complex appears to play a fundamental role in DNA repair process (Martin, Hamilton and Schilder, 2008; Galluzzi *et al.*, 2012).

More than 90% of the metastatic testicular cancer is cured by cisplatin. These tumours are associated with low levels of the NER proteins (XPA, XPF, ERCC1) and this might explain the testicular cell line higher sensitivity to cisplatin therapy in comparison to cell line from other tumour (Welsh *et al.*, 2004). However, a GOG study failed to demonstrate an association between ERCC1 and platinum sensitivity, overall survival and progression free survival in OC patients (Deloia *et al.*, 2012; Rubatt *et al.*, 2012).

MMR is another mechanism of DNA repair. This mechanism is divided into three steps, initiation, excision and resynthesis and involves several proteins including MLH1, MSH2, MSH3, MSH6, and PMS2. The marker of the MMR deficiency is microsatellite instability which is the occurrence of variable length of unpaired deletions in mono and dinucleotide repeats (Helleman *et al.*, 2006). MMR inactivation has been associated with the resistance to cisplatin therapy in OC (Vasey, 2003). The *MLH1* gene appear to be essential for normal physiological function of MMR system (Martin, Hamilton and Schilder, 2008). Cisplatin resistance in a significant proportion of OC patients is correlated with *MLH* silencing induced by methylation (Agarwal and Kaye, 2003).

1.1.7.4. Evasion of apoptosis

Apoptosis, or programmed cell death, is an irreversible process characterized by chromatin condensation, nuclear fragmentation, membrane blebbing and cell shrinkage, and formation of apoptotic bodies (Kerr, 2002). The ability to evade programmed cell death is a hallmark of the human cancer and is implicated in resistance to chemotherapy (Hanahan and Weinberg, 2011).

The intrinsic apoptotic pathway (Figure 1-11) is regulated by Bcl-2 family proteins and includes mitochondrial outer membrane depolarization, release of cytochrome c from the mitochondria, apoptosome formation and the subsequent activation of the caspase cascade

(Ichim and Tait, 2016). Lethal stimuli induce the interaction of Bid and Bim with Bax and Bak inducing their activation and oligomerization in the mitochondrial membrane to form pores that allow cytochrome c and other proteins including Smac and Omi to be released from the mitochondrial intermembrane space into cytoplasm. Cytochrome c binds to Apaf1 to form the apoptosome complex which activates caspase-9. In addition, Smac and Omi deactivate the IAP proteins, an inhibitors of caspases, to facilitate the commitment of cell death (Tait and Green, 2010).

In the extrinsic apoptotic pathway (Figure 1-11), the initiators are specific transmembrane death receptors triggered by their respective ligands. Apoptosis is initiated by the binding of death ligands, receptor, adaptor molecules (FADD) and caspase 8 to form the death-inducing signalling complex (DISC) (Lavrik, 2014). This complex triggers a series of events and causes activation of effector caspases and cell death (Ichim and Tait, 2016). The death receptor family is a main inducer of the pathway and include FAS (also named CD95 /APO-1), TNF-R1, TRAIL-R1,TRAIL-R2, DR3 and DR6 (Lavrik, 2014).

Many proteins are involved in apoptosis pathway regulation, which are tumour suppressor genes (such as *TP53*), oncogenes (such as Ras and Akt) and apoptotic machinery proteins (Bcl-2 Family) (Agarwal and Kaye, 2003). An increase in the anti-apoptotic proteins and/or reduction of the pro-apoptotic molecules can be a major cause of cancer cell resistance to cell death signals and consequently this may lead to tumour progression and development of clinical drug resistance (Fulda, 2009). Chemotherapeutic drug resistance has been correlated with expression of anti-apoptotic proteins (Bcl-2 and Bcl-X_L) and the caspase inhibitor, X-linked inhibitor of apoptosis protein (XIAP) in OC cell lines (Yang *et al.*, 2004, 2005; Williams *et al.*, 2005).

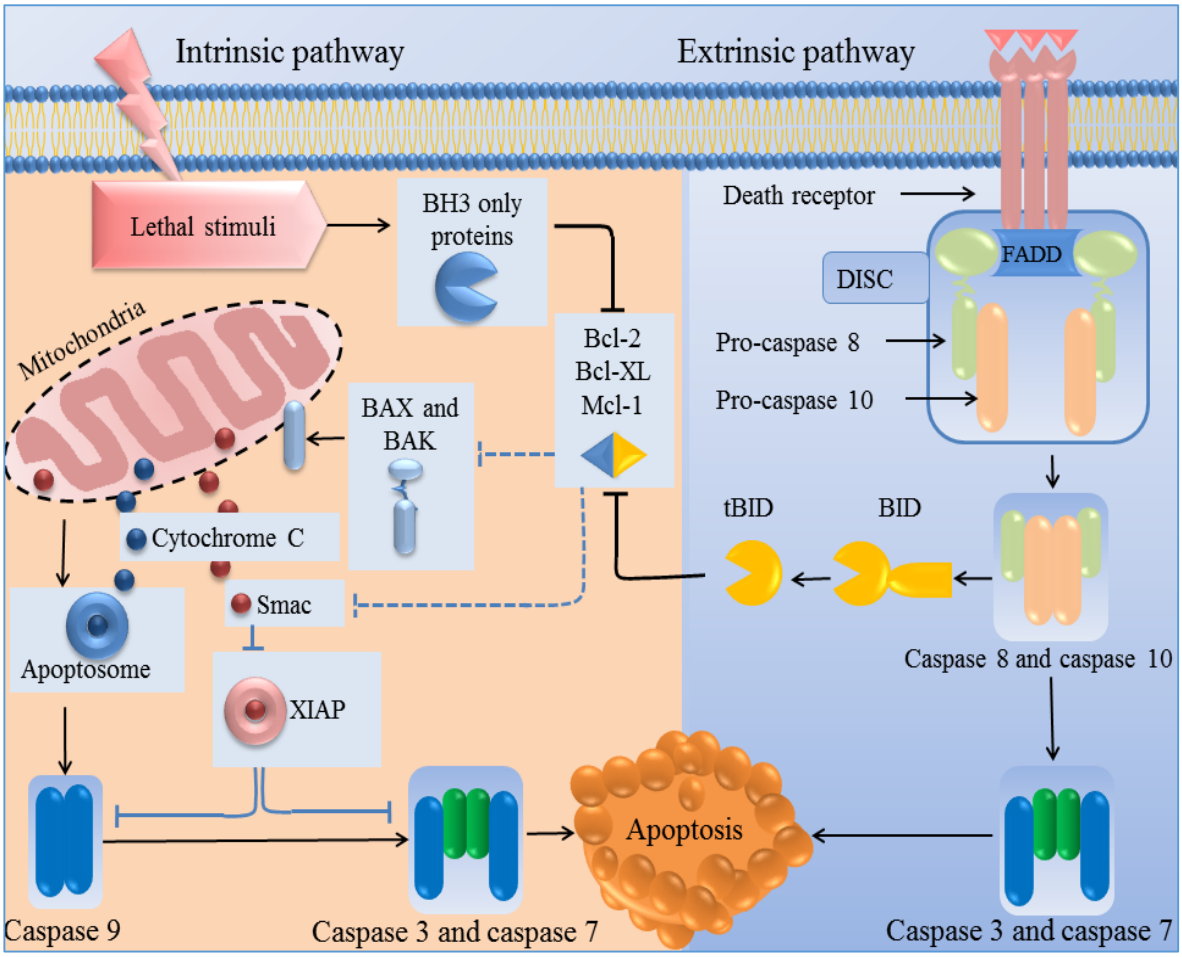


Figure 1-11 Extrinsic and intrinsic apoptotic signalling pathways

In the extrinsic apoptotic pathway, the stimulation of death receptor by ligand can activate initiator caspases (caspase 8 and caspase 10) by dimerization mediated by adaptor proteins (FAS-associated death domain protein (FADD)). The effector caspase 3, 7 is activated by caspase 8 and 10 which eventually lead to apoptosis induction. The intrinsic (or mitochondrial) pathway of apoptosis is more complicated than the extrinsic pathway and needs mitochondrial outer membrane permeabilization (MOMP). BAX and BAK activation process triggered by cell stress activation of BH only protein stimulation leading to MOMP. However, Bcl-2 family proteins counteract this process. Subsequently, cytochrome c and mitochondria-derived activator of caspases (SMAC) released from the mitochondrial intermembrane space into cytosol. SMAC release facilitates apoptosis by opposing the caspase inhibitor X-linked inhibitor of apoptosis protein (XIAP). Triggering of apoptosome formation by interaction of cytochrome C with apoptotic protease activating factor 1 (APAF1) lead to activation of caspase cascade and finally induction of cell death. Caspase-8 cleavage of the BH3-only protein BH3-interacting death domain agonist (BID) allows crosstalk of the extrinsic and intrinsic apoptotic pathways. ER, endoplasmic reticulum; MCL1, myeloid cell leukaemia 1; tBID, truncated BID. Reproduced with permission form (Ichim and Tait, 2016).

1.2. Mevalonate pathway

The diversity of the products of the mevalonate pathway (MP) and their biological activity provides an insight into the importance of this pathway in health and disease (Burg and Espenshade, 2011; Ghavami *et al.*, 2017). The cornucopias products of MP, about 30,000 compounds identified to date and many new chemical structures being described annually, are involved in crucial functions necessary for life. For instance, dolichol, ubiquinone, heme A, vitamin D, cholesterol, bile acid and steroids are some products of the pathway. These metabolites play a fundamental role as mating pheromones, in reproductive hormones synthesis, membrane structure and signal transduction (Sacchettini and Poulter, 1997; Osmak, 2012; Dhar, Koul and Kaul, 2013; Likus *et al.*, 2016). In particular the, MP intermediate metabolites regulate diverse cellular functions by controlling the function of small GTPases proteins (Mullen *et al.*, 2016; Brandi *et al.*, 2017). Disruption in the MP has been associated with a number of disorders such as autoinflammatory disease and atherosclerosis (Thurnher, Gruenbacher and Nussbaumer, 2013).

1.2.1. Biochemistry of mevalonate pathway

The MP can be divided into three main stages. The 20 enzymes of the MP catalyse the biochemical reactions which culminate in the formation of the 27 carbon atom structure of tetracyclic molecule, cholesterol (Figure 1-12) (Burg and Espenshade, 2011).

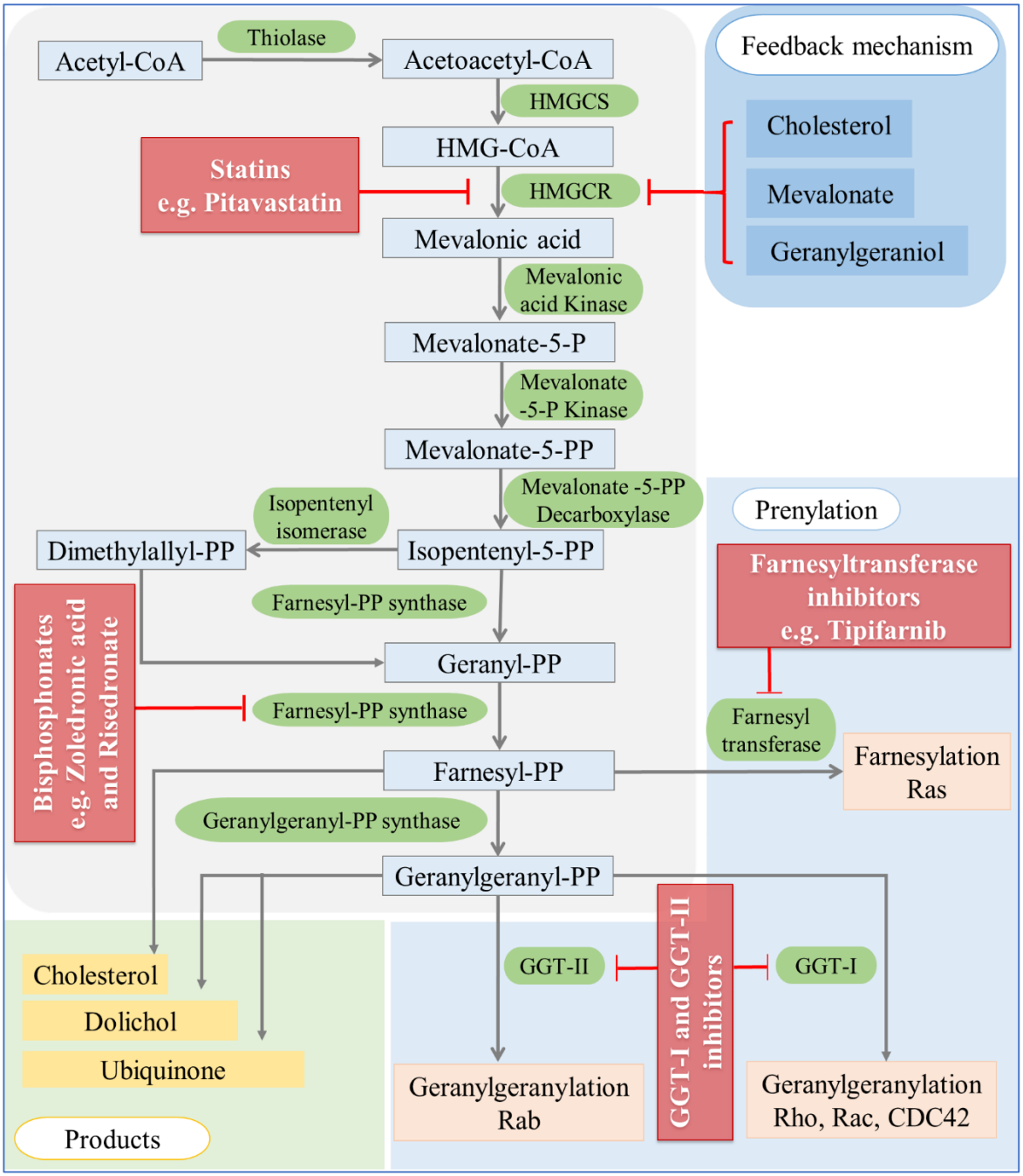


Figure 1-12: Mevalonate pathway.

The figure shows the details of the mevalonate pathway biosynthesis. The pathway started by condensation of Acetyl-CoA to HMG-CoA, which in turn reduced to Mevalonate. The later product is decarboxylated to isopentenyl which involve in synthesis of other intermediate metabolite of the pathway. It also shows some of the inhibitors of pathway enzymes such as statins and bisphosphonates. HMGCS, Hydroxymethylglutaryl-CoA synthase; HMGCR, 3-Hydroxy-3-Methylglutaryl-CoA Reductase; GGT-I, geranylgeranyl transferase-I; GGT-II, geranylgeranyl transferase-II.

The first stage of mevalonate biosynthesis pathway occurs in the cytoplasm while the remaining stages take place in the endoplasmic reticulum. In the first stage, acetyl-CoA, derived from carbohydrate, amino acid and fatty acid metabolism, supplies two carbon atoms for conversion by condensation reactions into the six carbon atom compound 3-hydroxy-3-methyl-glutarate conjugated to coenzyme A (HMG-CoA). The condensation reaction is catalysed by Acetyl-CoA thiolase and HMG-CoA synthase (HMGCS). Further reduction of HMG-CoA produces mevalonate and this reaction is catalysed by the enzyme HMG-CoA reductase (HMGCR) and is considered the rate limiting step of the MP. HMGCR is extensively regulated at multiple levels of transcription, translation and degradation.

The second stage, which is considered as a branching step leading to the synthesis of many intermediate metabolites, involves phosphorylation and decarboxylation of the mevalonate molecules to yield isopentenyl pyrophosphate via the action of the mevalonate kinase and mevalonate decarboxylase. This molecule is a fundamental precursor to many biological compounds required by animals and plant cells. Subsequently, farnesyl pyrophosphate (FPP) is produced from the condensation of three isopentenyl moieties (isopentenyl pyrophosphate and dimethyl allyl pyrophosphate) in a reaction catalysed by farnesyl synthase. Next, geranylgeranyl pyrophosphate synthase (GGPPS) catalyses the addition of isopentenyl moiety to farnesyl pyrophosphate to yield geranylgeranyl pyrophosphate (GGPP).

In the third stage, squalene synthase facilitates the condensation of two molecules of FPP to produce squalene, a 30-carbon atom compound with a half dozen double bonds. Finally, cholesterol, *"the most highly decorated small molecule in biology",* is synthesised from lanosterol by three decarboxylation reactions (Hooff *et al.*, 2010; Burg and Espenshade, 2011; Berg, Tymoczko and Stryer, 2013; Dhar, Koul and Kaul, 2013; Nelson and Cox, 2013; Likus *et al.*, 2016).

1.2.2. Regulation of mevalonate pathway

The MP is precisely regulated at multiple levels (Goldstein and Brown, 1990; Likus *et al.*, 2016). This is not surprising because cholesterol synthesis requires carbon atoms and large amount of energy (ATP) expenditure. The balance of cholesterol synthesis is maintained by controlling both cholesterol synthesis and uptake in which the cholesterol itself is the main mediator of this regulation. Cholesterol synthesis is also regulated by negative feedback inhibition of *HMGCR*, since a high intracellular concentration is toxic and associated with several disorders (Sharpe and Brown, 2013).

Cholesterol is mainly produced by the liver and transported to other tissue via low-density lipoproteins. This lipoprotein in turn reduces the activity of HMGCR to 10% which is only required to maintain production of non-sterols by the MP (Likus *et al.*, 2016). Indeed, HMGCR activity is regulated not only by cholesterol concentration but also by other intracellular factors (sterol and non-sterol products of the MP) and extracellular factors (insulin, tri-iodothyronin, glucagon and cortisol) (Räikkönen *et al.*, 2009; Burg and Espenshade, 2011).

HMGCR is one of the most tightly controlled enzymes in human body and its activity regulated by several mechanisms (Goldstein, DeBose-Boyd and Brown, 2006; DeBose-Boyd, 2008; Kamisuki *et al.*, 2009; Sato, 2010; Burg and Espenshade, 2011; Berg, Tymoczko and Stryer, 2013; Spann and Glass, 2013; Luu, Gelissen and Brown, 2017): Sterol Regulatory Element Binding Protein (SREBP), a transcription factor that regulates the rate of *HMGCR* expression, acts on sterol response element (SRE), a short sequence of nucleotides in the reductase gene, to initiate gene transcription (Figure 1-13). There are three mammalian SREBP isoforms (SREBP-1a, SREBP-1c and SREBP-2) which are encoded by SREBF1 and SREBF2 genes. They have distinct but overlapping lipogenic transcriptional

function. SREBP-1a activates fatty acid and cholesterol biosynthesis, SREBP-1c enhance fatty acid biosynthesis, and SREBP-2 regulate cholesterol biosynthesis and uptake (Shao and Espenshade, 2012).

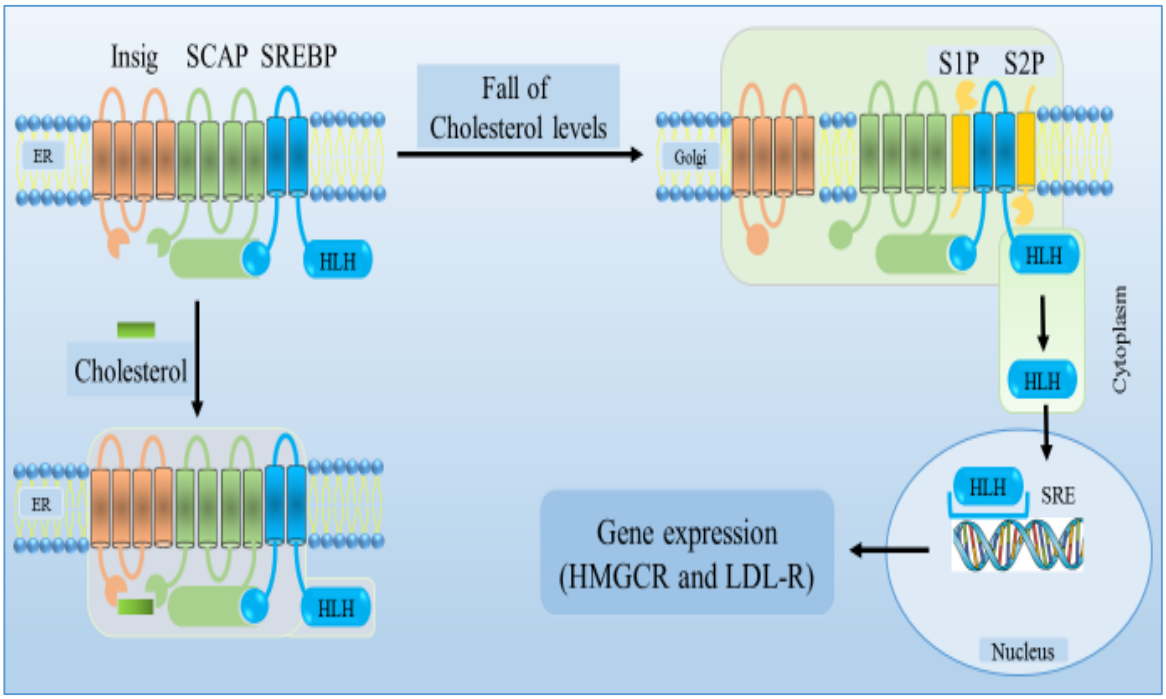


Figure 1-13 Regulation of cholesterol synthesis.

Cholesterol level controls the rate of HMGCR and LDL-R expression. Insulin-induced gene (Insig); sterol response element- binding proteins (SREBP); SREBP cleavage-activating protein (SCAP); endoplasmic reticulum (ER); site 1 protease (S1P); site 2 protease (S2P); helix–loop–helix domain of SREBP (HLH); sterol regulator element (SRE). Reproduced with permission from (Cyster *et al.*, 2014).

In low cholesterol conditions, SREBP cleavage-activating protein (SCAP) escorts SREBP in small membrane vesicles to the Golgi apparatus. Activation of the SREBP requires the activity of two proteolytic enzymes, namely S1P and S2P. The first protease enables the dissociation from SCAP and the second one permits the cleavage and release of SREBP from the vesicles membrane. The amino-terminal domain, containing a basic helix-loop-helix (HLH) transcription factor, is transported to the nucleus and binds to SRE in target genes encoding proteins required for cholesterol synthesis (HMGCR) and uptake (LDL receptor)

which results in their transcriptional activation. At the same time, LXR-RXR transcription factor heterodimers recruit corepressor complexes and actively repress genes encoding molecules that facilitate cholesterol efflux, such as ABCA1, and decrease degradation of the LDL receptor which result in higher cellular concentrations of cholesterol.

In contrast, during high intracellular levels of cholesterol, cholesterol and desmosterol cause retention of SCAP-SREBP by binding to SCAP. This induces conformational change in SCAP which in turn facilitate its binding to another ER protein called Insig. This protein prevents the release of SCAP-SREBP complex from the ER. The proteolytic activity of the S1P and S2P enzymes is blocked and SREBP nuclear translocation is prevented. Oxysterols and desmosterol bind to LXRs, which causes dissociation of corepressors and recruitment of coactivators that induce the transcription of target genes, such as those encoding ABCA1 which cause lower cellular concentrations of cholesterol.

In addition to sterol regulation of HMGCR expression, its turnover is also regulated. HMGCR comprises 2 domains, the cytoplasmic domain which provides the catalytic activity of the enzyme and membrane domain which senses the signals from outside that participate in the regulation of reductase degradation. Lanosterol and 25-hydroxycholesterol trigger conformational changes of the reductase membrane domain that permits the binding to a subset of Insig proteins and ubiquitination of HMGCR. Degradation of the reductase is a result of polyubiquitination and dissociation from membrane.

Lastly, phosphorylation also regulates the reductase enzyme. At low level of ATP, AMP-protein kinase phosphorylates the reductase enzyme and consequently inhibits cholesterol synthesis (Figure 1-14).

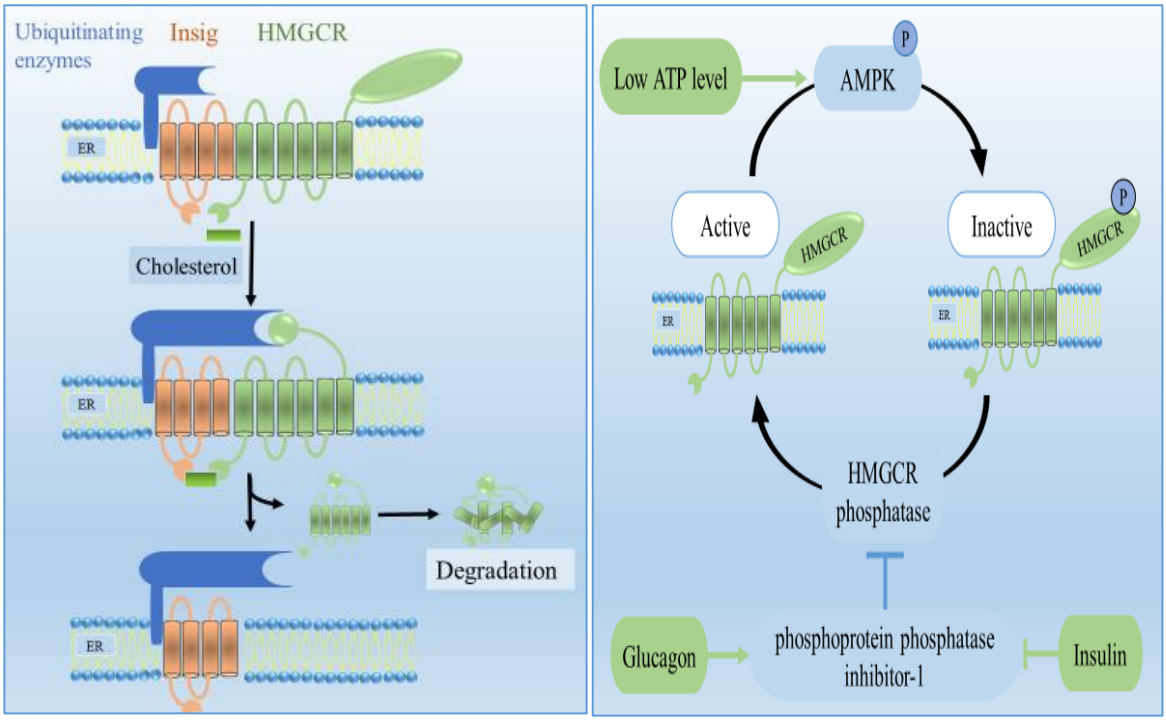


Figure 1-14 Regulation of HMGCR.

The HMGCR level is regulated by multiple mechanisms. The left figure shows the process of the HMGCR degradation by high cholesterol level. The right figure shows the changes in the HMGCR status between active and inactive state by AMPK and HMGCR phosphatase. Reproduced with permission form (DeBose-Boyd, 2008).

1.2.3. Deregulation of the mevalonate pathway in cancer

Deregulation of MP was first reported around 50 years ago in mouse hepatomas (Siperstein and Fagan, 1964). Several reports suggest that HMG-CoA reductase play an important role in human cancer. Many cancers have been shown to exhibit either increased expression and activity of HMGCR or deficient feedback control of the HMG-CoA reductase activity (Clendening *et al.*, 2010). The increase cholesterol synthesis may depend on the availability of MP precursors such as acetyl-CoA (Cruz *et al.*, 2013). Cancer cells require *de novo* lipid synthesis for growth. It has been found that increased lipid synthesis participates in the pathogenesis of cancer, including ovarian neoplasm (Pyragius *et al.*, 2013). The earliest evaluation the of MP in cancer was by Fumagalli et al., (1964) (Fumagalli *et al.*, 1964) who

reported that human glioblastomas cells synthesize large quantities of cholesterol. In addition, the failure of the pathway's negative feedback inhibition in mouse hepatoma cells was reported at the same time by Siperstein and Fagan (Siperstein and Fagan, 1964). Furthermore, it has been shown that exogenous mevalonate administration promotes tumour growth in xenograft-bearing mice (Duncan, El-Sohemy and Archer, 2004).

HMGCR is considered as metabolic oncogene as the ectopic expression of full length HMGCR increases anchorage dependent growth of cells and cooperates with Ras to transform cells (Clendening *et al*. 2010). The expression of SREBP-2, a transcription factor that regulates several MP genes, correlated with viability of prostate tumour cells (Krycer, Phan and Brown, 2012). In addition, it was reported that 40% of genes of the MP are either amplified or show increased expression (Bell *et al.*, 2011). In contrast, overexpression of HMGCR has been correlated with prognosis in ovarian, breast and colorectal cancers (Borgquist *et al.*, 2008; Brennan *et al.*, 2011; Bengtsson *et al.*, 2014). Intermediate products of the MP (GGPP and FPP) are involved in post-translational modification of several important proteins implicated in cell signals, proliferation and differentiation. This includes the RAS superfamily of GTPases, a large family of proteins encompasses of more than 150 members. Ras itself is mutated in about 20% of human cancers. Mutated Ras can be stabilized in a constitutively active conformation and efforts are ongoing to develop novel therapies that inhibit Ras actions (Konstantinopoulos, Karamouzis and Papavassiliou, 2007; Zhang *et al.*, 2013). It has been observed that neoplastic tissue can show not only an increase in the rate of cholesterol synthesis but also in the HMGCR activity. Collectively, this has led to the proposal that inhibition of sterol synthesis can impede tumour growth (Thibault *et al.*, 1996). Taken together, these findings pointed to the importance not only the cholesterol but also reflect the activity of the MP, as driver in cancer.

1.2.4. Protein prenylation

Isoprenylation ("prenylation") is considered as a key physiological process and one of the most important functions of the MP (Wang and Casey, 2016). Many prenylated proteins functions as signalling molecules which are activated by extracellular stimuli to control intracellular activities. This can include regulation of gene transcription which influences diverse biological processes including differentiation, cell division, cell proliferation, vesicle transport, nuclear assembly, and cytoskeleton reorganization (Heasman and Ridley, 2008; Karnoub and Weinberg, 2008; Nilsson, Huelsenbeck and Fritz, 2011). These post-translational modifications are essential for protection of Ras superfamily from proteolytic degradation, facilitate protein-protein interaction and the most importantly assist anchoring to membrane and subcellular localization (Schafer and Rine, 1992; Konstantinopoulos, Karamouzis and Papavassiliou, 2007).

The prenylation process is catalysed by three prenyl transferase enzymes (farnesyl transferase and Geranylgeranyl transferase-I and II) and involves the covalent addition of the isoprene moiety to the C termini of proteins (details of protein prenylation in Figure 1-15 and Figure 1-16) (Vinet and Zhedanov, 2010; Li and De Souza, 2011). The addition of 15-carbon atom farnesyl moiety to proteins (Ras, RhoB and HDJ2) is called farnesylation and catalysed by the enzyme farnesyl transferase. In contrast, geranylgeranylation, which include the addition of one 20 or two 20 C-atom molecules to proteins (Rab, Rap1A, RhoA, Rac1 and Cdc42), is catalysed by geranylgeranyl transferase-I (GGT-I) and geranylgeranyl transferase-II (GGT-II), respectively (Swanson and Hohl, 2006; Gao, Liao and Yang, 2009; Rogers *et al.*, 2011).

The terminal tetrapeptide amino acid sequences of the protein (CAAX box) is the main determinant of the prenylation type. In the CAAX motif, C represent the cysteine residues,

A represents an aliphatic amino acid and X a range of other amino acids (Winter-Vann and Casey, 2005). Ras farnesylation arises when X is glutamine, methionine or serine while geranylgeranylation occurs when X is leucine or phenylalanine amino acid. However, NRas and KRas4A can be farnesylated or geranylgeranylated. Rho GTPases are geranylgeranylated if X is either leucine or phenylalanine and farnesylated if X are other amino acids. In contrast, HRas is only farnesylated. In addition, the CAAX motif is replaced by CXC or CC in case of substrates of GGT-II (Shimoyama, 2011; Holstein and Hohl, 2012).

After prenylation, the AAX tripeptide undergo proteolytic removal by Ras-converting CAAX endopeptidase 1 (RCE1) and carboxymethylation by isoprenylcysteine carboxylmethyltransferase (ICMT) in the endoplasmic reticulum. They are palmitoylated in the Golgi apparatus and then anchored by farnesyl or geranylgeranyl, and palmitoyl moieties to plasma membrane. KRas4B does not require palmitoylation to anchor it to the plasma membrane. Rho is associated with guanine nucleotide dissociation inhibitors (GDIs), which deliver them to their membrane locations (Konstantinopoulos, Karamouzis and Papavassiliou, 2007; Wang and Casey, 2016).

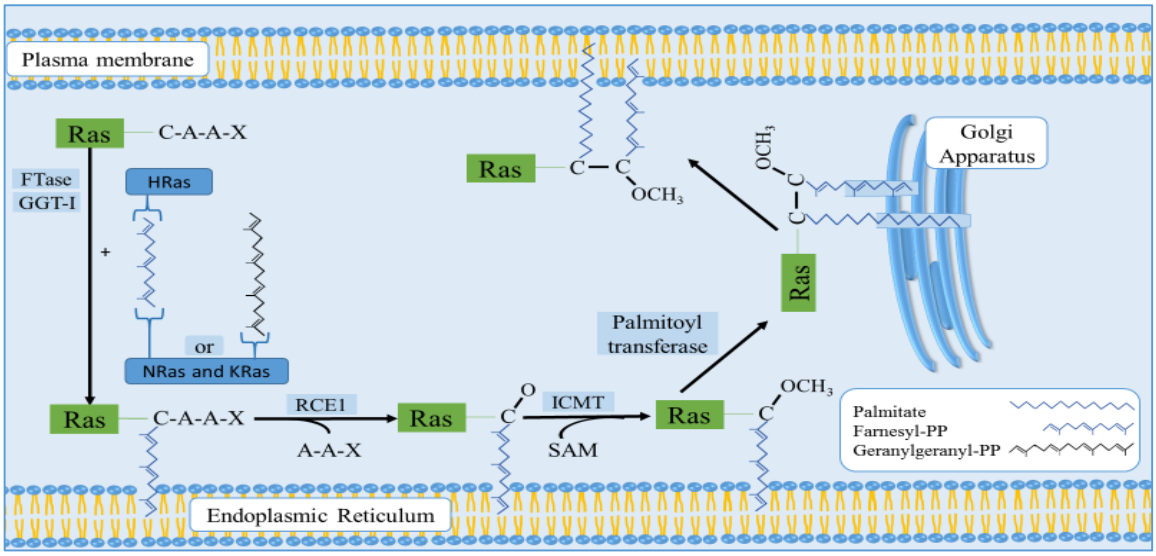


Figure 1-15 Prenylation of Ras GTPases.

Prenylation process of Ras GTPase involve the addition of either geranylgeranyl or farnesyl and palmitate moiety. FTase, farnesyltransferase; GGT-I, geranylgeranyl transferase I; SAM, S-adenyosyl methionine; RCE1, Ras-converting CAAX endopeptidase 1; ICMT, carboxymethylation by isoprenylcysteine carboxylmethyltransferase.

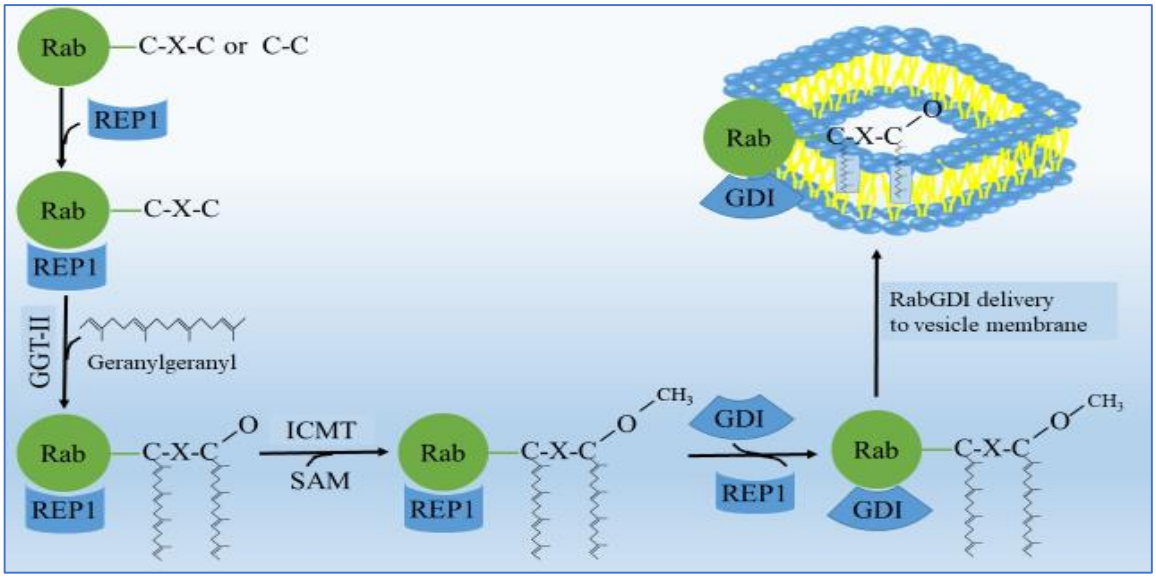


Figure 1-16 Prenylation and post-prenylation reactions of Rab GTPases.

Rab with C terminal C-X-C or C-C residues are geranylgeranylated in both C residues by GGT-II. Unprenylated Rab are presented by a REP1 (Rab escort protein 1) to GGT-II. Subsequently only RAB GTPases ending in C-X-C undergo carboxymethylation by ICMT. Lastly, these RabGTPases bind to RabGDIs, which recognize their two geranylgeranyl moieties and deliver them to their membrane locations. RCE1, Ras-converting CAAX endopeptidase 1; ICMT, carboxymethylation by isoprenylcysteine carboxylmethyltransferase; SAM, S-adenosylmethionine. Reproduced with permission from (Konstantinopoulos, Karamouzis and Papavassiliou, 2007).

1.2.5. Activation of Ras superfamily proteins

Ras superfamily proteins possess the same molecular switch system despite the functional and structural diversity of their members (Reuther and Der, 2000). The switching system of these proteins form is firmly controlled by a complex regulatory network. GTPase activating proteins (GAPs), guanine nucleotide exchange factors (GEFs) and guanine nucleotide dissociation inhibitors (GDIs) are classes of proteins that control activation of the inactive GDP-bound to active GTP-bound conformation (Wennerberg, 2005; Ahearn *et al.*, 2012). The activation cycle induces conformational changes resulting in modulation of binding affinities to the effector proteins (Agarwal *et al.*, 2009).

GEFs and GAPs regulate the cycling of Rho and Rab GTPases between active and inactive status (Bos, Rehmann and Wittinghofer, 2007). However, the extraction and delivery of prenylated inactive Rab and Rho from Rab escort protein 1 (REP1) is catalysed by GDIs (Alexandrov *et al.*, 1994). GDI displacement factors (GDFs) are another family of protein that interact with Rab and Rho proteins to regulate their detachment from GDIs and subsequently transfer to subcellular membranes (O'Neill *et al.*, 2012). In addition, heat-shock protein 90 (HSP90) involve in the extraction of Rab proteins from membrane by stimulating the activity of GDI. The series of activation and inactivation process of Ras superfamily is associated with the transduction of an upstream signal to activation of downstream effectors by activation of second messenger cascades (Konstantinopoulos, Karamouzis and Papavassiliou, 2007).

1.2.6. Prenylated proteins overview of the classical families

Guanine nucleotide-binding proteins (G proteins) comprise of two groups of proteins, the heterotrimeric G proteins (large G-proteins) and the monomeric small G-proteins (small GTPases) (Konstantinopoulos, Karamouzis and Papavassiliou, 2007). The Ras superfamily

of small GTPases, a large family of proteins encompasses more than 150 members, which accounts for about 0.5-2% of all human proteins (Reigard *et al.*, 2005; McTaggart, 2006; Gao, Liao and Yang, 2009; Vigil *et al.*, 2010). The human Ras superfamily can be grouped into five major branches (Figure 1-17) (Colicelli, 2004; Vigil *et al.*, 2010). Ras, Rho, Rab, Ran and Arf are major subfamilies of the Ras superfamily. The family is involved in regulation of crucial biological processes such as intracellular signal transduction (Ras), reorganization of the cytoskeletal (Rho), gene expression (Ras, Rho), trafficking of the intracellular vesicle (Rab), organization of microtubules (Ran) and nucleocytoplasmic transport (Ran) (Takai, Sasaki and Matozaki, 2001). The role of the prenylated proteins is now evident in the pathogenesis and progression of cancer and similarly in atherosclerosis and Alzheimers' disease (McTaggart, 2006). So, it is reasonable to consider MP as potential target in cancer therapy by virtue of its ability to affect the function of Ras family members through prenylation (Dudakovic *et al.*, 2008).

The Ras superfamily is the most studied group of small GTPase proteins. Historically, the Ras family, which comprising HRas, KRas, and NRas, were the founding members of the Ras-related superfamily. 25 years ago, Valencia et al.,(Valencia *et al.*, 1991) proposed the first classification of this family of proteins which included about 30 family member. Since then, the studies of the family have increased exponentially due to the fact that Ras superfamily has a great impact on human diseases and also provided a promising target for drug development (Rojas *et al.*, 2012).

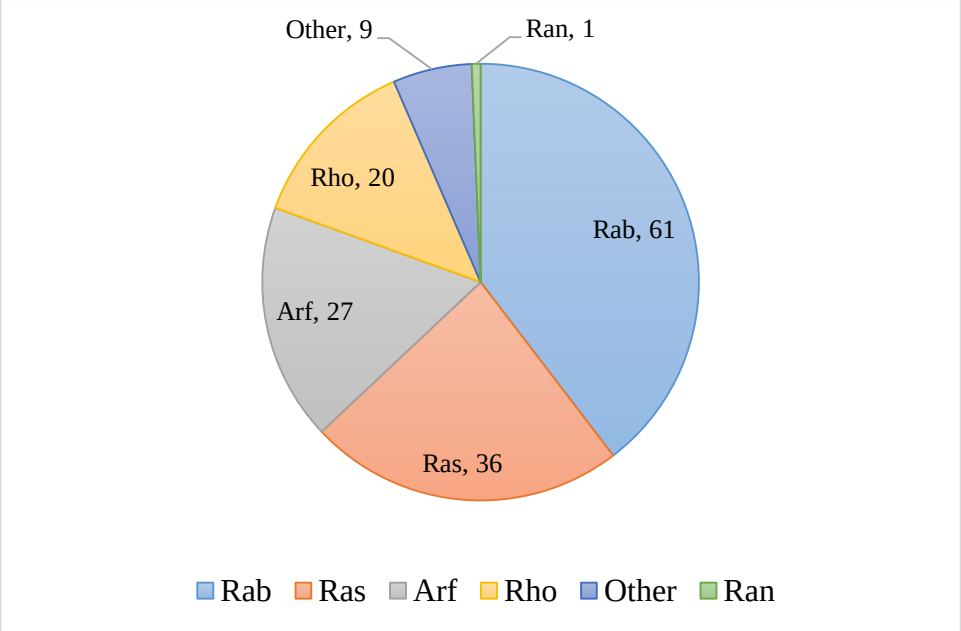


Figure 1-17 Ras superfamily proteins.

1.2.6.1. Ras GTPases

Ras GTPases affect several different signalling pathways (Figure 1-18) (Coleman, Marshall and Olson, 2004). This family consists of 36 members (Vigil *et al.*, 2010) and the most well know of these are H-Ras, N-Ras and K-Ras which have been reported to be frequently mutated in human cancer (Gysin *et al.*, 2011). Ras proteins are mutated in about 30% of all human cancers and up to 90% in pancreatic cancer (Goldfinger and Michael, 2017). 43-65% of mucinous OCs were reported to contain mutations in K-Ras proteins (McCluggage, 2011). It has been claimed that K-Ras and N-Ras are overexpressed and mutated in OC (Cho and Shih, 2009; Gysin *et al.*, 2011; Emmanuel *et al.*, 2014). However, the activity of wild-type Ras is also increased in cancer due to other genetic lesions such as mutational activation or increased expression of tyrosine kinases receptors (Fiordalisi, Der and Cox, 2006). Activation of these oncogenes result in prolonged activation of Ras proteins and the downstream effector signalling pathways which in turn might cause malignant transformation (Gysin *et al.*, 2011).

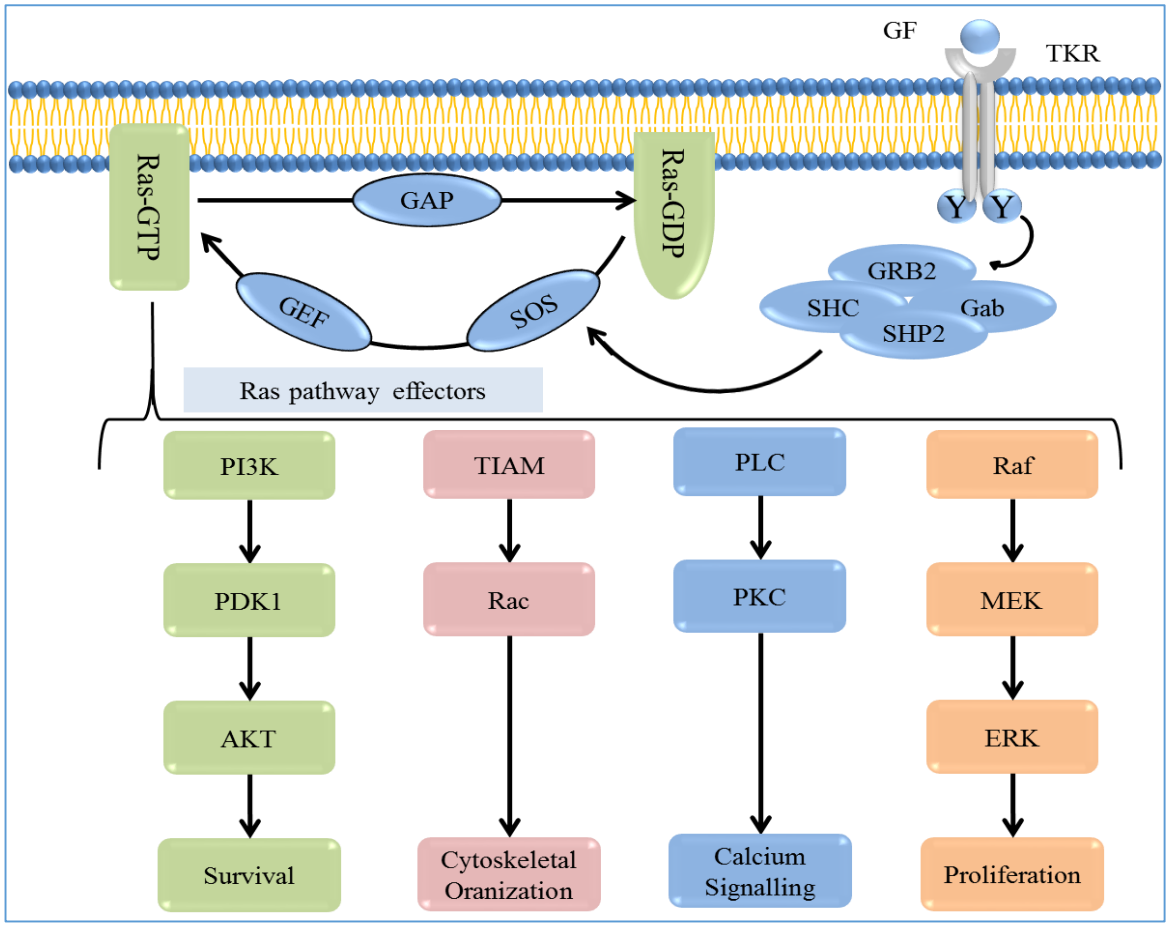


Figure 1-18 Ras signalling pathway

RAS proteins are activated by guanine nucleotide exchange factors (GEFs). This enzyme catalyses the exchange of GDP for GTP. These proteins are inactivated by the GTPase activating proteins (GAPs) which stimulate the intrinsic RAS GTPase activity. The cell surface receptor complex is activated by binding of growth factors. The activated complex which include multiple adapters like GRB2 (growth-factor-receptor bound protein 2), SHC (SH2-containing protein) and Gab (GRB2-associated binding) proteins, recruit SHP2 and SOS. Ras-GTP level is increased by the recruitment of SOS which in turn catalyses exchange of GDP nucleotides for GTP on Ras. Conversely, Ras-GTP binds to GTPase-activating protein (GAP) such as neurofibromin (NF1) which terminates the signalling by accelerating the conversion of the Ras-GTP to Ras-GDP. The Raf-MEK-ERK cascade which is usually deregulated in cancer, controls the rate of proliferation. In addition, Ras activates the PI3K-Akt pathway which regulate the cell survival. TIAMI are exchange factor for the Rac which in turn regulates actin dynamics and, thus cytoskeleton. Lastly, Ras activate phospholipase Cε (PLCε) to produce IP3. The hydrolytic product of this enzyme, which regulates calcium signalling and diacylglycerol regulates the protein kinase C family (PKC). TKR, tyrosine kinase receptor; GF, growth factor; P, phosphate; Y, tyrosine residue. Reproduced with permission from (Schubbert, Shannon and Bollag, 2007).

1.2.6.2. Rho GTPases

Rho GTPase subfamily consist of about 20 proteins such as RhoA, RhoB, RhoC, Rac1 and Cdc42 (Vigil *et al.*, 2010; Hodge and Ridley, 2016). This family of proteins are geranylgeranylated with the exception of RhoB which can be either geranylgeranylated or farnesylated (Sarrabayrouse *et al.*, 2017). Active Rho triggers signalling networks to direct cellular responses by binding to different effectors molecules. The most important of these cellular processes are actin and microtubule cytoskeleton organization, cell division, cell adhesion, motility, vesicular trafficking, phagocytosis and transcriptional regulation (Figure 1-19)(Jaffe and Hall, 2005; Vega and Ridley, 2008).

It has been shown that Rho GTPase contribute to the survival in some cancer cell type. They are also involved in transformation and angiogenesis but unlike Ras GTPases, mutation in the Rho subfamily in cancer are rare (Bryan and D'Amore, 2007). Rho expression is increased in number of cancers such as skin, liver, colon and ovarian (Vega and Ridley, 2008; Karlsson *et al.*, 2009). Specifically, RhoA, RhoC and Cdc42 are reported to increase their activity and/or expression, while RhoB is down regulated in human tumours (Vega and Ridley, 2008; Arias-Romero and Chernoff, 2013; D. Yang *et al.*, 2017). In addition, the increased expression of RhoA, Rac and Cdc42 is correlated with prognosis, recurrence and progression as well (Kamai *et al.*, 2004; Karlsson *et al.*, 2009).

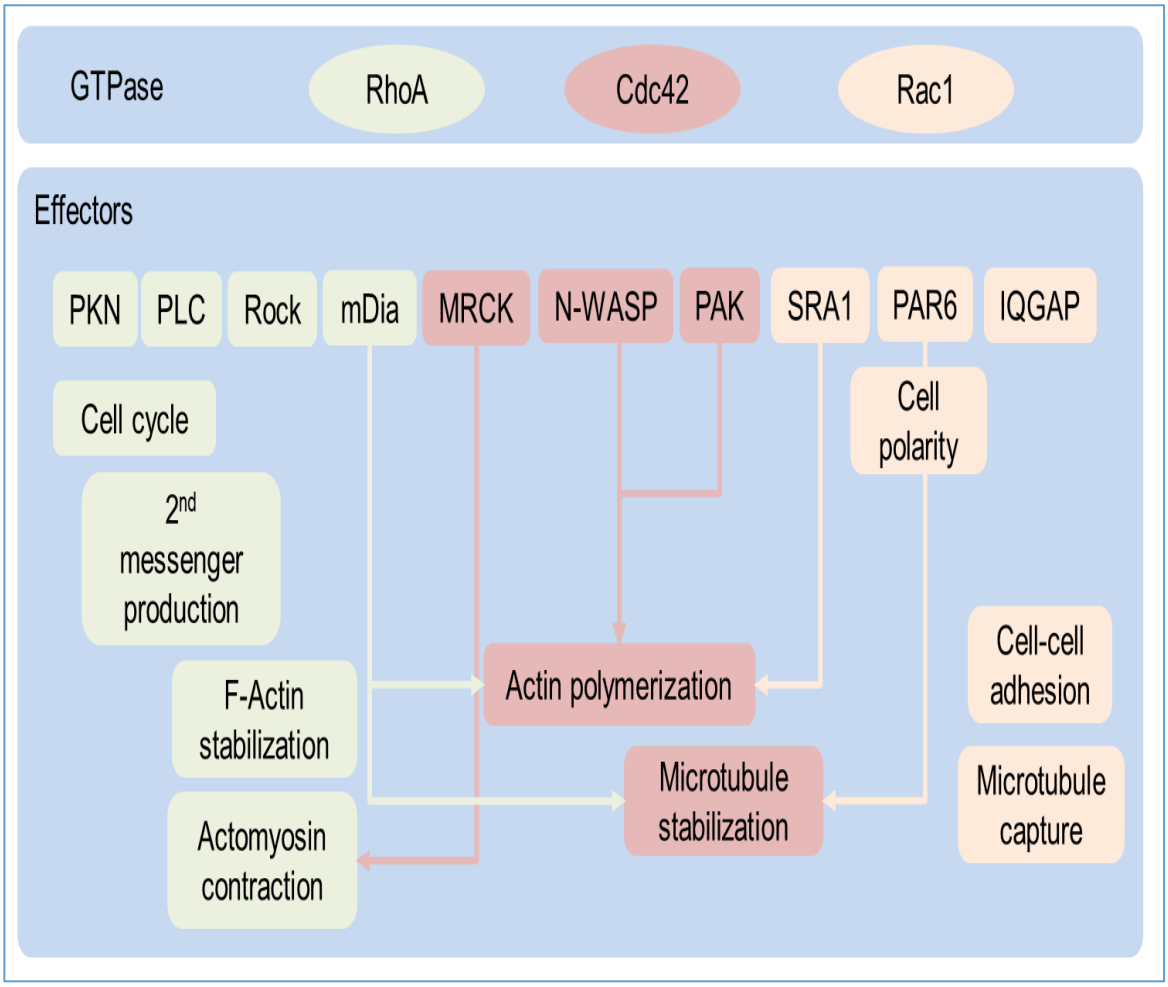


Figure 1-19 Effectors of Rho GTPases

Rho family proteins are stimulated by receptors such as receptor tyrosine kinases, G-protein-coupled receptors and adhesion receptors (integrins and cadherins). Cell-cell adhesion and cell polarization is mediated by effectors of active Cdc42 and Rac1. The activity of Rho proteins is accomplished by actin polymerization at cell protrusions, stabilization and capture of microtubules and positioning of the cytoskeleton and organelles (such as Golgi, centrosomes and nucleus). One of the important mediators of the cytoskeleton organization is p21-activated kinase (PAK) which is an effector of Cdc42 and Rac. The active RhoA regulates a number of downstream signalling process include membrane retraction by actinomycin based stress fibre contraction, cell division and cell cycle progression. The assembly of the proteins machineries which is required for actin polarization are initiated by RhoA binding to mDia or Cdc42 binding to N-WASP. MRCK, myotonic dystrophy kinase-related Cdc42-binding kinase; F-actin, filamentous actin; IQGAP, IQ motif-containing GTPase-activating protein; PAR6, partitioning defective-6; PKN, protein kinase N; PLC, phospholipase C; SRA1, specifically Rac1-associated protein-1; mDia, formin mammalian diaphanous; N-WASP, neural Wiskott–Aldrich syndrome protein. Reproduced with permission from (Iden and Collard, 2008).

1.2.6.3. Rab GTPases

There are more than 60 members of Rab subfamily (Vigil *et al.*, 2010). The main function of Rab GTPase is to control vesicle trafficking between organelles which regulates protein secretion, endocytosis, recycling and degradation (Figure 1-20) (Recchi and Seabra, 2012; Srikanth, Woo and Gwack, 2017). Rab GTPases have been demonstrated to contribute to tumour-stromal cell communication and cell cycle progression in some tumour types (Recchi and Seabra, 2012). In addition, Rab proteins have been implicated in cancer progression and metastasis (Yang et al. 2017). Rab25 has been found to promote migration and invasion of cancer and its over-expression also correlates with poor survival in OC (Cheng *et al.*, 2004; Caswell *et al.*, 2007). Lastly, a number of proteins from this family are also involved in drug resistance. For example, Rab4a and Rab6, are underexpressed in MDR cells while their overexpression is associated with increased sensitivity of cancer cells to cytotoxic drugs as a result of increased intracellular accumulation. In contrast, Rab8 overexpression in sensitive cancer cells enhances their resistance to cisplatin (Recchi and Seabra, 2012).

1.2.6.4. The Arf family

The Arf family consist of about 27 proteins and there are three classes of Arf proteins, class I (Arfs1–3), class II (Arfs 4–5), and class III (Arf6). They had been implicated in number of cellular processes such as vesicle membrane traffic, morphology, metabolism, actin cytoskeleton, endocytosis and exocytosis (Kahn, 2003). Despite being a part of the Ras superfamily, Arfs are not subjected to prenylation, instead they are localized to membrane by the addition of 14 C-atom myristate fatty acid (Konstantinopoulos, Karamouzis and Papavassiliou, 2007). The Arf family play a critical role in cancer progression and might be a prognostic factor for cancer patients. The aberrant activity or expression of Arf family proteins has been shown to have a role in migration, invasion and proliferation (Casalou,

Faustino and Barral, 2016). The expression of some Arf proteins were found to be upregulated in breast cancer cell lines (Schlienger *et al.*, 2016). Lastly, it was recently reported that Arf is negatively correlated with miR-221-3p, whose higher expression is associated with better overall survival in epithelial OC patients (Q. Wu *et al.*, 2017).

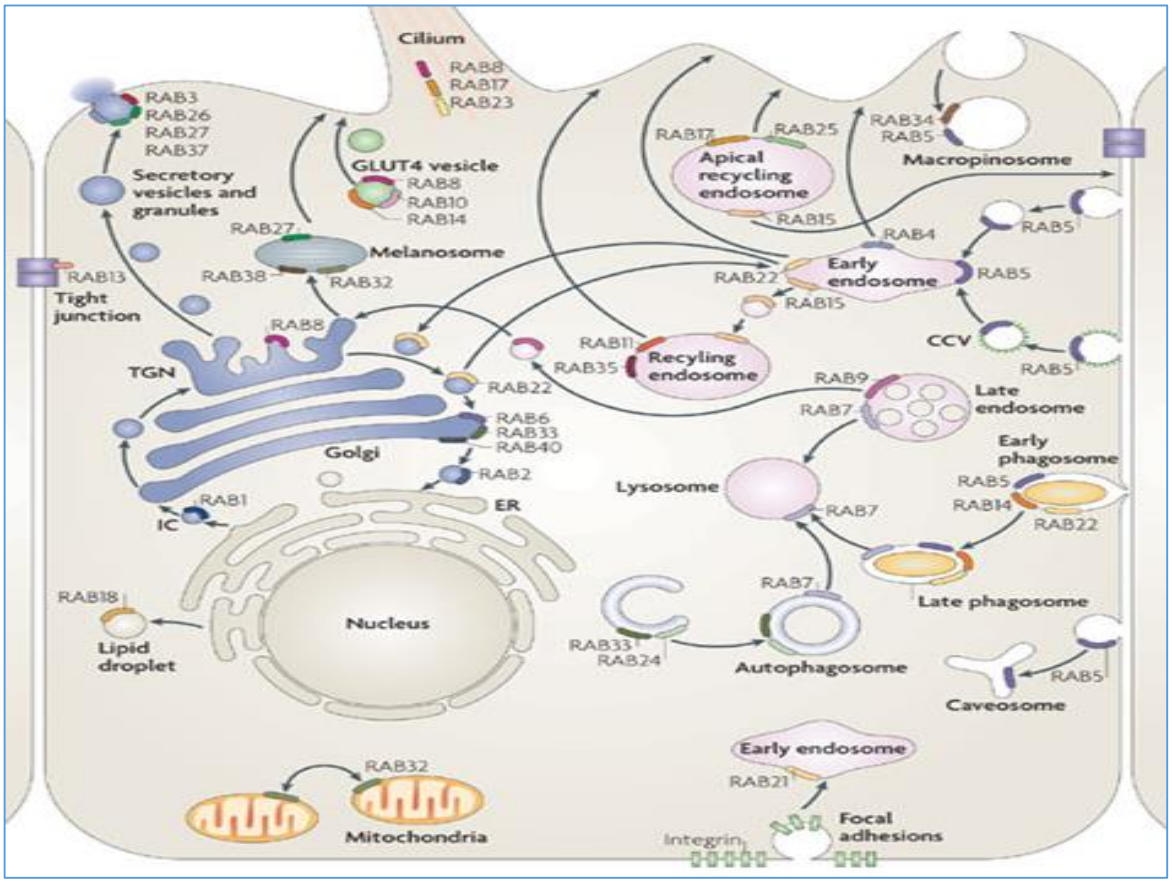


Figure 1-20 The functions and localization of Rab-GTPase.

This figure shows epithelial cells and the localization of selected Rab proteins in the vesicle transport pathways. Rab1 is located at the ER (blue) exit sites and the Golgi (grey) intermediate compartment and is responsible for mediating the trafficking from the ER to the Golgi apparatus. Rab6, 33 and 40 mediate trafficking within the Golgi apparatus. The formation of autophagosomes is controlled by Rab33 together with Rab24. Lipid droplet formation is mediated by Rab18 and the trafficking between the Golgi network and early endosomes is mediated by Rab22. Biosynthetic trafficking from Golgi apparatus to plasma membrane is mediated by Rab8. In addition, Rab8 together with Rab10 and Rab14 involved in GLUT4 vesicle translocation and with Rab17 and Rab23 in ciliogenesis. Rab3, 26, 27 and 37 mediate translocations of melanosomes to the cells periphery and also mediate several types of regulated exocytic events. The assembly of tight junction between epithelial cells are regulated by Rab13. Rab32 and 38 participate in biosynthesis of melanosomes and Rab32 also

regulates mitochondrial fission. Rab5 is positioned to early endosomes, phagosomes, caveosomes and the plasma membrane stimulate endocytosis and endosome fusion of the clathrin coated vesicles (CCVs). Rab5 together with Rab34 mediate micropinocytosis and with Rab14 mediate maturation of early phagosomes. Integrin endocytosis is mediated by Rab21. Rab4 mediate the fast-endocytic recycling whereas Rab11 and 35 mediate the slow endocytic recycling from early endosomes. Rab15 is participated in the trafficking from the apical recycling endosomes to the basolateral plasma membrane and in trafficking from early endosomes to recycling endosomes. The trafficking form the apical recycling endosomes to the apical plasma membrane is regulated by Rab17 and Rab25. Maturation of the late endosomes and phagosomes and their fusion with lysosomes is mediated by Rab7. Lastly, Rab9 regulate trafficking form the late endosomes to the Golgi networks. Reprinted with permission from (Stenmark, 2009).

1.2.6.5. Ran GTPase

The fundamental cellular function of the Ran GTPase is nucleocytoplasmic transport (Figure 1-21). The Ran protein is involved in mitotic spindle assembly, microtubule nucleation and dynamics and post-mitotic nuclear assembly (Dasso, 2002). A number of studies have showed that Ran GTPase is implicated in cancer cell growth, tumour transformation, resistance to apoptosis, tumour aggressiveness and increased metastasis in several types of cancers (Abe *et al.*, 2008; Kurisetty *et al.*, 2008; Xia, Lee and Altieri, 2008; Ly *et al.*, 2010; Yuen *et al.*, 2012). Ran is differentially overexpressed in cancer tissue as compared with normal tissues and its expression is correlated with tumour progression (Kau, Way and Silver, 2004). Acute silencing of Ran in cancer cells induces mitochondrial dysfunction and causes cell death. Therefore, these evidences suggest that Ran pathway might be an important target for cancer treatment (Xia et al. 2008).

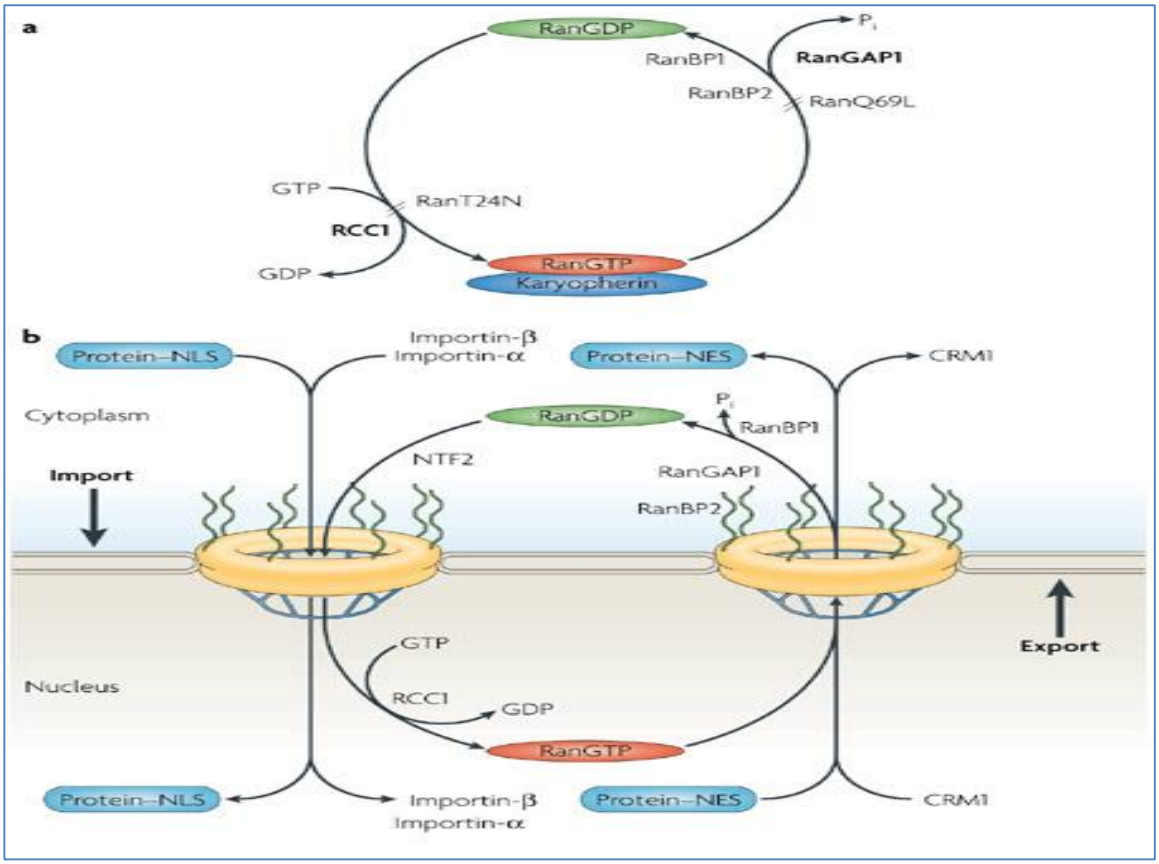


Figure 1-21 Nucleocytoplasmic Transport by Ran GTPase.

(a) the GTP (active) and GDP (inactive) cycle of Ran. Guanine nucleotide-exchange factor and Regulator of chromosome condensation 1 (RCC1) catalyse the conversion of Ran GDP to Ran GTP, which in turn interact with the Karyopherins, a transporter factor for the family of importin-β. In contrast, Ran-binding protein-1 (RanBP1) or RanBP2 stimulate Ran GTPase-activating protein, RanGAP1 to induce the hydrolysis of GTP to GDP which does not interact strongly with karyopherin. There are number of mutation that prevent GTP-GDP cycle of Ran such as RanT24N and RanQ69L.

(b) The nuclear transport factor-2 (NTF-2) facilitates the active transport of Ran through the nuclear pores across the nuclear membrane. In the nucleus, the chromatin-bound RCC1 catalyses the generation of high concentration of the Ran GTP by nucleotide exchange. High concentration of Ran GTP induce the separation of the imported complexes by binding to importin-β and ejection of the cargo which carry the nuclear localization signal (NLS). On the contrary, the assembly of the export complexes, which contain protein with a nuclear export signal (NES), is promoted by binding of the RanGTP to the chromosome-region maintenance protein-1 (CRM1). In cytoplasmic compartment, RanGTP hydrolysis is stimulated by RanGAP1 and RanBP1 or RanBP2 to release the exported complexes. Reprinted with permission form (Clarke and Zhang, 2008).

1.2.7. Clinical implication

A significant body of research has demonstrated the importance of the MP in health and disease. Inactivation of the MP appears to be fatal in some animal models. Mice deficient in HMGCR stop developing at the blastocyst stage, whereas, mice that have squalene synthase deficiency demonstrate retardation in growth and defects in neural tubes (Ohashi *et al.*, 2003). In addition, gunmetal mice, a GGT-II deficient mouse, matures normally but display a defect in platelets function and prolongation of the bleeding time (Zhang *et al.*, 2002).

Mevalonic aciduria (MAU) and Hyperimmunoglobulinaemia D syndrome (HIDS) are two inherited disorder caused by mutation in the mevalonate kinase (MK) gene which lead to a disruption in the activity of MK, one of the crucial enzyme in MP (Haas and Hoffmann, 2006). MAU is a rare autosomal recessive inborn disorder associated with multiple abnormalities (Normand *et al.,* 2009). The main sign and symptoms of the disorder are recurrent febrile crises and inflammatory episodes, which are also associated with psychomotor retardation, failure to thrive, ataxia, cataracts, retinitis pigmentosa, uveitis, hepatomegaly, lymphadenopathy, vomiting, diarrhoea, arthralgia, myopathy, skin rash and mucosal ulcers. MAU has a poor prognosis and the patients usually die in early childhood (van der Burgh *et al.*, 2013).

In contrast to MAU, in which MK activity is completely abolished, the residual activity of the MK is 5-15% in HIDS which is characterized by early childhood onset of recurrent febrile attacks, triggered by infection, trauma, stress, surgery and vaccination. Some patients may develop neurological symptoms, mental retardation, ataxia, epilepsy and ocular disorder. The defect in the MK leads to increases in the concentration of mevalonic acid in plasma. In addition, the accumulation of mevalonic acid in plasma is accompanied by a

shortage in the production of the downstream products of the MP in both MAU and HIDS (Haas and Hoffmann, 2006; Buhaescu and Izzedine, 2007).

Scientists have failed to produce effective treatment for these disorders in spite of the huge improvement in understanding of the molecular pathogenesis of both disorders. However, two options are available, etanercept and anakinra, but these drugs have unreliable beneficial effects. The counter intuitive use of statins in MAU and HIDS is support by limited and conflicting data. Lovastatin can provoke a severe clinical crisis while, simvastatin shows a reduction of the febrile attacks in HIDS patients (Buhaescu and Izzedine, 2007). However, the clinical crisis might be resolved within one day of corticosteroid administration. Administration of vitamin C, vitamin E and ubiquinione-50 for long-term have a stabilizing effect on the clinical course and improve the development (Haas and Hoffmann, 2006).

1.2.8. Mevalonate pathway as therapeutic target

The foregoing discussion illustrates that pharmacological manipulation of the MP is a novel, attractive and promising therapeutic target for the treatment of many disorders such as autoimmune disorder, Alzheimer's disease, atherosclerosis and cancer (Swanson and Hohl, 2006; Buhaescu and Izzedine, 2007). Statin administration to mice with multiple sclerosis (MS), an autoinflammatory disease, at earlier stage reverses the symptoms and prevents the progression to recurrent or chronic paralysis (Weber *et al.*, 2006). In transgenic mice of Alzheimer disease, the use of statins assists in inverse learning and memory deficits (Li *et al.*, 2006). In addition, the immunomodulatory effects of statin might reverse neurodegeneration in MS, Alzheimer's and Parkinson disorders, through reduction of protein isoprenylation (Butterfield, Barone and Mancuso, 2011).

There are multiple pharmacological targets in the MP that might be targeted for treatment of cancer (Figure 1-12). Beside statins and nitrogenous bisphosphonates (NBPs) which inhibits

the HMG-CoA reductase and Farnesyl pyrophosphate synthase respectively, there are number of other drugs which modulate the MP (Table 1.5). In addition, toxic effects in non-diseased cells might result from complete inhibition of single enzyme activity or from off-target effects. Hence, targeting more than one enzyme in the MP might be a superior option to overcome toxicity and being more effective for the treatment of cancer. Moreover, many studies show that the synergistic effect of MP inhibitors with conventional anticancer drugs might augment the therapeutic outcomes (Swanson and Hohl, 2006).

1.2.9. Mevalonate pathway enzymes inhibitor

1.2.9.1. Statins

In 1971, Endo, the father of statins, started searching thousands of fungi with the hope of discovering a compound that will be able reduce cholesterol level in human. By the end of one year of searching, he found a compound called compactin or mevastatin that inhibits HMGCR activity with high potency. However, the compound was soon withdrawn from the market because of the suspected adverse effect. In the 1990, the development of the analogs of mevastatin opened the way for the marketing authorization of statins such as lovastatin, simvastatin and pravastatin (Endo, 2010).

Globally, statins ranked as the most commonly used drug (Collins *et al.*, 2016). Their main indication is to treat hypercholesterolemia particularly for preventing myocardial infarction, ischemic heart disease and peripheral arterial disease (Taylor-Harding *et al.*, 2010; Osmak, 2012). Nonetheless, due to the pleiotropic properties of statins, their beneficial effect have been demonstrated in other disease, for instance Alzheimer's, multiple sclerosis and ischemic stroke disorders (Sławińska-Brych, Zdzisińska and Kandefer-Szerszeń, 2014). Statins have anti-inflammatory and immunomodulatory effects, neuro-protective effects, positive effects on bone metabolism and improve the prognosis in chronic kidney disease.

Therefore, statins might be a potential therapeutic option for different disorders (Gazzerro *et al.*, 2012).

Table 1-5 Targets in the MP with anticancer activity.

Drugs	Site of action	Mechanism of action	References
Statins (Simvastain, Fluvastatin, Atorvastatin, Lovastatin)	HMGCR	-Cell cycle arrest -Induction of apoptosis and autophagy	(Liu *et al.*, 2009; Li *et al.*, 2010; Martirosyan *et al.*, 2010; Scoles *et al.*, 2010; Taylor-Harding *et al.*, 2010; Robinson *et al.*, 2014)
Apomine	HMGCR	-Down regulation of the HMGCR by increase degradation -Induction of apoptosis -Cell cycle arrest	(Edwards *et al.*, 2007; Roelofs *et al.*, 2007; Moriceau *et al.*, 2010, 2012)
NBP (Aldronate, Ibandronate, Clodronate, Zoledronic acid)	FPPS	-Cell cycle arrest -Induction of apoptosis -Inhibition of proliferation, migration and invasion -Antiangiogensis	(Hashimoto *et al.*, 2007; Stresing *et al.*, 2007; Mahtani and Jahanzeb, 2010; Clézardin, 2011; Gnant and Clézardin, 2012; Winter and Coleman, 2013; Okamoto *et al.*, 2014)
Zaragozic acid	Sequalene synthase	-Reduction of cholesterol synthesis -Accumulation of the FPP products	(Brusselmans *et al.*, 2007; Henneman *et al.*, 2011)
FTase inhibitors (Tipifarnib, Lonafarnib)	FTase	-Inhibition of protein farnesylation which play crtical role in growth and proliferation	(Buhaescu and Izzedine, 2007; Holstein and Hohl, 2012; Meier *et al.*, 2012; Abuhaie *et al.*, 2013; Volpe *et al.*, 2013)
GGT-I inhibitors (GGTI-2133)	GGT-I	-Cell cycle arrest -Inhibition of migration and invasion of tumour cells	(Swanson and Hohl, 2006; Buhaescu and Izzedine, 2007)
GGT-II inhibitors	GGT-II	-Interruption of Rab protein function	(Swanson and Hohl, 2006; Sane *et al.*, 2010; Wasko, Dudakovic and Hohl, 2011)
Dualprenyltransferase inhibitors	FTase & GGT-I	-Inhibition of both farnsylation and geranylgeranylation of Ras GTPase proteins	(Lobell *et al.*, 2002; Zhang *et al.*, 2009)

Statins are either naturally derived or chemically synthesized (Shah *et al.*, 2015). Statins are structurally are composed of two components (Figure 1-22), the dihydroxyheptanoic acid which is the pharmacophore and the ring system with different substituents. Statins competitively inhibit HMGCR enzyme in dose-dependent and reversible manner. The stereoselectivity of the HMGCR enzyme to statins depends on chemical structure of the pharmacophore which are either an inactive closed lactone ring or active open hydroxy carboxylic acid moiety (Gazzerro *et al.*, 2012). The binding affinity of statins is 1000-fold more than the natural substrate for HMGCR enzyme (Clendening and Penn, 2012). The function of the ring system is to prevent statins displacement from the enzyme by the natural substrate. In addition, the substituents of the ring system are one of the major determinants of the pharmacokinetic characteristics of the statins (Wong, Dimitroulakos and Penn, 2002; Martirosyan *et al.*, 2010).

Generally, statins are well tolerated (Collins *et al.*, 2016). The most frequently reported adverse effects of the standard doses are mild, such as constipation, flatulence, nausea and gastrointestinal pain. Rhabdomyolysis, myopathy, myositis, autoimmune disorders, cardiac dysfunctions and elevated liver enzymes are less common but potential serious adverse effects of this family of drugs (Gazzerro *et al.*, 2012; Moon *et al.*, 2014). Several factors have been recognized to increase the incidence of statins adverse effects such as the age, gender, family history, concomitant disease and drug-drug interactions (e.g. cimetidine, clarithromycin and erythromycin) (Ahmad, 2014). Even though the safety profile is understood, statin therapy requires careful monitoring.

The antitumour activity of statin is a result of suppression of proliferation, cell cycle arrest and induction of apoptosis (Hindler *et al.*, 2006; Matusewicz *et al.*, 2015). Statins induces cell cycle arrest at G_1/S phase boundary by up-regulation of the cell-cycle inhibitors

$p21^{WAF1/CIP1}$ and/or $p27^{KIP1}$ and reduce the expression of the CDK2, CDK4, Cyclin D1 and Cyclin E (Wong, Dimitroulakos and Penn, 2002; Morgan *et al.*, 2005; Sleijfer *et al.*, 2005; Clendening and Penn, 2012; Gazzerro *et al.*, 2012). In addition, others have also reported G_2/M phase cell cycle arrest in lymphoma cells and breast cancer cells (Sánchez CA *et al.*, 2008).

In contrast, statins activation of the extrinsic apoptotic pathway appears to be dependent on tumour type. Statins stimulate caspase-8 activation which amplifies the executioner caspases activity in myeloma cells (Cafforio *et al.*, 2005). In prostate cancer cells, simvastatin cause activation of caspase-8 through upregulation of TNF-α and FasL (Chapman-Shimshoni *et al.*, 2003; Goc *et al.*, 2012). It has been suggested that statins activate the death receptor signalling pathway by interruption of cholesterol synthesis which leads to change the organization of cholesterol rich membrane raft. Mevastatin administration cause spontaneous ligand-independent clustering of Fas and formation of the Fas-FADD complexes which led to activation of caspase-8 and apoptosis (Gniadecki, 2004).

Numerous preclinical studies have evaluated statin activity against various cancers. It has been demonstrated that statins exhibit significantly different cytotoxic potentials. For instance, Wong, et al., (Wong *et al.*, 2001) evaluated the antitumour activity of different statins (fluvastatin, atorvastatin, and cerivastatin) on human acute myeloid leukaemia cells. He found that AML cells are at least ten-fold more sensitive to cerivastatin-induced apoptosis in comparison with other statins. Furthermore, Glynn, et al. (Glynn *et al.*, 2008) tested the statins' (simvastatin, lovastatin, mevastatin and pravastatin) ability to inhibit tumour growth of lung carcinomas, breast carcinomas and malignant melanomas cell lines. Simvastatin had the highest potency compared with lovastatin and mevastatin. In contrast, pravastatin was ineffective for inhibiting proliferation. However, cell lines from lung and

malignant melanoma were more sensitive to statins in comparison to breast cancer cell lines (Swanson and Hohl, 2006). It has also been concluded that lipophilicity is one of the major determinants of statins activity against cancer, as more potent effects are observed with lipophilic statins (Martirosyan *et al.*, 2010; Corcos and Le Jossic-Corcos, 2013; Zhang *et al.*, 2013).

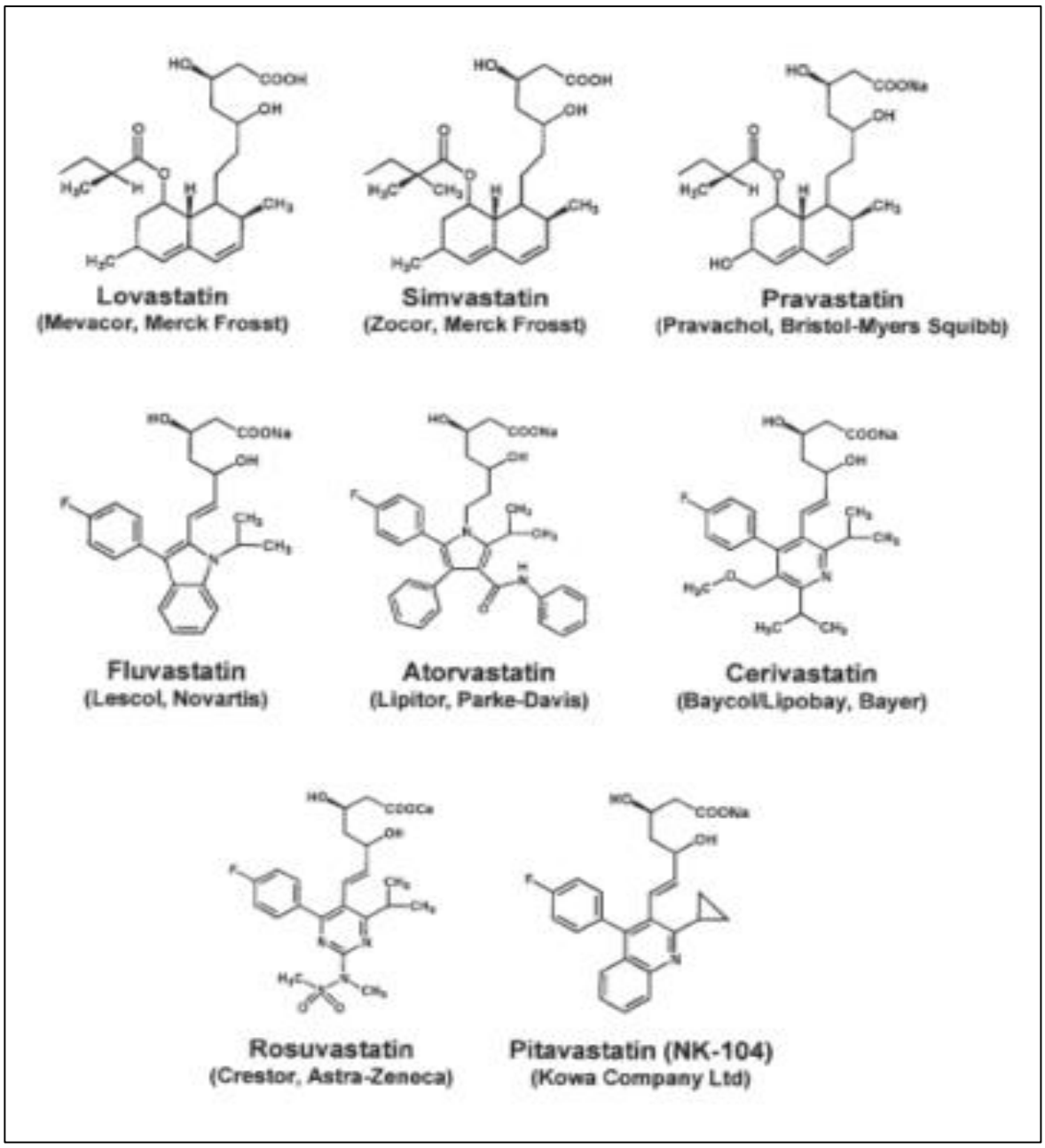


Figure 1-22 Chemical structures of the statin family are shown in their open ring active form (Wong, Dimitroulakos and Penn, 2002)**.**

Retrospective clinical studies had shown that statins have pleiotropic and multi-favourable effects in biological behaviour of tumour (Altwairgi, 2015). Several studies consistently demonstrate that patients using statins to control cholesterol have reduced cancer risk and cancer related mortality (Cuello F *et al.,* 2013). Meta-analysis and retrospective cohort studies revealed that statins improve the overall survival of patients with kidney cancer, pancreatic cancer and OC (Habis *et al.,* 2014; Huang *et al.*, 2017; Nayan *et al.,* 2017). In prostate cancer, Jespersen et al., (Jespersen *et al.,* 2014) state that statin use is associated with reduced risk and Yu et al., (Yu *et al.,* 2014) reported that statins use after cancer diagnosis correlated with a decreased risk of mortality. Statins also enhance the therapeutic activity and overall survival lung cancer patients receiving EGFR-TK inhibitors therapy (Hung *et al.,* 2017). Randomized controlled trial in advanced hepatocellular carcinoma patients treated by transcatheter arterial embolization and 5-flurouracil or transarterial chemoembolization shows that the median survival time is doubled upon the addition of the statin to the previous treatment modality (Kawata *et al.,* 2001; Graf *et al.,* 2008).

The Cancer in The Ovary and Uterus Study (case-control study) evaluated the effect of statins prior to and following diagnosis of ovarian and endometrial cancers in a subset of 424 patients and 341 cases as controls. It found that the use of statins for more than 1 year before diagnosis was associated with a reduction in cancer risk and prolonged survival was noted among those patients when statins were prescribed after cancer diagnosis (Lavie *et al.,* 2013). In addition, it is observed that OC risk was reduced by the use of statins and the effect was stronger in patients using statins for a prolonged period (Liu *et al.,* 2014). An improvement in survival was observed in epithelial OC patients using statin after the diagnosis (Elmore *et al.,* 2008). In contrary, others had found no association between statin use and cancer incidence. Several meta-analysis did not support a protective effect of statins on cancer incidence such as lung cancer, bladder cancer and skin cancer (Bonovas *et al.,*

2005; Kuoppala, Lamminpää and Pukkala, 2008; da Silva *et al.*, 2013; Li, Wu and Chen, 2014).

Evaluation of the antitumour activities of statins in clinical trials have had conflicting results and are faced by number of difficulties. In 1996, Thibault et al., (Thibault *et al.*, 1996) investigated the effect of 2-45 mg/kg/day orally-administered lovastatin in four divided doses for one week course in monthly bases. It was found that lovastatin in a dose of 25 mg/kg/day was well tolerated but only 1 minor response out of 88 patients was observed. In addition, the author observed that 35 mg/kg was associated with transient adverse effects such as myalgia and elevated level of serum creatinine phosphokinase. However, ubiquinone co-administration did not prevent the occurrence of the adverse effect but decreased their severity. In addition, pravastatin was investigated in advanced hepatocellular carcinoma. Random allocation of the patients to either pravastatin group or control group, after standard therapy, revealed that statin group had significantly longer median survival as compared to the control group (Kawata *et al.*, 2001). Simvastatin (15 mg/kg/day) administered orally for multiple myeloma patients on days 1-7 was followed by intravenous infusion of vincristine, doxorubicin and dexamethasone orally on day 7 to 10. Haematological (neutropenia and thrombocytopenia) and gastro-intestinal toxicity but not rhabdomyolysis was reported. The study stopped as the response was insufficient and it was suggested that although simvastatin is very effective in vitro, its short half-life might be the main cause for failure of the study. In addition, it was proposed that statins with long half-life and continuous administration are required to maintain high plasma level in patients which is essential to induce cell death (Van Der Spek *et al.*, 2006; van der Spek *et al.*, 2007).

Lastly, the data from experimental studies illustrate that stains had little toxicity on cells from normal tissue (Martirosyan *et al.*, 2010; Corcos and Le Jossic-Corcos, 2013; Zhang *et*

al., 2013). Likewise, some statins might have protective effect against doxorubicin induced toxicity on liver, renal and cardiac tissue (El-Moselhy and El-Sheikh, 2014). Statins, in combination with radiotherapy in clinical trial, demonstrated a reduction in rectal toxicity and also delay the intestinal damage in animal models (Begg, Stewart and Vens, 2011). It has been reported that normal cells, for example, ovary, neuronal, lung fibroblast and endothelial cells, might be more resistant to collateral damage produced by statins than cancer cells (Hindler *et al.*, 2006; Liu *et al.*, 2009; Clendening and Penn, 2012; Sławińska-Brych, Zdzisińska and Kandefer-Szerszeń, 2014). Taken together, it appears reasonable to propose that statins might be potential candidates for repurposing in cancer therapy (Konstantinopoulos, Karamouzis and Papavassiliou, 2007; Gazzerro *et al.*, 2012).

1.2.9.2. Nitrogenous bisphosphonates

Nitrogenous bisphosphonates (NBPs) drugs are commonly indicated for management and prevention of bone disease such as postmenopausal osteoporosis, corticosteroid-induced bone loss, Paget's disease and skeletal lesions due to malignancy (Issat *et al.*, 2007; Bosch-Barrera *et al.*, 2011). The therapeutic effect of NBPs is mainly through inhibition of bone resorption mediated by osteoclasts and reduction of calcium release and other minerals from bone. Although, NBPs are widely used, their adverse effects are not well defined. However, jaw osteonecrosis is a rare but reported adverse effect (Rennert, Pinchev and Rennert, 2010).

Bisphosphonates can be classified in to two classes according to structure and mechanism of action. The first class is pyrophosphate-resembling bisphosphonates (e.g. clodronate, etidronate), which are converted into cytotoxic non-hydrolyzable ATP analogues products which decrease mitochondrial membrane potential by inhibiting the ATP-dependent enzymes to produce cytotoxic effects. The second class is nitrogen-containing bisphosphonate (e.g. alendronate, pamidronate, risedronate, ibandronate and zoledronic

acid) which inhibit a crucial enzyme, FFP synthase, in the mevalonate biosynthetic pathway (Okamoto *et al.*, 2014; Zekri, Mansour and Karim, 2014). Therefore, NBPs deplete the isoprenoid pools and prevent the prenylation process of GTP proteins (Manoukian *et al.*, 2011).

Several studies emphasize the ability of NBPs use to reduce the risk of several cancer types such as colon and breast cancer (Chlebowski *et al.*, 2010; Rennert, Pinchev and Rennert, 2010). In addition, evidence suggest that NBPs have direct antitumour activity against number of transformed cell lines for instance myeloma, breast, pancreatic, prostate, ovarian, colon and hepatic cancer (Siddiqui *et al.*, 2014). The antitumour effects of NBPs include inhibition of proliferation by induction of cell cycle arrest and programmed cell death (Shai *et al.*, 2014). Furthermore, combining NBPs with the standard anticancer agents enhance the therapeutic activity of several regimens *in vitro* (Horie *et al.*, 2007).

1.2.9.3. Prenyltransferases inhibitors

There is major challenge associated with development of direct pharmacological inhibitors of Ras. The picomolar affinity of Ras for GTP makes the intervention of the nucleotide-binding pocket of Ras much more difficult than interfering with ATP-binding pocket of kinases (Bommi-Reddy and Kaelin, 2010; Samatar and Poulikakos, 2014) with exception of sorafenib which has some Ras kinase inhibitor activity (Asati, Mahapatra and Bharti, 2016). It is known that proper Ras function requires prenyl transferase enzyme activity (Maynor *et al.*, 2008). This has been prompted the development prenyl transferase inhibitors as potential anticancer drugs (Maynor *et al.*, 2008).

The crystal structure of FTase revealed that the enzyme has two binding sites which are involved in recognition of the FPP and of the CAAX box of the protein substrate (Appels, Beijnen and Schellens, 2005). Therefore, several strategies have been developed to inhibit

farnesylation of Ras and inhibitors can be divided into three main categories based on site and mechanism of action. These are FPP competitive inhibitors, CAAX competitive inhibitors and bi-substrate analogue compounds that inhibit both FPP and CAAX (Crul *et al.*, 2001; Downward, 2003; Wasko, Dudakovic and Hohl, 2011). However, there are major concerns about the action of these inhibitors because Ras proteins can be geranylgeranylated when the FTase enzyme is inhibited. Thus, the potential of cross prenylation of Ras by GGTI might reactivate these proteins and cause failure of the therapy (Brunner *et al.*, 2003; Appels, Beijnen and Schellens, 2005).

Several FTase inhibitors have been identified in each category and have undergone extensive assessment both *in vitro* and *in vivo*. The antitumour effects of FTase inhibitors in laboratory studies has been associated with induction of programed cell death, inhibiting angiogenesis and cell cycle arrest of some cancer cell lines regardless of Ras mutational status (Sebti and Hamilton, 2000; Wang, Yao and Huang, 2017). In addition, these inhibitors showed activity *in vivo* on xenografts of nude mice (Yeganeh *et al.*, 2014). However in clinical trials FTase inhibitors have generally performed poorly except in myeloid leukaemia and pituitary adenomas (Bell, 2004; Asati, Mahapatra and Bharti, 2016).

Inhibitors of geranylgeranyl transferases (GGTIs) enzymes was not initially considered as a promising target due their expected toxic effects (Yeganeh *et al.*, 2014). Like FTase inhibitors, GGTIs have displayed a promising result *in vivo* and *in vitro* (Berndt, Hamilton and Sebti, 2011). These inhibitors induce apoptosis, reduce tumour invasiveness, induce cell cycle arrest in several cell lines through preventing Rho isoprenylation, and some of these inhibitors act on N-Ras and Rab GTPase as well (Coxon *et al.*, 2001; Kusama *et al.*, 2003). Administration of GGTI-2154 to mouse mammary tumour virus Ha-Ras mice, a breast cancer model, causes tumour regression by induction of apoptosis (Sun *et al.*, 2003).

Additionally, treatment of lung cancer mouse model with GGTI-297 or GGTI-2154 decrease the cancer tumour volume by 40 and 60%, respectively (Sun *et al.*, 1999).

1.3. Background of *TP53*

Thirty years ago, an approximately 53 kDa protein which complexes with the viral SV40 T-antigen was proposed as proto-oncogene in the first decade after its discovery (Brosh & Rotter 2009; Lane & Crawford 1979; Linzer et al. 1979). Later on, the potential of increased tumourgenicity by overexpression of mutant p53 in p53 null cells was demonstrated (Wolf, Harris and Rotter, 1984). In 1989, the Levine laboratory revealed the tumour suppressive activity of wild-type p53 overexpression in rat embryo fibroblast and they suggested that p53 might block transformation (Finlay, Hinds and Levine, 1989). Therefore, the initial view of p53 mislead researchers to consider it as an oncogene because they analysed mutated forms of *TP53* isolated from tumour cells (Brosh and Rotter, 2009).

The *TP53* gene is widely acknowledged as one of the most frequent genetic alteration in all of human malignancies (Levine and Oren, 2009; Oren and Kotler, 2016; Napoli and Flores, 2017). *TP53* mutations range from 5-80% and depend on type, stage, and aetiology of cancers (Petitjean *et al.*, 2007) and it is found in a broad range of cancers with highest rate in OC (47%), colorectal (43%), head/neck (42%), and oesophageal cancers (41%) (Kandoth *et al.*, 2013; Sorrell *et al.*, 2013; Cole *et al.*, 2016). Over 96% of high-grade serous OC displaying *TP53* mutation (Cole *et al.*, 2016; Oren and Kotler, 2016). In addition, *TP53* mutation might be an early molecular event associated with malignant transformation of fallopian tube cells to OC (Lee *et al.*, 2007). Li-Fraumeni syndrome patients, an inherited germline mutation in p53 allele, are more prone to develop a variety of cancer such as tumour of breast, brain, bone, bladder and soft tissue sarcoma (Olive *et al.*, 2004; Brosh and Rotter,

2009). These data supported the conclusion that p53 was in fact a tumour suppressor (Levine and Oren, 2009).

1.3.1. Function of wild type p53

The tumour suppressor p53, guardian of the genome, is a central hub in living cells, which is allied to a complex network of signalling pathways. "The functional complexity of p53-dependent events is the reflection of the complexity of p53 as a protein" (Laptenko and Prives, 2006; Goldstein *et al.*, 2010). Functionally, p53 is a stress response protein that is activated by different cellular insults such as oncogenic activation and DNA damage (Silwal-Pandit, Langerød and Børresen-Dale, 2017). Activation of p53 initiates cell cycle arrest, senescence or apoptosis by up-regulation or down-regulation of numerous target genes (Harris and Levine, 2005; Beckerman and Prives, 2010; Lane, Cheok and Lain, 2010).

The activities of p53 as well as its half-life are regulated by site-specific modification of certain p53 residues (Laptenko and Prives, 2006). Structurally, p53 can be post-translationally modified by phosphorylation, acetylation and ubiquitination. These modifications add another layer of complexity to p53 (Laptenko and Prives, 2006). For instance, p53 protein is very short lived under unstressed condition due to its continual ubiquitination by MDM2 and proteasomal degradation. In contrast, the half-life is extended in stressful condition by phosphorylation to promotes its stabilization and subsequently lead to accumulation of p53 protein (Brosh and Rotter, 2009; Beckerman and Prives, 2010).

The activation of p53 arises after DNA double-strand breaks trigger activation of ataxia-telangiectasia mutated (ATM), a kinase that phosphorylates the CHK2 kinase, or after collapse of the DNA replication forks enrols ATM and RAD3-related (ATR), which phosphorylates CHK1 (Kastan and Bartek, 2004). P53 is phosphorylated by both ATM and ATR, either directly or through CHK1 and CHK2 (Lavin and Gueven, 2006). This post-

translational modification prevents the interaction of p53 negative regulators MDM2 and MDM4 with activation domains of p53. Thus this post translational modification preventing the ubiquitination and turnover of p53 (Wade, Wang and Wahl, 2010). P53 is also activated by hyperproliferative signals through stimulating the transcription of ARF which in turn antagonize MDM2 by inhibiting the enzyme's ubiquitin ligase activity (Karni-Schmidt, Lokshin and Prives, 2016).

Activation of the P53 (Figure 1-23) has been proposed as model for cisplatin-induced cytotoxicity through up-regulation of pro-apoptotic and down-regulation of the anti-apoptotic proteins (Agarwal and Kaye, 2003). In contrast, inactivation of the p53 is one of mechanisms of cytotoxic drug resistance (Bast, Hennessy and Mills, 2009). Hence, the efficacy of cisplatin chemotherapy is higher in OC patients with wild-type *TP53* than patients with *TP53* mutations (Galluzzi *et al.*, 2012). Lastly, it is well known that cell-cycle arrest and apoptosis are the main prominent consequences of p53 activation (Chen, 2016).

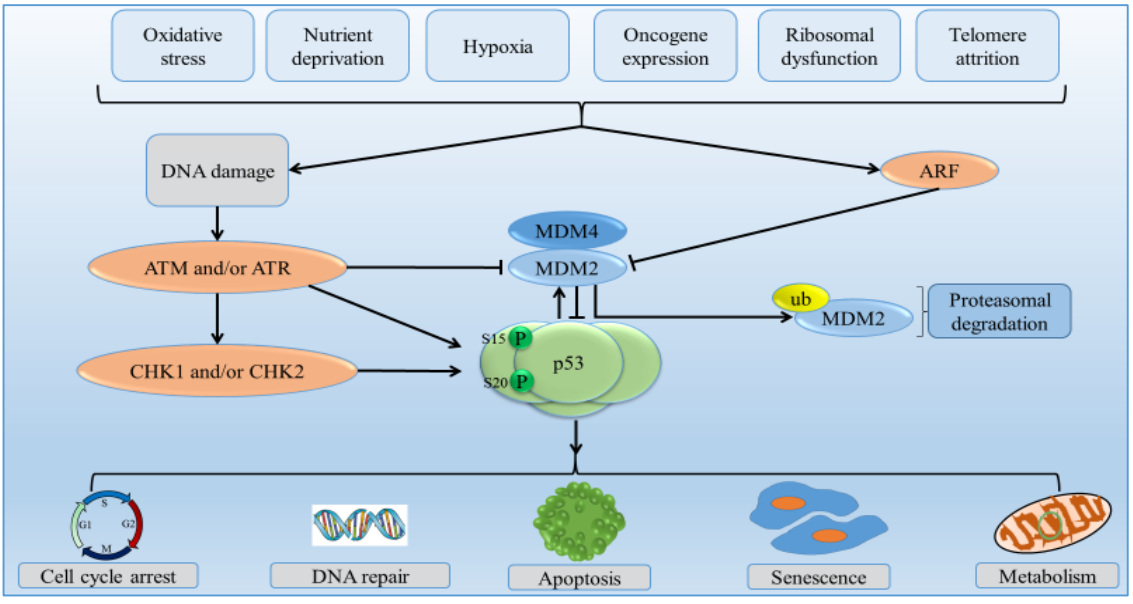


Figure 1-23 The classical view of p53 activation and response

P53 is activated in response to different stimuli that induce either DNA damage or Arf stimulation and lead to activation of several cellular responses. Reproduced with permission from (Bieging, Mello and Attardi, 2014).

1.3.2. P53 and cell cycle arrest

Multiple checkpoint have evolved to monitor and respond to cellular distresses, arresting cellular progression until defects are repaired or the environment becomes acceptable to the faithful transmission of genetic information (Bartek and Lukas, 2007). Perturbations in checkpoint mechanisms not only harmful to the integrity of the genome, but also stimulate tumourigenesis and considerably affect the efficacy of cytotoxic drugs (Abbas and Dutta, 2009).

The cell cycle is a complex process which is strictly synchronized by multiple factors. Cyclins, cyclin dependent kinases (Cdks) and CDK inhibitors are fundamental regulatory machine of this process. There are multiple checkpoints to regulate progression through cell cycle phases, when DNA damage is detected. P53 mediates cell cycle arrest by transcriptional activation of $p21^{WAF1}$, a negative regulator of cell cycle, which in turn binds to and inhibit cyclin E/Cdk2 and cyclin D/Cdk4 complexes to cause cell cycle arrest at G_1 (Chen, 2016). Inhibition of Cdk2 and Cdk4 impedes phosphorylation of Rb and promotes its physical association with E2F1. Therefore, blocking the ability of E2F1 to activate gene expression that encodes products essential for DNA replication and cell cycle progression (Giacinti and Giordano, 2006). P21 also inhibits cellular proliferation by preventing DNA replication which is vital for S phase cell cycle progression (Abbas and Dutta, 2009). Additionally, P53 is mediator of cell cycle arrest at G2/M phases by inhibiting cyclin B and Cdk2 (Deng *et al.*, 1995; Russell *et al.*, 1999). Consequently, p53 maintains genomic stability and improves the survival of damaged cells by allowing time for repairing of potentially lethal DNA damages (Gatz and Wiesmuller, 2006).

1.3.3. P53 and apoptosis

The earliest evidence of p53 acting as mediator of apoptosis was provided by study of temperature-sensitive mutant p53 which is discovered by accident owing to a misadjusted incubator. At 32ºC, the mutant p53 has a wild type activity but this activity is lost at 37ºC (Zhang *et al.*, 1994). It has noted that M_1 leukaemia cell line exhibit a typical feature of apoptosis upon reactivation of temperature sensitive p53 (Yonish-Rouach *et al.*, 1991). In addition, stable transfection of wild-type p53 in human colon tumour-derived cell line EB promote morphological features of apoptosis (Shaw *et al.*, 1992). These studies presented the role of p53 as apoptosis stimulator for preventing tumour development (Levine and Oren, 2009). P53 has the ability to induce a vast number of genes which contribute to different steps of apoptosis signalling (Riley *et al.*, 2008). This includes activation of the extrinsic apoptotic pathway through the induction of death receptor, such as Fas and DR5, stimulating the dimerization of the receptor, activation of procaspase-8 and activation of caspase-3/7. In contrast, stimulation of the Bcl-2 family proteins induces permeabilization of the mitochondrial outer membrane, which is the main step in the intrinsic apoptotic pathway (Tait and Green, 2010).

1.3.4. The p53 family: p63 and p73

Two p53 family gene products were identified decades after discovery of p53, namely p63 and p73. Both of these genes are ancestors with structural and functional similarity to p53 (Collavin et al. 2010; Li & Prives 2007). The existence of several variant isoforms of p63 and p73, which have a vastly different activity, made these members more complex than p53 itself (Flores *et al.*, 2005). P63 and p73 contain 2 promoter region and as a consequence two classes of proteins are encoded: one comprising the N-terminal transactivation domain (TAp63, TAp73 which act similarly to wild-type p53) and the other which lack N-terminal

transactivation domain (ΔNp63, ΔNp73) that act primarily in dominant-negative fashion to regulate the p53 family (Inoue and Fry, 2014).

Many studies demonstrate the acquisition of the binding capability of p63/p73 with mutant p53. It has been shown that p63 and p73 can associate with each other to form homo- or hetero-tetramers. However, both p63 and p73 do not interact with wild-type p53 (Davison *et al.*, 1999; Bensaad *et al.*, 2003) but they do interact with mutant p53 (Irwin *et al.*, 2003). This interaction has been connected to the ability of some tumour-derived p53 mutants to promote resistance to chemotherapy, metastasis and invasion (Flores *et al.*, 2005). The binding of p63 and/or p73 isoforms might explain several gain-of-function properties of mutant p53 (Adorno *et al.*, 2009; Muller *et al.*, 2009). Genetic studies in mice revealed that p63 and p73 have an important function in normal development. In addition, the activity of both has been connected to induction of program cell death (Di Agostino *et al.*, 2008). However, the precise role of p63 and p73 in tumourigenesis are unclear because they are rarely mutated in cancer, albeit with some exceptions (Fridman and Lowe, 2003).

1.3.5. Therapeutic targeting of p53 in cancer

The crucial role of p53 in regulation of cellular growth and apoptosis led to its consideration as an interesting target for therapy (Zeimet and Marth, 2003). Activation of p53 function of various established tumour in animal models can cause tumour regression (Lane, Cheok and Lain, 2010). Thus, many studies have investigated methods to restore p53 tumour suppressor function. These include wild type p53 gene therapy, reactivation of mutant p53 and relief of wild type p53 from overexpressed MDM2.

The main aim of gene therapy is to re-introduce wild type p53 into tumour cells using several approaches. A viral vector is used to introduce an intact cDNA copy of the p53 gene into the tumour (Wang and Sun, 2010). Adenovirus-mediated transfer of the p53 gene to reconstitute

the wild type activity has shown some efficacy in clinical trial. P53 gene therapy stabilizes tumour growth or even cause regression without major toxic effects (Wiman, 2006). A combination of p53 gene therapy with traditional chemotherapy might enhance the clinical efficacy (Nemunaitis *et al.*, 2000; Swisher *et al.*, 2003). Despite this, adenovirus p53 therapy had been restricted to localized intratumoural injection. Therefore, novel method are required to overcome the systemic delivery problems for targeting disseminated disease and neutralizing antibody against viral antigens (Wiman, 2007; Lane, Cheok and Lain, 2010).

P53 Reactivation and Induction of Massive Apoptosis (PRIMA-1) and Mutant p53 Reactivation and Induction of Rapid Apoptosis (MIRA-1) (Lambert *et al.*, 2009) are compounds found to transactivate p53 target genes through restoring the sequence-specific DNA binding and mutant p53 conformation to wild type. This restores the expression of p21, MDM2 and PUMA *in vitro* and inhibit tumour growth *in vivo* (Bykov *et al.*, 2002, 2005). Another molecule, CP-313198, activates p53 and inhibit tumour growth *in vivo* as well. This compound increases the level of p53 by prevent its ubiquitination independently of MDM2 and p53 phosphorylation. However, CP-313198 molecules might have p53 independent activity (Selivanova and Wiman, 2007).

Blocking the interaction between p53 and MDM2, which is frequently overexpressed in tumours, is an effective approach to prevent wild-type p53 degradation (Bullock and Fersht, 2001). Several compounds had been identified to do so, such as Nutlin-3a, which efficiently stimulate apoptosis in acute myeloid leukaemia and in colon and breast cancer cell lines (Wang and Sun, 2010). In addition, Nutlin act synergistically with ABT-737, Bcl-2 antagonist, to activate the intrinsic apoptotic pathway and induce apoptosis (Kojima *et al.*, 2006).

1.4. Ovarian cancer experimental models

1.4.1. Ovarian cancer cell lines

Ovarian cancer cell lines were frequently used tumour models employed to study anti-cancer agents in OC (Konstantinopoulos and Matulonis, 2013). Cell lines can provide an insight of the molecular diversity and histological subtypes of cancer tissues. In addition, they are inexpensive, easy to manage in the laboratory and culture condition of the cell lines can be controlled easily. However, there is a lack of some aspects of representation by cell lines of an authentic tumour such as the heterogeneity of the population (Sandberg and Ernberg, 2005). Usually the OC cell lines are grown attached to the surface of the culture vessels in a flat layer. This monolayer form of growth is advantageous for the functional assay and microscopic study but generally it is considered not to be entirely physiologically relevant. Therefore, the use of spheroids might be an alternative model to evaluate therapy.

1.4.2. Spheroids

Spheroids, which are free-floating multicellular aggregate, are thought to more precisely represent OC *in vivo*. It has been shown that these aggregates have similar cellular, molecular and biochemical properties to OC (Zietarska *et al.*, 2007; Ahmed and Stenvers, 2013). For example, monolayer cell culture was found to be considerably more sensitive to chemotherapy than spheroid cancer cells, which is consistent with the observed chemoresistance to metastatic cancers (Kobayashi *et al.*, 1993; Achilli, Meyer and Morgan, 2012). The central region of tumours is poorly vascularized and exhibits an oxygen/nutrient and proliferation gradient, which might be associated with accumulation of catabolites and limited access to essential component needed for growth (Carlsson and Acker, 1988; Friedrich *et al.*, 2009). Aggregates (spheroid) also provide a 3D architecture that is lacking in monolayer cultures and which can be used for evaluation of drug combination efficacy

(Petrik, 2013). Thus, spheroid culture might provide a model which more closely resembles tumours in a clinical setting.

Chapter Two

Aims and Objectives

2. Aims and Objectives

The aims of this thesis were in three-fold. Firstly, to establish a link between the mevalonate pathway and *TP53* which is the most frequently mutated gene in OCs. Secondly, to test the antitumour activity of the previously described mevalonate pathway inhibitors in combination with pitavastatin. Lastly, to identify additional compounds which are synergistic with the anticancer activity of pitavastatin. To achieve this, the following objectives were set.

1- Expression and repression of wild-type and mutant *TP53* to evaluate their effect on the expression of MP enzymes (HMGCR, GGTI, GGTII and FTase).
2- Evaluate the anti-cancer effects of combinations of zoledronic acid, risedronate or GGTI-2133 with pitavastatin and investigate the mechanism of synergy between these agents.
3- Evaluate whether the antitumour activity of pitavastatin can be potentiated by combination with off-patent orally available drugs by screening a library of such drugs.

Chapter Three

Materials and Methods

3.1. Cell culture

3.1.1. Ovarian cell lines

A panel of ovarian cancer (OC) cells were used in these studies which were either selected previously as part of the "NCI-60" panel of cells or because they were subsequently shown to be better representative in vitro models of high grade serous ovarian cancer (HGSOC) (Table 3-1) (Domcke *et al.*, 2013).

Table 3-1 Cell line representation of HGSOC

Cell line	Representation of the HGSOC
Cov-362 Ovcar-4 Cov-318 Ovsaho	Likely
Ovcar-3 Ovcar-8	Possibly
Skov-3 A2780	Unlikely
Igrov-1	Hypermutated

A2780 (cisplatin sensitive) and Cis-A2780 (cisplatin resistant) cell lines (ATCC) were derived from a human ovarian serous carcinoma (Langdon, 2004; Cree, 2011). CisA2780 cells were developed by exposing A2780 cell line to increasing concentration of cisplatin. CisA2780 are 14 fold more resistant to cisplatin compared to the mother cell line (Behrens *et al.*, 1987).

Cov-318 and Cov-362 cells (European Collection of Authenticated Cell Cultures (ECACC) /Sigma Aldrich) were derived from peritoneal ascites and pleural effusion of patients

diagnosed with serous carcinoma and endometrioid cancer, respectively (van den Berg-Bakker *et al.*, 1993).

Ovcar-3 (ATCC) and Ovcar-4 (NCI) cell lines originated from ascites of patients refractory to treatment with cyclophosphamide, adriamycin and cisplatin. Ovcar-3 and Ovcar-4 cell line were developed after the diagnosis of progressive poorly differentiated papillary adenocarcinoma and adenocarcinoma of the ovary, respectively. The cytological features of these cells were consistent with the primary tumour (Hamilton *et al.*, 1983; Louie *et al.*, 1985).

Ovcar-5 (ATCC) cell line was derived from patient ascites with ovarian adenocarcinoma but prior to the chemotherapy treatment (Hamilton, Young and Ozols, 1984).

Ovcar-8 and Skov-3 (ATCC) cell lines were derived from carcinoma which is resistant to carboplatin and cisplatin, respectively (Hamilton, Young and Ozols, 1984; Buick, Pullano and Trent, 1985).

Igrov-1 cell line (ATCC) was obtained from ovarian carcinoma patient (Bénard *et al.*, 1985).

Normal Human Ovarian Epithelial (HOE; Applied Biological Materials Inc.) cells originated from normal ovarian epithelium and immortalized using SV40 large T antigen (Tsao *et al.*, 1995). All these cell lines are adherent cells that require trypsin for detachment and sub-culturing from the culture flask.

3.1.2. Cell lines growth conditions

Ovarian cancer cell lines (A2780, CisA2780, Cov-318, Cov-362, Ovcar-3, Ovcar-4, Ovcar-5, Ovcar-8, Igrov-1 and Skov-3) and HOE cells were grown as monolayer in a humidified incubator (NAPCO water jacketed incubator, Precision Scientific) at 37 °C in 5% CO_2.

Roswell Park Memorial Institute (RPMI 1640; Lonza) medium was used for all cell lines, except Cov-318 and Cov-362, for which Dulbecco's Modified Eagle Medium (DMEM, Lonza) was the base medium of choice as recommended by ECACC. The base medium of all cell lines were supplemented with 10 % (v/v) fetal bovine serum (FBS) (Lonza), 2 mM L-Glutamine (Lonza) and 50 IU/mL of Pen-Strep solution (penicillin/streptomycin; Lonza). However, Ovcar-3 cells were additionally supplemented with 0.01 mg/mL bovine insulin (Lonza) and 1 mM sodium pyruvate (Lonza).

3.1.3. Ovarian cells subculture

Cell lines in a T25 culture flask were inspected regularly using light microscopy (Olympus CKX41). Once confluent, the monolayer cells were rinsed with 2 mL of phosphate buffer saline (PBS) (Lonza) and exposed to 0.01% (v/v) trypsin in PBS and incubated at 37°C for 2–5 minutes. When the cells had detached, the trypsin was neutralized with 1 mL cell culture medium containing 10 % (v/v) FBS. Cells were then centrifuged (150 g, 3 min) and re-suspended in fresh growth medium and reseeded in T25 or T75 culture flask or tissue culture plate for experimentation. Cells were usually subcultured at 1:4 ratios. After several passages, cells were discarded (3-4 months) and replaced by new cells from liquid nitrogen stocks (Mitry and Hughes, 2012).

3.1.4. Cryopreservation

Healthy, contamination free, 50-80 % confluent and low passage number cells in T75 flask were collected by trypsinization. The cells pellet was resuspended in chilled growth medium containing 8 % (v/v) dimethyl sulfoxide (DMSO, Sigma-Aldrich). 0.3 mL Aliquots were transferred into 2 mL cryovials. The cryovials were slowly frozen in freezing container containing isopropanol in a Nuaire -80 °C Ultralow freezer (Parker, 2011) and next day transferred into liquid nitrogen.

To resuscitate cells, frozen cells were thawed rapidly at 37 °C. The medium was removed by centrifugation and the cells were resuspended in fresh medium and transferred into T25 tissue culture flask. After overnight incubation, the growth medium was again replaced to remove residual DMSO and dead cells. Cells were grown to an appropriate density for experimentation or sub-culture.

3.1.5. Three-dimensional spheroid culture

Spheroids, a free-floating multicellular aggregate, are considered more closely represent OC *in vivo*. Multicellular GravityTRAP ULA Plates (InSphero) were used to growth spheroids. The plates were wet with 40 µL of medium before seeding cells. The medium was removed from the plates and 500 Ovcar-4 or Cov-362 cells in 70 µL growth medium added per well followed by brief centrifugation (ALC PK120 Centrifuge) for 1 min at 900 rpm. After 3-5 day, spheroids could be observed. Thereafter, 30 µL of medium containing vehicle, pitavastatin (10µM or 7µM), prednisolone (70µM) or a combination with prednisolone were added. Ovcar-4 or Cov-362 cells were incubated for 72 or 120 hours, respectively. Intracellular ATP level was quantitated using the cell Titer-Glo Luminescent assay reagent (Promega, Madison, WI, USA) as described in 3.5.2. The Bliss independence criterion was calculated to determine the expected effect of the drug combination as described in section 3.7.2 and this was compared to the observed effect of the combination.

3.2. Compounds

Pitavastatin (Livalo, Adooq), Zoledronic acid (Selleckchem), Risedronate (Selleckchem) and GGTI-2133 (Sigma-aldrich) Tipifarnib (APExBIO), Prednisolone (Sigma) were prepared as 20mM solutions in DMSO except zoledronic acid which was dissolved in H_2O. The library of compounds evaluated in chapter 6 was a generous gift form Dr. Farahat Khanim, School of life Science- Birmingham University.

3.3. Cell proliferation assay (sulforhodamine B assay)

5000 cells (or 2500 cells of A2780, CisA2780 and Ovcar-8) were seeded in 80 µL medium per well of a 96 well plate. The cells were allowed to adhere for 24 hours. Then, 20 µL of 18 serial dilutions of individual drugs were added to cells. The cells were incubated for 72 hours, except for the Cov-318 and Cov-362 which were incubated for 120 hours. Subsequently, cells were fixed by adding 100 µL of 10 % (w/v) trichloroacetic acid (Sigma-Aldrich) and incubated on ice bucket for 30 minutes. The plates washed three times by immersion in cold water. After drying, cells were stained with 100 µL of 0.4 % (w/v) sulforhodamine B (SRB, Sigma-Aldrich) for 30 minutes. The plate wells washed three times in 1 % (v/v) acetic acid (Sigma-Aldrich) to remove excess SRB. Lastly, the dye was solubilized in 100 µL Tris base (10mM, pH not adjusted) and the optical density measured at 570nm with a microplate reader (Synergy 2 Multi-Mode Microplate Reader-BioTek). Graphpad prism 6 (Graphpad Software, Inc.) was used to analyze the data obtained from the cell proliferation assay by fitting a four parameter sigmoidal dose response curve for each drug to calculate the $IC_{50.}$ The IC_{50} values obtained from each repeated experiment were used to calculate the mean IC_{50} and associated standard deviation.

3.4. Cell growth assay for the screening of the library with pitavastatin

A panel of 100 off-patent licensed orally-bioavailable drugs were tested alone and in combination with pitavastatin in cell growth assays. The experimenter was “blind” to compounds names which were labeled with numbers from 1-100.

Ovcar-4 cell line were seeded (5000 cells/well) overnight in 96-well plate in 80 µL of growth medium. The next day, the cells were exposed to vehicle, pitavastatin (10 µM), library

compounds, or a combination of pitavastatin and a compound from the library. After 72 hour incubation, cells were fixed same as previous described in section 3.3 and relative cell number were estimated by measuring the optical density at 570 nm using plate reader as described in Section 3.3. Each drug was tested in triplicate in two independent experiments. The Bliss independence criterion (Section 3.7.2) was used to analyse the drug combination data (Goldoni and Johansson, 2007; Zhao *et al.*, 2014) and allows calculation of the expected effect from the drug combination if their activity were additive. The "Bliss excess" was calculated by subtracting the measured effect of combination from the expected additive effect.

3.5. Cell viability assay

3.5.1. Trypan blue assay

To evaluate the effect of drug combination on cell viability using the Trypan blue exclusion assay, A2780, Ovsaho or Skov-3 cells were seeded at density of 2×10^5 cells/2mL/well in 6 well plate. The next day, 20 µL of medium containing vehicle or pitavastatin or zoledronic acid or a combination of both were added to the indicated final concentration. After incubation for 72 and 96 hours, adherent cells were collected gently by trypsinization and combined with the medium that contain non-adherent floating cells. The cells were centrifuged at 150 g for 3 minutes and the pellet was re-suspended in 0.5 mL of medium then equal volume of cell suspension mixed with equal volume of 0.4 % (v/v) trypan blue (Sigma-Aldrich). The viable and non-viable cells were counted by Neubauer hemocytometer.

3.5.2. Cell Titer-Glo luminescent assay

A2780, Ovsaho and Skov-3 cells were seeded in 96 well plates as described in the cell proliferation assay (Section 3.3). The next day, 20 µL of medium containing vehicle, or pitavastatin or zoledronic acid or a combination of both were added to the cells at the indicated final concentration. After 72 hours, 25 µL of CellTiter-Glo reagent was added to the sample in each well of the 96 well plates. After 2 min shaking (Platform shaker STR6, Stuart scientific) and 30 min incubation in dark at room temperature, 100 µL was transferred to opaque-walled multiwell plates. The luminescence was measured in a Synergy 2 Multi-Mode Microplate Reader (BioTek).

3.5.3. Assessment of apoptosis by Annexin V-FITC/Propidium iodide staining

To measure the effect of drug combination on apoptosis, annexin V and propidium iodide (PI) labelling was used. For siRNA transfection studies (Section 3.10), Ovcar-4 cells were seeded at a density of 1×10^5 cell/mL/well in antibiotic free medium in 12-well plates and incubated overnight. The cells were transfected with 100 nM of siGGTI-β #6, #7, #8, #9 or GGTII-β #5, #6, #7, #8 or non-targeting#1 (NT#1) (Sequences are in Table 3.3) using 1% (v/v) Dhamafect-1 (Dharmacon). The next day, the medium was replenished with fresh antibiotic free medium and the cells exposed to pitavastatin (10µM) prepared in 20 µL of medium and incubated for additional 48 hours.

To measure the effect of pitavastatin-prednisolone combinations on apoptosis, Ovcar-4 or Cov-362 cells were seeded at density of 2×10^5 cells/2 mL/well in 6-well plate and incubated overnight. The medium was replenished and 20 µL of medium containing vehicle or pitavastatin or prednisolone or a combination added to the indicated final concentration. Ovcar-4 and Cov-362 cells were incubated with drugs for 48 and 72 hours, respectively.

For flow cytometry, the cells were labelled using a Annexin-V FITC kit (Miltenyi biotech) following the manufacturer's instructions. After collection of the media from each well, the cells were trypsinized and washed in ice-cold PBS and centrifuged at 300 g for 5 minutes. The pellets were re-suspended in 1 mL of annexin V binding buffer and centrifuged for 10 minutes at 300 g. Again, the pellets re-suspended in 100 µL of annexin V binding buffer and 10 µL of Annexin V were added and incubated for 10 minutes in dark at room temperature. The cells were once more washed with 1 mL of annexin V binding buffer. Lastly, cells were centrifuged and the pellet was re-suspended in 500 µL Annexin V Binding Buffer and 5 µL of propidium iodide (1µg/mL) were added at least 5 min before the analysis by flow cytometry. The viability of cells was defined as live (annexin V-negative and PI-negative), early apoptotic cells (annexin V-positive and PI-negative), late apoptotic cells or dead cells (annexin V-positive and PI-positive) and necrotic cells.

3.6. Apoptosis assay

3.6.1. Caspase -3/7, -8 and -9 assay

Caspase-Glo -3/-7, -8, -9 assay kits were used to determine Caspase-3/7, -8 and -9 activity according to the manufacturer's instructions (Promega, Madison, WI, USA). A2780, Ovsaho, Skov-3, Ovcar-4 and Cov-362 5000 cells/80 µL of medium were seeded in 96 well plate and incubated overnight. The next day, 20 µL of medium containing vehicle, pitavastatin, zoledronic acid, prednisolone or combination were added to each well to the indicated final concentration.

Cells were incubated with drugs for 48 hours or 72 hours for Cov-362 cells. 25 µL of the reagent were added to each sample and incubated for 1 hour in dark, with continuous shaking. Next, 100 µL of the medium transferred to 96 opaque-walled multiwell plates. Lastly, Caspase activities were measured using a microplate reader.

A parallel plate was prepared and treated with drug in the same way as that used for the caspase assay and this was stained with SRB as described in section 3.3. The results of the caspase assay were normalized to the protein content (SRB assay) of the sample.

3.7. Drug combination analysis

Drug combinations are common strategy indicated for immune disease, infection and cancer treatment. There are several methods for quantitative measurement of the effects of drug combinations (Bijnsdorp, Giovannetti and Peters, 2011). An effect-based Strategy can be achieved by calculating the expected effect of the combination using the Bliss independence criterion; a dose based strategy can be achieved by measuring the concentration of drugs in a combination required to have the same effect as the single agents and calculating a combination index (Foucquier and Guedj, 2015).

3.7.1. Combination index

The combination index is the most widely used method for evaluation of the drug combination (CI) was developed by Chou and Talalay to allow a quantitative measurement of synergy, additivity or antagonism between two agents.

$$CI = \frac{(D)_1}{(D_x)_1} + \frac{(D)_2}{(D_x)_2}$$

$(D)_1$, indicate the doses of first drug; $(D)_2$, indicate the doses of second drug; D, the dose of the drug in combination which inhibit cell growth by X%; $(D_x)_1$ is for $(D)_1$ "alone" that inhibits a system X%; $(D_x)_2$ is for $(D)_2$ "alone" that inhibits a system X%.

The combination index was quoted at fraction affected $f_a = 0.5$ (at which 50% of cells were apparently affected) and were calculated and compared to deviation from unity using a paired *t*-test. If the CI = 1, then the interaction between the two drugs is considered additive,

whereas, if the CI value is greater than 1 the interaction is antagonistic, but if the CI value is smaller than 1, then the interaction is considered synergistic.

3.7.2. Bliss independence

Bliss independence (Goldoni and Johansson, 2007) effects were calculated according to the following equation to evaluate drug interactions when full dose response curves were not performed (e.g. for trypan blue assays and ATP-assay).

$$E_{(X,Y)} = E_{(X)} + E_{(Y)} - E_{(X)}E_{(Y)}$$

$E_{(X)}$ and $E_{(Y)}$ are the fractional effect (between 0 and 1) of drug x and y measured by the assay. $E_{(X,Y)}$ is the expected effect of the drug assuming Bliss independence. The calculated additive effect calculated using the Bliss independence criterion was compared to the observed effect of the drug combination measured to quantify the drug interaction.

3.8. Western blot analysis

3.8.1. Whole cell lysate

Ovarian cancer cell lines, A2780, Skov-3, Ovsaho, Ovcar-4 and Cov-362 were seeded in 6 well plates at a density of 2×10^5 cell/2 mL medium/ well (same as section 3.5.1). The cells were incubated with vehicle, different concentration of the drugs alone, and in combinations of the indicated final concentration for 48 hours. After trypsinization, the cells washed with ice cold PBS and lysed in a modified Radio immunoprecipitation assay (RIPA) buffer (20 mM Hepes (CalbioChem), 150 mM sodium chloride (NaCl, Sigma-Aldrich), 2 mM ethylene-diamino-tetraacetic acid (EDTA, Sigma-Aldrich), 0.5% sodium deoxycholate (Sigma-Aldrich), 1% NP40 (Sigma-Aldrich)). Additionally, 120 µM leupeptin (Sigma-Aldrich), 10 µM pepstatin (Sigma-Aldrich) and 1 mM phenylmethanesulfonyl fluoride

(PMSF, Sigma-Aldrich) were added as protease inhibitors. The lysate was centrifuged at 14,000 RPM for 15 minutes at 4°C, and the supernatant was collected and stored at -80°C.

3.8.2. Cytoplasmic and membrane protein fractionation

A2780 and Skov-3 2×10^5 cells/ 2 mL medium were seeded in 6 well plates and incubated overnight. The next day, 20 µL of medium containing vehicle or pitavastatin or zoledronic acid or a combination of both were added to the indicated final concentration (section 3.5.1). Membrane and cytoplasm proteins were separated using Mem-PER™ Plus Membrane Protein Extraction Kit (Thermofisher) according to the manufacturer's protocol. The cells washed twice with 2 mL of ice cold washing buffer, re-suspended in 0.25 mL cell permeabilization buffer and incubated for 15 minutes with constant mixing. The mixture was centrifuged at 16000 xg for 15 minutes at 4 °C and the supernatant collected in a new eppendorf tube (cytoplasmic fraction). The pellets were re-suspended again in 0.16 mL of solubilization buffer and incubated for a further 30 minutes at 4 °C with constant shaking. After centrifugation at 16000 x g for 15 minutes at 4 °C, the supernatant was collected (membrane fraction) and both the cytoplasmic and membrane fraction were stored at -80°C waiting for analysis.

3.8.3. Bicinchoninic acid protein assay

The bicinchoninic acid (BCA) protein assay used to determine total protein concentration of the samples. Eight standards of Bovine serum albumin (BSA, Sigma-Aldrich) were prepared at concentrations between 0.1 and 2 mg/mL. Copper (II) sulphate pentahydrate solution (4%) (Sigma-Aldrich) were added to BCA solution (bicinchoninic acid, sodium carbonate, sodium tartate and sodium bicarbonate in 0.1 M sodium hydroxide) at a ratio of 1:50 to prepare the BCA reagent. 10 µL of each BSA standard or duplicate of each cells lysate (10 µL) were added to 100 µL of the BCA reagent. After 30-minute incubation at 37 °C, the absorbance

for standards and samples were measured using a plate reader. A calibration curve was fitted to the data obtained with BSA using linear regression and used to determine the concentration of protein in each sample lysate.

3.8.4. Gel electrophoresis and immunodetection of proteins using SDS-page and western transfer

Equal amount of protein from each sample cell lysate were first mixed with 5 µL of NuPAGE sample buffer (Invitrogen) containing 5 % (v/v) β-mercaptoethanol (Sigma-Aldrich) and samples denatured at 70 °C for 15 minutes. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was used to separate the protein according their molecular weight. PageRuler Plus Prestained Protein Ladder (10 to 250 kDa) (Thermo Scientific) and denatured samples were added to Tris-Glycine polyacrylamide gradient gel (4-20%) (Nusep) in an XCell SureLock Mini Cell (Invitrogen) with Hepes running buffer (100 mM Hepes, 100 mM Tris and 1% sodium dodecyl sulphate (SDS, Sigma-Aldrich)), and electrophoresis performed at 65V for 2 hours. Subsequently, the proteins were transferred from the gel to PVDF Amersham Hybond membrane (Hybond-ECL, GE Healthcare) using a freshly prepared ice cold transfer buffer (25 mM Tris, 200 mM glycine (Sigma- Aldrich), 0.075 % (w/v) SDS and 10 % (v/v) methanol (Sigma-Aldrich)) at 30 V for 2 hours. Next, 5% (w/v) skimmed milk powder in Tris-Buffered Saline with Tween (TBST) buffer was added to the PVDF membrane as a blocking solution and incubated on a shaker (Stuart Scientific Platform, STR6) for an hour at room temperature. The membrane was then incubated overnight with primary antibody at 4 °C with continuous shaking. The antibodies used are described in Table 3-2. The following morning, the membrane was washed five times (5 minutes each) in TBST buffer and incubated with secondary antibody for an hour at room temperature with continuous shaking. The membrane was again washed five times (5 minutes each) with TBST buffer and proteins visualized using UptiLight HRP US

chemiluminescent substrate (Interchim) and a FluorChem M Imager. AlphaView SA software (Protein Simple) was used to quantify protein bands by measure the total signal in the selected area after subtracting the mean background per pixel. The results were then normalized to the GAPDH loading control.

Table 3-2 The primary and secondary antibodies used for protein immunodetection

Antibody	Dilution	Product code	Supplier
Anti-Actin	1/5000	#4968	Cell signalling Technology
Anti-CDC42	1/10000	Ab187643	Abcam
Anti-RAB6A	1/500	Ab95954	Abcam
Anti-Ras	1/10000	Ab52939	Abcam
Anti-RhoA	1/5000	Ab187027	Abcam
Anti-FDPS	1/5000	Ab153805	Abcam
Anti-GAPDH	1/5000	Mab374	Millipore
Anti-GGTII-β	1/1000	Sc365901	Santa cruz
Anti-GGTI-β	1/1000	Sc376854	Santa cruz
Anti-HMGCR	1/1000	Ab173830	Abcam
Anti-IDI1	1/1000	Ab97448	Abcam
Anti-MVD	1/5000	Ab129061	Abcam
Anti-P53	1/5000	Ab179477	Abcam
Anti-NaK ATPase	1/100000	Ab167390	Abcam
Anti-PARP	1/2000	#95425	Cell signalling Technology
Anti-mouse secondary antibody	1/2000	#7076	Cell Signalling Technology
Anti-rabbit secondary antibody	1/2000	#7074	Cell Signalling Technology

3.9. Transient gene transfection

3.9.1. Plasmid preparation

Plasmids encoding *TP53* and *TP53* variants, pCMV-Neo-Bam (vector), pCMV-Neo-Bam p53 WT, pCMV-Neo-Bam p53 R175H, pCMV-Neo-Bam p53 R273H, pCMV-Neo-Bam, pCMV-Neo-Bam p53 R248W, were obtained from Addgene.

Luria-Bertani (LB) Agar was prepared by adding Bacto-tryptone (10 g), yeast extract (5 g) and NaCl (10 g) to 800 mL of distilled H_2O. The pH was adjusted to 7.5 with NaOH and then 15 g of agar were added to the medium. Lastly, the volume was adjusted to 1L with dH_2O and the LB agar sterilized by autoclaving. In addition, LB medium was prepared by mixing Bacto-tryptone (10 g), yeast extract (5 g) and NaCl (10 g) in 800 mL of distilled H_2O. The pH was adjusted to 7.5 with NaOH and the volume adjusted to 1 L with dH_2O. The LB medium was sterilized by autoclaving. Ampicillin antibiotic was added immediately prior to use to a final working concentration of 100 µg/mL.

Sterile spreaders were used to spread the stab culture of *E. Coli* carrying the plasmid on a Luria-Bertani (LB) Agar. After overnight incubation at 37 °C, picked colonies were used to inoculate a sterile conical flask containing 100 mL of LB medium. The flasks were place in incubator at 37 °C with continuous shaking (300 RPM) for 20 hours.

The plasmid was purified from the culture using the HiSpeed Plasmid Midi Kit (Qiagen). The collected culture medium was centrifuged at 6000 xg for 15 minutes at 4 °C. The pellets were re-suspended in 6 mL of buffer P1 and 6 mL of buffer P2 was added and the tube contents mixed by gentle inversion several times. After 5 minutes incubation at room temperature, 6 mL of buffer P3 was added and followed by gentle inversion to mix. The lysate was added to the barrel of the QIAfilter cartridge and incubated for 10 minutes at room

temperature. 4 mL of Buffer QBT was used to equilibrate a HiSpeed Tip. The lysate was allowed to pass through the HiSpeed Tip which was subsequently washed with 20 mL of buffer QC. The DNA was eluted by 5 mL of buffer QF and precipitated by adding 3.5 mL of isopropanol. After incubation for 5 minutes, the eluate was collected with a QIAprecipitator and washed with 2 mL of 70 % ethanol. Once dried, the DNA was eluted with 1 mL of buffer TE and the concentration of plasmid was determined by spectrophotometer (Nanodrop2000) before storage at -20 °C.

3.9.2. Plasmid transfection

Expression studies were approved by the Keele genetic modifications of microorganisms committee. 32,000 Skov-3 cells per well of a 24 well plate were seeded in 500 µL antibiotic free RMPI medium. After an overnight incubation, the medium was replaced with 400 mL of fresh antibiotic free medium. Cells were transfected with 0.1 % CMV-Neo-Bam (vector), pCMV-Neo-Bam p53 WT, pCMV-Neo-Bam p53 R175H, pCMV-Neo-Bam p53 R273H, pCMV-Neo-Bam p53 R248W (Addgene) and 0.2 % of Lipofectamine 2000. The transfection mixture was prepared by adding 0.55 µg of DNA to 50 µL of Optimem media and 1.1 µL of lipofectamine 2000 (Invitrogen) to a separate aliquot of 50 µL of Optimem. The lipofectamine and DNA solutions were mixed and incubated for 30 minutes at room temperature to allow DNA-lipofectamine 2000 complex formation. 100 µL of the transfection mixture were added to each well of the plate and next day, the medium was changed and the cells incubated for a further 48 hours. The transfection efficiency was measured using western blotting to assess the expression of p53 level.

3.10. Small interfering ribonucleic acid (siRNA) transfections

5000 Ovcar-4 and Ovcar-3 cells were plated in 80 µL of antibiotic-free growth medium per well in a 96 well plate and incubated overnight. A solution of 1% DharmaFECT-1 was prepared in Optimem (Invitrogen) and incubated for 10 minutes at room temperature. The siRNA oligos (Table 3-3) were prepared at 10 times the final concentration required at well (100 nM) and then mixed with 1% DharmaFECT-1 solution. The mixture was incubated for 30 minutes at room temperature to facilitate complex formation between the siRNA and liposomes. The growth media on cells was replaced with 80 µL of fresh antibiotic free growth media, then 20 µL of siRNA and DharmaFECT-1 mixture were added to each well. The same process was made for the non-targeting siRNA control to demonstrate if there are any off-target effects on gene expression (off-target gene silencing). The next day, the media were replenished with 100 µL of fresh antibiotic free growth media and cells incubated for additional 48 hours. Subsequently, cells processed same as in section 3.8.1 for whole cell lysate and gene expression was assessed by immunoblotting.

Table 3-3 siRNA transfection Oligos

Gene Name	Used Concentration	Target Sequence
P53#smart pool	100nM	-
P53#1	100nM	GAAAUUUGCGUGUGGAGUA
P53#2	100nM	GUGCAGCUGUGGGUUGAUU
P53#3	100nM	GCAGUCAGAUCCUAGCGUC
P53#4	100nM	GGAGAAUAUUUCACCCUUC
GGTI-β#6	100nM	CGACUUAAGCCGAGUAAAU
GGTI-β#7	100nM	GAGACAAGCAGGUUGACAA
GGTI-β#8	100nM	GGAUAAAGAGGUGGUGUAU
GGTI-β#9	100nM	CCACAUGAAUGAUUUUAGA
GGTII-β#5	100nM	UUACUUGGCUGGUGGCUUU
GGTII-β#6	100nM	GGAAUAAGUGCUAGUAUCG
GGTII-β#7	100nM	UCUGAGUAUUUGAGAAUGA
GGTII-β#8	100nM	UGGAAUAUGUUAAAGGUCU
NT#1	100nM	UGGUUUACAUGUCGACUAA
NT#2	100nM	UGGUUUACAUGUUGUGUGA

3.11. Quantitative reverse transcriptase polymerase chain reaction

3.11.1. RNA extraction from culture cells

5000 Ovcar-3 and Skov-3cells were plated in 80 µL of antibiotic free medium per well in 96 well plate and incubated overnight. Cells were harvested using RNeasy mini kit (Qiagen) following the manufacturer instruction. 175 µL of RTL buffer, supplemented with 1% β-mercaptoethanol, were added to each well (usually 2 well were combined to create one sample) and pipetted up and down several times to ensure cell lysis. Lysates were centrifuged for 3 min at maximum speed (16000xg). The supernatant transferred to a new 1.5 mL tube and mixed with 350 µL of 70% ethanol by pipetting up and down several times. Next, approximately 700 µL of each sample was transferred to mini spin column placed in 2 mL tube and centrifuged for 15 second at 8000 xg and the flow through discarded. The centrifugation process was repeated with another 700 µL of RW1 buffer and subsequently twice more with 500 µL RPE buffer. The column was dried by centrifugation for 2 minutes. Lastly, the spin column was placed in new 1.5 mL tube and 50 µL of RNase free water added, incubated for 2 minutes and then centrifuged for 1 minute at 8000xg in order to elute the RNA. The purity and concentration of the extracted RNA were measured using a Nano-Drop2000 spectrophotometer (Thermo fisher scientific). The extracted RNA placed in -20 °C.

3.11.2. Synthesis of cDNA by reverse transcriptase

SuperScript™ III Reverse Transcriptase was used to generate the complementary DNA strand (cDNA). The reaction mixtures were maintained in ice during the procedure. A mixture of 11.5 µL of RNA extracted from cells and 1.5 µL (0.5 µg) of Oligo $(dT)_{20}$ (50 µM) were mixed and denatured at 65ºC for 5 minutes in thermal cycler (MJ Research PTC-200

Thermal Cycler, USA) and stored on ice. The reaction mixture for each sample was prepared by mixing 4 µL of 5X reverse transcriptase RT reaction buffer, 1 µL of dNTP mix (10 mM), 1 µL of reverse transcriptase and 1 µL of DTT (0.1 M). The reaction mixture (7 µL) was added to already prepared RNA mixture. The tubes were exposed to pulse spin using centrifuge and transferred to thermal cycler again which include incubation for 5 minutes at 65 ºC, 30 minutes at 50 ºC, 5 minutes at 85 ºC and lastly cooling down to 4 ºC.

The concentration and purity of the cDNA was measured using Nanodrop2000 spectrophotometer. The DNA concentration is dependent on absorbance at 260 nm. The ratio of the 260/280nm were used to assess the purity of DNA. The reaction product was diluted 5 times by adding 80 µL of water and the sample were stored in -20.

3.11.3. qRT-PCR

A master mix was prepared by adding 6.25 µL of syber green (Absolute SYBR Green ROX mix Thermo Scientific), 0.125 µL of forward and reverse primer (10 mM) (Table 3-4) and 1.125 µL of nuclease free water for each sample and kept on ice. The master mix was mixed gently and collected by brief centrifugation. 7.5 µL reaction mixture were added to each wells of optical 8-tube strips, then 5 µL of DNA sample or dH_2O were added to each tube in duplicate. The optical strips were briefly vortexed to remove any air bubbles and centrifuged to collect the reaction mixture at the bottom of the wells. A Stratagene Mx3005P thermal cycler (Agilent Technologies) was used to conduct the analysis (Table 3-5). To confirm that only one amplicon was detected the dissociation curves were analyzed for each sample. In addition, the efficiency of genes was confirmed by measuring the CT value of 4 serial of 4-fold dilution of samples using qRT-PCR. The standard curve produced from the measured C_T data and the log of the dilution factors were analyzed by liner regression and implemented in the following equation to measure the efficiency.

$$\text{Efficiency} = (10^{-1/\text{slope}})-1$$

A comparative cycle threshold (CT) method was used to analyses the data, which compares the CT value of target gene to the CT value of the reference gene.

$$\Delta C_T = C_T \text{ (target gene)} - C_T \text{ (reference gene)}$$

$$\Delta\Delta C_T = 2^{\wedge(-\Delta CT)}$$

$$\text{Ratio (Fold changes)} = \frac{\Delta\Delta\text{CT of treated sample}}{\Delta\Delta\text{CT of control sample}}$$

Table 3-4 Primer sequences

Primer Name	Forward (5' → 3')	Reverse (5' → 3')
HMGCR	CAGAATTACGTCAACTTGGATC	AGAAGTGATGACAACTGTACTG
GGTI-β	GGATTTCTTACGGGATCGGC	TTGTCAACCTGCTTGTCTCG
GGTII-β	CTGGTGGCTTTGTGAACGAC	CAGGACCCACCATGAGTAGC
FT-β	CTTTTTGCCTCTATCCGCTCG	TGTGTAGGACTCTGCTTCGT
β-actin	GCAAAGTTCCCAAGCACA	AAGCAAGCAGCGGAGCAG

Table 3-5 Thermal cycling profile for qRT-PCR

	First segment	Second segment	Third segment
	Denaturation of DNA strands at 95°C for 15 minutes	Denaturation of the DNA at 95°C for 30 second	For dissociation curve at 95°C for 1 minutes, 55°C for 30 second and 95°C for 30 second
		Primer annealing at 60°C for 1minute	
		DNA strand elongation at 72°C for 30 second	
Cycle Number	1	40	1

3.12. Statistical analysis

Number of experiments (n) of independent biological replicate were employed to calculate means and standard deviations (mean±SD). GraphPad Prism version 6.0 (GraphPad Software, San Diego, USA) was used to generate sigmoid dose response curves and to calculate the IC_{50}s. *t*-tests were performed using Excel to compare two variables. One-way ANOVA followed by Tukeys post hoc was used to compare the mean of more than two variables. Results were considered statistical significance at $P < 0.05$.

Chapter Four

TP53 and mevalonate pathway interplay in ovarian cancer cell lines

4.1. Introduction

The early observation of p53 oncogenic properties was misled by the use of mutated clones of p53 extracted from cancer cells. However, Shaulsky, et al., 1991 and Dittmer, et al., 1994 were the first to report the gain of new functions of mutant p53 (Shaulsky, Goldfinger and Rotter2, 1991; Dittmer *et al.*, 1993). While most tumour suppressor genes are inactivated by frameshift or nonsense mutation, missense is a common *TP53* mutation that is associated with cancer and which often generates high levels of a stable mutant p53 (Olivier, Hollstein and Hainaut, 2010; Oren and Rotter, 2010; Goh, Coffill and Lane, 2011). Mutated *TP53* not only abrogates the tumour suppressor function of the wild type *TP53* allele via dominant-negative mechanism, but also endows abnormal oncogenic gain-of-function (GOF) properties. This results in enhanced proliferation, metastasis and chemoresistance (Heublein and Sabine, 2016; Hientz *et al.*, 2016; Oren and Kotler, 2016).

TP53 mutation (90%) cluster in the DNA binding domain and about 1300 different single base-pair substitution of the core domain of p53 protein have been reported in cancer. *TP53* missense mutation tend to cluster at hot-spot codons in the DNA binding domain (Figure 4-1) (Goldstein *et al.*, 2010). The six most common hotspot mutations are R175, G245, R248, R249, R273, and R282 (Mello and Attardi, 2013) and ~20% of *TP53* mutation occur at *R273*, *R248* and *R175* in ovarian cancer (OC) (Brachova, Thiel and Leslie, 2013). Mutations outside the DNA binding domain are also reported (Joerger and Fersht, 2007; Olivier, Hollstein and Hainaut, 2010). Even in cancers where *TP53* mutation is rare, *TP53* function is inactivated by different mechanism such as nuclear exclusion, inactivation of $p19^{ARF}$ and interaction with over expressed MDM2 or with a viral protein (Soussi and Béroud, 2001).

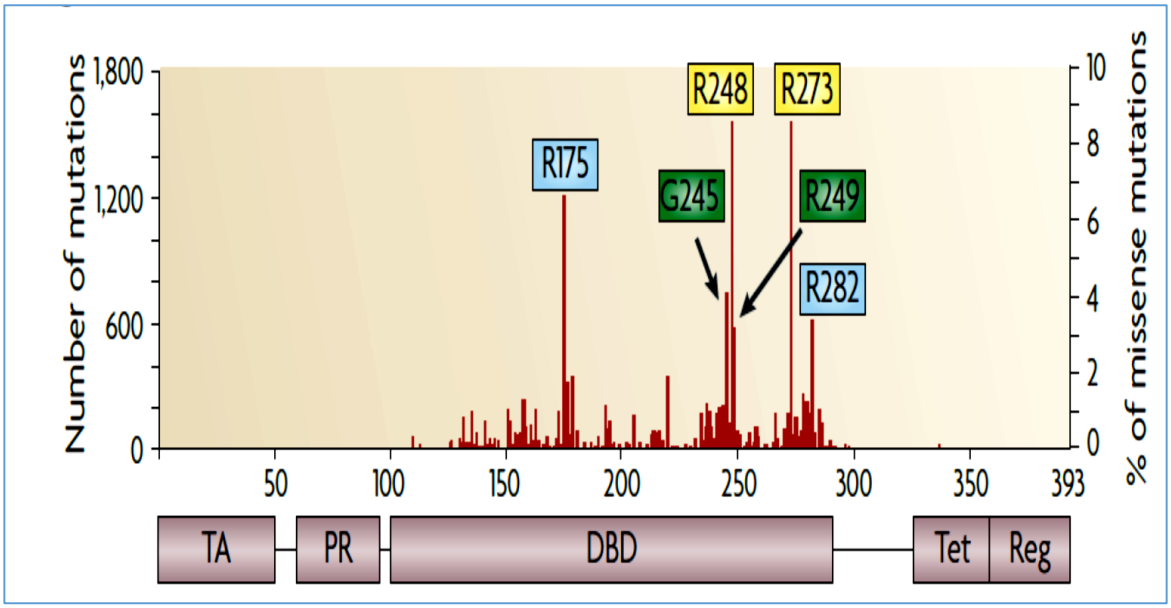


Figure 4-1 Distribution of TP53 missense mutation

TP53 mutation mostly located at the DBD with the six most common hotspot mutations are R175, G245, R248, R249, R273, and R282. PR, proline-rich domain; Reg, carboxy-terminal regulatory domain; TA, transactivation domain; Tet, tetramerization domain. Reprinted with permission from (Brosh and Rotter, 2009).

TP53 mutation can be categorized according to the effect of mutation on the thermodynamic stability of the protein. These mutations can be classified in two groups, DNA contact and conformational mutations. The DNA contact group comprise mutations in residues that are involved in DNA binding (R273H and R248Q), while the second conformational group include mutation that led to global (R175H and R282W) or local (R249S and G245S) conformational distortion (Brosh and Rotter, 2009).

Whereas, several GOF phenotypes have been noted, our understanding of the underlying mechanism remains incomplete (Hanel *et al.*, 2013). The nature of the mutation at a particular residue can have a profound effect on the phenotype that is observed. For example, the $p53^{R248Q}$ mutant is linked with metastasis, whereas the $p53^{R248W}$ mutant is less metastatic (Mullany *et al.*, 2015). In addition, the $p53^{R175C}$ mutant was wild-type in its phenotype;

R175K, R175P, R175I and R175S trigger only cell cycle arrest; R175N and R175T stimulate cell cycle arrest and partially stimulate programed cell death; and R175Y, R175W, R175D and R175F do neither (Goh, Coffill and Lane, 2011). The wide spectrum of *TP53* mutations led to a widely established notion that not all mutations are equivalent in their structural and functional effects (Mullany *et al.*, 2015).

TP53^{R273} mutations are the most frequent mutation in OC. In this mutation, the amino acid residues at position 273 is altered to either histidine (46%) or cysteine (39%), the protein DNA target sequence is altered but without deforming the structure of p53 protein and thus maintaining its DNA binding ability (Wong *et al.*, 1999; Joerger *et al.*, 2009; Eldar *et al.*, 2013). *In vitro* and *in vivo* studies showed that R273 mutation increase cellular proliferation in culture and the liability to develop carcinomas in mice (Olive *et al.*, 2004). Furthermore, resistance to cisplatin developed following ectopic overexpression of R273H on a *TP53* null background cells (Chang and Lai, 2001). The interaction of p53^{R273H} with several proteins such as NF, SP1, p63 and SREBP might be the likely cause of tumour progression and increased resistance to chemotherapy (Brachova, Thiel and Leslie, 2013). R273H also promote TGF-β induced metastasis (Adorno, et al. 2009).

The second most commonly transformed codon in OC is R248 in which the arginine amino acid is replace by tryptophan or glutamine (R248W or R248Q) (Brachova, Thiel and Leslie, 2013). This alteration affects the interaction of p53 with DNA binding response element without significant changes to the structural conformation of p53 (Wong et al. 1999). Acceleration of the tumour onset and shortened survival is reported in p53^{R248Q} knock-in mice (Hanel *et al.*, 2013). In addition, upregulation of the multidrug resistance gene (MDR1) mediated the resistance to doxorubicin and paclitaxel in hepatocellular carcinoma cells which harbour p53^{R248Q} (Chan and Lung, 2004).

The third well characterized hotspot mutation is R175H, in which the arginine is replaced by histidine. The connection between R175H and tumourigenic potential and chemoresistance had been reported (Blandino, Levine and Oren, 1999). Transgenic expression of $p53^{R175H}$ enhances the proliferation capacity and increases the tumourigenic potential (Olive *et al.*, 2004). The interaction of R175H with a number of proteins might mediate changes in proliferation, metastasis and chemoresistance. Overexpression of $p53^{R175}$ mediates resistance to cisplatin, etoposide and paclitaxel (Blandino, Levine and Oren, 1999).

In OC, the most frequent hereditary genetic aberrations are germline mutation in BRCA1 and BRCA2. In contrast, *TP53* mutation are a common alteration in sporadic epithelial OC (Corney *et al.*, 2008). There is little evidence of p53 mutation in benign and borderline epithelial ovarian tumours. Only one in a series of 48 borderline tumours was reported to have *TP53* mutation and 2 of 48 cases were found to overexpress p53 (Russell, 2001). Mutant p53 had been associated with poor clinical outcome in ovarian carcinomas (Corney *et al.*, 2008). High grade serous OC patients with *TP53* mutation are more likely to exhibit distant metastases and develop resistance to platinum chemotherapy (Kang *et al.*, 2013; Zhang *et al.*, 2016). In addition, progression-free survival is significantly shorter in patients with p53 mutation compared to patients with wild type p53 activity (Russell, 2001). Mutant TP53 is also considered as a poor prognostic factor in colorectal, prostate, lung and breast cancer (Yemelyanova *et al.*, 2011; Mantovani, Walerych and Sal, 2016).

A precise control of metabolism is vital for a normal cellular activity. It is increasingly evident that cellular metabolism is aberrant in cancer and plays a key role in maintaining the malignant state (Vousden and Ryan, 2009). Recently, several studies had identified a number of mevalonate pathway (MP) enzymes as crucial for the survival of different transformed cells by supplying sterol and isoprenoids and other products (Blomen *et al.*, 2015; Hart *et*

al., 2015; Wang *et al.*, 2015). In addition, the involvement of MP in several aspects of carcinogenesis led to it being considered as a target for therapy (Wong, Dimitroulakos and Penn, 2002; Clendening *et al.*, 2010; Clendening and Penn, 2012). In addition, an emerging role of mutant *TP53* in regulation of MP in breast cancer cells had been reported recently. Gain-of-function mutants of p53 interact with the SREBP to stimulate the transcription of genes involved in MP activity regulation in breast cancer cell lines (Freed-Pastor *et al.*, 2012). Therefore, it seems reasonable to evaluate the contribution of *TP53* in regulation of MP in OC to support the application of statins to the treatment of OC.

4.2. Aims

The goal of this chapter was to evaluate the contribution of *TP53* to the regulation of MP. To do this the effect of ectopic expression of *TP53* and siRNA directed to mutant *TP53* on the expression of MP enzymes, HMGCR, GGTI-β, GGTII-β and FT-β, was examined.

4.3. Results

4.3.1. The expression of HMGCR in a panel of ovarian cancer cell lines

It has been demonstrated that the activity of MP is deregulated in several different tumours. In order to study the activity of this pathway in OC cell lines, the level of HMGCR was measured to confirm if this correlated with mutational and expression of *TP53*. HMGCR enzyme were measured using western blot in a panel of OC cell lines, and compared to the level of HMGCR in normal human ovarian epithelial cells (HOE). Quantification of HMGCR enzyme levels reveals that all OC cell lines had a higher level of HMGCR protein expression than the normal HOE cells and it was statistically significant in A2780, CisA2780, Cov-318, Cov-362, Ovcar-3, Ovcar-5, Skov-3 cell lines compared to HOE cells (Figure 4-2 A and B).

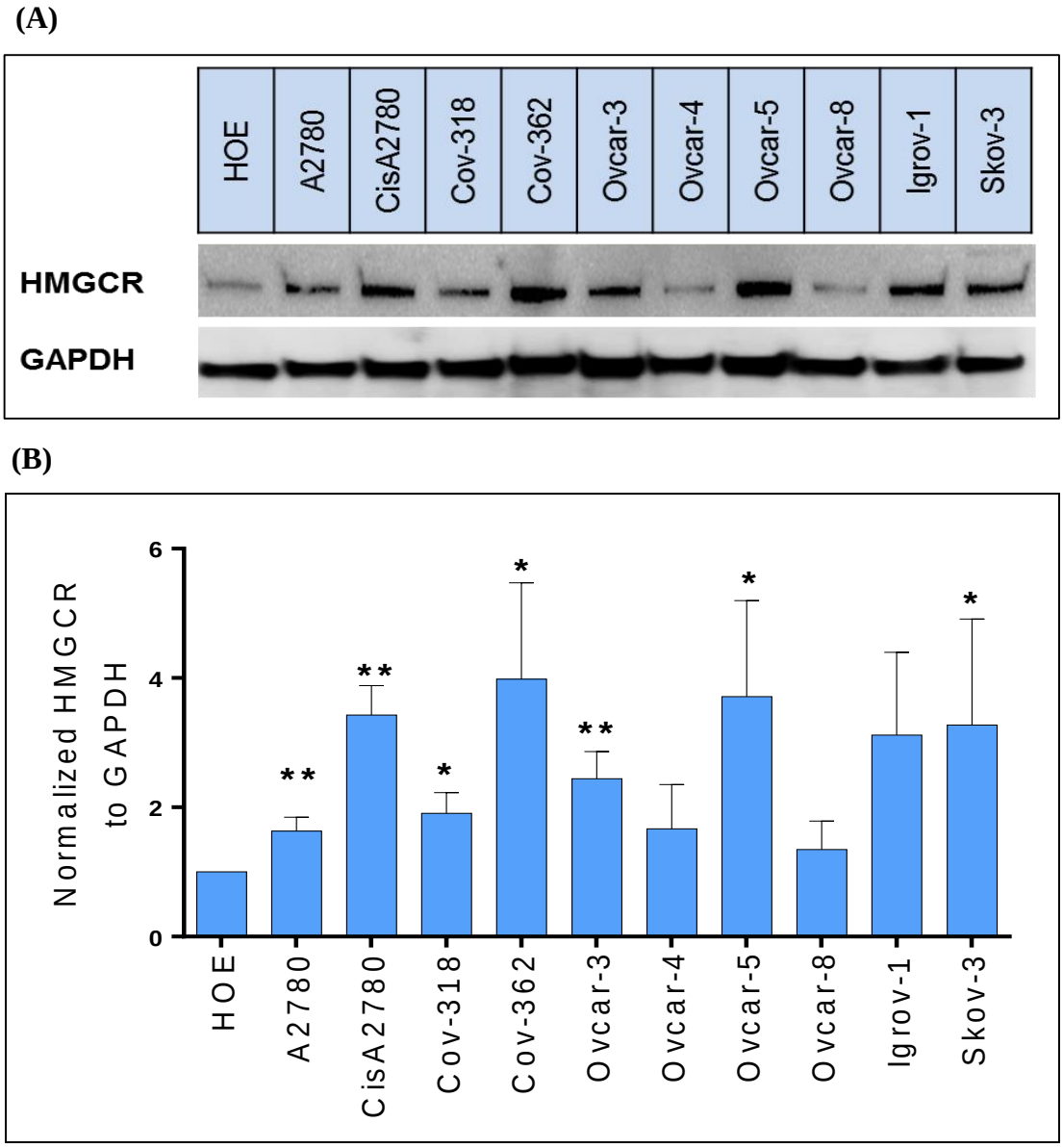


Figure 4-2 The level of HMGCR protein in panel of ovarian cancer cell lines.

The level of HMGCR was measured using western blot (A) in a panel of OC cell lines and normal human epithelial ovarian cell line and quantified (B). GAPDH was used as loading control. HMGCR protein were normalized to GAPDH (n = 3, *, P <0.05; **, P <0.01; paired t-test).

4.3.2. The expression of GGTI-B and GGTII-B in a panel of ovarian cancer cell lines

In addition to HMGCR, the level of the GGTI-β and GGTII-β enzymes of the MP were determined by immunoblotting in a panel of OC cell lines (Figure 4-3 A) and quantified (Figure 4-4 A and B). The results showed that GGTI-β and GGTII-β protein are overexpressed in subset of OC cell lines compared to HOE. However, the differences in expression were statistically significant in A2780, CisA2780, Ovcar-4, Ovcar-8, Igrov-1 and Skov-3 cell line for GGTI-β and A2780, Ovcar-5, Ovcar-8 and Igrov-1 cell line for the GGTII-β.

(A)

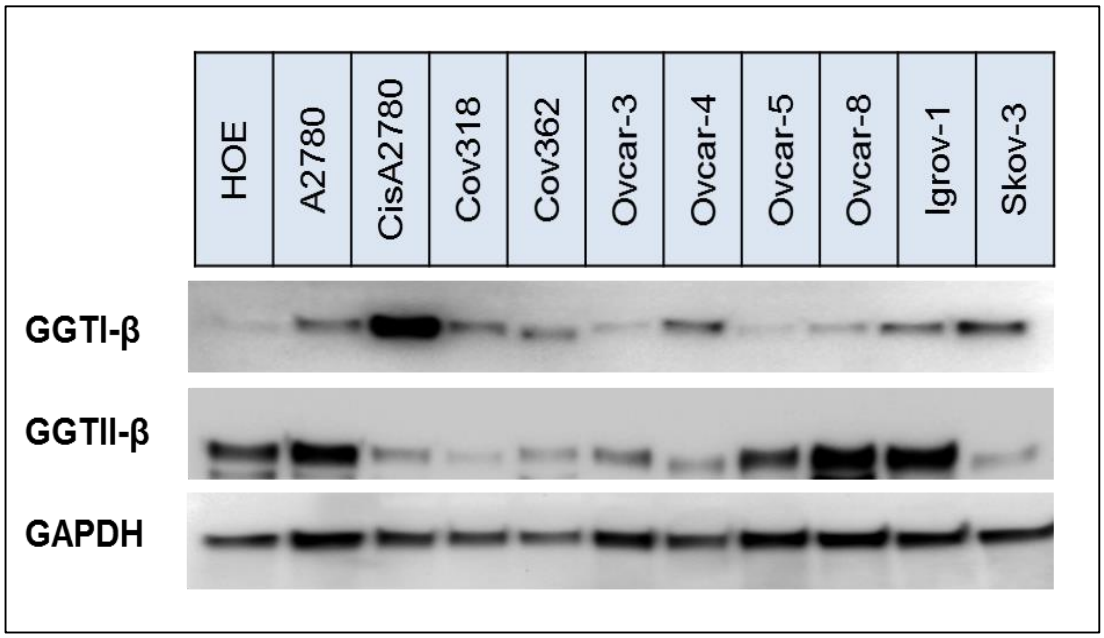
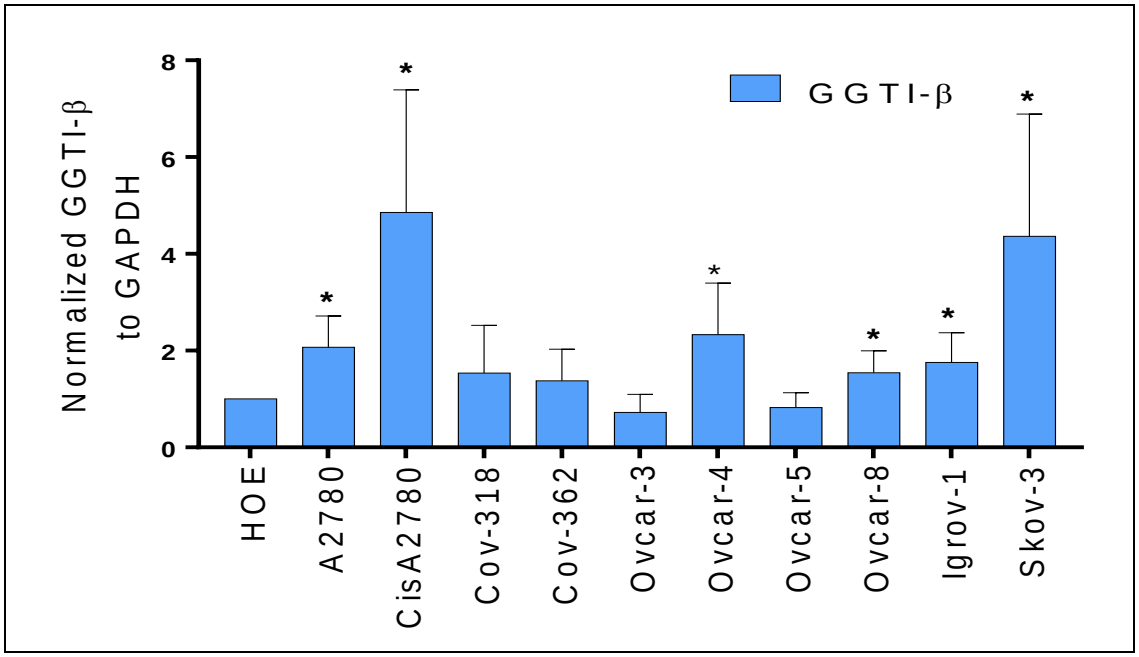


Figure 4-3 GGTI-β and GGTII-β level in panel of ovarian cancer cell lines.
The level of GGTI-β and GGTII-β was measured using western blot in a panel of OC cell lines and HOE. GAPDH was used as loading control (n = 3).

(A)



(B)

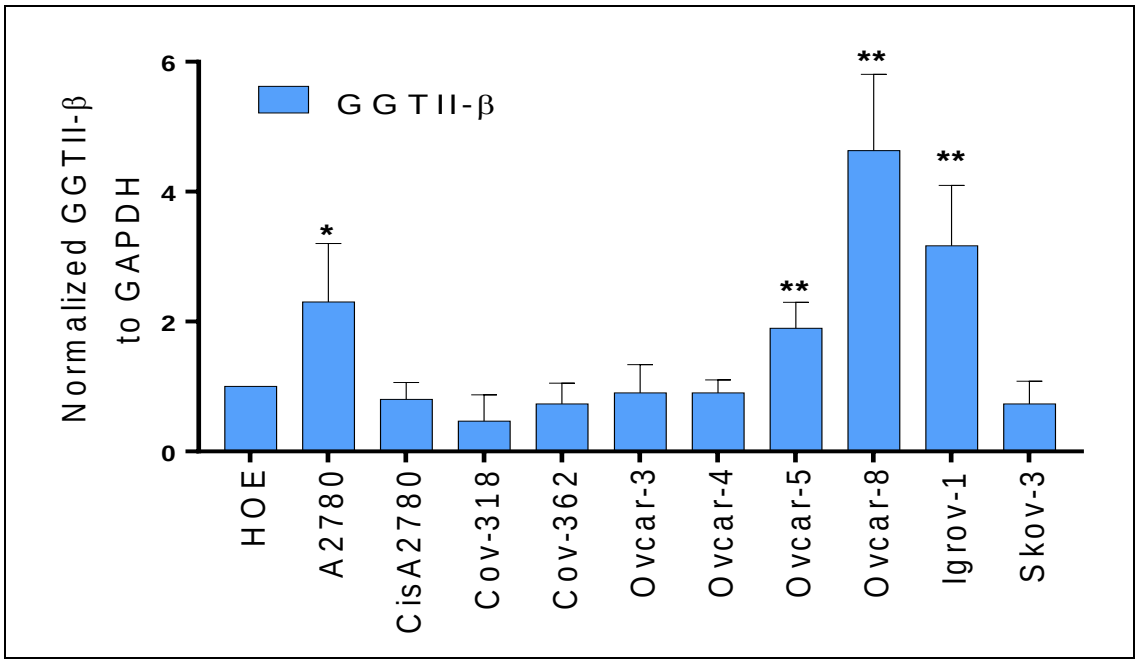


Figure 4-4 Quantification of GGTI-β and GGTII-β level in panel of ovarian cancer cell lines.

The level of GGTI-β and GGTII-β was measured using western blot in a panel of OC cell lines and HOE and quantified (B and C). GAPDH was used as loading control. GGTI-β and GGTII-β were normalized to GAPDH (*, P <0.05; **, P <0.01; paired t-test).

4.3.3. P53 status in panel of ovarian cancer cell line

To investigate the role of TP53 in regulation of MP enzymes in OC, the status of *TP53* mutations was first obtained from a public database (Bouaoun *et al.*, 2016) (Table 4-1). Most of the cell lines harbour a mutation in *TP53*, only HOE and A2780 cell lines have a wild type *TP53*. In common with other cancers, the mutations were mostly in the DNA binding domain (Figure 4-5) (Bieging and Attardi, 2012). Ovcar-3 cells had a mutation R248Q, which is one of most frequent hotspot mutations in p53 and is considered a gain of function mutation. In contrast, Skov-3 cell line is an established cell line that does not express p53 at protein level (Mullany *et al.*, 2015).

Table 4-1 *TP53* status in a panel of ovarian cancer cell lines (Bouaoun *et al.*, 2016)**.**

Cell line	TP53 status	p53 Mutation
HOE	Wild-type	-
A2780	Wild-type	-
CisA2780	Mutated	Q136
Cov-318	Mutated	I195F
Cov-362	Mutated	Y220C
Ovcar-3	Mutated	R248Q
Ovcar-4	Mutated	L130V
Ovcar-5	Mutated	-
Ovcar-8	Mutated	Y126C
Igrov-1	Mutated	Y126C
Skov-3	Null	-

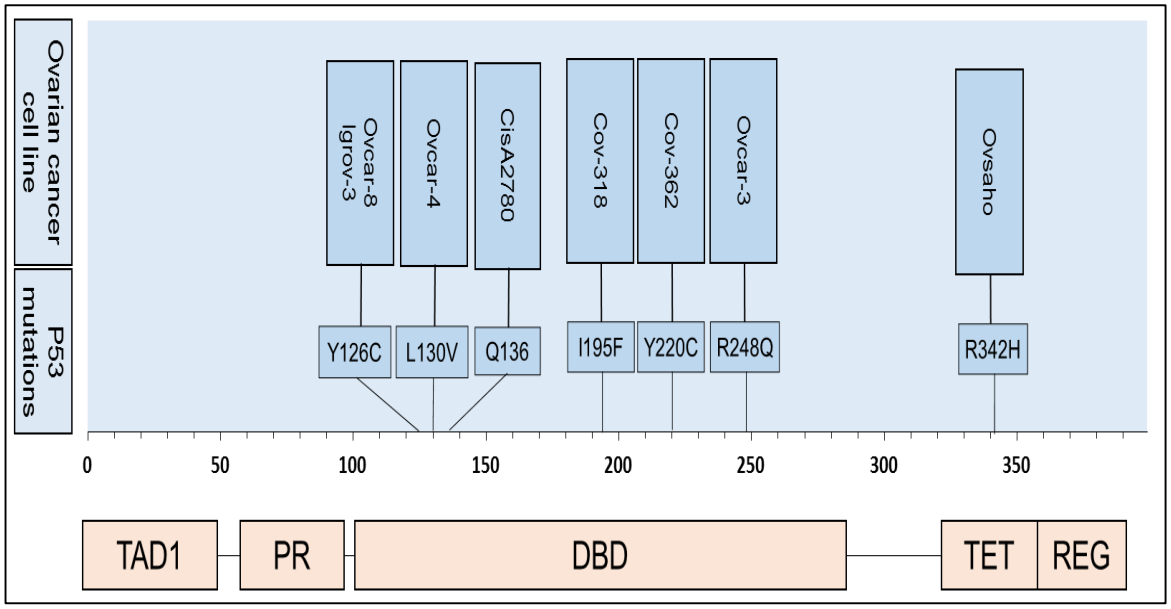
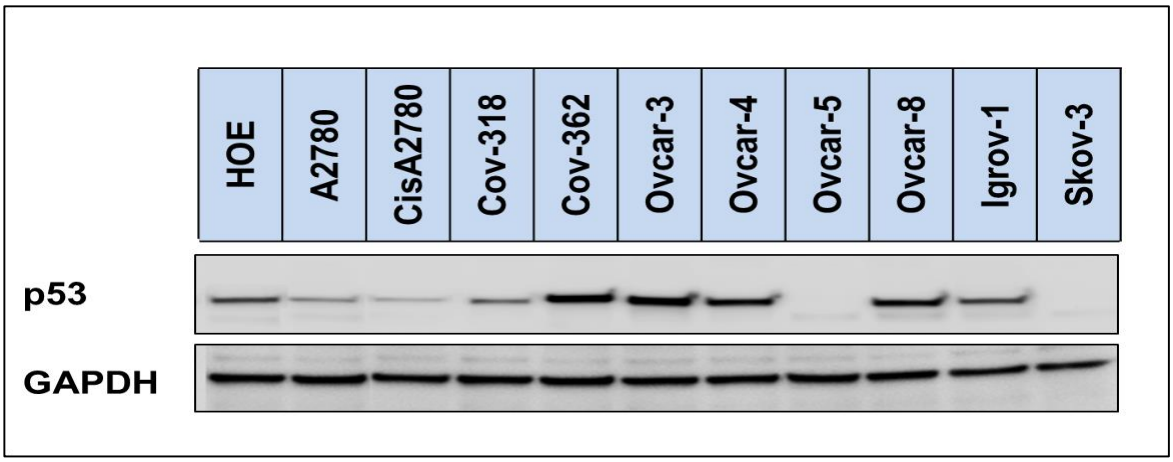


Figure 4-5 Sites of p53 mutation on ovarian cancer cell line.

Schematic representation of the 393 amino acid domain structure of human p53 showing the sites of mutation in OC cell lines. N-terminal transactivation domain (TAD); proline-rich domain (PR); tetrameriszation domain (TET); C-terminal regulatory domain (REG).

In addition, the expression of p53 was examined in panel of OC cell lines by western blotting (Figure 4-6). The result showed that p53 was most highly expressed in Cov-362, Ovcar-3, Ovcar-4 and Ovcar-8 cell lines and to lesser extent in A2780, CisA2780, Cov-318 and Igrov-1 cell lines. P53 expression was not been detected in Ovcar-5 and Skov-3 cell lines as previously reported (Mullany *et al.*, 2015).

(A)



(B)

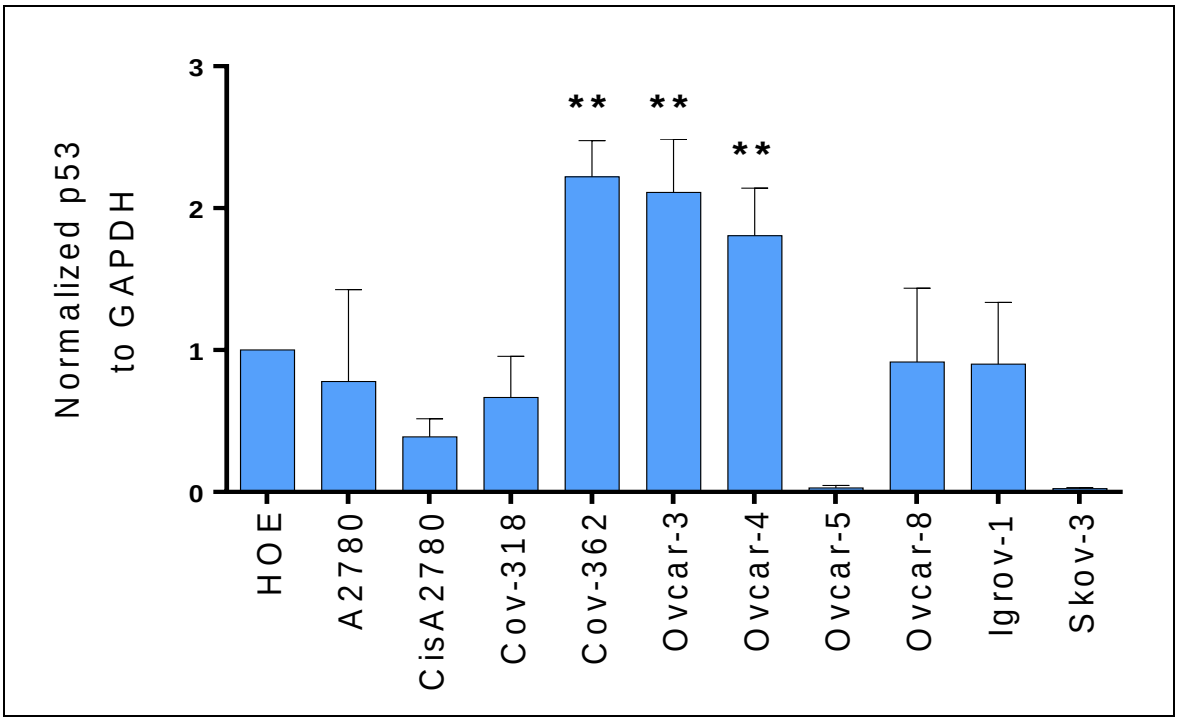


Figure 4-6 the expression of p53 in a panel of ovarian cell lines.

The level of p53 was measured using western blot (A) in a panel of OC cell lines and normal HOE cell line and quantified (B). GAPDH was used as loading control. P53 protein were normalized to GAPDH (n=3, **, P <0.01; paired t-test).

The cells were classified according to the *TP53* mutational status into wild type and mutated *TP53* and the level of MP enzymes determine by western blot in section 4.3.1 and 4.3.2 were used to compared between these two groups. The results showed that HMGCR level (Figure 4-7) in cell line that harbour *TP53* mutation was significantly higher than in cell lines with wild type *TP53*. However, there were no significant differences in GGTI-β and GGTII-β expression between cell lines with wild type *TP53* and mutant *TP53* (Figure 4-8).

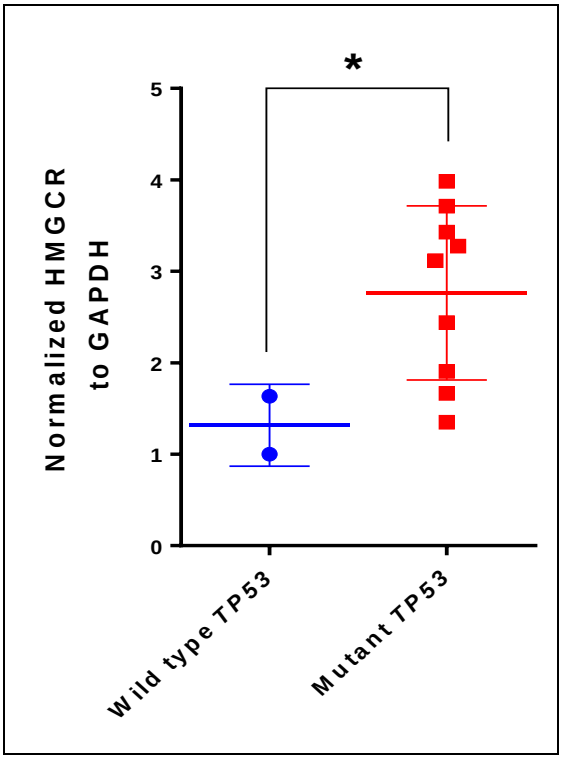


Figure 4-7 Comparison of the HMGCR expression in cells with wild type and mutant TP53.

The cell lines were classified according the *TP53* status into wild type and mutant TP53 and the level of the HMGCR were compared between these two groups of cell lines (Mean ± SD; * $P < 0.05$; unpaired *t*-test).

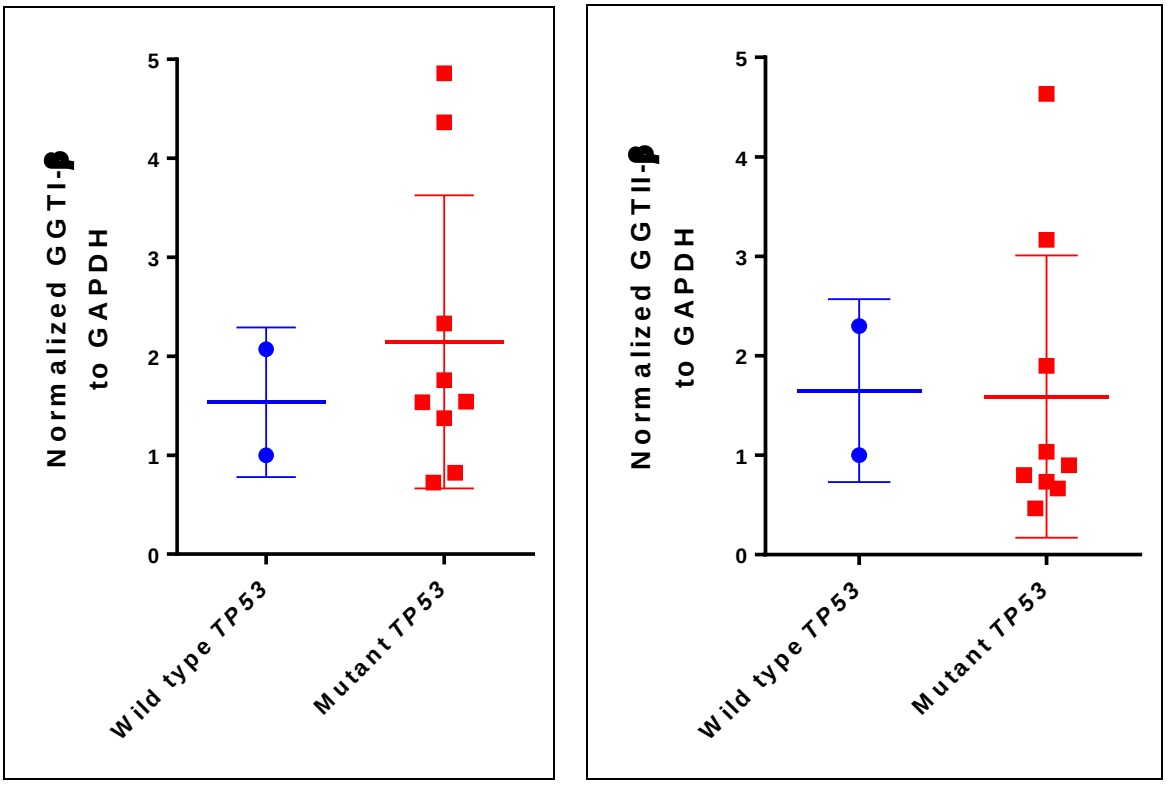


Figure 4-8 Comparison of the GGTI-β and GGTII-β expression in cells with wild type and mutant TP53.

The cells were classified according the TP53 status into wild type and mutant TP53 and the level of the GGTI-β and GGTII-β were compared between these two groups of cell lines (Mean ± SD; * $P < 0.05$; unpaired *t*-test).

The expression of the MP enzymes was compared with that of mutant p53 in CisA2780, Cov-318, Cov-362, Ovcar-3, Ovcar-4, Ovcar-8 and Igrove-1 OC cell lines. There was no significant correlation between p53 and HMGCR and GGTII-β expression. In contrast, the GGTI-β were significantly correlated with p53 expression ($R^2 = 0.213$; $p < 0.05$) (Figure 4-9). In addition, when the expression of MP enzymes was compared with sensitivity to pitavastatin IC_{50} in cell growth assays, only GGTI-β showed a statistically significant correlation ($R^2 = 0.463$; $p < 0.05$) (Figure 4-10). Although these correlations were statistically significant the correlation coefficient were weak to moderate linear correlation.

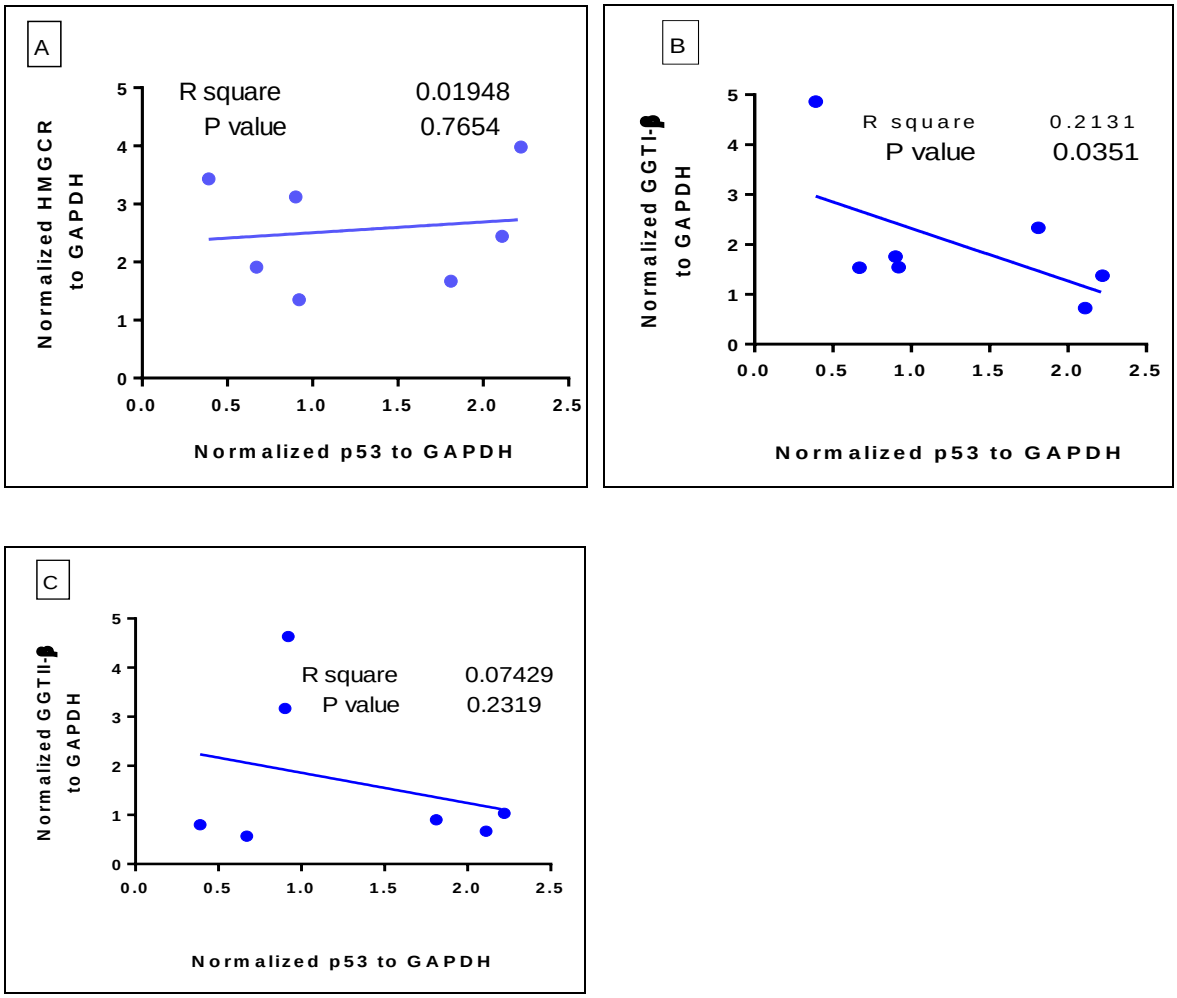


Figure 4-9 Relationship between the expression of mutant p53 and the expression of the mevalonate pathway enzymes in ovarian cancer cell lines.

The expression of the p53 was correlated with HMGCR (A), GGTI-β (B) and GGTII-β (C) expression which are quantified from blotting in CisA2780, Cov-318, Cov-362, Ovcar-3, Ovcar-4, Ovcar-8 and Igrov-1 OC cell lines.

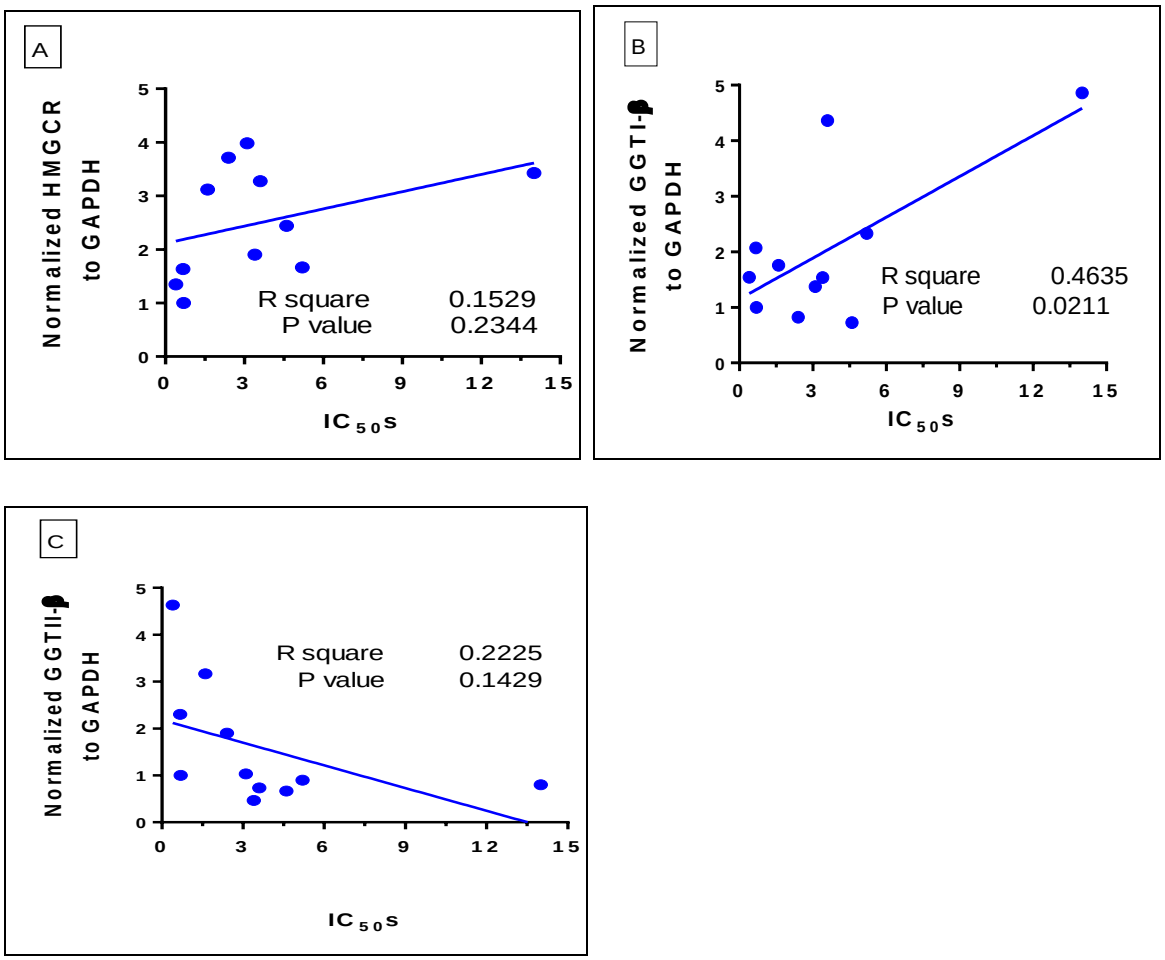


Figure 4-10 Relationship between sensitivity to pitavastatin and the expression of mevalonate pathway enzymes in ovarian cancer cell lines.

Pitavastatin IC_{50}s correlated to the expression of HMGCR (A), GGTI-β (B) and GGTII-β (C) which is quantified from blotting in a panel of OC cell lines.

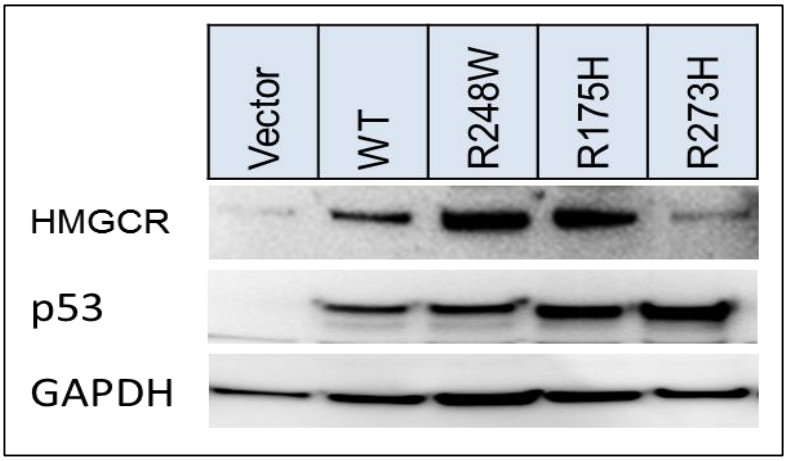
4.3.4. The effect of p53 overexpression on mevalonate pathway

4.3.4.1. The effect p53 overexpression on HMGCR in Skov-3 cell line

It has been proposed that to understand mutations in *TP53*, cells lacking p53 can be used to overexpress of mutant *TP53*; alternatively, siRNA can be used to knockdown the *TP53* in cells harbouring mutated p53 (Brachova, Thiel and Leslie, 2013). Therefore, Skov-3 (*TP53* null) and Ovcar-3 (*TP53* R248Q) cells were tested to explore the effect p53 on the MP enzymes.

In order to evaluate the effect of p53 in MP in OC cell lines. Skov-3 were transiently-transfected with a plasmid encoding mutated *TP53* variants R284W, R175H and R273H which are the most frequently reported mutation in OC (Brachova, Thiel and Leslie, 2013). Skov-3 cells were also transfected with a plasmid encoding wild-type *TP53* variant. The expression of HMGCR and p53 was monitored after 48 and 72 hours because transient ectopic expression was used in these experiments. The result showed that there is marked increase in level of expression of all p53 variants after transfection compared with cells transfected with vector. In addition, all p53 variants caused an increase in the level of HMGCR expression. However, only wild type and R248W gain of function variant led to a significant increase in the expression of HMGCR compared to cells transfected with vector measured by immunoblotting after 48 hour of transfection (Figure 4-11). 72 hours after transfection p53 was still detectable but the changes in the level of HMGCR were no longer significantly different to cells transfected with the empty vector (Figure 4-12).

(A)



(B)

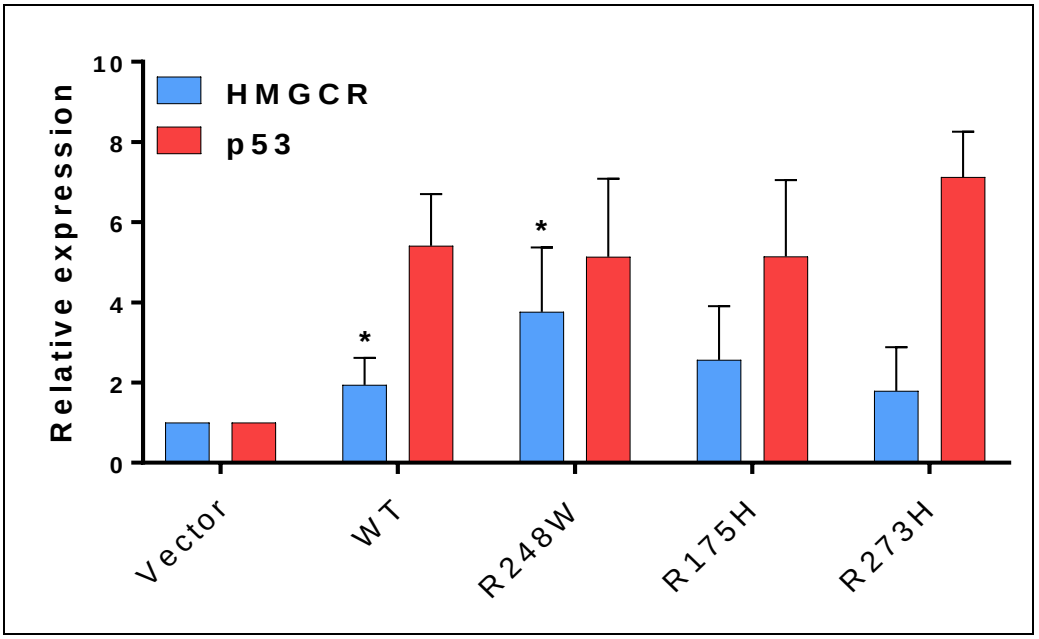
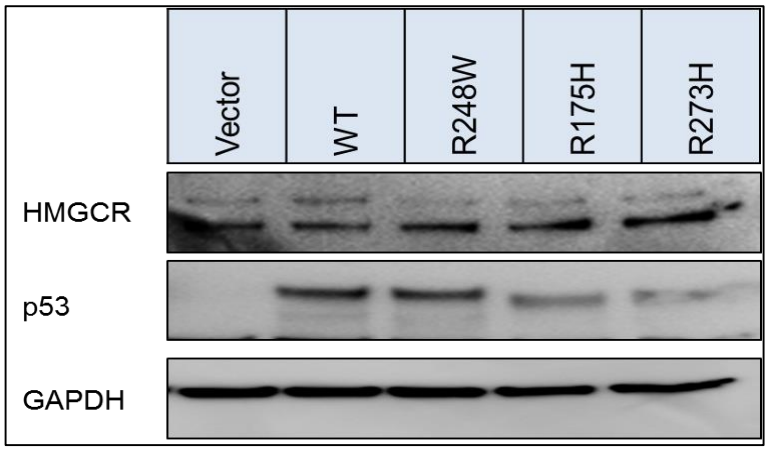


Figure 4-11 The level of HMGCR and p53 in Skov-3 cell line transfected with p53 after 48 hour incubation.

Wild type (WT) and p53 variants were over expressed in Skov-3 cell lines. HMGCR and p53 measured by immunoblotting (A) after 48 hours of incubation and proteins were quantified (B). HMGCR were significantly different to expression in cells transfected with vector where shown (mean ± S.D., n=3, *, P <0.05; paired t-test).

(A)



(B)

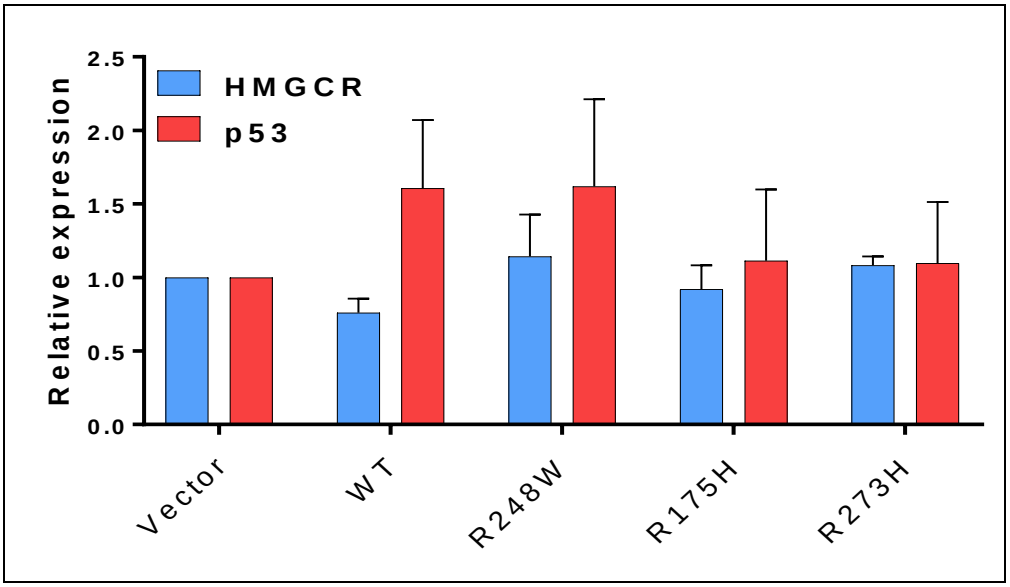


Figure 4-12 The level of HMGCR and p53 in Skov-3 cell line transfected with p53 after 72 hour incubation.

Wild type (WT) and p53 variants were over expressed in Skov-3 cell lines. HMGCR and p53 measured by immunoblotting (A) after 72 hours of incubation and proteins were quantified (mean ± S.D., n=3) (B). HMGCR expression was not significantly different to cells transfected with the vector.

4.3.4.2. The effect of ectopic p53 transfection on mevalonate pathway genes expression

To confirm that the increase in HMGCR protein was due to increased transcription of *HMGCR*, its mRNA was measured by QPCR. In parallel the expression of genes encoding farnesyl and geranylgeranyl transferases GGTI-β, GGTII-β and FT-β was also measured. The results showed that there was an increase in mRNA level of all tested MP enzymes in Skov-3 cells which were transiently-transfected with plasmids encoding wild type *TP53* variant and mutated *TP53* variants R284W, R175H and R273H compared to cells transfected with vector. The expression of *HMGCR* were significantly increased by ectopic expression of the wild type *TP53* variant and mutant *TP53* variants. The increase in HMGCR mRNA was most pronounced in cells transfected with R248W variant, in agreement with our previous observation of increased HMGCR protein measured by western blotting (Section 4.3.4.1). GGTI-β expression increased in a pattern similar to that of HMGCR expression with R248W variant inducing higher expression than other *TP53* variants. In contrast, the expression of GGTII-β was consistently and significantly induced by all *TP53* variants. Lastly, the expression of FT-β were also significantly elevated except in cells transfected with R248W, in which the change was not statistically significant (Figure 4-13).

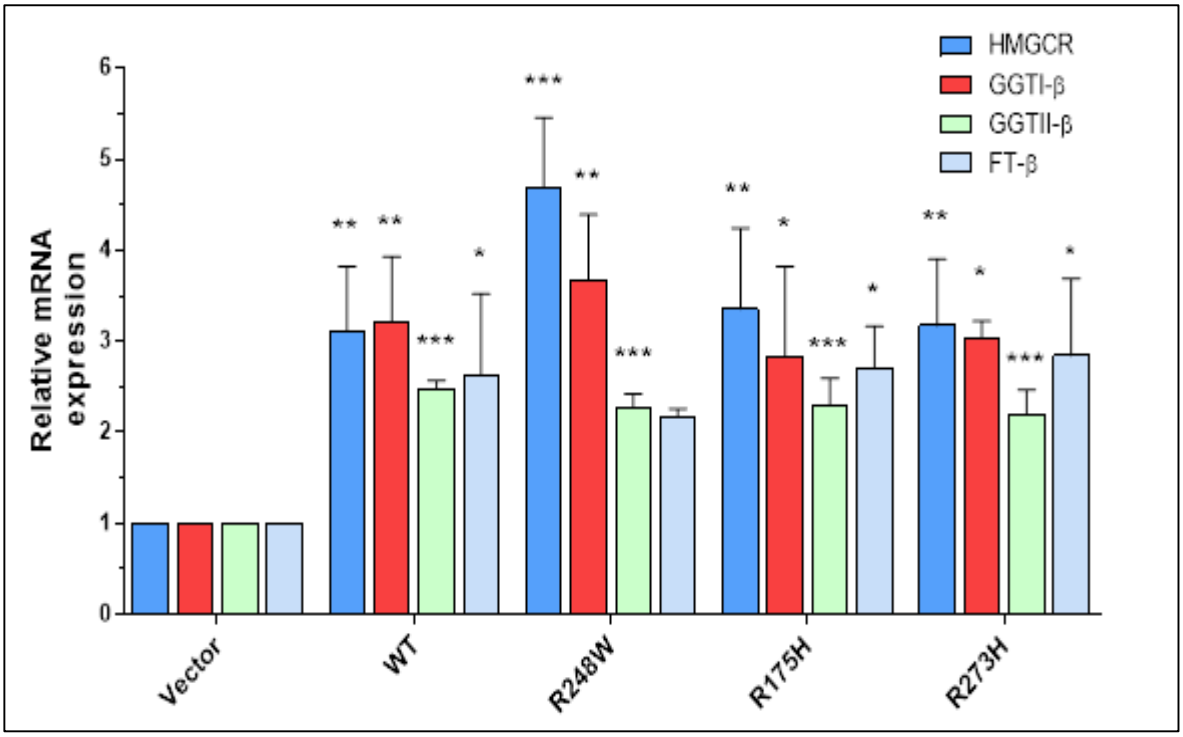


Figure 4-13 mRNA expression of HMGCR, GGTI-β, GGTII-β and FT-β genes of Skov-3 cell line transfected with p53.

Wild type (WT) and p53 variants were over expressed ectopically in Skov-3 cell lines. Relative mRNA expression of HMGCR, GGTI-β, GGTII-β and FT-β genes were measured using QPCR after 48 hour of transfection. Relative mRNA expression of genes was significantly different compared to cells transfected with vector (mean ± S.D., n=3, *, P <0.05, **, P <0.01; ***, P <0.001; one-way Anova followed by Tukeys post-hoc).

4.3.5. The effect of p53 knockdown on mevalonate pathway

4.3.5.1. The effect of p53 siRNA transfection on HMGCR expression in Ovcar-3 cell line

The results showed that ectopic overexpression of p53 in Skov-3 cell line cause significant increase in level of MP gene expression. To determine if the pre-existing mutation in *TP53* also regulate MP genes, the expression of *TP53* in Ovcar-3 cells (which contain a mutation in *TP53* encoding R248Q) was repressed using four different siRNA oligo's directed to p53 mRNA. First, the p53 knockdown was confirmed by western blotting. All of the *TP53* siRNAs showed a reduction by more than 65 % of the p53 protein level compared to cells transfect with non-targeting-1 siRNA. Secondly, HMGCR protein levels were decrease significantly after transfection with each of thep53 siRNA (Figure 4-14 A). However, the quantification revealed that p53#2 and p53#4 oligos induce a reduction in HMGCR level which was statistically more significant than p53#1 and p53#3 oligos (Figure 4-14 B).

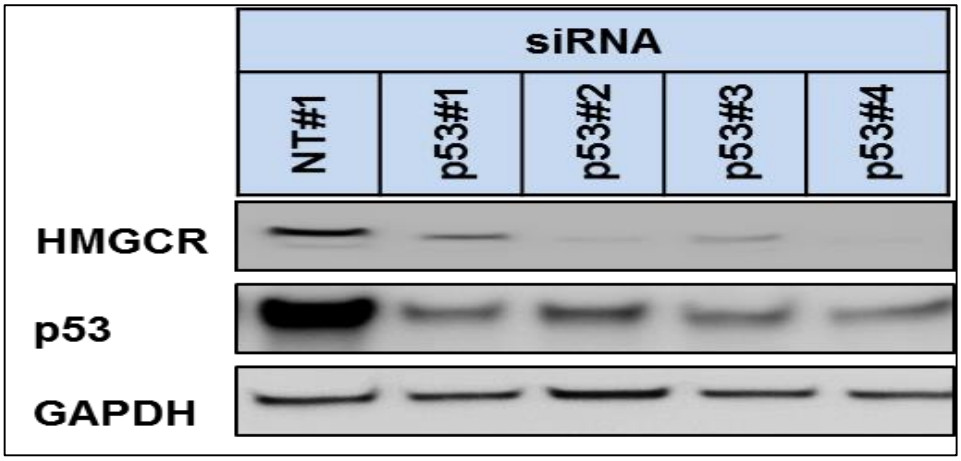
4.3.5.2. The effect of p53 siRNA transfection on mevalonate pathway genes expression.

RT-QPCR was used to confirm that the knockdown of p53 protein by siRNA directed to *TP53* reflected reduced transcription of HMGCR. In parallel the expression of GGTI-β, GGTII-β and FT-β enzymes was also measured. The expression of HMGCR, GGTI-β, GGTII-β and FT-β genes were markedly reduced by transfection of Ovcar-3 cells with four separate siRNA directed to *TP53* compared to cells transfected with NT#1 siRNA (Figure 4-15).

Lastly, it can be summarized that MP enzymes are deregulated in OC. Particularly, HMGCR level is higher in OC cell line in comparison to normal human ovarian epithelial cells. In addition, p53 play a central role in regulation of MP. Exogenous transfection of Skov-3 cell

line with p53 upregulate the expression of key enzymes of MP, such as HMGCR, GGTI-β, GGTII-β and FT. in contrast, knockdown of p53 using siRNA downregulate the expression of these MP enzymes.

(A)



(B)

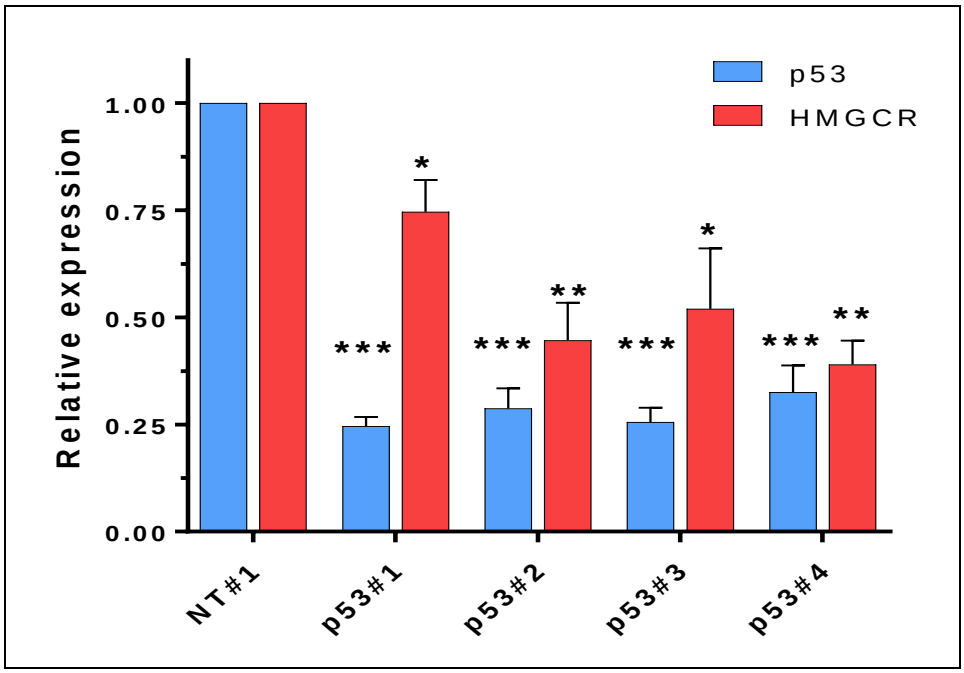


Figure 4-14 The level of p53 in Ovcar-3 cell line transfected with non-targeting (NT) siRNA or p53 siRNA.

p53 measured by immunoblotting (A) and quantified (B) after 48 hour of transfection of Ovcar-3 cell line with Non-targeting#1 siRNA (NT#1) or 4 different p53 siRNA (#1, #2, #3, #4). p53 were significantly different to expression in cells transfected with NT#1 where shown (mean ± S.D., n=3, *, *P* <0.05; **, *P* <0.01; ***, *P* <0.001; paired *t*-test).

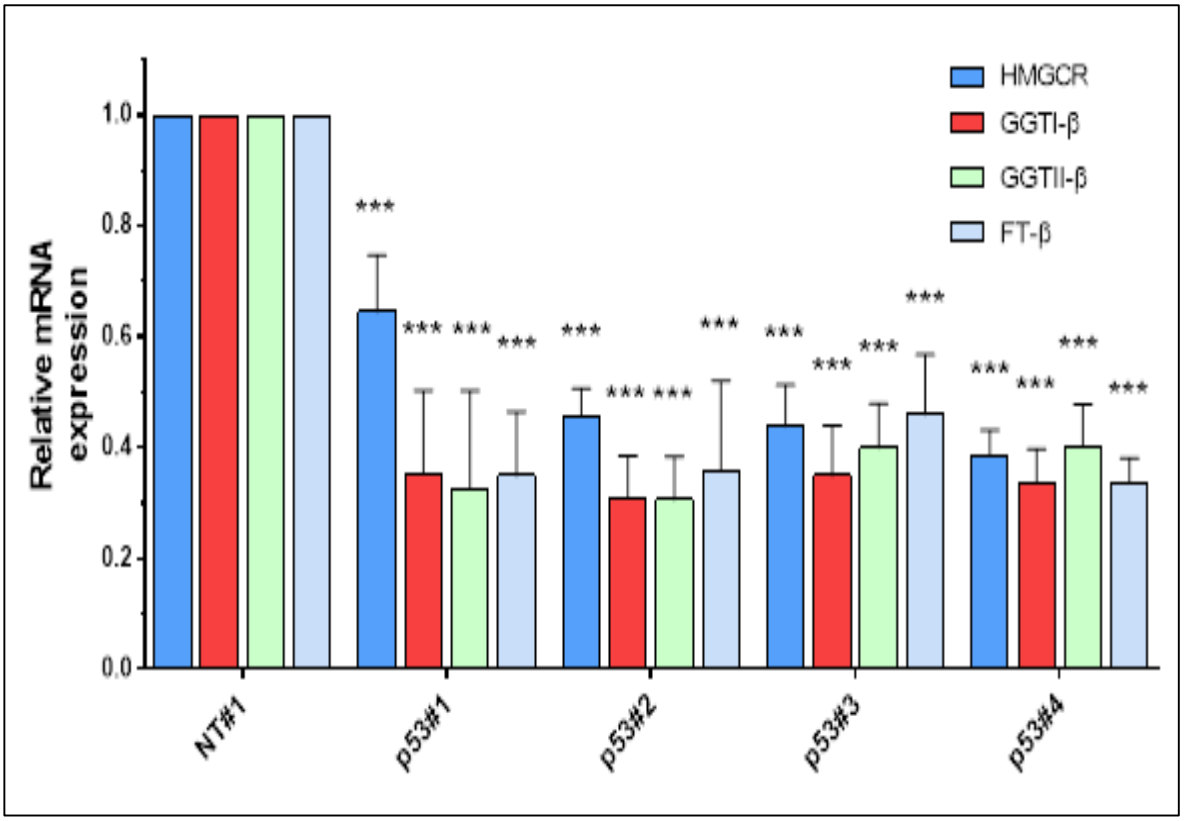


Figure 4-15 mRNA expression of HMGCR, GGTI-β, GGTII-β and FT-β genes of Ovcar-3 cell line transfected with p53 siRNA.

p53 knockdown using four p53 siRNA and NT#1 siRNA in Ovcar-3 cell lines. Relative mRNA expression of HMGCR, GGTI-β, GGTII-β and FT-β genes were measured using QPCR after 48 hour of transfection. Relative mRNA expression of genes was significantly different compared to cells transfected with NT#1 siRNA (mean ± S.D., n=3, ***, *P* <0.001; one-way Anova followed by Tukeys post-hoc).

4.4. Discussion

Approximately 75% of OC deaths is caused by HGSOC (Bowtell *et al.*, 2015; Lee *et al.*, 2015). The tumour suppressor *TP53*, which is frequently mutated in OC, and up to 99 % in HGSOC (Fleury *et al.*, 2015), is considered as a master regulator of diverse cellular process in health and disease (Farnebo, Bykov and Wiman, 2010). Once mutated, it is involved in several aspect of malignant transformation and resistance to cancer therapy but the actual mechanism remains incompletely understood (Brosh and Rotter, 2009). This study describes the possible role of p53 in regulation of MP. Exogenous transfection of p53 upregulate the level of MP enzymes while knockdown of mutant p53 down regulate the level of MP enzymes. Therefore, studying p53 is still an important target to identify their role in oncogenesis.

The expression of MP enzymes, HMGCR, GGTI-β and GGTII-β were determined and the result showed high HMGCR expression and to leaser extent of GGTI-β and GGTII-β in a panel of OC cell line compared to normal cells. Cancer evolution has been linked with metabolic processes that tumour cells successfully hijacks to assist malignant transformation (Clendening *et al.*, 2010). For example, ectopic expression of HMGCR promotes transformation which led to considered it as a metabolic oncogene (Clendening *et al.*, 2010). This increased expression of the HMGCR and other MP enzymes provide the fast-proliferating malignant cells with copious amount of products which are principally used for biosynthesis of the cells component (Parrales and Iwakuma, 2016) to maintain the growth and development machinery (Clendening and Penn, 2012). However, there are several mechanisms for the regulation of the HMGCR. HIF-1 alpha accumulation increase the level and activity of HMGCR by stimulating it is transcription (Pallottini *et al.*, 2008). In addition, sterol mediated degradation of HMGCR has been shown to be inhibited by mutations which led to increase its enzymatic activity (Lee, Nguyen and Debose-Boyd, 2007). In addition,

recent study in our laboratory has defined a promising role for statins in OC (De Wolf *et al.*, 2017). Regardless of the mechanism by which deregulated HMGCR expression occurs, better understanding of the contribution of genes regulating isoprenoid metabolism might lead to improved cancer patients care (Clendening and Penn, 2012) and provide a rational for the use of the pathway inhibitors as anticancer therapy.

It was also observed that p53 level were higher than normal cells at least in a subset of the OC cell lines. In addition, the status of the p53 has been retrieved form public database and this analysis showed that p53 was mutated in most of OC cell lines. Most of the mutation are missense and located at DNA binding domain which produce a full-length protein with prolonged half-life (Rivlin *et al.*, 2011). In contrast to normal cells, there is a copious production of p53 protein in many tumours (Rotter, Abutbul and Ben-Ze'ev, 1983) and this tends to accumulate until reach steady state (Rotter, 1983). The accumulation of p53 protein causes defect in activation/ repression of target genes to ablate p53-induced apoptosis and maintains the malignant phenotype (Wiman, 2007). However, increase of p53 level might be a response to stimuli such as DNA damage and hypoxia (Strano *et al.*, 2007; Sionov, Hayon and Haupt, 2013). Therefore, it might be suggested that the accumulation is a consequence of prolonged stability and extended half-life of the p53 protein by point mutation, or it is just a normal physiological response of cells to stimuli in course of carcinogenesis process.

In this study, wild type and several mutant variants of *TP53* has been studied to understand the relationship between MP and *TP53* in OC. The result suggested that p53 controls at least four important enzymes of the pathway. Two lines of evidence point to the regulation of MP by *TP53*. Firstly, the ectopic expression of wild type and several mutant p53 variant led to increase the expression of HMGCR, GGTI-β and GGTII-β and FT-β in p53 null background

OC cell line. Secondly, siRNA directed to R248Q *TP53* mRNA significantly decreased the level of HMGCR, GGTI-β, GGTII-β and FT-β enzymes. A new role of *TP53* has emerged in mediating cancer development through regulating the MP. It is found that several enzymes of the MP controlled by gain of function of mutant *TP53* in breast cancer cells (Freed-Pastor *et al.*, 2012; Sorrentino *et al.*, 2014). The result showed that ectopic expression of *TP53*, particularly gain of function variant, increased expression of HMGCR and is likely to be common in OC. It reported that gain-of-function mutants p53 prompt the synthesis of cholesterol (Laezza *et al.*, 2015; Napoli and Flores, 2017). This hypothesis is supported by immunohistochemical studies which have identified HMGCR in 65% of OCs (Brennan *et al.*, 2010) as well as other cancer types such as breast, colorectal and gastric tumours (Bengtsson *et al.*, 2014; Gustbée *et al.*, 2015; Chushi *et al.*, 2016). In contrast, depletion of mutant p53 leads to reduced expression of seven MP genes -*HMGCR, MVK, MVD, FDPS, SQLE, LSS, DHCR* (Freed-Pastor *et al.*, 2012). In addition, the normal morphology phenotype of breast cancer cells is restored by knock down of mutant p53 or by inhibition of MP by pharmacological agents such as statins (Freed-Pastor *et al.*, 2012).

The mechanism of the p53 regulation of the MP is not fully understood but much evidence points to a role for SREBP, although a direct link between mutant p53 and SREBP has not been established. It has been reported that mutant p53 is recruited to gene that encode of the MP pathway enzymes by SREBPs to upregulate their expression in breast cancer cells (Freed-Pastor *et al.*, 2012). In addition, p53 supresses the expression of the SREBP1c, a transcription factor involves in the expression of the two lipogenic enzymes (fatty acid synthase and ATP citrate lyase) that regulate fatty acid synthesis. Augmentation of fatty acid synthesis in different cancers type has been linked to overexpression of the activity of lipogenic enzymes and their inhibition is associated with repression of the cell transformation and oncogenesis (Freed-Pastor *et al.*, 2012). Furthermore, another

mechanism by which p53 control SREBP are by inhibiting mTOR and activating AMPK. The mTOR, which is frequently deregulated in cancer, increases the transcription of SREBP, and conversely AMPK inactivates HMGCR by phosphorylation (Budanov and Karin, 2008; Mullen *et al.*, 2016). The PI3K/AKT signalling pathway is one of the most frequently altered pathway in cancers and especially OC (Sain *et al.*, 2006; Fruman and Rommel, 2014) and has also been implicated in regulating the mevalonate pathway. It is activated by multiple molecular defects, mainly *PIK3CA* mutation or amplification (30%) and *PTEN* loss (40%) (Glaysher *et al.*, 2013). The PI3K/AKT pathway can activate the MP by upregulating the transcription, increasing stability or inhibiting the metabolism of the SREBP (Mullen *et al.*, 2016). Additionally, p53 induction reciprocally downregulates PI3K/AKT activity by binding to the *PTEN* promoter (Singh *et al.*, 2002). These data might provide a further link for the p53 role in regulation of MP.

Interestingly, the data showed that both wild type p53 and mutant p53 increase the expression of the MP which might suggest that both the level of the p53 expression and the mutational status of p53 are determinant of the pathway activity. It has also been proposed that mutant *TP53* might retain or exaggerate certain p53 function whereas evading certain wild type p53 tumour suppressive activity. Therefore, it might be a remnant of unrecognized wild type p53 function is responsible for the maintaining of high level of MP through SREBP transcription (Freed-pastor, 2012). In line with this proposition, it is reasonable to propose that both wild-type and mutant p53 have a significant role in regulating the expression of sterol biosynthesis genes.

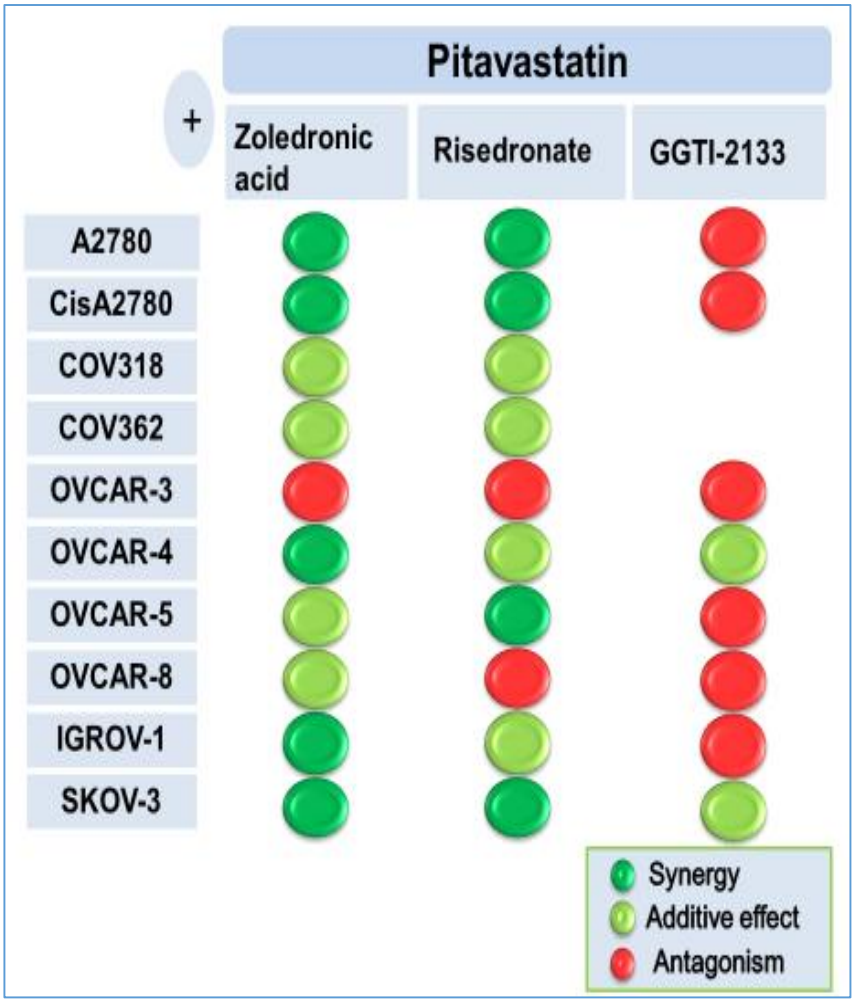
It is well known that mutant p53 cannot activate the expression of its negative regulator MDM2, consequently, mutant p53 protein is stabilised and accumulated (Flöter, Kaymak and Schulze, 2017). It is possible that this is more pronounced in case of mutant “gain-of

function" form which are not subjected to ubiquitination by MDM2. A feed-forward loop is formed in cancer cells which harbour a mutant p53. MP enzymes, including HMGCR, are upregulated through activation of SREBP transcription by mutant p53. The increase in pathway activity and productivity in turn lead to stabilizing the mutant p53 function (Freed-Pastor and Prives, 2016). In addition, it has recently been reported that statins enhances the degradation of mutant p53 protein, a process which is independent on the inhibition of protein prenylation (Parrales et al. 2016), supporting the use of statins in the treatment of cancers with mutated p53.

In conclusion, the result suggested that the upregulation of HMGCR enzymes in OC cell lines might be involved in malignant transformation in OC. P53, is a pivotal transcription factor for control of MP enzymes function in OC and the interplay between p53 and the MP suggests that pharmaceutical inhibition of the pathway with statins may be a novel therapeutic approach for tumours and warrant a promising role in treatment of OC.

Chapter Five

Modulation of mevalonate pathway to potentiate the activity of pitavastatin against panel of ovarian cancer cell lines



5.1. Introduction

Ovarian cancer (OC) is the 5th leading cause of death in women with more than 14,000 deaths reported annually in United States (Siegel, Miller and Jemal, 2016). The disease responds initially to treatment but most patients relapse after a period of remission (Vaughan *et al.*, 2011). Therefore, new therapeutic agents or treatment strategies are required.

The results of previous chapter suggested that the deregulation of MP might be involved in malignant transformation in OC. It is also proposed that p53, which is frequently mutated in OC, might be essential transcription factor for control of MP enzymes function in OC and the interplay between p53 and the MP suggests that pharmaceutical inhibition of the pathway with statins is a novel therapeutic strategy and warrant a promising role in treatment of OC.

The mevalonate biosynthetic pathway is responsible for the synthesis of several important metabolites, producing cholesterol, dolichol, ubiquinone and the isoprenoids farnesol (FOH) and geranylgeraniol (GGOH). The rate limiting step in the mevalonate pathway (MP) is hydroxymethylglutaryl coenzyme A reductase (HMGCR) which catalyses the production of mevalonate (Brennan *et al.*, 2010) and HMGCR has been identified as metabolic oncogene which promotes xenograft growth (Clendening *et al.*, 2010; Martirosyan *et al.*, 2010) and this has raised interest in the MP as a potential target in oncology.

Several studies have demonstrated that statins inhibit cell growth and induce apoptosis *in vitro* in cell lines from a range of cancer types (Swanson and Hohl, 2006; Gazzerro *et al.*, 2012; Osmak, 2012). It has also been reported that statins inhibit tumour xenograft growth in mice (Kobayashi *et al.*, 2015; Tsubaki *et al.*, 2015) and recently publish data from our laboratory have demonstrated that pitavastatin causes tumour regression in mice fed a controlled diet (De Wolf *et al.*, 2017). However, relatively high doses of statins are likely to be necessary to achieve an adequate plasma concentration of drug in patients (Dudakovic *et*

al., 2008; Robinson *et al.*, 2014) and this raises concerns about the potential risk of myopathy, a side effect commonly associated with statins (Likus *et al.*, 2016). Therefore, it desirable to identify drugs which synergize with statins and potentially reduce the dose of statin that is necessary to treat patients.

Bisphosphonates (e.g. zoledronic acid, risedronate) are drugs which are already approved for the management and prevention of bone disease and bone metastasis (Stresing *et al.*, 2007). Bisphosphonates can also inhibit the MP enzyme farnesyl diphosphate synthase (Wasko, Dudakovic and Hohl, 2011). Inhibition of farnesyl diphosphate synthase depletes both farnesyl diphosphate and geranylgeranyl diphosphate which in turn are required for protein isoprenylation of small G-proteins (Gnant and Clézardin, 2012). Bisphosphonates have shown potential anti-cancer activity in different cancer cell lines including ovarian, colon and hepatic cells (reviewed in (Stresing *et al.*, 2007)). In addition, several studies showed that bisphosphonate use correlates with reduced cancer risk (Rennert, Pinchev and Rennert, 2010; Rennert *et al.*, 2014). Bisphosphonates can also enhance the anticancer activity of several chemotherapeutic agents *in vitro* (Jagdev *et al.*, 2001; Neville-Webbe *et al.*, 2005; Horie *et al.*, 2007; Hafeman, Varland and Dow, 2012).

There are several reasons to believe that drug combinations may be particularly useful in the treatment of cancer. Firstly, tumours represents a heterogeneous group of diseases with several different pathological mechanisms participating in their evolution (Bertolini, Sukhatme and Bouche, 2015). As a consequence, drug combinations inhibiting different underlying pathways might theoretically be more effective than single agents because multiple cell populations may be simultaneously affected by the drugs (Rodon, Perez and Kurzrock, 2010). Secondly, drug combinations can concurrently affect different signalling pathways in individual cancer cells. These drugs may act synergistically to increase the efficacy of the treatment above that which would be achieved by the single agents alone.

Thirdly, during cancer pharmacotherapy, mutation and epigenetic change can activate multiple compensatory pathways in cancer cells, leading to the emergence of drug-resistant subpopulations. Therefore, drug combinations or multi-targeted drugs may offer a better chance of obtaining a sustained clinical response (He *et al.*, 2016; Han *et al.*, 2017). Lastly, there is a historical precedent for the use of drug combinations and many traditional chemotherapeutic regimens incorporate several different drugs.

Recent results from our laboratory have suggested that pitavastatin is superior to other statins for use in oncology because it is the only statin that is both lipophilic, rendering it more potent than hydrophilic statins, and has a suitably long half-life ($t_{1/2}$ ~11 hour) (Robinson *et al.*, 2013; Jiang *et al.*, 2014; Zhang *et al.*, 2016; De Wolf *et al.*, 2017). The latter property is important because it has been shown continual inhibition of HMGCR is necessary to induce cell death and the troughs in plasma drug concentration between prolonged dosing intervals using short half-life statins are likely to compromise the activity of statins (Robinson *et al.*, 2013). To reduce the dose of pitavastatin necessary in patients, and potentially minimize adverse effects, zoledronic acid, risedronate or the geranylgeranyl transferase I inhibitor, GGTI-2133 was investigated to determine if they potentiate the activity of pitavastatin. Any effective combinations identified could subsequently be evaluated in clinical trials.

5.2. Aims

To identify drugs which may reduce the dose of pitavastatin necessary to treat OC, a combinatorial drug approach was used. The following objectives were addressed:

1. Does zoledronic acid, risedronate and GGTI-2133 potentiate the activity of pitavastatin against OC cell lines ?

2. Investigate the mechanism of action of pitavsatstin and the mechanism underlying the synergy between it and bisphosphonates.

5.3. Results

5.3.1. Antiproliferative activity of pitavastatin, zoledronic acid, risedronate and GGTI-2133 against panel of ovarian cancer cell lines

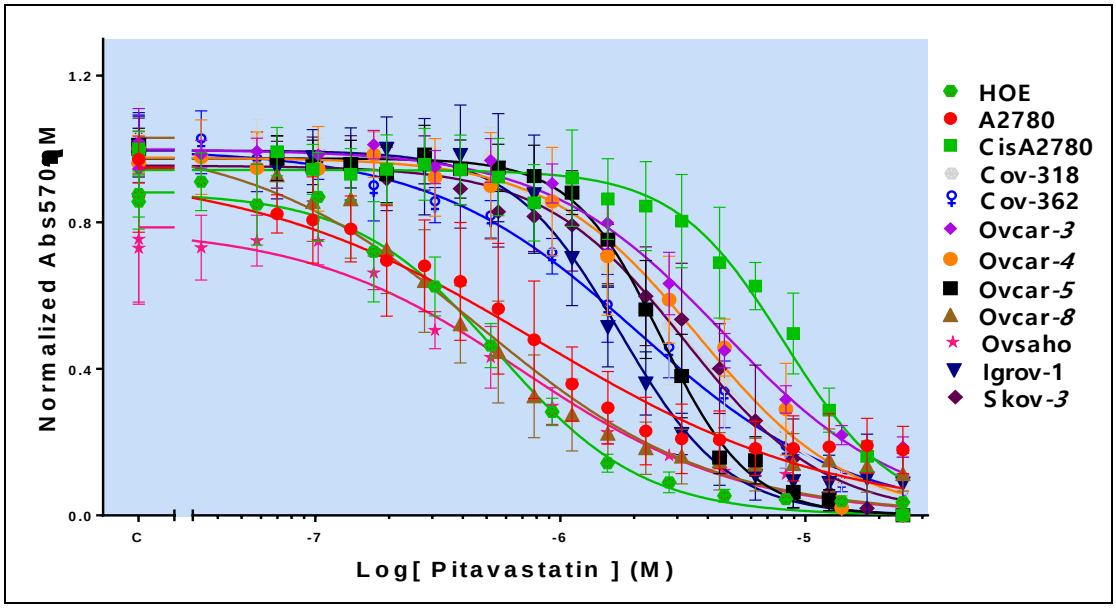
The potential growth inhibitory activities of pitavastatin, zoledronic acid, risedronate and GGTI-2133 as single agents were first determined against a panel of OC cell line in order to subsequently evaluate them in drug combination studies. Pitavastatin as a single agent displayed concentration- and time- and cell line specific growth inhibitory activity against tested cell lines with an IC_{50}s ranging from 0.6-14 µM (Table 5-1) (Figure 5-1 A). Pitavastatin's potency was comparable to its reported activity against breast and brain cancer cell lines (Jiang *et al.*, 2014). Zoledronic acid displayed lower potency compared to pitavastatin and it showed concentration-dependent growth inhibition activity with an IC_{50}s ranging from 21-60 µM (Table 5-1) (Figure 5-1 B). It has been reported that zoledronic acid inhibits cancer cell growth (10-100 µM) (Tamura *et al.*, 2011). In contrast, risedronate (IC_{50} >100 µM) (Figure 5-2 A) and GGTI-2133 (IC_{50} > 25 µM) (Figure 5-2 B) did not show significant activity against OC cell lines at the concentrations tested and an accurate estimation of IC_{50}s could not be made.

Table 5-1 IC_{50}s of pitavastatin and zoledronic acid.

IC_{50} (μM)		
Cell line	**Pitavastatin (n)**	**Zoledronic acid (n)**
HOE	0.59 ± 0.16 (6)	57 ± 6 (5)
A2780	0.67 ± 0.34 (9)	29 ± 4 (4)
CisA2780	14.0 ± 7.00 (9)	36 ± 6 (8)
Cov-318	3.40 ± 1.40 (8)	28 ± 2 (4)
Cov-362	3.10 ± 0.70 (8)	42 ± 4 (4)
Ovcar-3	4.60 ± 0.90 (6)	60 ± 4 (6)
Ovcar-4	5.20 ± 1.20 (4)	51 ± 7 (4)
Ovcar-5	2.40 ± 1.30 (9)	30 ± 6 (9)
Ovcar-8	0.40 ± 0.10 (4)	21 ± 3 (4)
Igrov-1	1.60 ± 0.10 (9)	43 ± 8 (7)
Skov-3	3.60 ± 1.00 (5)	26 ± 5 (5)
Ovsaho	0.69 ± 0.12 (5)	44 ± 7 (3)

Pitavastatin and zoledronic acid IC_{50}s (mean ± S.D.) were calculated from the indicated number (n) of experiments.

(A)



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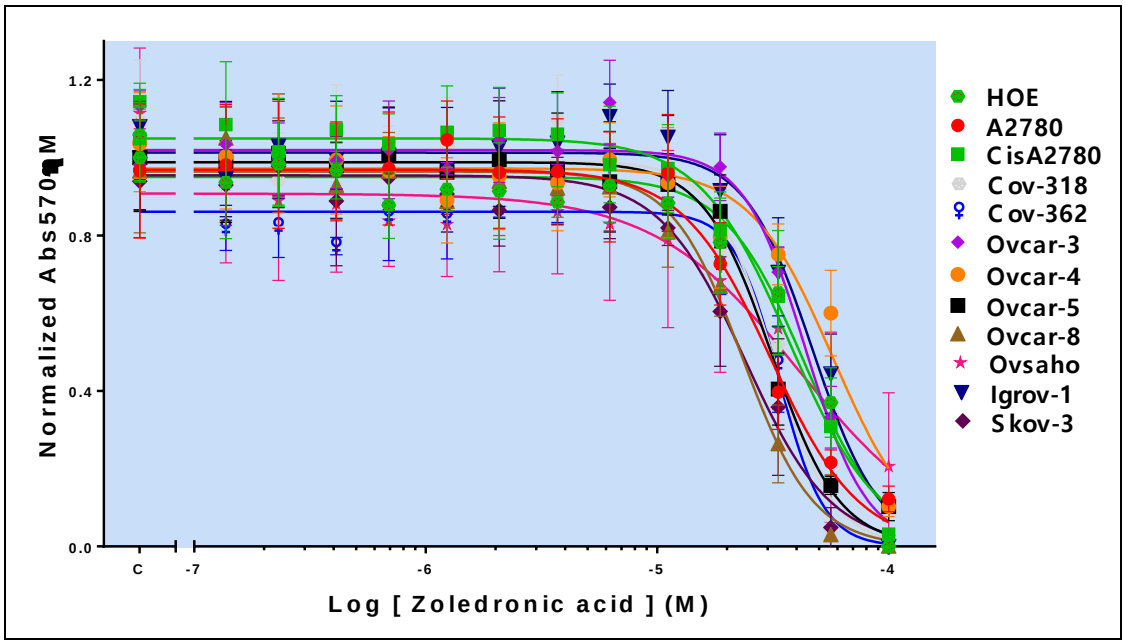
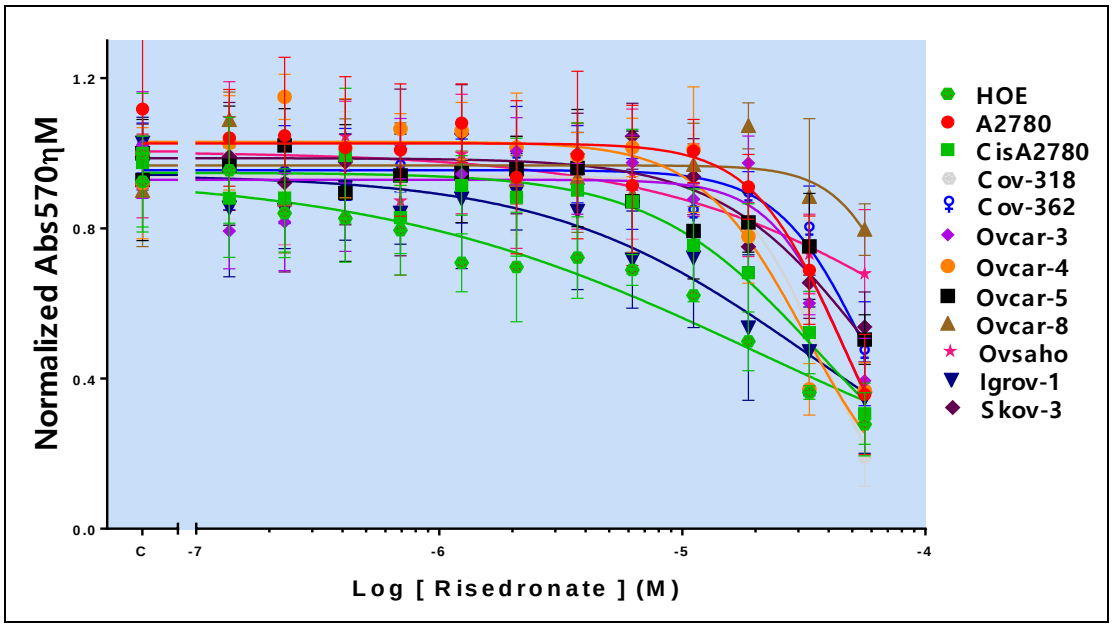


Figure 5-1 Dose response curve of pitavastatin and zoledronic acid against a panel of ovarian cancer cell lines.

Cells were exposed to a range of concentrations of pitavastatin (A) or zoledronic acid (B) for 72 hours, except for the slow growing cell lines Cov-318 and Cov-362 (120 hours). The numbers of surviving cells were estimated using SRB assay. Dose response curve expressed as a fraction of the top of the curve which was recognised by curve fitting (mean ± SD, n ≥ 3). "C" on the x-axis indicates control samples measured in the absence of the drug.

(A)



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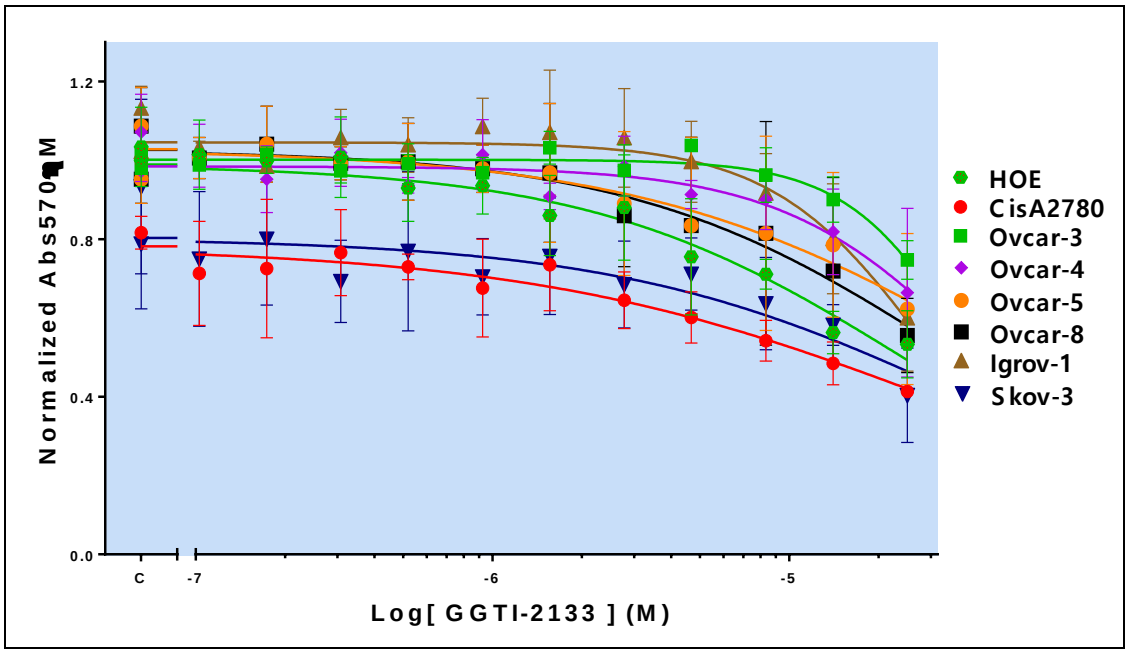


Figure 5-2 Dose response curve of risedronate and GGTI-2133 against a panel of ovarian cancer cell lines.

Cells were exposed to a range of concentrations of risedronate (A) or GGTI-2133 (B) for 72 hours, except for the slow growing cell lines Cov-318 and Cov-362 (120 hours). The numbers of surviving cells were estimated using SRB assay. Dose response curve expressed as a fraction of the top of the curve which was recognised by curve fitting (mean ± SD, n ≥ 3). "C" on the x-axis indicates control samples measured in the absence of the drug.

5.3.2. Drug combination

Cell growth assays were employed to assess the drug combinations in a panel of OC cell lines and combination index were calculated as an indicator of synergy and antagonism.

5.3.2.1. Pitavastatin and zoledronic acid synergistically inhibit the growth of ovarian cancer cell lines

The lack of potent activity of both bisphosphonates and GG-2133 led to the evaluation of these drugs at fixed concentrations, as suggested by (Bijnsdorp, Giovannetti and Peters, 2011), in combination with a range of concentrations of pitavastatin in cell growth assays. Pitavastatin and zoledronic acid (10µM) displayed synergistic activity in 8 of 11 cell lines tested (A2780, CisA2780, Cov-362, Ovcar-4, Ovcar-5, Ovsaho, Igrov-1 and Skov-3 cells), additive activity was observed in two cell lines (Cov-318 and Ovcar-8 cells) and antagonism was observed in one cell line (Ovcar-3 cells). When pitavastatin was combined with risedronate, additive or synergy was observed in 9 of 11 cell lines, although the synergy only reached statistical significance in 3 of the cell lines (A2780, Ovcar-5 and Skov-3 cells). An antagonistic interaction was observed in two cell lines (Ovcar-3 and Ovcar-8). In contrast, most of the cell lines showed an antagonist interaction when GGTI-2133 (5µM) was combined with pitavastatin (Figure 5-3). These data suggest that zoledronic acid might be a suitable option for the combination with pitavastatin and led to focus on this combination in further studies.

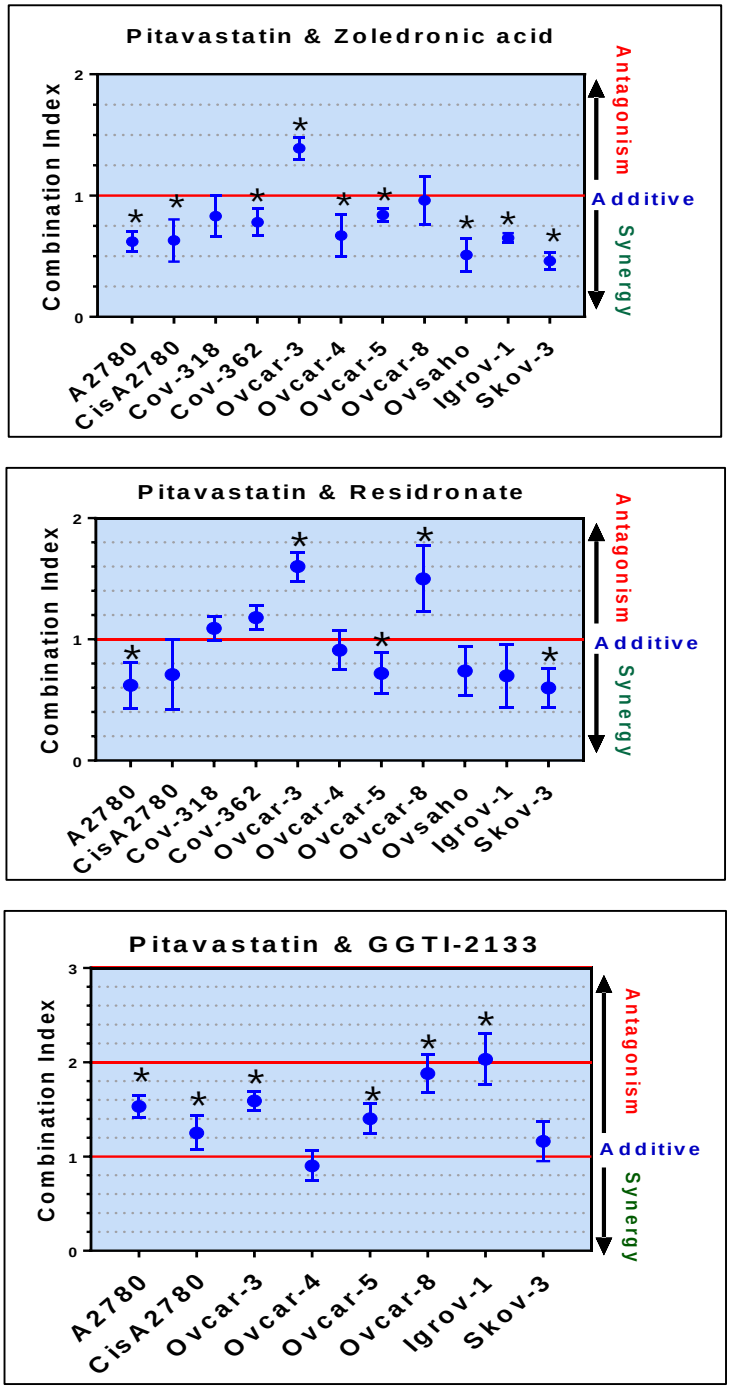


Figure 5-3 The effect of pitavastatin combinations in cell growth assays.

To measure the activity of pitavastatin in combination with other agents, the indicated cells were simultaneously exposed to a range of pitavastatin concentrations with fixed concentration of zoledronic acid (10 µM), or risedronate (10 µM) or GGTI-2133 (5 µM). Combination indices (CI) (mean ± S.D., n=3-4) are quoted at a fraction affected of 0.5 and differed significantly from unity where indicated (*, $P \leq 0.05$; paired *t*-test).

5.3.2.2. Confirmation of antiproliferative synergistic effect of pitavastatin and zoledronic acid combination

In order to confirm the synergy observed between pitavastatin and zoledronic acid, A2780, Skov-3 and Ovsaho cell lines were tested because the most significant synergy was observed in these cell lines. Cell death was first assessed by staining with trypan blue. The combination of pitavastatin with zoledronic acid resulted, in all three cell lines, in significantly more cell death after 72 and 96 hours of drug exposure than would have been expected from an additive effect calculated using the Bliss independence criterion (Figure 5-4). To confirm these results, a separate measurement of cell viability was used by measuring intracellular ATP level. After 72 hours of drug exposure, significantly less ATP was measured in cells exposed to the drug combination than that expected effect from an additive effect calculated using the Bliss independence criterion (Figure 5-5).

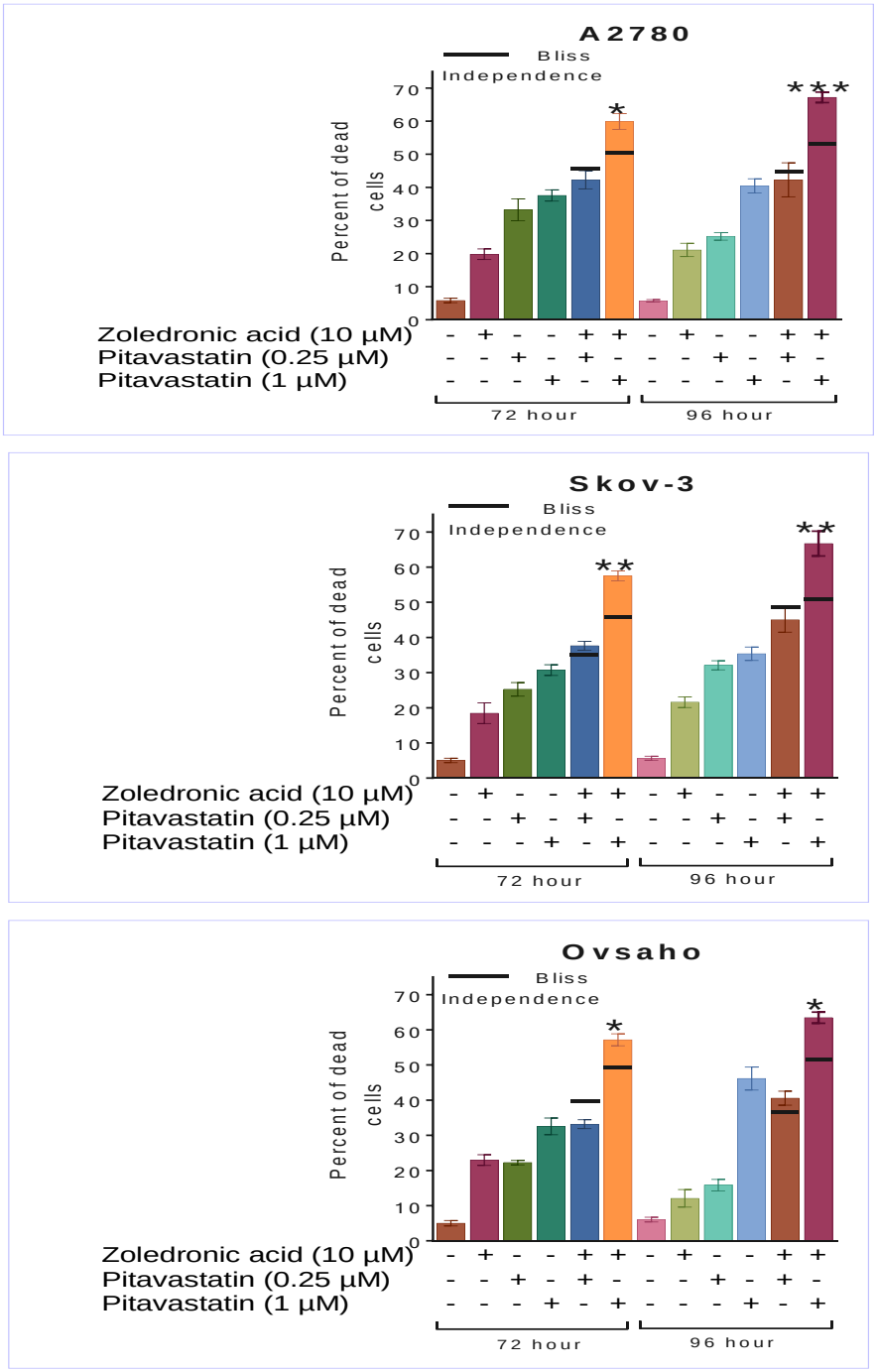


Figure 5-4 The effect of pitavastatin-zolendronic acid combinations on cell death.

Dead cells were measured by trypan blue staining after 72 and 96 hours of exposure to the indicated drug concentration. The results (mean ± SD; n = 3) were compared to the effect expected for an additive interaction calculated using the Bliss independence criterion (solid line for each drug combination) and determined using the measured effect of the individual drugs in each individual experiment. Results were significantly different from the expected Bliss effect where shown (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$, paired *t*-test).

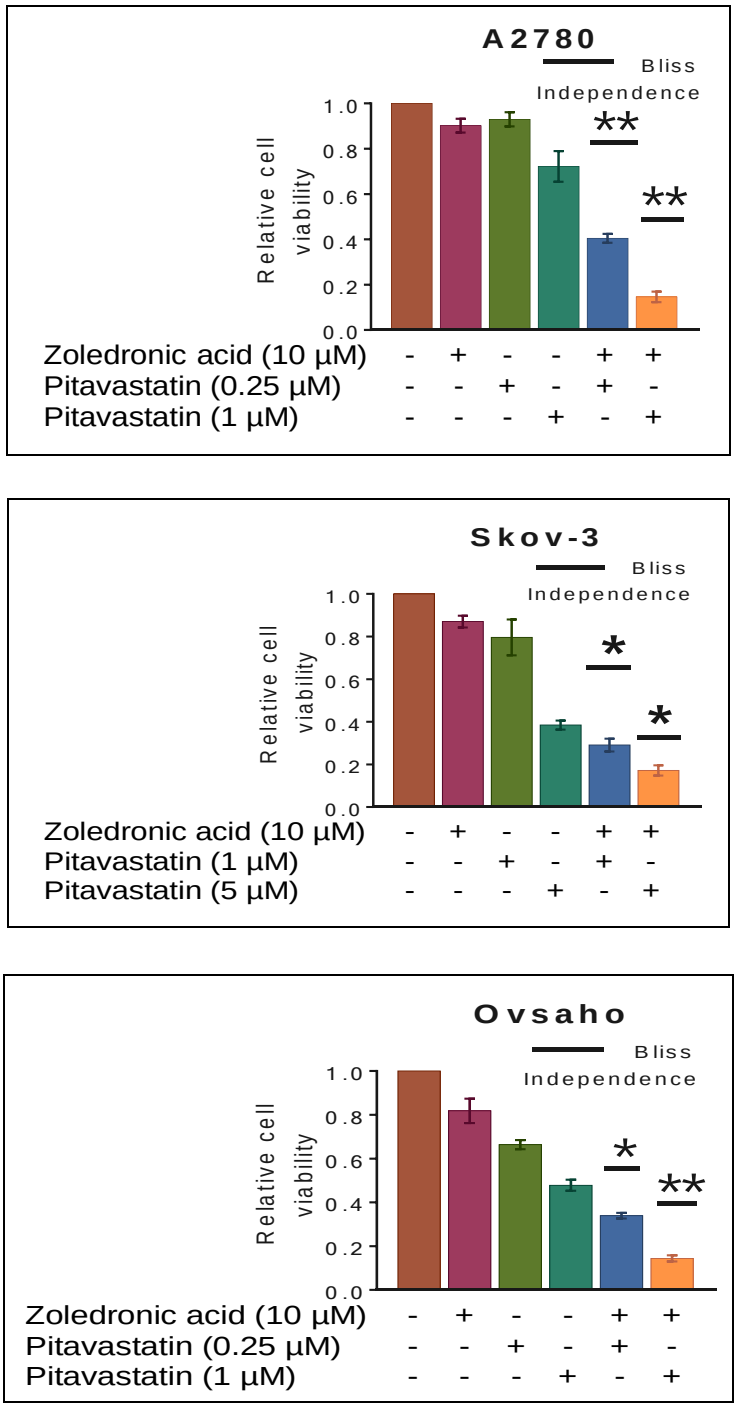


Figure 5-5 The effect of pitavastatin-zolendronic acid combinations on cell viability.

Relative cell viability was measured by ATP-celltiter-Glo assay after 72 hour exposure to the indicated drug concentration. The results (mean±SD; n = 3) were compared to the effect expected for an additive interaction calculated using the Bliss independence criterion (solid line for each drug combination) and determined using the measured effect of the individual drugs in each individual experiment. Results were significantly different from the expected Bliss effect where shown (*, $P < 0.05$; **, $P < 0.01$; paired *t*-test).

5.3.2.3. The pitavastatin and zoledronic acid combinations synergistically induce apoptosis

Previous studies have shown that statins and bisphosphonates induce apoptosis in cancer cell lines (Liu *et al.*, 2009; Tamura *et al.*, 2011). To confirm that the reduction in cell viability and growth is attributable to apoptosis, the effects of drugs alone and in combination on caspase activity and PARP cleavage were assessed. The combination of zoledronic acid and pitavastatin caused activation of the caspase-8 (Figure 5-6) and caspase-9 (Figure 5-7) as well as the effector caspases-3/7 (Figure 5-8). In all three cases, the caspase activation elicited by the combination was significantly higher than that of pitavastatin alone. (Calculation of the expected effect of the combination using the Bliss criterion was not possible in this experiment because of difficulties in accurately measuring maximum caspase activation, required to calculate the fractional effect of each drug). Subsequently, immunoblot analysis demonstrated that the combination resulted in accumulation of cleaved PARP that was greater than that observed with each single agent (Figure 5-8). However, it is noticed that pitavastatin alone at both tested concentrations were able to produce significant PARP cleavage in Ovsaho cell line in comparison to A2780 and Skov-3 cell line. This might indicate that Ovsaho cell line are more sensitive to pitavastatin induced PARP cleavage than other cell lines and that the activity of this drug is cell line dependent. Importantly, the addition of geranylgeraniol, but not farnesol, blocked the cleavage of PARP induced by pitavastatin or the combination (Figure 5-9). Finally, phase contrast microscopy revealed that untreated cells remain attached to culture plate and maintained their original morphology. In contrast, more pronounced rounding, blebbing or detachment from the plate was observed in cells treated with the drug combination than in cells treated with the single agents (Figure 5-10).

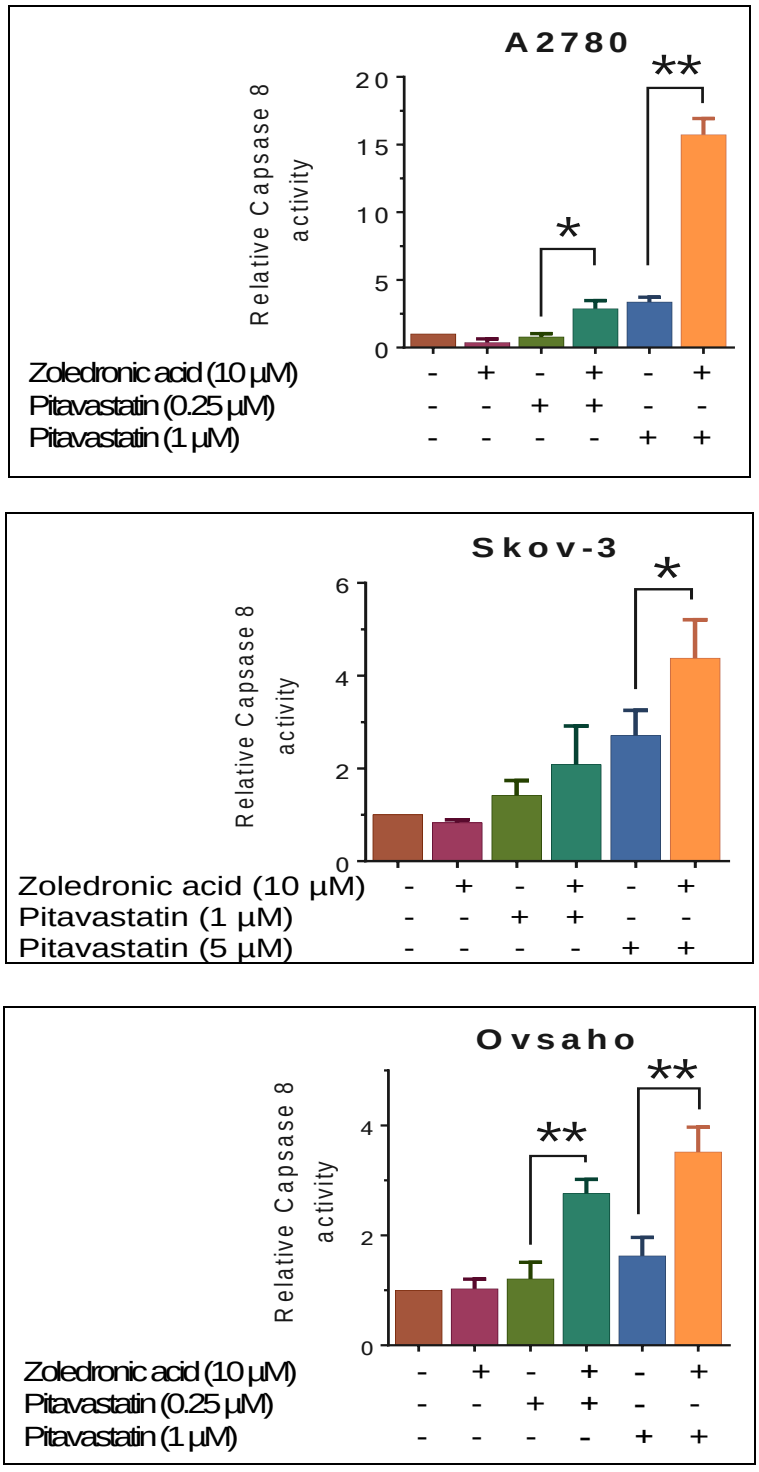


Figure 5-6 The effect of pitavastatin-zolendronic acid combinations on caspase 8 activity.

Caspase 8 activity of A2780, Skov-3 and Ovsaho cell lines were measured by Caspase-Glo assays. Cells were treated with the indicated concentrations of pitavastatin and zoledronic acid for 48 hour. Drug combinations effects were compared to the effect of the pitavastatin (Mean ± SD; n = 3; *, $P < 0.05$; **, $P < 0.01$; paired *t*-test).

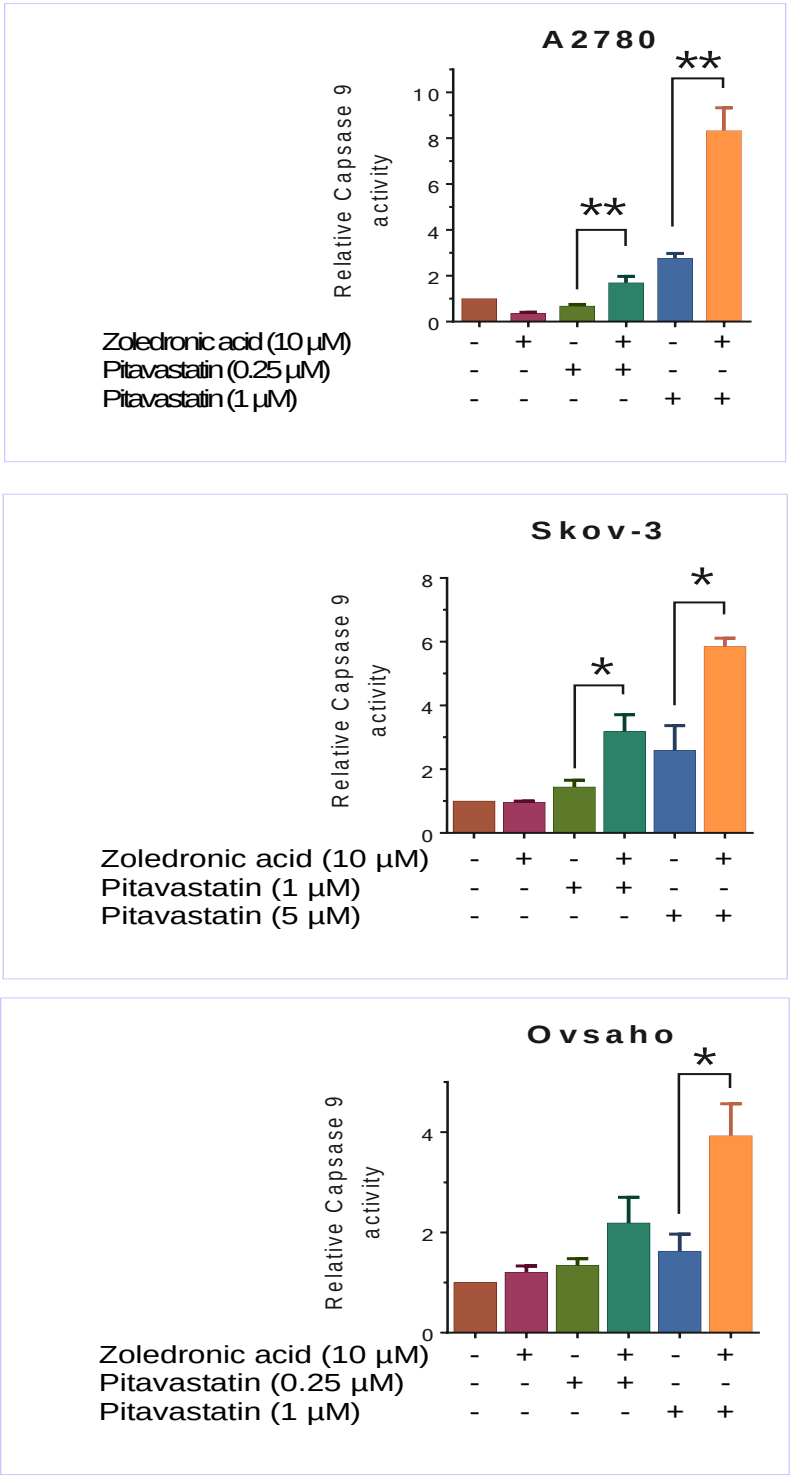


Figure 5-7 The effect of pitavastatin-zolendronic acid combinations on caspase 9 activity.

Caspase 9 activity of A2780, Skov-3 and Ovsaho cell lines were measured by Caspase-Glo assays. Cells were treated with the indicated concentrations of pitavastatin and zoledronic acid for 48 hour. Drug combinations effects were compared to the effect of the pitavastatin (Mean ± SD; n = 3; *, $P < 0.05$; **, $P < 0.01$; paired *t*-test).

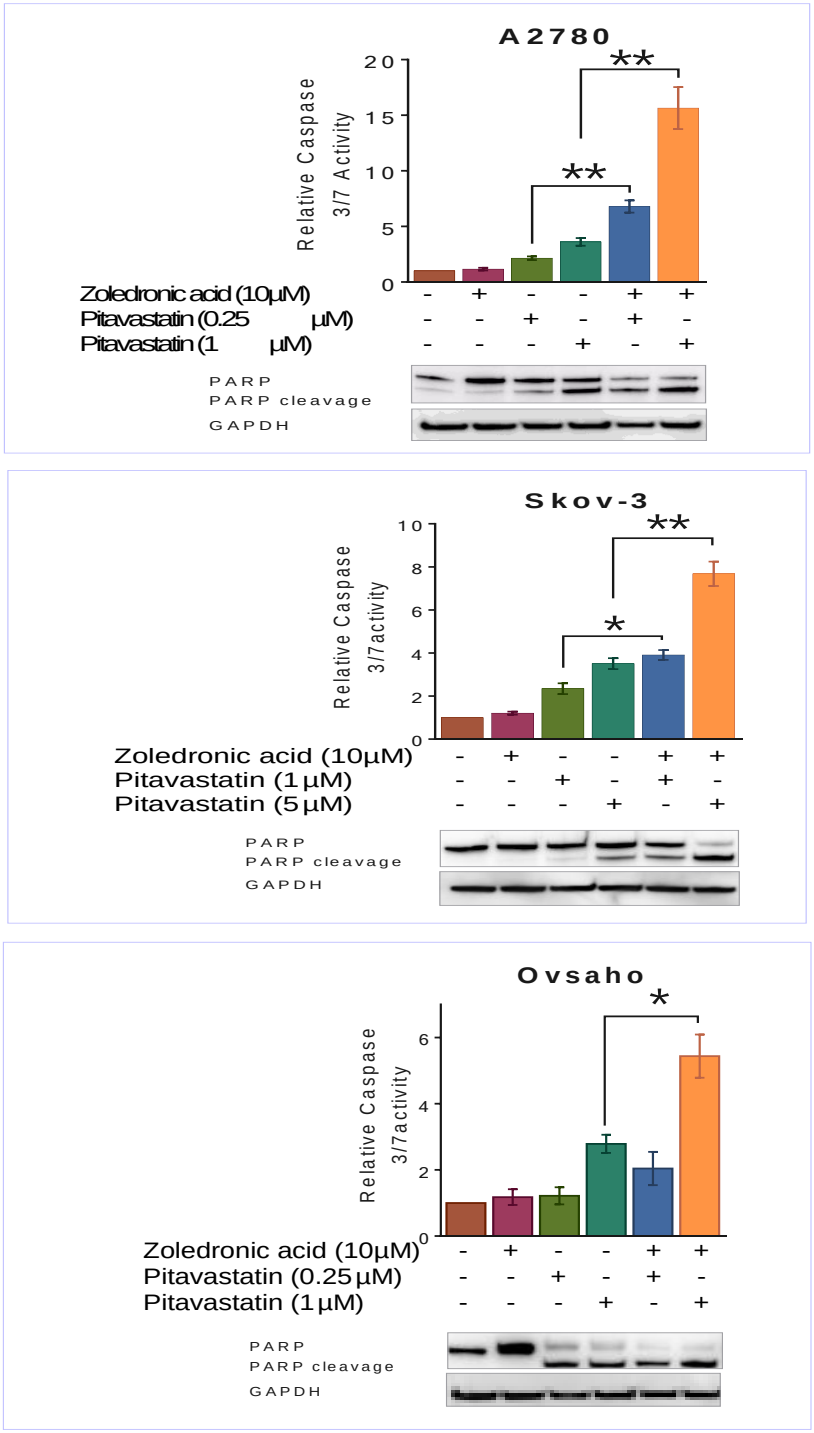


Figure 5-8 The effect of pitavastatin-zolendronic acid combinations on caspase 3/7 activity and PARP cleavage.

Caspase 3/7 activity of A2780, Skov-3 and Ovsaho cell lines were measured by Caspase-Glo assays. Cells were treated with the indicated concentrations of pitavastatin and zoledronic acid for 48 hour. Drug combinations effects were compared to the effect of the pitavastatin (Mean ± SD; n = 3; *, $P < 0.05$; **, $P < 0.01$; paired *t*-test). PARP and PARP cleavage were measured by western blot analysis (n = 3).

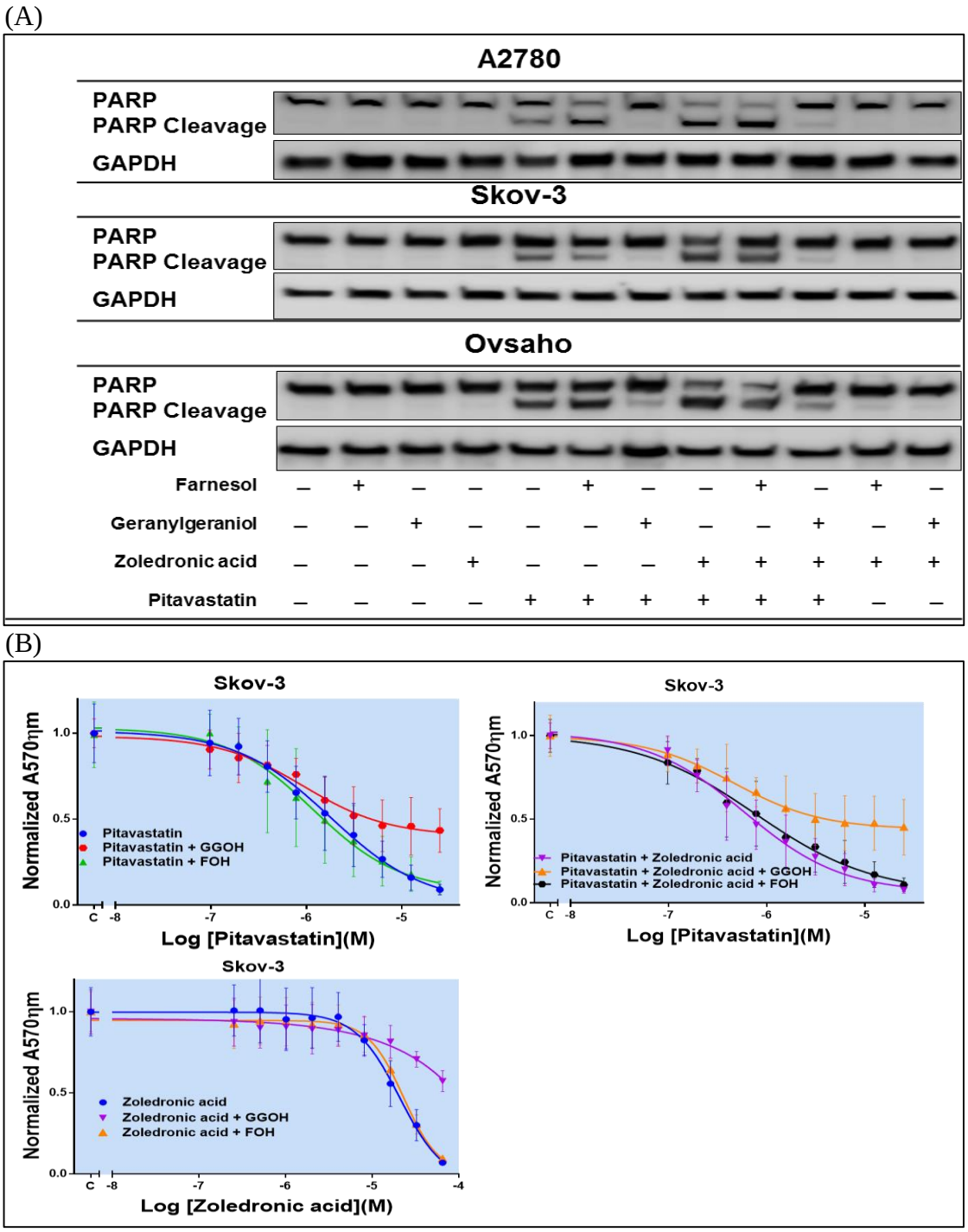


Figure 5-9 The effect of the pitavastatin-zolendronic acid drug combination is blocked by geranylgeraniol.

Cells treated with pitavastatin (A2780 and Ovsaho 1 µM and Skov-3 5µM) and farnesol (10µM) geranylgeraniol (10µM) zoledronic acid (10µM) for 48 hour. PARP cleavage was assessed by immunoblotting. The results are representative of 3 experiments. (B) Skov-3 cell line was treated with pitavastatin or pitavastatin and zoledronic acid (10µM) and GGOH (10 µM) and FOH (10 µM) and after 72 hour relative cell number was determined by staining with SRB.

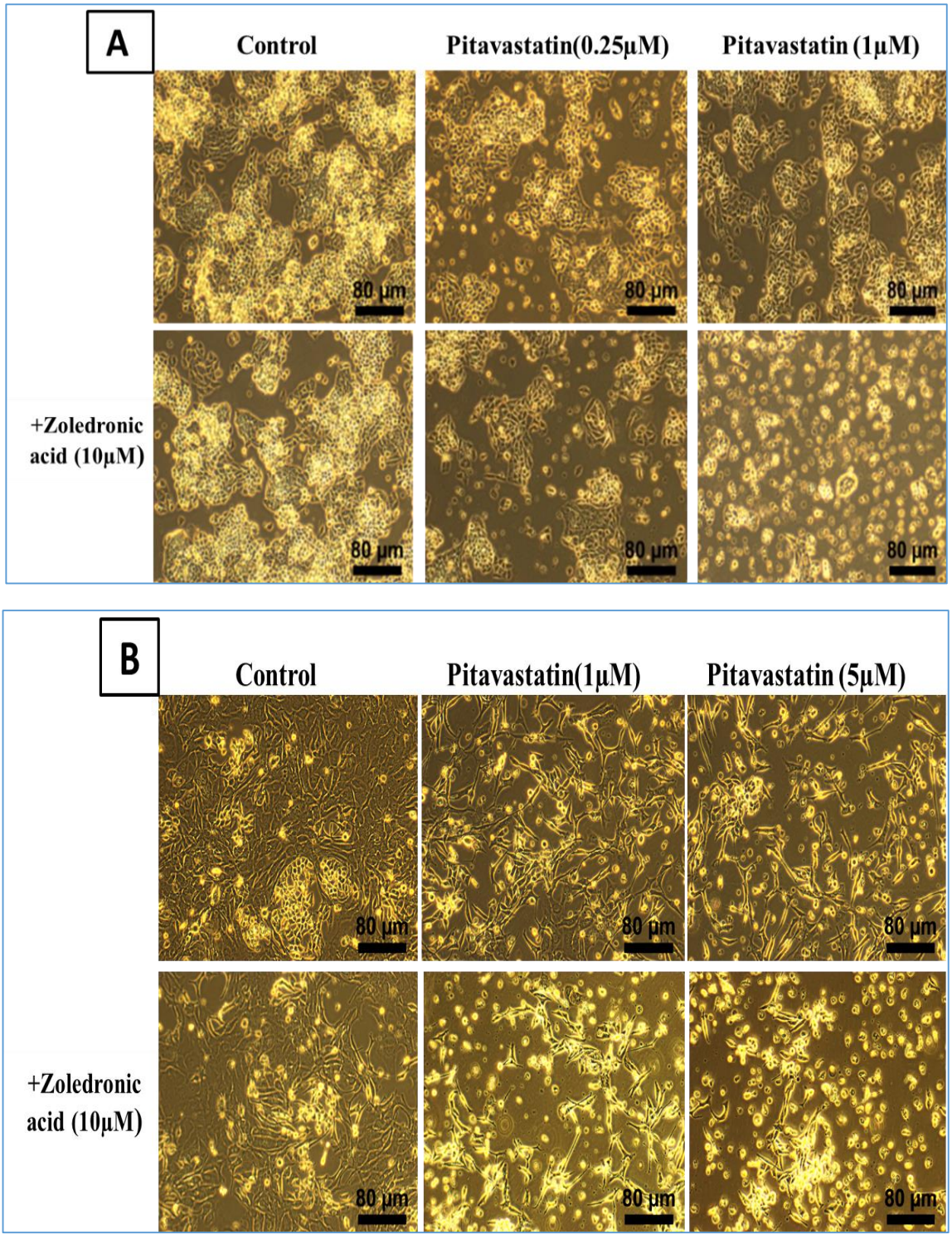


Figure 5-10 Morphological changes of A2780 and Skov-3 cell lines

morphological changes of A2780 (A) and Skov-3 (B) cell line treated with indicated drug concentration for 72 hour were visualized by phase contrast light microscope which revealed that untreated cells were attached to culture plate and maintained their original morphology. In contrast, it revealed a more pronounced rounding, blebbing or detachment from the plate in cells treated with the drug combination than in cells treated with the single agents.

5.3.3. The effect of pitavastatin and zoledronic acid on mevalonate pathway enzymes and p53 level

In order to further explore the mechanism of the drug combinations, the effect of the drug combination on MP enzymes using western blotting was assessed. The ability of GGOH to suppress the effects of pitavastatin led to the hypothesis that the geranylgeranylation branch of enzymes of the pathway are more important targets affected by pitavastatin. Pitavastatin decreased the level of GGTII-β in A2780 and Ovsaho cell line but without a noticeable change in the level of this enzyme in Skov-3 cell line. The reduction in level of GGTII-β was blocked by the inclusion of GGOH but not FOH. This might indicate that the pitavastatin effects on reduction of GGTII-β is cell line dependent. In contrast, pitavastatin did not a significantly affect the level of HMGCR, GGTI-β and p53 levels in A2780, Skov-3 and Ovsaho cells. Zoledronic acid, at the concentration tested, did not show significant effect on level of the MP enzymes. The combination of pitavastatin and zoledronic acid also reduced the level of GGTII-β and this was also ameliorated by the inclusion of GGOH (Figure 5-11).

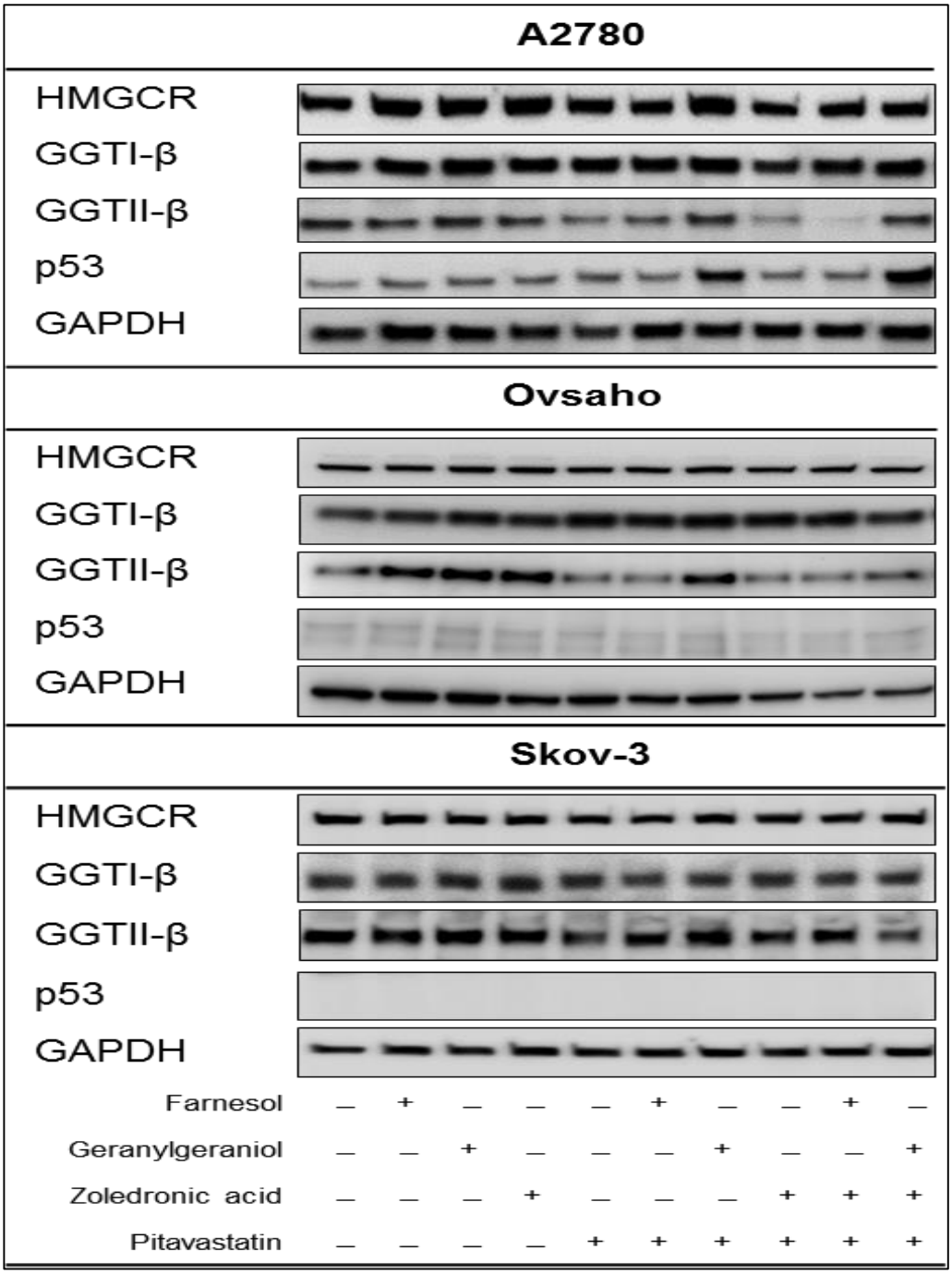


Figure 5-11 The effect of pitavastatin and pitavastatin-zolendronic acid on geranylgeranyl transferases.

A2780, Skov-3 and Ovsaho cell lines were exposed to pitavastatin (1μM, 5μM and 1μM, respectively) and zoledronic acid (10μM) with and without geranylgeraniol (10μM) and farnesol (10μM) for 48 hours. The levels of HMGCR, GGTI-β, GGTII-β and p53 were measured by immunoblotting of whole cell lysate. GAPDH was used as a loading control. The results are representative of 3 experiments.

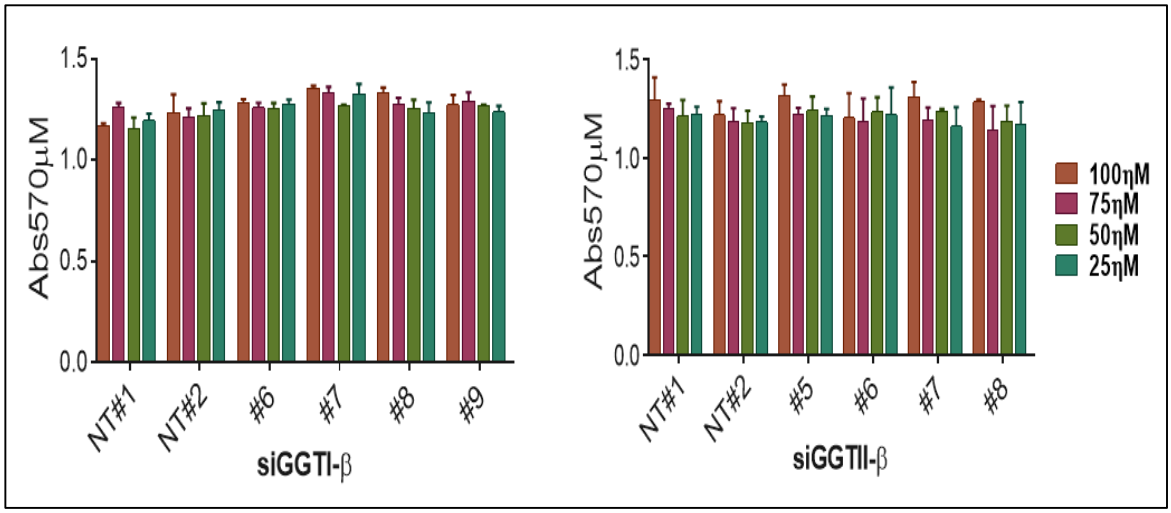
5.3.4. Inhibition of both GGTI-β and GGTII-β in combination with pitavastatin

Next the mechanism of action of the drug combinations was considered and in particular whether the effect of pitavastatin and pitavastatin drug combinations was mediated through inhibition of GGTI or GGTII. It was hypothesized that if inhibition of prenylation by one or both of geranylgeraniol transferases was essential for the cytotoxic activity of pitavastatin, then knockdown of either or both of them should increase the potency of pitavastatin. This could potentially provide information about which geranylgeranyl modified proteins are most crucially affected by pitavastatin. For these studies, Ovcar-4 cells were used because it has suggested they are more representative of high grade serous ovarian carcinoma (Domcke *et al.*, 2013).

5.3.4.1. Knockdown of GGTI-β and GGTII-β

To measure the effects of knockdown of geranylgeranyl transferase, the toxicity of different siRNAs targeting GGTI-β and GGTII-β were tested before determining the efficiency of knockdown of these enzymes. Ovcar-4 cells were exposed to four different concentrations (25, 50, 75 and 100ηM) of siRNA GGTI and GGTII enzymes for 72 hours. It was observed that there was no significant effect on cell growth at any of the concentrations tested. A concentration of 100nM were used in subsequent studies (Figure 5-12 A). Next, the effect of knockdown of GGTI-β and GGTII-β were tested. All 4 siRNAs targeting each enzyme inhibited the expression of these transferases when measured at 72 and 96 hours after transfection. (Figure 5-12 B).

(A)



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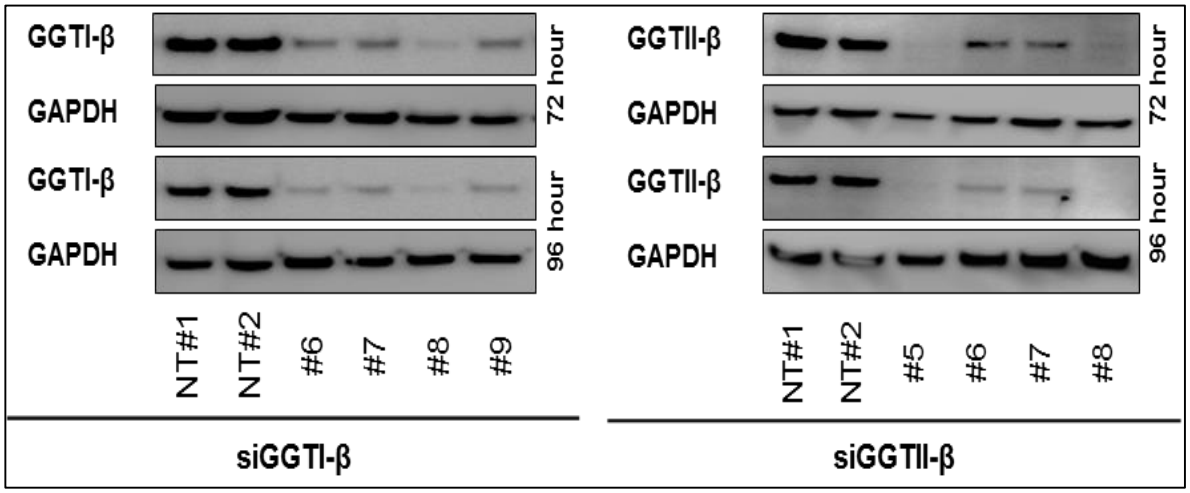


Figure 5-12 The kinetic of knockdown of GGTI-β and GGTII-β.

Ovcar-4 cells exposed were exposed to 4 different concentrations (25, 50, 75 and 100ηm) of each siRNA to GGTI-β and GGTII-β for 72 hours. Cell growth assay were used to determine relative cell numbers by staining with SRB (Mean±SD; n=3). (B) Ovcar-4 cells were transfected with the indicated siRNA. The level of GGTI-β and GGTII-β expression measured by immunoblotting after 72 and 96 hours.

5.3.4.2. The effect of knockdown of GGTI-β and GGTII-β on sensitivity to pitavastatin

Next the effect of knockdown on GGTI and GGTII on the sensitivity to pitavastatin was measured. Knockdown of either GGTI-β or GGTII-β alone using 3 separate siRNA did not significantly increase the potency of pitavastatin against Ovcar-4 OC cell line. However, inhibition of both GGTI-β and GGTII-β simultaneously using 3 separate siRNA combinations resulted in a significant increase in sensitivity to pitavastatin, shown by a significant decrease in pitavastatin IC_{50} compared to control cells exposed to non-targeting siRNA (Figure 5-13). Non-targeting siRNA had no significant effect on the sensitivity to pitavastatin.

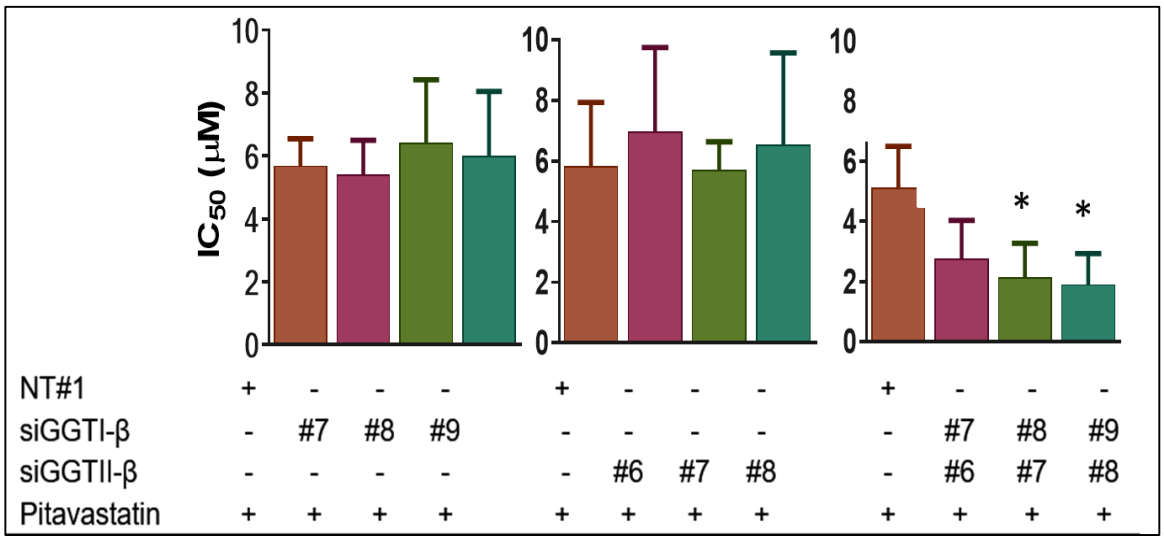


Figure 5-13 The effect of GGT-Iβ and GGT-II β knockdown on potency of pitavastatin.

Ovcar-4 cell line was transfected with siRNA of GGT-Iβ (100nM) or GGT-IIβ (100nM) oligos for 24 hours before exposed to serial drug dilution of pitavastatin (starting concentration 25μM) for additional 72hour. The IC_{50} were calculated from DRC which were determined by Graphpad Prism, (one-way Anova followed by Tukeys post-hoc *, $P < 0.05$) (mean ± S.D., n = 3).

5.3.4.3. Confirmation of the synergy of GGTI-β and GGTII-β knockdown by flow cytometry and PARP cleavage

To confirm the synergy observed following combined knockdown of both geranylgeranyl transferases and exposure to pitavastatin, the effect of the combination on apoptosis was measured by flow cytometry. Ovcar-4 cell line were incubated with GGTI-β, GGTII-β for 24 hours alone then exposed to pitavastatin for additional 48 hours. Flow cytometry analysis to measure apoptosis by Annexin V/propidium iodide staining revealed that inhibition of each the geranylgeranyl transferase enzymes alone or in combination with pitavastatin did not alter the sensitivity of Ovcar-4 cells to the drug. Similarly, knockdown of either transferase did not augment PARP cleavage induced by pitavastatin. However, when the cells were exposed to pitavastatin with concomitant inhibition of both geranylgeranyl transferases, there was significantly more Annexin V/PI labelling and more PARP cleavage (Figure 5-14 and Figure 5-15 A) compared to treatment of cells with pitavastatin alone. In contrast, inhibition of farnesyl transferase with tipifarnib did not augment the activity of pitavastatin in cell growth assays because an additive interaction was observed (Figure 5-15 B).

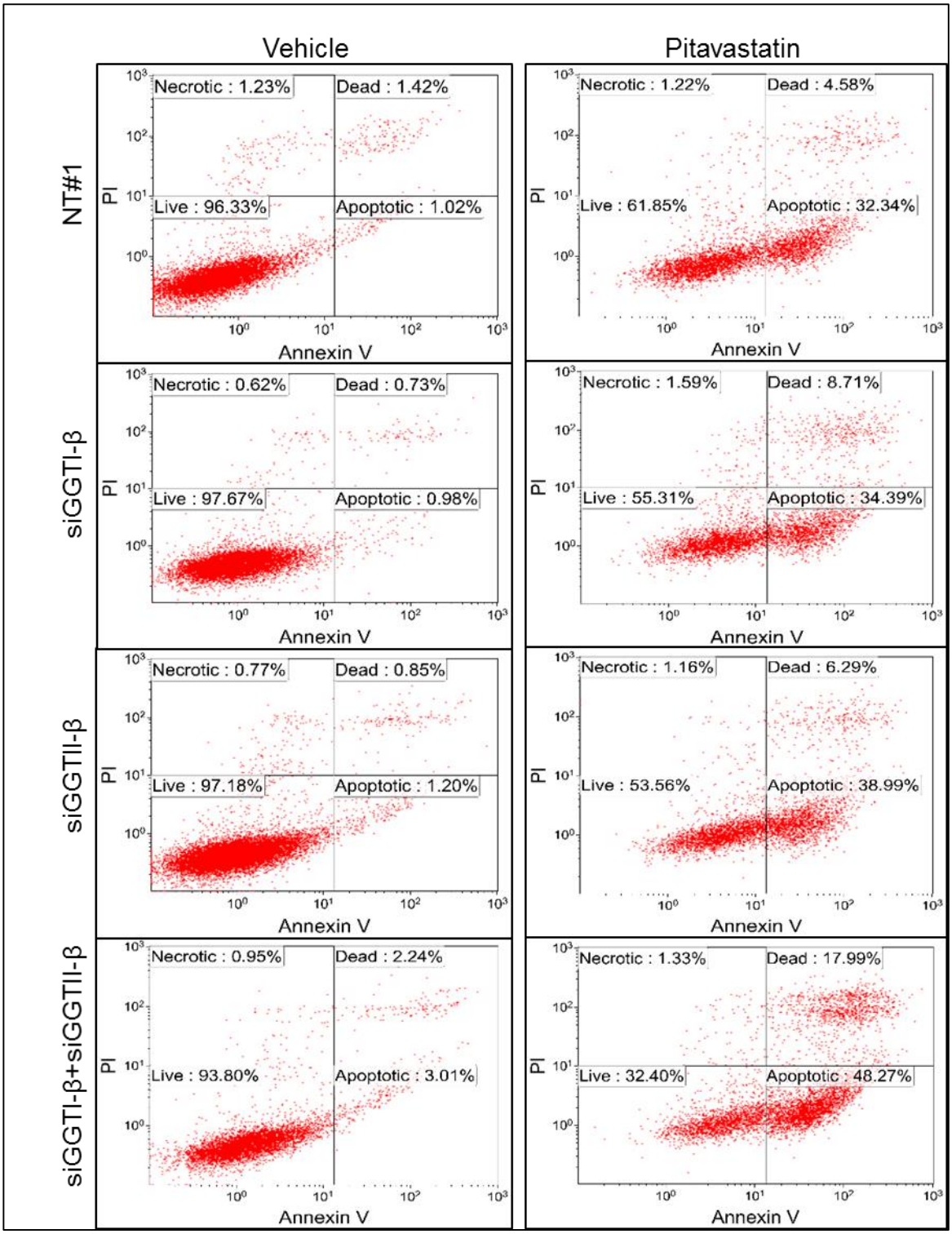
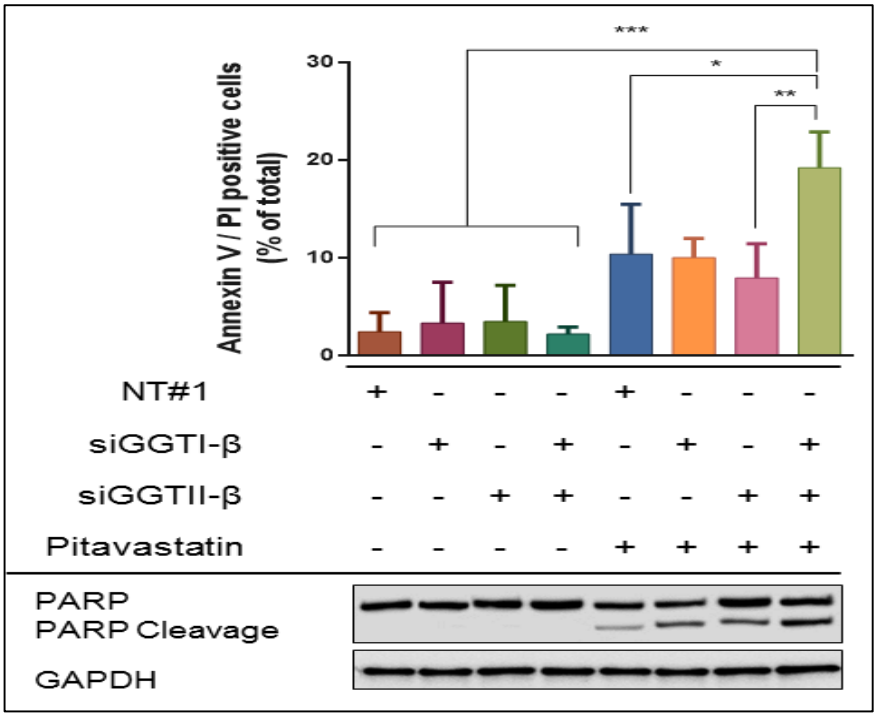


Figure 5-14 The effect of pitavastatin and pitavastatin–siGGTI-β and siGGTII-β combinations on apoptosis.

Ovcar-4 cells were transfected with siRNA to GGTI-β and GGTII-β and exposed to pitavastatin (10μM) for 48 hours. After labelling with annexin V/propidium iodide the cells were analysed by flow cytometry (results shown are from single representative experiment, n=3).

(A)



(B)

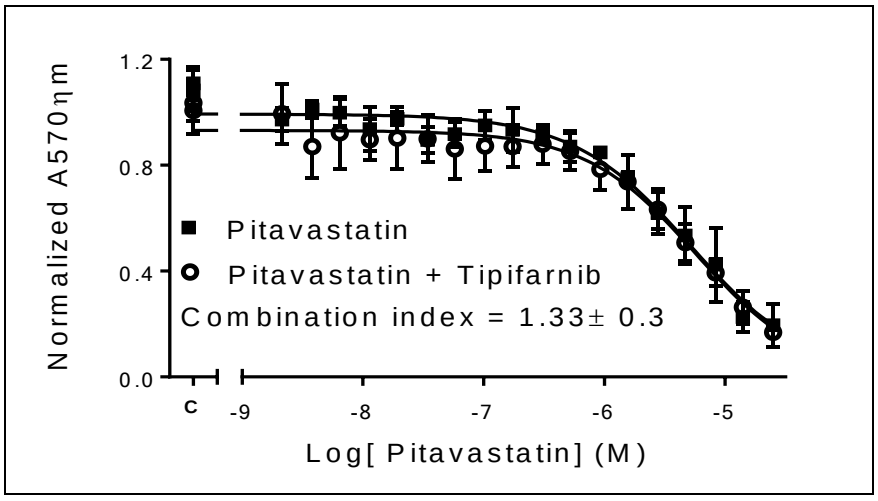


Figure 5-15 the apoptosis induction by geranylgeranyl transferase knockdown with pitavastatin in Ovcar-4 cell line.

(A) The annexin V and propidium iodide positive cells were quantified (mean ± SD, n=3) and were significantly different from cells transfected with non-targeting siRNA where shown (one-way Anova followed by Tukeys post-hoc *, $P < 0.05$; **. $P < 0.01$; ***, $P < 0.001$). In parallel, PARP cleavage determined by western blotting. (B) The activity of pitavastatin in a cell growth assays were measured in the absence and presence of tipifarnib (0.25 µM) and combination index calculated.

5.3.5. The effect of pitavastatin and pitavastatin-zolendronic acid on the subcellular localization of small GTPases

The data suggested that blocking geranylgeranylation may be crucial to the cytotoxic activity of drug combinations involving pitavastatin. Attachment of geranylgeraniol to small GTPases is necessary for their membrane localization and activity. This suggested that the drug combination would alter the subcellular localization of small GTPases. A2780 and Skov-3 Cells were treated with pitavastatin and/or zoledronic acid for 48 hours, the cells fractionated into cytoplasmic and membrane fractions and the distribution of RhoA, CDC42, Rab6A and Ras was examined using western blotting. Actin and NaK ATPase were measured as loading markers of the cytosolic and membrane fractions respectively. Although zoledronic acid used as a single agent did not affect the membrane localization of these small GTPases, pitavastatin decreased the proportion of RhoA, CDC42 and Ras proteins found in the membrane fraction and also caused a reciprocal increase in the cytosolic fraction. When cells were treated with pitavastatin and zoledronic acid, the loss of small GTPases from the membrane fraction to the cytosolic fraction was augmented by this combination (Figure 5-16).

Finally, it can be summarized that zoledronic acid potentiate the antiproliferative activity of pitavastatin in panel of OC cell lines in several cell growth and apoptosis assays. It is also found that inhibition of both geranylgeranyl transferase enzymes are essential to potentiate the cytotoxic activity of statins against OC cell lines.

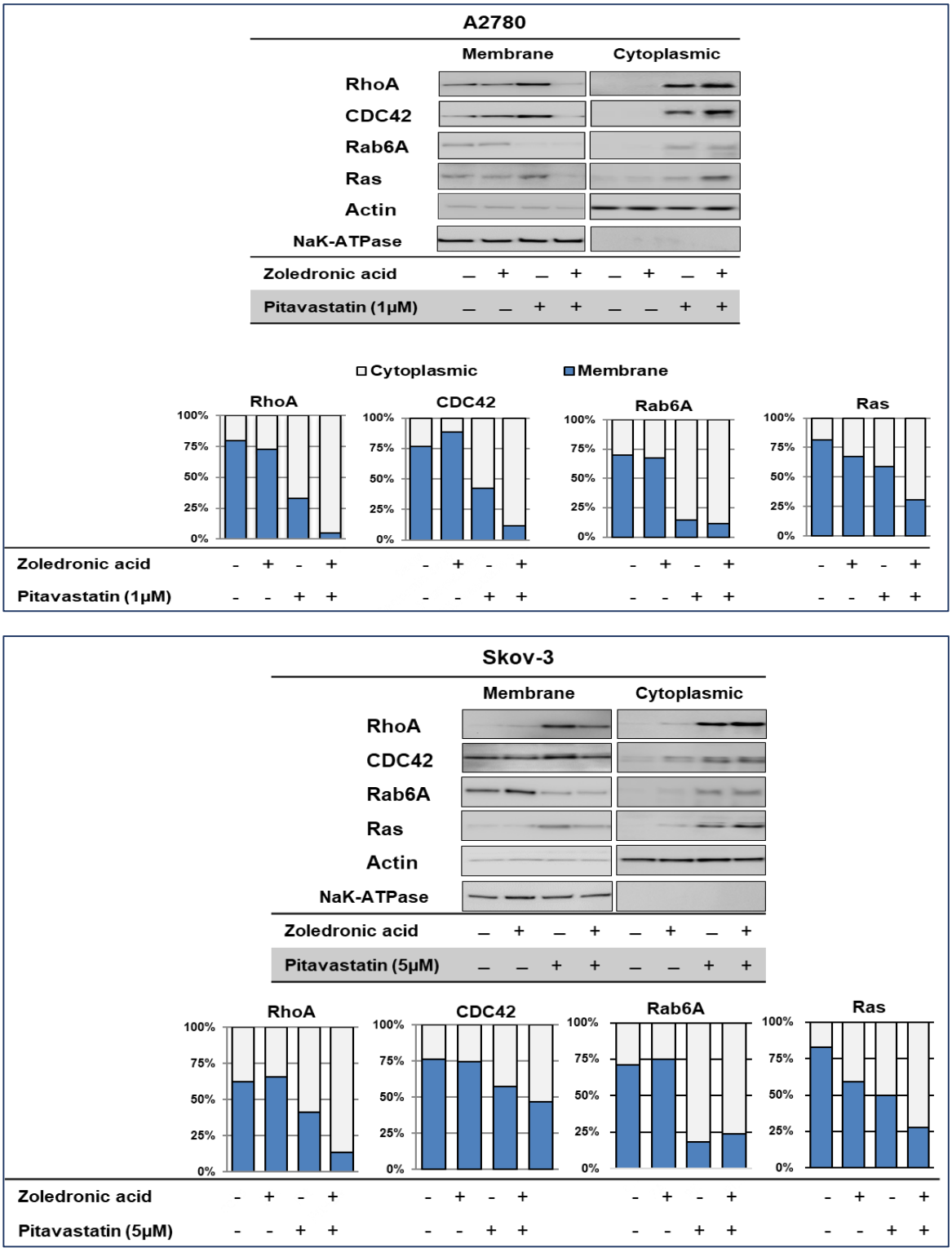


Figure 5-16 The effect of pitavastatin and pitavastatin-zolendronic acid combination on the subcellular localization of small GTPases.

Lysates of A2780 and Skov-3 cells that had been treated with indicated drugs for 48 hours were fractionated into cytoplasm and membrane and analyzed by immunoblotting. The graphs show the fraction recovered in the cytosolic or membrane fractions (n=3).

5.4. Discussion

Failure of cancer treatment is a significant challenge to the modern medicine (Colombo *et al.*, 2014). Regrettably, side effects, toxicity to normal tissue and drug resistance limit the activity of chemotherapy. Therefore, multi-drug regimens are prescribed for cancer to improve the efficacy and reduce the requirement of high drug doses (Marczak, Bukowska and Rogalska, 2014). At the same time, it is well known that cancer is a heterogeneous cluster of disorders with different molecular mechanisms of pathogenesis. Therefore, targeting multiple pathways is beneficial for inhibition of tumour growth and improving survival (Clendening and Penn, 2012; Yeganeh *et al.*, 2014). This study indicated that a combination of MP inhibitors potentiates the antiproliferative activity of pitavastatin and combined inhibition of geranylgeranyl transferase enzymes are required to potentiate the cytotoxic activity of statins in OC.

5.4.1. MP inhibitors single agent activity

It has been established that pitavastatin is the statin most likely to be effective in the treatment of OC (Robinson *et al.*, 2013; De Wolf *et al.*, 2017). Although repurposing statins for use in oncology is attractive, there are legitimate concerns about the potential for myopathy (Saito, 2011) and this makes it desirable to identify drugs which could potentially reduce the dose of pitavastatin administered to patients.

The result revealed that pitavastatin has concentration-, time- and cell-dependent growth inhibitory effects against a panel of OC cell lines. Pitavastatin was more potent than zoledronic acid while risedronate and GGTI-2133 were the least effective agents. Beside the restriction of the pathway products by MP inhibitors, NBPs may also act by causing the accumulation of metabolite of MP upstream of the site of inhibition (Okamoto *et al.*, 2014).

Accumulation of metabolites such as isopentyl diphosphate, caused by inhibition of the FPP synthase leads to the production of another metabolite, ApppI (triphosphoric acid 1-adenosin-5'-yl ester 3-(3-methylbut-3-enyl) ester), an ATP analogue which is toxic and can induce apoptosis by inhibiting mitochondrial ADP/ATP translocase protein (Räikkönen *et al.*, 2009). GGTI-2133, an inhibitor of GGTI enzyme, restrains the activity of small GTP proteins. GGTI-2133 did not display a potent growth inhibitory activity compared to pitavastatin. It has an IC_{50} for the enzyme inhibition about 40 and 5400 nM for GGTI and FT, respectively (Vasudevan *et al.*, 1999). In these studies, concentrations up to 25μM were tested, with only modest growth inhibitory effects. This suggests that inhibition of GGTI on its own does not have a profound effect on cell growth. This was subsequently confirmed in siRNA experiments in which knockdown of GGTIβ did not appreciably affect cell growth.

5.4.2. Pitavastatin and MP inhibitors combination activity

In this study, it was observed that zoledronic acid act synergistically with pitavastatin using several different assays and in several different cell lines. The drug combination was synergistic when assessed in two cell growth assays, a cell survival assay and also in several assays measuring apoptosis. It is observed that PARP cleavage in Ovsaho cell line are more prominent than other tested cell lines exposed to pitavastatin alone and in combination with zoledronic acid. In addition, the discrepancy between the caspase activity and PARP cleavage might be related to the method used for the measurement. This is significant because zoledronic acid is a drug in clinical use and so may reasonably be combined with pitavastatin in the treatment of cancer patients. Pitavastatin inhibits HMGCR, reducing the supply of mevalonate that is used to synthesize isoprenoids and zoledronic acid is known to inhibit farnesyl diphosphate synthase (Mukhtar, Reid and Reckless, 2005; Thurnher, Nussbaumer and Gruenbacher, 2012; Dhar, Koul and Kaul, 2013; Okamoto *et al.*, 2014)

which is also part of the MP. Previous studies have identified synergy between statins and zoledronic acid (Budman and Calabro, 2006; Dudakovic *et al.*, 2008; Rogers *et al.*, 2015; Elsayed *et al.*, 2016; Göbel *et al.*, 2016). The underlying mechanism of this synergy has not been fully elucidated but it likely that statin augment the activity of the FPPS inhibitors by additionally restraining protein geranylgeranylation by inhibiting the supply of isoprenoid substrates (Issat *et al.*, 2007). In this study, the mechanism underlying synergy between pitavastatin and zoledronic acid was further explored. In particular, it was found that pitavastatin reduces levels of GGTII-β. This is significant because it demonstrates that the drug combination inhibits the MP at three different points. The drug combination also profoundly reduces the amount of small GTPases associated with the cell membrane, suggesting a potential mechanism by which the drugs trigger apoptosis. It was also noticed that simultaneous inhibition of both GGT-I and GGT-II, but not either transferase individually, potentiates the activity of pitavastatin. This has significant implications for understanding the mechanism by which pitavastatin induces cell death and for drug discovery programmes to identify compounds which inhibit geranylgeranyl transferases synergistically with pitavastatin. Drugs which inhibit both transferases are likely to be necessary.

This study and several previous studies, have suggested that the cytotoxic effect of statins in cancer cells result from inhibiting the synthesis of geranylgeraniol (Zhong *et al.*, 2003; Gazzerro *et al.*, 2012; P Jiang *et al.*, 2014). Indeed, the importance of this isoprenoid in oncogenesis is underlined by the observation that geranylgeraniol promotes tumour growth in xenograft-bearing mice (Duncan, El-Sohemy and Archer, 2004). In this study, geranylgeraniol was able to inhibit the cytotoxic effects of the pitavastatin-zoledronic acid drug combination in wild-type *TP53* (A2780 cells), mutated *TP53* (Ovsaho) and cells lacking *TP53* (Skov-3). This observation is significant because it was proposed that

relatively high doses of statins will be necessary to treat cancer to provide an adequate plasma concentration (microMolar) of the drug in patients, leading to the concern that high concentrations of pitavastatin might be cytotoxic by inhibiting target other than HMGCR. This data provides several other lines of evidence in support of pitavastatin exerting its effect through inhibition of HMGCR. Firstly, the observation that geranylgeraniol, a product of the MP, suppresses the effects of pitavastatin support pitavastatin working through an "on-target" mechanism. Secondly, our observation of synergy between two sets of drugs inhibiting the same pathway (pitavastatin and bisphosphonates) is also consistent with the effect of pitavastatin being mediated by HMGCR. Finally, it was also noticed that siRNA directed to geranylgeranyl transferases, a part of the MP, potentiated the activity of pitavastatin. In summary, the synergy between pitavastatin and several reagents targeting the MP strongly supports the argument that pitavastatin, even at microMolar concentrations, acts principally through inhibition of HMGCR and the MP. This conclusion is of crucial importance to the design of clinical trials, because understanding the mechanism of action of a drug is essential for selecting which patients should receive the drug.

5.4.3. Role of GGTI-B and GGTII-B knockdown in potentiation of pitavastatin activity

The suppression of the activity of pitavastatin-zoledronic acid combinations by geranylgeraniol suggested that inhibition of the production of this isoprenoid was central to the effect of the drug combination. However, this observation did not indicate whether the effect of pitavastatin reflects inhibition of geranylgeranylation of a crucial subset of proteins or whether inhibition of protein prenylation more broadly underlies the effect of pitavastatin. This is not a trivial issue to tackle because around 2% of mammalian proteins undergo post-translational prenylation (Nguyen *et al.*, 2009). Although, Ras superfamily GTPases are

obvious candidates affected by pitavastatin, the sensitivity of multiple myeloma cells to lovastatin was not modulated by ectopic expression of individual constitutively active Ras, RhoA, RhoB, Rac1, and Cdc42 small GTPase proteins (Wong *et al.*, 2007). To begin to address this, it is first considered which geranylgeranyl transferases might be most significantly affected by pitavastatin. It was been hypothesized that if the effects of pitavastatin were mediated by preventing the prenylation of a substrate of either GGTI-β or GGTII-β, then synergy would be observed between pitavastatin and siRNA to one of these geranylgeraniol transferases. It was anticipated that this information could be used to focus on substrates for one transferase to search for the proteins whose geranylgeranylation is affected by pitavastatin and pitavastatin-zoledronate combinations and which is necessary for the cytotoxic activity of these drugs. However, it is found that siRNA to either one of the transferase alone was insufficient to potentiate the activity of pitavastatin in both cell growth assays and in two apoptosis assays. However, when different siRNA combined to simultaneously repress both geranylgeraniol transferase I and II, the potency of pitavastatin was increased. This was observed using three separate siRNA combinations. In contrast, inhibiting farnesyltransferase by tipifarnib was not synergistic with pitavastatin. This confounded an approach to understanding the mechanism of action of pitavastatin and pitavastatin/zoledronate because these results did not implicate one single geranylgeranyl transferase. Instead, these data suggest that pitavastatin does not exert its cytotoxic activity by preventing the geranylgeranylation of a small number of key proteins, rather that inhibition of geranylgeranylation of several proteins, whose prenylation is catalysed by GGTI-β and GGTII-β, contributes to pitavastatin's activity. It is likely that these same proteins are affected by the pitavastatin-zoledronic acid combination. It cannot rule out, however, that the activity of the pitavastatin-zoledronic acid combination depends on blocking the prenylation of a small subset of unidentified proteins that can be redundantly

isoprenylated by either GGTI-β or GGTII-β. Redundancy between these prenyl transferases explains why inhibition of both GGTI-β and GGTII-β was found to be necessary for synergy with pitavastatin because one transferase can compensate for the depletion of the other. The idea of redundancy between the transferases is plausible because these enzymes do not exhibit absolutely inflexible substrate specificity and geranylgeranylation has even been reported as a mechanism of resistance to farnesyl transferase inhibitors (Park *et al.*, 2014). The apparent redundancy observed between GGT-I and GGT-II also provides important information for drug discovery programmes designed to identify compounds which are synergistic with pitavastatin. The data suggests that targeting selectively either GGT-I or GGT-II may be futile because one transferase may compensate for inhibition of the other. Compounds which inhibit both transferases may be necessary. Indeed, the synergy has not been observed when pitavastatin combined with GGTI-2133 which inhibits GGTI-β but not GGTII-β. Rather, GGTI-2133 was antagonistic with pitavastatin, although this may reflect off-target effects of this compound (Vasudevan *et al.*, 1999; Delarue *et al.*, 2007). It has been previously shown that MP inhibitors can disrupt cellular signalling mechanism through Ras and Rho proteins. RhoB contributes to the process of apoptosis in cancer cells. Apoptosis is suppressed upon deletion of this protein and sensitisation of cells to apoptotic stimuli following stable expression of RhoB has been reported (Morgan *et al.*, 2005). Therefore, inhibition of GGTI by GGTI-2133 might inactivate RhoB and inhibit the induction of apoptosis and lead to the antagonism with pitavastatin.

5.4.4. The effect of drug combination on protein prenylation

To confirm that pitavastatin, zoledronic acid and the combination of the two drugs resulted in altered protein prenylation, the effect of these drugs on several small GTPases were measured. It seems reasonable to consider these as relevant targets affected by pitavastatin

because small GTPases proteins are involved in regulation of several signalling pathways involved in cell growth and survival (Rogers *et al.*, 2011) Pathways known to be regulated by small GTPases include the PI3K/AKT and Raf/Mek/MAPK/ERK pathways which regulate in cell cycle progression and apoptosis (Swanson and Hohl, 2006). Substrates of GGTI-β (RhoA, CDC42) or GGTII-β (Rab6A) as well as of farnesyltransferase (Ras) were selected (Park *et al.*, 2014) to evaluate the effect of the pitavastatin-zoledronic acid combination. Pitavastatin increased the proportion of all four small GTPases that was found in the cytosolic fraction, consistent with inhibition of prenylation. In both cells lines pitavastatin also increased the amount of RhoA, Cdc42 and Ras found in the cell membrane, suggesting that loss of prenylation may lead to an increase in the abundance of these small GTPases. Upregulation of Ras and Rho by statins has been observed previously (Mo and Elson, 2004; Göbel *et al.*, 2016) as a result of increase translation or reduced turnover (Mohamed, Smith and de Chaves, 2015). In contrast, there appeared to be a reduction in the total amount of Rab6A, consistent with our previous results (Robinson *et al.*, 2013). The combination of zoledronic acid with pitavastatin increased in most cases the proportion of small GTPases found in the cytosolic fraction. Taken together, this study suggests that the synergy between pitavastatin and zoledronic acid inhibits the MP at multiple points and leads to a profound reduction in the membrane localization of small GTPases. Since several of these GTPases regulate cell survival and proliferation, the loss of membrane localization of these proteins is likely to contribute to the synergistic inhibition of cell growth and survival. It cannot be ruled out, however the possibility that the cytosolic form of these proteins inhibits cell growth and survival (Dunford *et al.*, 2006).

It was observed that pitavastatin, alone and in combination with zoledronic acid, decreases the level of GGTII-β. Thus, the pitavastatin-zoledronic acid drug combination inhibits at least three points on one biosynthetic pathway and it is likely that this contributes to the

synergy which it has been observed in almost all cell lines tested. This is also significant because it suggests that reduced GGTII-β is likely to contribute to the activity of these drugs, although the mechanism underlying the reduction in GGTII-β is not yet clear. MP enzymes are regulated by feedback and feedforward mechanisms (Katz, 2005; Henry *et al.*, 2015). The reduced supply of geranylgeraniol may cause changes in the level of the enzyme for which it is a substrate. This may explain why pitavastatin altered the level of GGTIIβ. This observation also raises the possibility that pitavastatin may be particularly useful in cancers in which GGTII-β is either abundantly expressed or mutated such as OC (Lackner *et al.*, 2005; Ageberg *et al.*, 2011). In addition, overexpression of GGTII enzyme substrate such as Rab25, Rab5 and Rab7, has been reported in breast, ovarian, prostate and bladder cancers, and some of these substrate mutation is determinant of aggressiveness of cancer and predictors of poor outcome (Watanabe *et al.*, 2008).

Finally, it can be concluded that inhibition of farnesyl diphosphate synthase by zoledronic acid offers a promising strategy to increase the efficacy of statins in cancer patients. Statins and bisphosphonates generally have a good safety profile and are available clinically in relatively cost-effective generic forms (Manzoni and Rollini, 2002; Katz, 2005; Chen and Sambrook, 2011), making this approach particularly attractive. The inclusion of zoledronic acid alongside pitavastatin in clinical trials of patients with OC warrants urgent consideration. Although, it has been recently suggested that statins' adverse effect might not be dependent on the inhibition of protein prenylation (Gee *et al.*, 2015) but these trials will need to evaluate whether the inclusion of zoledronic acid potentiates the efficacy of pitavastatin without an increased risk of myopathy which is associated with statin use.

Chapter Six

Screening a library of compounds reveals a novel synergistic drug combination: pitavastatin and prednisolone

6.1. Introduction

The fundamental goal of cancer chemotherapy is eliminating malignant tissues. Combining drugs is important strategy for cancer treatment after the failure of single agents (Devita, Young and Canellos, 1975). The main rationale for drug combinations involving cytotoxic chemotherapy is that this targets several cellular components simultaneously. This offers several advantages including augmenting tumour cell killing and correspondingly therapeutic efficacy, reducing the occurrence of resistance and potentially minimizing the toxicity associated with high dose chemotherapy (Rodon, Perez and Kurzrock, 2010; Al-Lazikani, Banerji and Workman, 2012).

The high cost of new molecularly-targeted agents is likely to put significant strain on health care budgets in most countries (Bertolini, Sukhatme and Bouche, 2015). The process of new drug discovery requires a long time and a considerable cost to translate the agent to the clinic. It is currently estimated that it requires approximately $2 billion to bring a new drug to market. This results in the average cost of the new targeted therapy being extremely high, with annual treatment costs frequently in the range $50,000-$100,000 per course of treatment (Pantziarka, Bouche, Meheus, Sukhatme, Sukhatme, *et al.*, 2014).

Since 1950, the number of new licensed drugs has fallen by half every decade. A recent report has described the process of drug development as a productivity crisis. The crisis is particularly problematic for oncology drugs discovery. The number of non-oncological drugs approved by FDA between 2003 and 2011 are double the number of oncological drugs that approved (Pantziarka, Bouche, Meheus, Sukhatme and Sukhatme, 2014). At the same time, the cost of cancer drug approval had increased dramatically and doubled several times from 1990 to 2011 (Bertolini, Sukhatme and Bouche, 2015). Therefore, drug redeployment is an attractive alternative strategy to develop new treatments.

Drug redeployment might be defined as the process of validating and marketing previously approved pharmaceutical active agent for new indications outside the scope of their original medical uses (Brown and Patel, 2017; Corsello *et al.*, 2017). This offers a promise of rapid clinical impact with a low development cost compared to de novo drug development (Corsello *et al.*, 2017). The goal of pharmaceutical companies in oncology drug development is to obtain market approval by demonstrating that the efficacy and superiority of their drug. Pharmaceutical companies may lack incentives to investment in drug combinations because this may involve working with competitor pharmaceutical companies. This problem is exacerbated in the case of drug repositioning where there may be only a limited period of patent protection remaining for their product which minimizes financial return (Keith, Borisy and Stockwell, 2005). Despite this, drug repositioning has a potential advantage of safer, cheaper and faster validation protocols (Bertolini, Sukhatme and Bouche, 2015). Repurposing of known drug with a history of clinical application also comes with a wealth of data that is readily available and which includes the pharmacokinetic, pharmacodynamics, toxicological and dosing schedules. The availability of this data reduces the duration of clinical drug development, which typically ranges from 5-7 years (Ashburn and Thor, 2004). The use of generic drugs with low cost is another key advantage of drug repurposing. It reduces the potentially high cost of therapy which can impose a substantial strain on the public health finance of the developed countries and it can be unaffordable for poor and middle income countries (Pantziarka, Bouche, Meheus, Sukhatme and Sukhatme, 2014).

There are many example of successful drug repositioning which bypass the hindrance associated with new drug development and hasten the potential therapeutic discovery (Langedijk *et al.*, 2015). One of the accounts of drug repositioning is "***The fall and rise of thalidomide"***, a drug which was originally prescribed to pregnant women for the management of morning sickness in 1957 in Germany and England (Raje and Anderson,

1999). The drug led to newborns with severe malformations such as deafness, blindness, limb growth defects in at least 15,000 children born to a mother who had used thalidomide during the first trimester of their pregnancy. In 1964, thalidomide was accidentally discovered to be effective for treatment of erythema nodosum laprosum (ENL) which is an inflammatory condition of leprosy. The drug was given to relieve the pain of critically ill patient with ENL in University Hospital of Marseille although thalidomide is contraindicated for pregnant women. The drug relieved not only the pain but also healed the patient's sores (Ashburn and Thor, 2004). Consequently, a follow up study sponsored by WHO of more than 4000 ENL patients showed complete remission of the disease within less than two week (Brynner and Stephens, 2001). Thalidomide was reported to inhibit the formation of new blood vessels induced in rabbit corneal model explaining the teratogenic effects of this drug (Iacopetta *et al.*, 2017). This activity is also clinically useful. In 1994, the antiangiogenic activity of thalidomide, which was discovered at Children's Hospital in Boston, allowed it to be redeployed as candidate in oncology and opened the way for its use in the treatment of cancers such as multiple myeloma and breast cancer (Ashburn and Thor, 2004).

Several other examples of successful drug repositioning such as aspirin, a cyclooxygenase inhibitors, to treat coronary-artery disease, sildenafil, a phosphodiesterase inhibitor, which is originally developed to treat hypertension and angina and then repurposed to treat erectile dysfunction, and the antibiotic erythromycin which is now prescribed for impaired gastric motility (Ghofrani, Osterloh and Grimminger, 2006; Corsello *et al.*, 2017). Drug screening against leukaemia in samples from patients with chronic lymphocytic and acute myeloid leukaemia as well as peripheral blood mononuclear cells for cytotoxic activity revealed that quinacrine, an antimalarial drug, exhibits anticancer activity by intercalating the double strand DNA (Eriksson *et al.*, 2015; Jones and Bunnage, 2017). An important lesson from drug repositioning stories is that the recognised mechanism of action of drug may not provide

a complete picture and drug repurposing may be achievable in a broad range of diseases (Ashburn and Thor, 2004).

Drug repurposing can also be used as a way to discover new drug combinations. Combinatorial drug screening had been applied to discover novel synergistic therapeutic prospects for treatment of cancer. Screening of drug combinations with ibrutinib, an inhibitor of tyrosine kinase, revealed that several PI3K inhibitors and the BCL-2 antagonist ABT199 (venetoclax) potentiate the activity of the ibrutinib against diffuse large B cell lymphoma and chronic lymphocytic leukaemia (Mathews Griner *et al.*, 2014; Cervantes-Gomez *et al.*, 2015). There are now systematic efforts to identify these potential new drug combinations (http://www.ecmcnetwork.org.uk/combinations-alliance-researchers).

6.2. Steroids

Steroid hormones can be classified as those having effects on metabolism, inflammation and immune function (glucocorticoids), those having salt-retaining activity (mineralocorticoids), and those having androgen, estrogen or progesterone activity (sex steroids). Steroids affect the major systems of the body, including the cardiovascular, nervous, musculoskeletal and immune system. Since 1940, steroids have been usually included in chemotherapy protocols for the treatment of hematopoietic malignancies such as acute lymphoblastic leukaemia, chronic lymphocytic leukaemia, multiple myeloma, Hodgkin's lymphoma, and non-Hodgkin's lymphoma. They also have some activity against some non-haematological malignancy such as prostate and breast cancer (Sionov *et al.*, 2008). Steroids reduce the adverse effects of radiotherapy and cytotoxic drug administration. The use of steroids is associated with improved appetite, reduced weight loss, decreased fatigue, diminished ureteric obstruction, reduced vomiting, diminished swelling and preventing severe immune reactions. In addition, they can effectively alleviate the pain associated with metastatic bone

disease by preventing the synthesis and release of prostaglandins (Lin and Wang, 2016; Sundahl *et al.*, 2016). In addition, steroids have anti-inflammatory and immunosuppressive properties, and they are indicated to control many chronic and acute conditions such as asthma, inflammatory bowel disease, rheumatoid arthritis, psoriasis, anaphylaxis and septic shock. Steroids showed excellent efficacy in the clinic in the treatment of these diseases. However, their usage is hampered because of adverse effects (Fleuren *et al.*, 2013).

The adverse effects of steroids included central adiposity, dyslipidaemia, impaired growth, hypokalaemia, myopathy, osteoporosis, glucose intolerance, insulin resistance, diabetes, increased risk of infections, pancreatitis, hypertension, cataract, gastrointestinal disease, psychologic and neurologic side effects (Fleuren *et al.*, 2013). Short term use of steroids is generally associated with mild adverse effects which is reversible upon its discontinuation. In contrast, long term use can be associated with more severe adverse effects (Buchman, 2001). For example, 50 % of patients using steroid for more than 12 months may develop osteoporosis because steroids inhibit osteoblast function (Becker, 2013).

6.2.1. Glucocorticoid receptor (GR)

There are several member of steroid receptor subfamily: estrogen, estrogen-related receptors 1 and 2, mineralocorticoids, androgens and progesterone receptors (Schmidt *et al.*, 2004; Kumar and Thompson, 2005). The actions of corticosteroids are mediated by glucocorticoid receptor (GR). The GR is a ligand-activated transcription factor of the superfamily of nuclear receptors. There are α and β isoforms of GR, which are generated by alternative splicing of a single gene. The α isoform, which is responsible for transcriptional activation of glucocorticoids target genes, is expressed in majority of tissues. In contrast, the function of the β isoform remains to be discovered (Lin and Wang, 2016). The conformational changes induced by ligand binding to the GR stimulates receptor dissociation and translocation into

the nucleus where it subsequently binds to glucocorticoid response elements in the regulatory regions of target genes. This is followed by either induction or repression of a set of genes in a tissue specific manner (Schoneveld, Gaemers and Lamers, 2004; Löwenberg *et al.*, 2008). The GR had also the ability to induce gene expression without direct binding to glucocorticoid-response elements through interaction of the GR with other transcription factors and this process is known as tethering (Ratman *et al.*, 2013).

It has been suggested that GR may have a role in tumourigenesis and tumour progression. Hence, the potential role of glucocorticoid receptor in non-hematopoietic cancers has been confirmed in several immunohistochemical studies. It is observed that about 50% of non-small cell lung cancer have positive GR immunoreactivity (Lu *et al.*, 2005) and an improved therapeutic outcome of non-small cell lung cancer patients has been associated with higher expression of GR (Lu *et al.*, 2006). A high percentage of oesophageal squamous carcinomas (98.1%) and hepatocellular carcinomas (92.9%) express GR and it has been suggested that this may cause dexamethasone induced resistance in those tumour types (Lien *et al.*, 2008). In addition, the overall survival in patients with GR positive adenocarcinoma tends to be shorter in comparison with GR negative adenocarcinomas (Sionov *et al.*, 2008). A much earlier study found that GR expression in 88 % of OC patients (Galli *et al.*, 1981). However, a recent study has highlighted that GR is expressed in 39 % of OC and the expression was associated with histologic subtype, higher grade, and advanced stage. It is also found that GR expression correlates with decreased PFS, in OC patients (Veneris *et al.*, 2017).

Glucocorticoids have been indicated for the treatment of hormone-refractory prostate cancer for many years. However, the alteration of GR expression, function and availability of their targets in prostate cancer cells limits the objective response to about 25% of patients. Reconstitution of the GR in prostate cancer cells using lentivirus increases the sensitivity of

those cells to glucocorticoid anti-proliferative activity. In contrast, alteration of GR function or loss of their expression might undesirably affect the sensitivity of cancer cells to the anti-proliferative effect of steroids (Yemelyanov *et al.*, 2007).

6.2.2. Glucocorticoids

Glucocorticoids hormones are derived from cholesterol and are secreted by the adrenal gland. The level of these hormones is regulated by the hypothalamic–pituitary–adrenal axis (Figure 6-1). At the tissue level, natural glucocorticoid availability is regulated by the corticosteroid-binding globulin level in serum and by the local expression of 11β-hydroxysteroid dehydrogenase (which converts cortisol to cortisone). Disturbance in glucocorticoid synthesis causes pathological conditions such as Cushing's syndrome and Addison's disease (Kadmiel and Cidlowski, 2013).

The hypothalamic–pituitary–adrenal (HPA) is a biological circuit capable of co-oridinating physiological signalling in response to stressful conditions such as severe infection and severe blood loss (Herman *et al.*, 2016). An extensive regulatory control is exerted on corticosteroid level (Coleman, 1992; McKay and Cidlowski, 2003). For example, in a stressed state, the cerebral cortex activates paraventricular nucleus of the hypothalamus to produce corticotrophin-releasing hormone (CRH) and vasopressin which are carried by the hypophyseal portal system to the pituitary gland (Goncharova, 2013). Both hormones act on pituitary to stimulate the release of adrenocorticotropic hormone (ACTH), which in turn acts on the adrenal cortex to promote steroid hormone secretion. Corticosteroids regulate vascular tone, and metabolic processes on variety of tissues. In addition, steroid hormones complete the negative feedback control mechanism by supressing the production of the CRH and ACTH from the hypothalamus and pituitary gland, respectively (Papadopoulos and Cleare, 2011; Boron and Boulpaep, 2012; Volden and Conzen, 2013). In contrast to natural

glucocorticoids, synthetic glucocorticoids such as prednisolone, dexamethasone, and budesonide, are artificial compounds that resemble natural glucocorticoids in functions but differ in their potency and metabolic clearance (Lin and Wang, 2016).

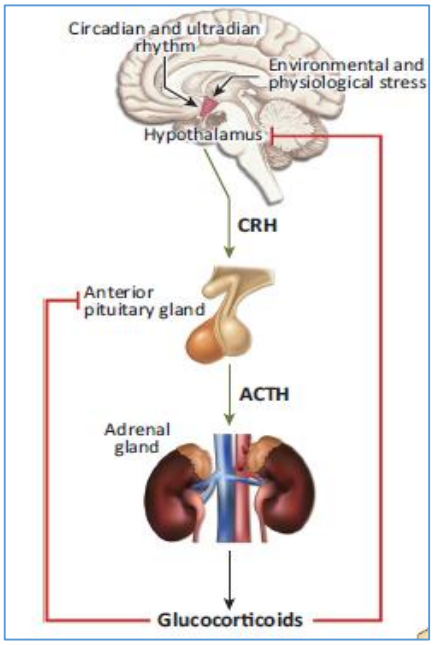


Figure 6-1 Schematic representation of glucocorticoid regulation by the hypothalamic–pituitary–adrenal axis. Synthesis and release of glucocorticoids is under regulation of the hypothalamus (Kadmiel and Cidlowski, 2013).

6.2.3. Glucocorticoids and cancer

Growth arrest and apoptosis induction are among several different functions mediated by glucocorticoids (Pufall, 2015). Activation of GR induces apoptosis in number of cells and tissues such as of osteoblasts, osteosarcoma, eosinophils, prostate, pancreatic β-islets and brain cells (Sionov *et al.*, 2008). There are two phases by which corticosteroids effects proceed. The first step is initial growth arrest followed by subsequent stage of cell death. *In vitro*, the initial phase lasts for 24 hours and with continuous administration it will progress to cell death which takes 2-3 days. The most commonly observed mechanism of cell death is apoptosis, while necrosis and necroptosis had been reported, as well (Pufall, 2015).

In haematological malignancies, the mechanism of glucocorticoid-mediated apoptosis appears to be complex and involving multiple signalling pathways. Despite studies that have lent some insight on the regulation of genes required to induce apoptosis, it is still not well defined (Ayroldi *et al.*, 2007). Steroids activate apoptotic pathways by tipping the balance of Bcl-2 family apoptosis regulators through upregulation of pro-apoptotic Bim protein and downregulation of the anti-apoptotic Bcl-2 proteins. Glucocorticoids have the ability to repress the activity of c-Myc and NF-κB, prosurvival transcription factors (Wang *et al.*, 2003). Progesterone use, which reduced the risk of OC, can induce programmed cell death in OC cells through stimulation of the apoptotic pathways (Han *et al.*, 2013) via activation of caspase-8 stimulated by Fas/ FasL signalling pathways (Syed and Ho, 2003). In addition, it was reported that apoptosis induced in thymocytes from p53 knockout mice by two pathways; the first one initiated by DNA damage which requires p53, and the second is stimulated by glucocorticoids and Ca^{2+} ionophores which is p53-independent (Clarke *et al.*, 1993). In contrast, the mechanisms of glucocorticoid-mediated growth arrest include downregulation of cyclin D1 and D3 and upregulation of $p21^{Cip1}$ and $p57^{Kip2}$ (inhibitors of cyclin-dependant kinases). In addition, steroids can inhibit the activity of the MAPK and Ras signalling pathway (Greenberg *et al.*, 2002; Ayroldi *et al.*, 2007).

The mechanism of resistance to steroids' antitumour activity may be provoked by activation of SGK1 and MKP1. The anti-apoptotic effects of glucocorticoids were reversed by knockdown these genes using siRNA directed to SGK1 or MKP1 in breast epithelial cells (Wu *et al.*, 2004). In addition, the expression of the small GTPase RhoB which is a negative regulator of proliferation, is stimulated by corticosteroids. RhoB drives inactivation of c-Myc facilitated by nuclear accumulation of GSK3, which in turn has direct involvement in apoptosis induced by steroids. Suppression of RhoB expression by siRNA also inhibits glucocorticoid mediated growth arrest (Sionov *et al.*, 2008). However, the corticosteroid-

induced resistance might be caused by downregulation of apoptosis pathway genes (Herr *et al.*, 2003).

In vitro and *in vivo* data on the effect of glucocorticoids on solid tumour are controversial, they may either promote or suppress tumour progression (Sundahl *et al.*, 2016). The activity of several first line therapy were antagonised by corticosteroids (Huang *et al.*, 2000; Lu *et al.*, 2006). An extensive statistical analysis of 150 cells derived from either primary malignant, solid tumour or established cell lines tested in cell culture or in tumour xenografts, suggested that glucocorticoids induced resistance toward cytotoxic therapies in majority (89%) of analysed tumour samples (Zhang *et al.*, 2007). In contrast, steroids might also inhibit tumour growth, metastasis and sensitize tumour cells to chemotherapy (Leo *et al.*, 2004; Palmiere *et al.*, 2005; Sundahl *et al.*, 2016). Dexamethasone was shown to potentiate the antitumour activity of carboplatin, gemcitabine and adriamycin possibly by increasing intracellular drug accumulation in several human cancer xenograft models, including breast, colon, lung and glioma cells (Wang *et al.*, 2004, 2007; Moutsatsou and Papavassiliou, 2008). Taken together, these data suggest that corticosteroids have dual actions with both pro and anti-apoptotic potential in many tissues in which the regulation of cell survival is cell type-dependent (Schmidt *et al.*, 2004; Wu *et al.*, 2004).

Steroids have been indicated for advanced breast cancer patients for many years. The doses of prednisolone evaluated varied between 10-100 mg daily, and the results reported were widely different, as well. Minton et al., (1981) tested prednisolone (15 mg daily) in 111 women over the age of 64 with advance breast cancer. Objective response to prednisolone therapy were found to be 13/91 assessable patients, and 19/91 achieved stable tumour for at least half a year. The median time to progression for responding patients was 15 months and for those with stable disease was 9 months (Minton *et al.*, 1981). In addition, administration

of 50 to 100 mg of prednisolone daily for three months to forty-five patients with disseminated breast carcinoma causes generalized tumour regression in eight patients and after one year, two patients are still in regression (Kofman *et al.*, 1958). There is uncertainty about the true value of prednisolone, which is a frequent component of combination chemotherapy regimen in advanced breast cancer. Despite that, prednisolone has a role in improving survival, reducing bone marrow toxicity, antiemetic effect and improving the sense of well-being of patients in general (Coleman, 1992).

6.2.4. Prednisolone

Prednisolone is a synthetic glucocorticoid. The pharmacokinetic properties of prednisolone are complex. It is rapidly absorbed from the gastrointestinal tract with maximum plasma level achieved after 1-2 hours of oral administration. Prednisolone has high systemic availability ranging from 75-98 % of administered oral dose (Frey, 1987). The drug is interconverted between the active form prednisolone and inactive form prednisone (Xu *et al.*, 2008). Elimination half-life varies from 2-4 hours. However, the biological half live of steroids are much longer and range from 18-36 hours (Becker, 2013). Hepatic metabolism by cytochrome P450 and renal excretion are the main routes of elimination. Steroids are largely present in a protein bound form (around 95%), bound to corticosteroid binding globulin and to less extent to albumin. The remaining 5% is free and available to exert effects on target cells (Katzung, B. G., Masters, S. B., & Trevor, 2012).

There is evidence that links prednisolone therapy with the MP, at least indirectly. Prednisolone presumably causes downregulation of Ras phosphorylation in prednisolone-sensitive cells. Inhibition of survival protein (AP-1) by prednisolone might be the mediators of apoptosis and this inhibitory activity of AP-1 is likely to be suppressed by Ras mutation in resistant cells (42% Ras mutation) (Ariës *et al.*, 2015). Prednisolone upregulates the

expression of TSC22D3 which in turn decreases the expression of antiapoptotic proteins Bcl-X_L and increase the activity of capase-8 and caspase-3 in transgenic mice. Furthermore, TSC22D3 negatively regulates Ras and Raf induced proliferation (Kajiyama *et al.*, 2010). It was observed that trametinib, a MEK1/2 inhibitors, potentiates the anti-proliferative activity of prednisolone on myeloma cells with K-Ras and N-Ras mutation (Ariës *et al.*, 2015).

Steroids have also been evaluated with statins to treat multiple myeloma in clinical trials. Simvastatin (15mg/kg/day) was administered orally on days 1-7 of the 28-day cycle, followed by intravenous infusion of vincristine, doxorubicin and dexamethasone orally on day 7 to 10. The study stopped as the response was insufficient and the reported adverse effect were haematological (neutropenia and thrombocytopenia) and gastro-intestinal toxicity but not rhabdomyolysis. It is believed that although simvastatin is very effective *in vitro*, its short half-life was the main cause for failure of the study (Robinson *et al.*, 2013). Statins with long half-life and continuous administration are required to maintain high plasma level in patients (Van Der Spek *et al.*, 2006; van der Spek *et al.*, 2007).

To reduce the risk of statins causing myopathy, it is desirable to identify drugs which are synergistic with pitavastatin. In 2011, Khanim, et al., (Khanim *et al.*, 2011) tested a library of 100 licensed oral drug for treatment of multiple myeloma. Drugs in the library were selected because of their oral availability, being off-patents with low toxicity. They found niclosamide, an anthelmintic drug, displayed significant antiproliferative activity against myeloma cell lines.

To utilize pitavastatin for cancer management, new synergistic combinatorial approaches are required. In this study, pharmacological screening of Dr. Khanim's library was conducted in combination with pitavastatin to identify a drug with synergistic activity against OC. The

results from the screen identified prednisolone as being synergistic with pitavastatin in a panel of OC cell lines.

6.3. Aims

To identify drugs which may reduce the dose of pitavastatin necessary to treat OC, a combinatorial drug screening approach was used. The growth inhibitory activity of pitavastatin was tested in combination with a library of 100-off patent orally available drugs against OC cell lines to identify effective combinations which could potentially be further evaluated in clinical trials for treatment of OC.

6.4. Results

6.4.1. Testing library of compound in combination with pitavastatin in ovcar-4 cell line

The Ovcar-4 cells line, which is considered representative of serous OC (Domcke *et al.*, 2013), was used to identify compounds that synergize with pitavastatin. A panel of 100 off-patent orally-bioavailable drugs were tested alone and in combination with pitavastatin in cell growth assays. To assess whether the compounds potentiated the activity of pitavastatin, the expected effect of the compounds if they acted additively was calculated using the Bliss independence criterion. Drugs were considered to potentiate the effect of pitavastatin if they exhibited a positive "Bliss excess"- those which resulted in more cell death than that expected from an additive interaction. Five compounds potentiated the effect of pitavastatin. These compounds are prednisolone (71.6 µM), rifampicin (12.2 µM), praziquantel (3.5 µM), flutamide (6.22 µM), mefenamic acid (41.4 µM). Six compound showed significant growth inhibitory activity against Ovcar-4 cell line when they were tested as single agents: zinc acetate (0.323mM), niclosamide (3.2 µM), mebendazole (1.69 µM), desferrioxamine mesilate (1.32 µM), methotrexate (1 µM) and bortezomib (0.291 µM) (Figure **6-2** and Appendix I).

Prednisolone, which is a glucocorticoid drug, showed the most significant synergistic effect in combination with pitavastatin with Bliss excess around 0.4. Therefore, it was chosen for further analysis in several authenticated OC cell lines (Cov-318, Cov-362, Ovcar-4, Ovcar-3, Ovsaho).

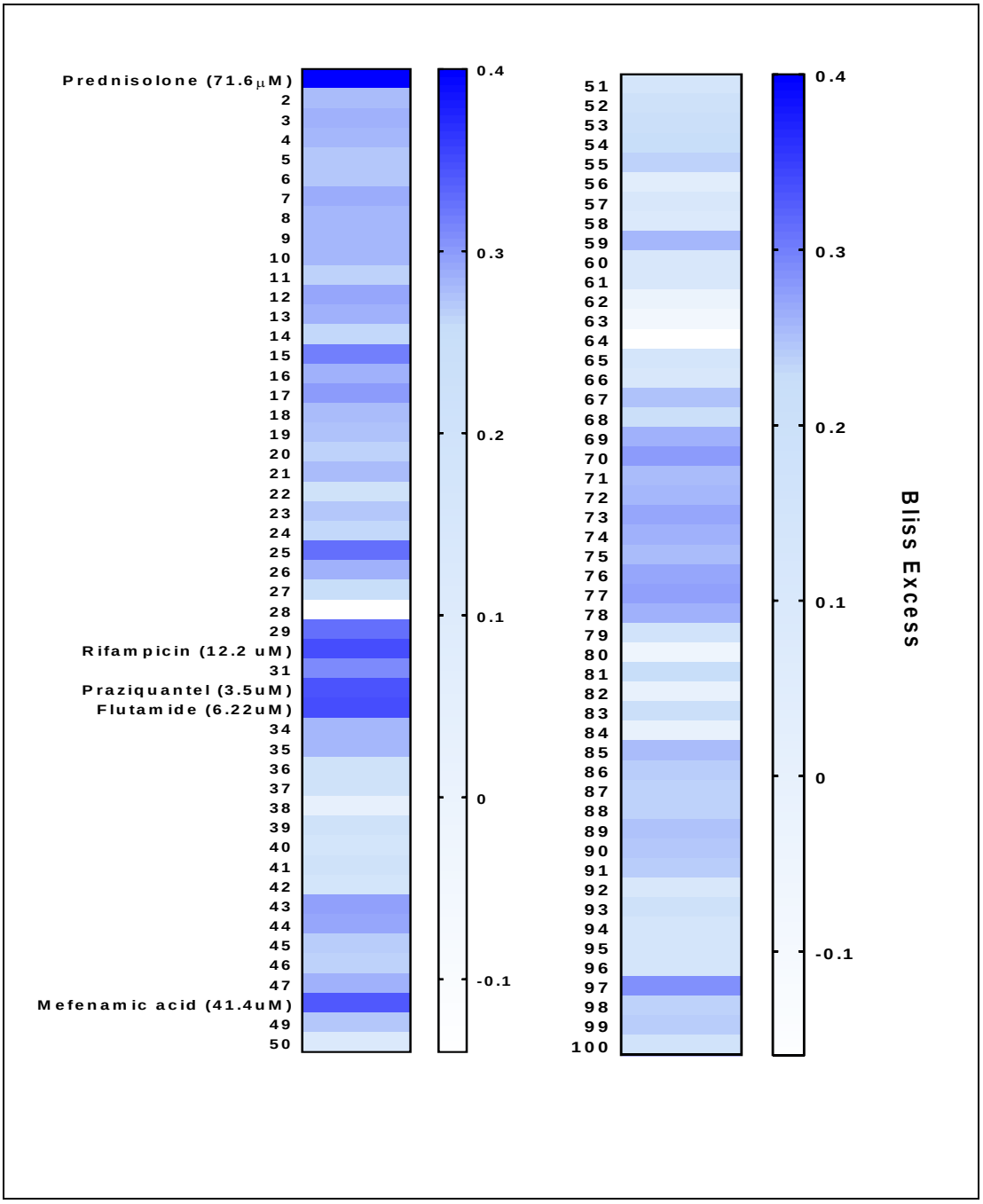


Figure 6-2 The heat map of the bliss excess

Ovcar-4 cells were exposed to the vehicle, pitavastatin (10μM), library compounds and to a combination of individual compound with pitavastatin. A triplicate of each drug addition was made and drugs effect measured using SRB assay. The heat map expressed as bliss excess which is calculated by subtraction of predicted bliss independence from the observed effects of drug combinations. Predicted bliss independence in turn, were calculated from the measured individual drug effects. The heat map expressed as mean of two independent experiments.

6.4.2. Prednisolone single agent activity in panel of ovarian cancer cell line.

The results of the screen were confirmed in cell growth assays. Firstly, the activity of prednisolone (and pitavastatin) used as a single agent was assessed using Ovcar-4, Ovcar-3 and Ovsaho, Cov-318 and Cov-362 OC cells growing in monolayer culture. Prednisolone, showed weak growth inhibitory activity using concentrations up to 500 µM. Complete concentration-response curves were not obtained hence IC_{50}s are reported as "approximately or greater than 500 µM" (Figure **6-3**). This agrees with other studies report that corticosteroids had insignificant activity against solid tumours (Lin and Wang, 2016; Sundahl *et al.*, 2016).

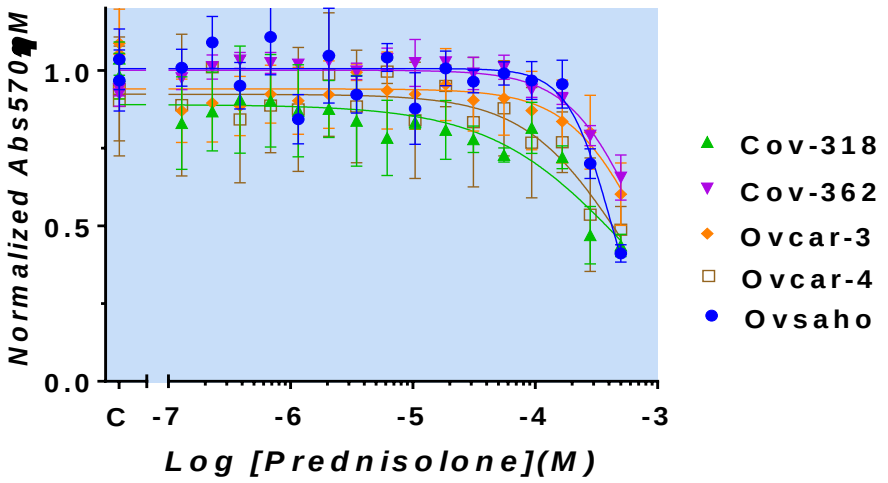


Figure 6-3 Dose response cure of prednisolone against a panel of ovarian cancer cell lines

To measure growth inhibitory activity of prednisolone, cells were exposed to the indicated concentration of prednisolone for 72 hours (Ovsaho, Ovcar-3, Ovcar-4) or 120 hours (Cov-318, Cov-362) and stained using SRB assay. The results are expressed as a fraction of the top of the curve which was determined by curve fitting (mean ± SD, n ≥ 3). "C" on the x-axis indicates control samples measured in the absence of the drug.

6.4.3. Pitavastatin single agent activity in panel of ovarian cancer cell line

Pitavastatin's activity against authenticated OC cell lines growing in monolayer cell culture plates were evaluated using SRB assay. Pitavastatin significantly inhibited the growth of all of the cells with a IC_{50}s ranging from (1.1 – 4.8µM) (Table 6-1and Figure 6-4).

Table 6-1 Single agent potency of pitavastatin in cell growth assays

Cell lines	Pitavastatin IC_{50} (µM)	N of experiments
Cov-318	3.1±0.55	4
Cov-362	3.3±0.73	4
Ovcar-3	4.1±0.12	3
Ovcar-4	4.8±0.56	3
Ovsaho	1.1±0.27	4

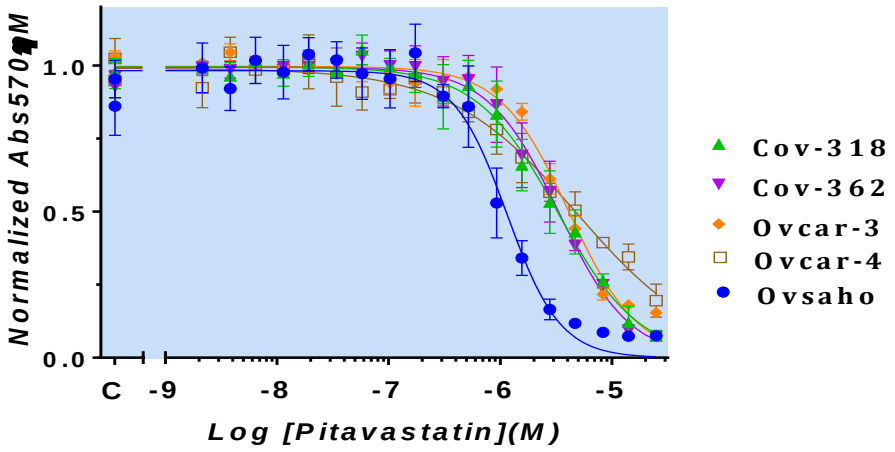


Figure 6-4 Dose response cure of pitavastatin against a panel of ovarian cancer cell lines

To measure growth inhibitory activity of pitavastatin, cells were exposed to the indicated concentration of pitavastatin for 72 hours (Ovsaho, Ovcar-3, Ovcar-4) or 120 hours (Cov-318, Cov-362) and stained using SRB assay. Dose response curve expressed as a fraction of the top of the curve which was determined by curve fitting (mean ± SD, n ≥ 3). "C" on the x-axis indicates control samples measured in the absence of the drug.

6.4.4. Pitavastatin combination with prednisolone

Drug combination studies were subsequently performed in cell growth assays using a fixed concentration of prednisolone (70µM) combined with a range of concentrations of pitavastatin. At this concentration, prednisolone has no measurable effect when used as a single agent, so any change in the pitavastatin IC_{50} is indicative of drug interaction. Prednisolone potentiated the activity of pitavastatin against OC cell line (Ovsaho, Cov-318, Cov-362, Ovcar-3 and Ovcar-4), with a significant reduction in pitavastatin IC_{50}s (Figure 6-5). To confirm this formally, combination indices were calculated. The combination showed significant synergy in tested cell lines with a combination index 0.43-0.65 calculated using the Chou and Talaly equation (Figure 6-6 and Figure 6-7).

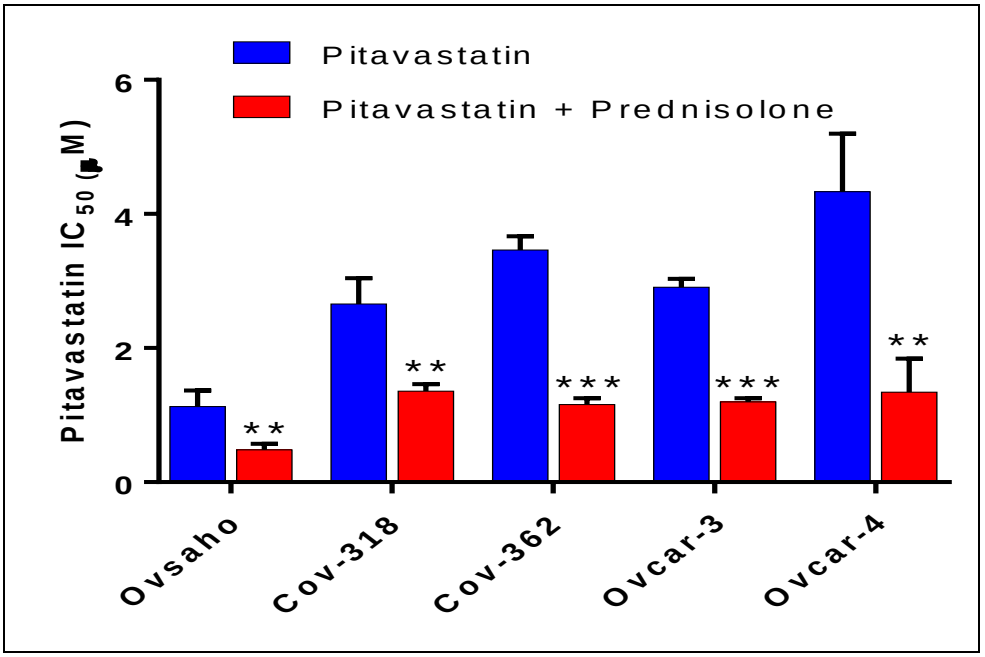


Figure 6-5 Pitavastatin IC_{50} changes after the addition of prednisolone

The figure shows the changes in pitavastatin IC_{50} after the addition of fixed dose prednisolone (70µM) in panel of OC cell line. All cell lines showed increased sensitivity to pitavastatin effect after the addition of prednisolone. The IC_{50} changes were significant in all the tested cell lines (n=3, **, $P < 0.01$; ***, $P < 0.001$, respectively, paired *t*.test).

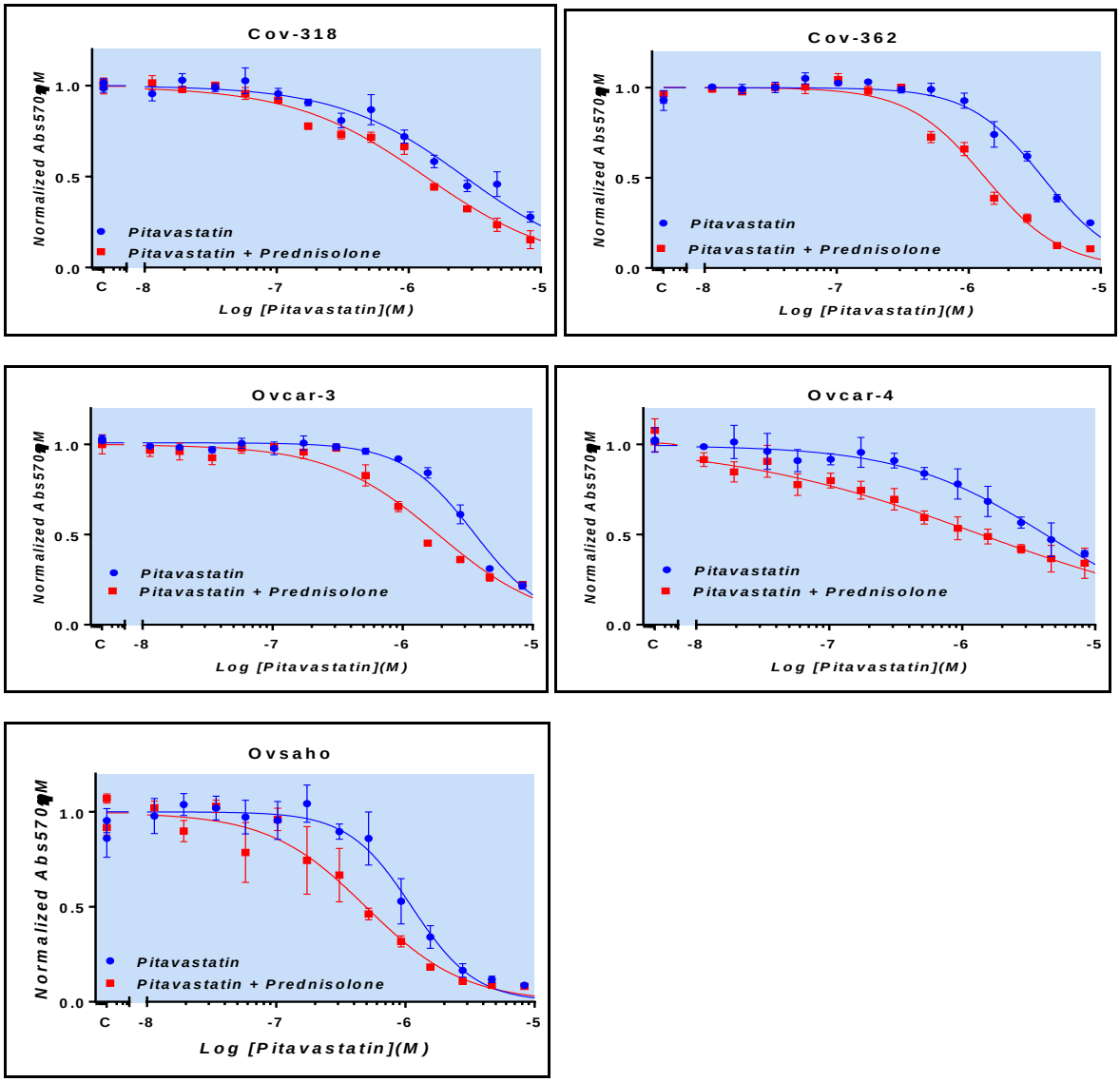


Figure 6-6 Pitavastatin combination with prednisolone in panel of ovarian cancer cell lines

To measure the activity of pitavastatin in combination with prednisolone, cells were exposed to indicated concentration of pitavastatin alone and in combination with fixed dose of prednisolone (70µM) for 72 hours (Ovsaho, Ovcar-3, Ovcar-4) or 120 hours (Cov-318, Cov-362) and stained using SRB assay. Dose response curve are expressed as a fraction of the top of the curve which was determined by curve fitting (mean ± SD, n = 3). "C" on the x-axis indicates control samples measured in the absence of the drugs.

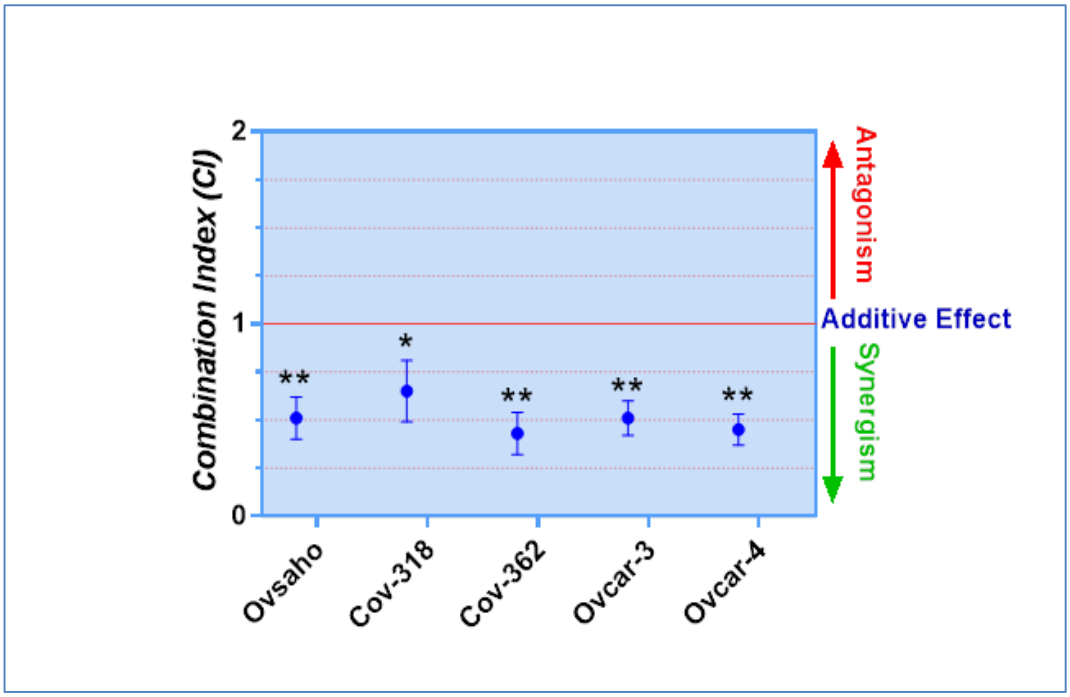


Figure 6-7 Combination indices of pitavastatin with prednisolone

Combination indices (CI) (Mean ± SD, n=3-4) are quoted at a fraction affected of 0.5. *, ** differed significantly from unity where indicated (*, $P \leq 0.05$; **, $P < 0.01$, paired *t*.test).

6.4.5. Effect of mevalonate pathway intermediate metabolites on the combination

To determine if the activity of pitavastatin and prednisolone combination in cell growth assays had resulted from inhibition of the MP, Ovcar-4 and Cov-362 cells were exposed to the combination supplemented with mevalonate, farnesol or geranylgeraniol. The addition of mevalonate to cells significantly reduced the growth inhibitory activity of drug combination. Furthermore, supplementing the combination with geranylgeraniol but not farnesol also significantly prevent growth inhibition (Figure 6-8). These results suggested that the growth inhibitory activity of combination is mediated mainly through inhibition of MP and most likely by inhibition of geranylgeranylation.

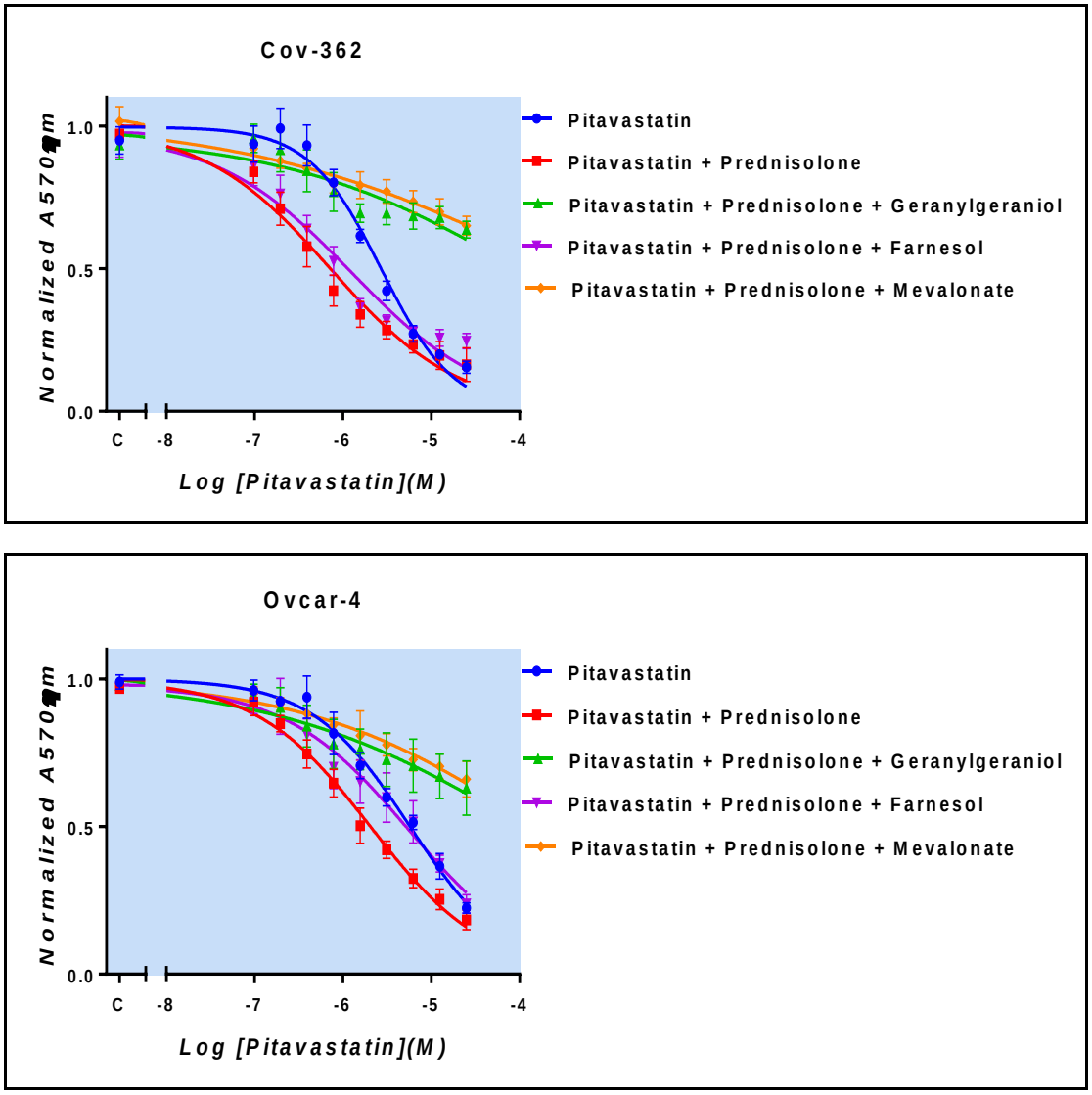


Figure 6-8 Addition of mevalonate pathway intermediate metabolite to pitavastatin prednisolone combination

Rescuing the effect of pitavastatin and prednisolone combination by the addition of geranylgeraniol (10 µM) and mevalonate (20 µM) but not farnesol (10 µM). Ovcar-4 and Cov-362 cell lines were exposed to serial dilution of pitavastatin in combination with prednisolone (70µM) for 72 and 120 hours, respectively. The data was represented as a fraction of the top of the curve which was identified by curve fitting (mean ± S.D., n = 3). "C" represents the control cells exposed to solvent alone.

6.4.6. ATP assay in spheroid cultures

To recapitulate a tumour environment more closely *in vitro*, multicellular aggregates (spheroid) were used to provide a 3D architecture of OC. The pitavastatin and prednisolone drug combination was evaluated using 3D cell culture of Ovcar-4 and Cov-362 by measuring ATP levels (Figure **6-9**). The combination of prednisolone and pitavastatin reduced ATP significantly more than would have been anticipated from the Bliss independence criterion if the two drugs had been acting additively (Figure **6-10**).

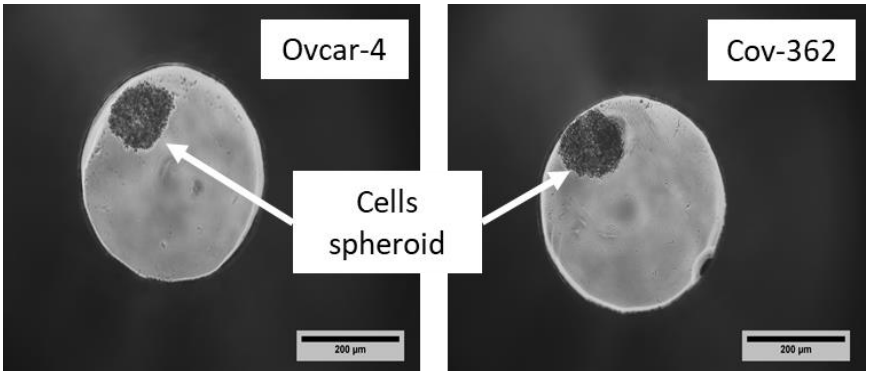


Figure 6-9 Phase contrast microscopy images of ovarian cancer cells aggregates

Ovcar-4 and Cov-362 (500 cell /well) were seeded in Gravity TRAP ULA Plates (InSphero) to build a spheroid structure from monolayer cells. The cells were observed under the phase contrast light microscope after 3-5days. Representative image for the Ovcar-4 and Cov-362 OC cell line grown on 3D culture are shown.

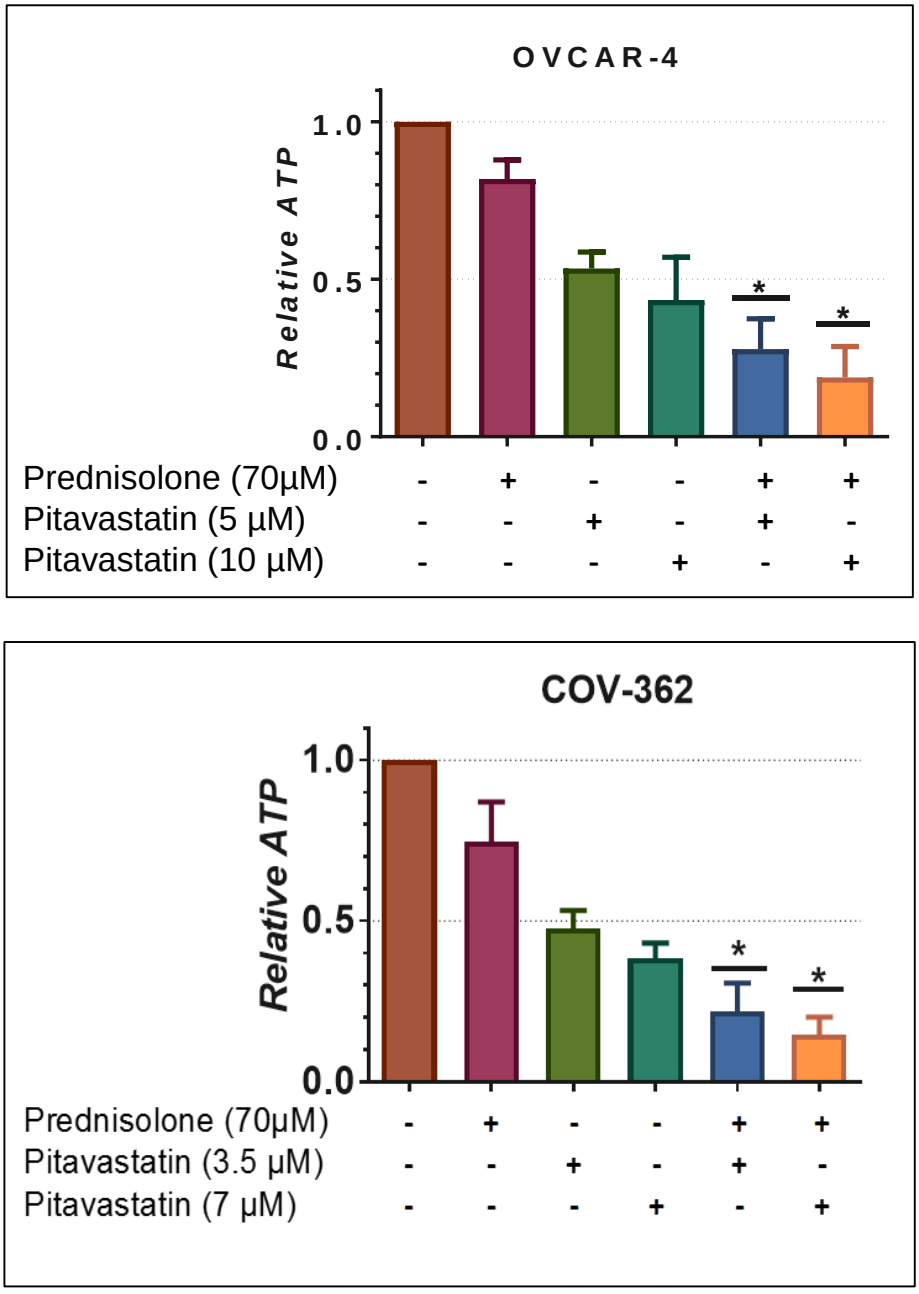


Figure 6-10 ATP assay for pitavastatin prednisolone combination

Cells were treated with the indicated drug concentration for 72 hours (Ovcar-4) or 120 hours (Cov-362). The relative viability of Ovcar-4 and Cov-362 spheroid cells were then measured by celltiter-Glo assay (relative ATP) and expressed as fraction of that measured in control samples treated with solvent (mean ± SD; n = 3). The observed drug combinations effects were compared to the effect expect if the drug effects were additive and calculated using the Bliss independence criterion (shown with a line for each drug combination) and calculated from the measured effect of the individual drugs in each individual experiment. The results were significantly different from the Bliss expected effect where shown (*, $P < 0.05$; paired t-test).

6.4.7. The synergistic antiproliferative effect of combination involve induction of apoptosis

To explore the mechanism of pitavastatin and prednisolone combination induced cytotoxicity, the cell death process was analysed. To determine whether the decrease in ATP activity after exposure to the combination was due to an apoptotic response, morphological assessment and annexin V staining was performed.

Morphological changes were observed under the phase contrast light microscope. Ovcar-4 cells were incubated with tested agents alone and in combination for 72 hours and compared to control, untreated cells. The cells treated with solvent or with prednisolone were attached and maintained their original morphology. In contrast, treated cells displayed dramatic morphological changes. Cells exposed to pitavastatin alone were detached from plate surface, round, shrinking and blebbing, and this was more pronounced in cells treated with the drug combination (

Figure **6-11**).

To assess Annexin V labelling as a marker of apoptosis, Ovcar-4 and Cov-362 cell lines were exposed to pitavastatin or prednisolone or to the combination of pitavastatin and prednisolone. On its own, prednisolone had no significant effect on the number of apoptotic cells. However, the number of live, early apoptotic and dead cells, defined by Annexin V and propidium iodide stained were significantly different from control in both cell lines and in both treatment regimens of pitavastatin alone and in combination with prednisolone. However, there were significantly more early apoptotic or dead cells in samples treated with the drug combination than in cells treated with pitavastatin alone (Figure 6-12, Figure 6-13).

Lastly, to confirm further that the reduction in cell viability and cells growth is attributed to induction of apoptosis, caspase-3/7 activity and PARP cleavage were assessed for each drug either alone and in combination. The combination caused significant activation of the effector caspases-3/7. Consistent with this, immunoblot analysis demonstrated that the prednisolone and pitavastatin combination caused significant accumulation of cleaved PARP that was greater than that observed with control and with each single agent (Figure 6-14).

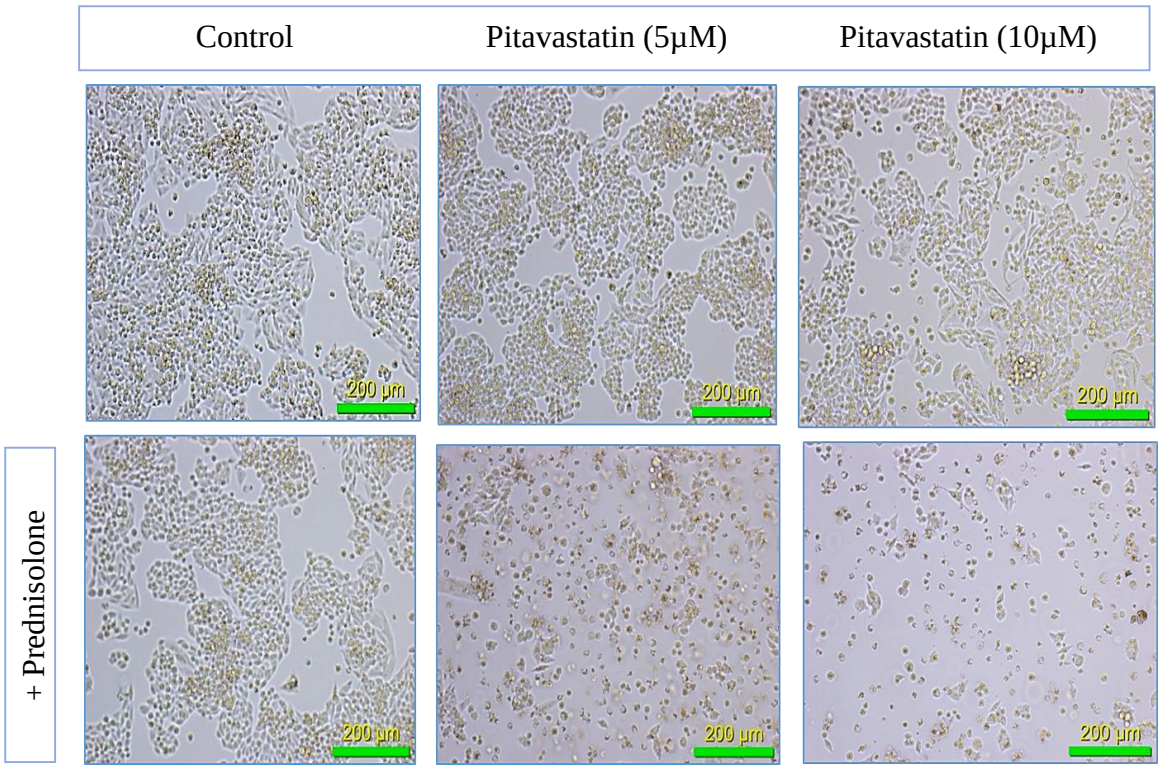
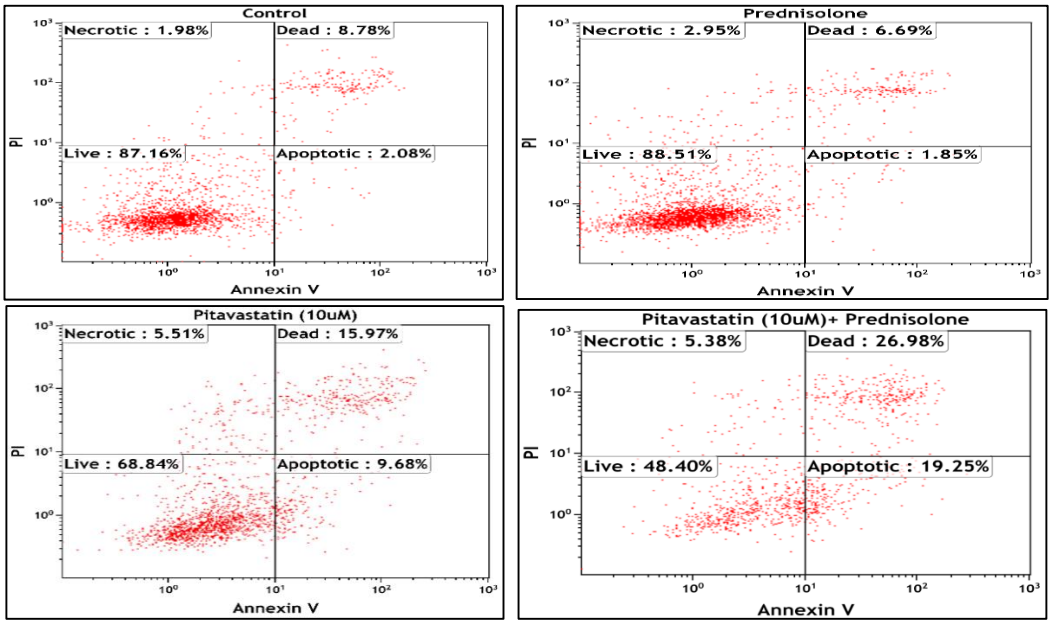


Figure 6-11 Phase contrast microscopy images of Ovcar-4 cell line

Ovcar-4 cell line exposed to the indicated drug concentration for 72 hours. Cells were exposed to vehicle, pitavastatin alone and in combination with prednisolone and visualized under phase contrast light microscope (representative of three experiments).

(A)



(B)

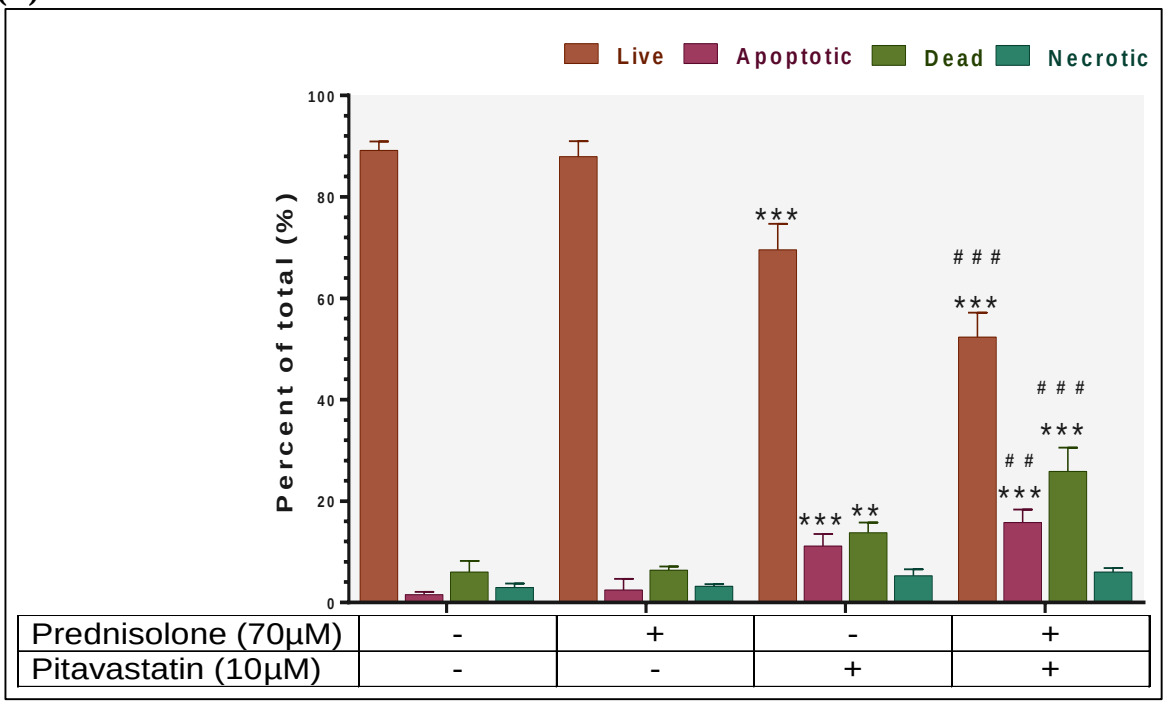
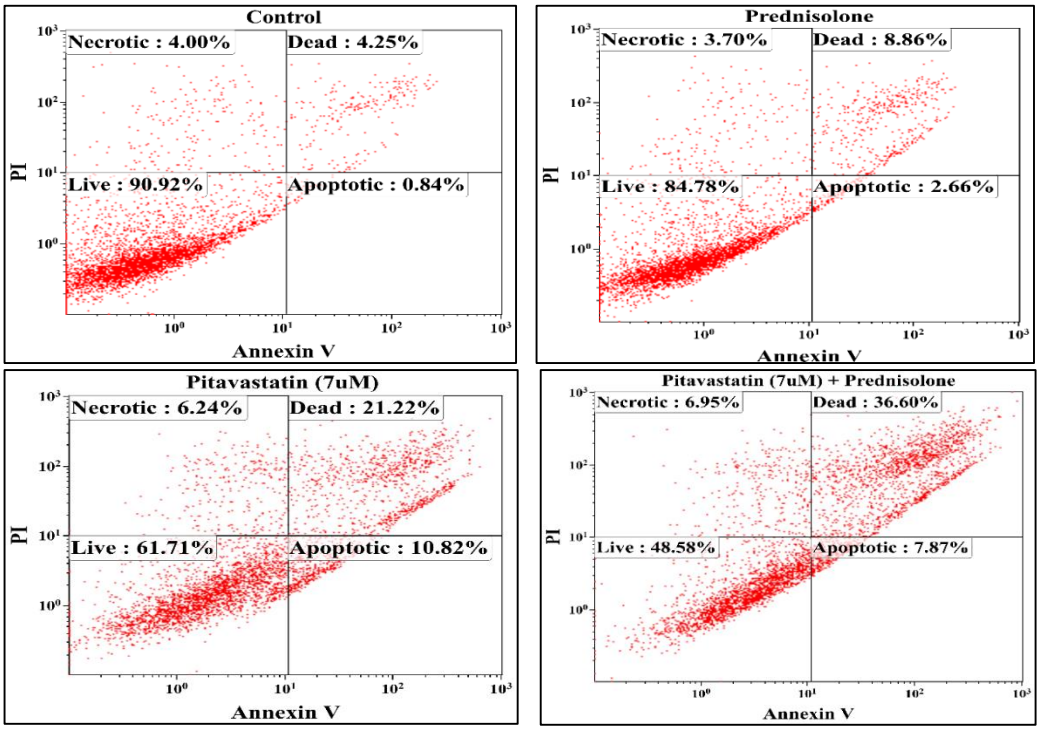


Figure 6-12 The effect of pitavastatin/ prednisolone combination on annexin V/propidium iodide staining on Ovcar-4 cell line

(A) Ovcar-4 cells were exposed to the indicated drug concentrations for 48 hours, the cells were labelled with annexin V and propidium iodide and assessed by flow cytometry. The results shown are representative of 3 experiments. (B) The graph shows the percent of cells and were compared with the control untreated cells (*) or with pitavastatin alone (#). The results (mean ± S.D., n= 3) were significantly different were indicated (**, ##, P <0.01; ***, ###, P < 0.001) (ANOVA test followed by Tukey's post hoc test).

(A)



(B)

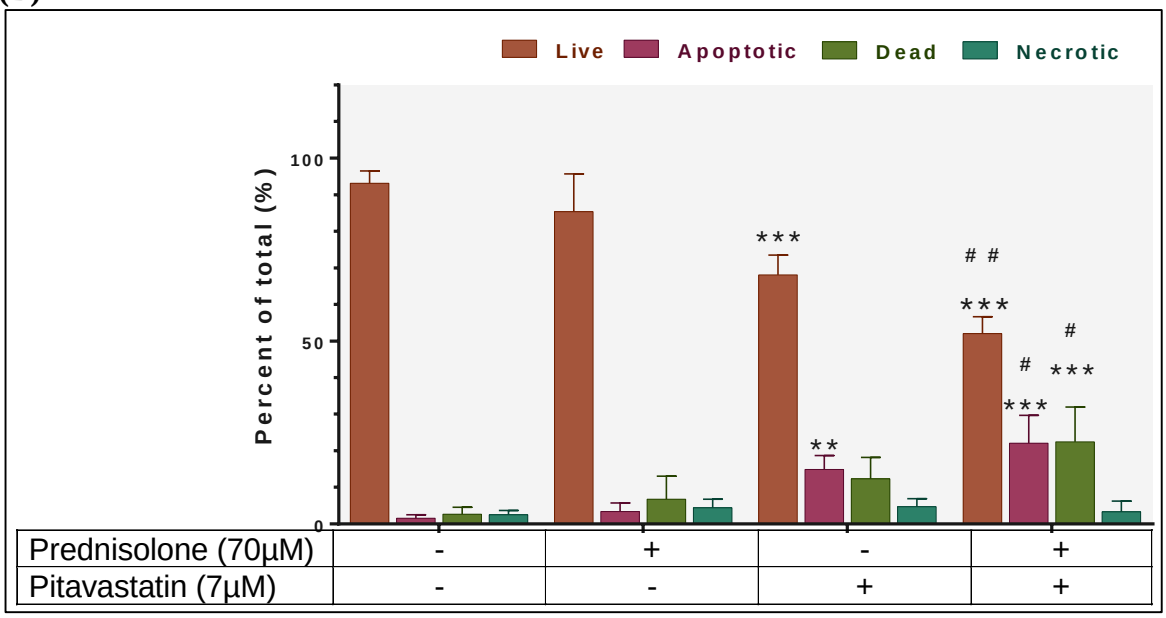


Figure 6-13 The effect of pitavastatin/ prednisolone combination on annexin V / propidium iodide staining on Cov-362

(A) Cov-362 cells were exposed to the indicated drug concentrations for 72 hours, the cells were labelled with annexin V and propidium iodide and assessed by flow cytometry. The results shown are representative of 3 experiments. (B) The graph shows the percent of cells and were compared with the control untreated cells (*) or with pitavastatin alone (#). The results (mean ± S.D., n= 3) were significantly different were indicated (#, $P < 0.05$; **, ##, $P < 0.01$; ***, $P < 0.001$) (ANOVA test followed by Tukey's post hoc test).

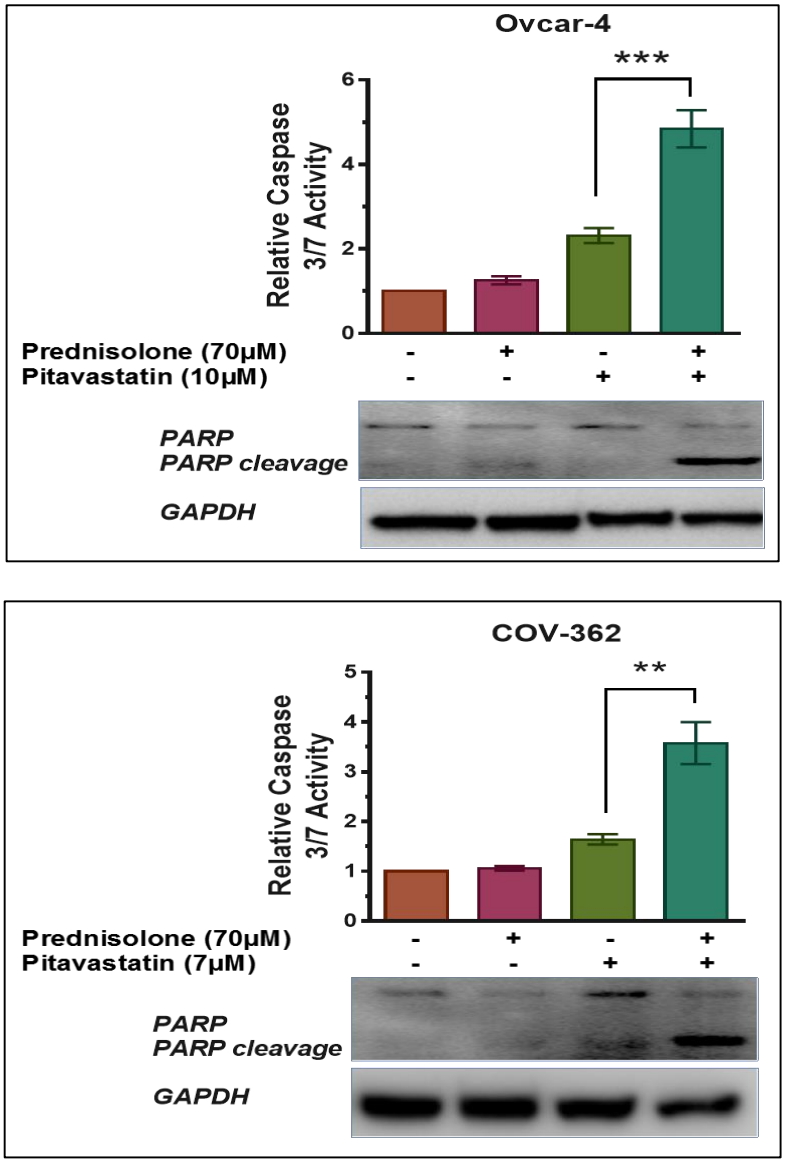


Figure 6-14 Caspase 3/7 and PARP cleavage of Ovcar-4 and Cov-362 cell lines after exposure to pitavastatin and prednisolone combination.

Caspase3/7 activity was measured by Caspase 3/7-Glo assay and the results expressed as fold of control (Mean ± SD; N=3). Cells were treated with the indicated concentrations of pitavastatin and prednisolone for 48 hours (Ovcar-4) or 72 hours (Cov-362). (n=3, **, P <0.01, ***, P < 0.001; Paired *t*-test). Note that it was not possible to calculate the expected additive effect from the Bliss independence criterion in these experiments because of the technical difficulty in accurately defining the maximum caspase 3/7 activity. PARP and PARP cleavage were measured for by western blot analysis (n=3).

6.4.8. The effect of pitavastatin/ prednisolone drug combination on mevalonate pathway

Prednisolone regulates the expression of genes by binding to the glucorticoid receptor. This raised the possibility that the synergy between pitavastatin and prednisolone occurred as a result of modulating the expression of MP genes. Previous work has identified genes whose expression is altered in 3T3-L cells exposed to prednisolone. HMGCR, GGTI, GGTII, isopentenyl diphosphate isomerase (IDI1), mevalonate decarboxylase (MVD) and farnesyl diphosphate synthase (FDPS) were reported (Fleuren *et al.*, 2013) to show decreased expression and so were selected for analysis by immunoblotting.

The result showed that neither pitavastatin nor prednisolone when used as single agents significantly altered the levels of HMGCR, FDPS, IDI1, MVD, GGTI-β. However, the combination causes significant reduction in level of HMGCR and FDPS enzyme when compared to the control of untreated cells. In contrast, GGTII-β was reduced upon exposure to either pitavastatin or prednisolone as single agents as well as in cells exposed to the combination of these two drugs (Figure **6-15**).

Lastly, it can be concluded that the antiproliferative activity of pitavastatin against OC cell lines is potentiated by prednisolone. The mechanism of this potentiation might be due to reduction of the level of HMGCR, FDPS and GGTII-β enzymes level.

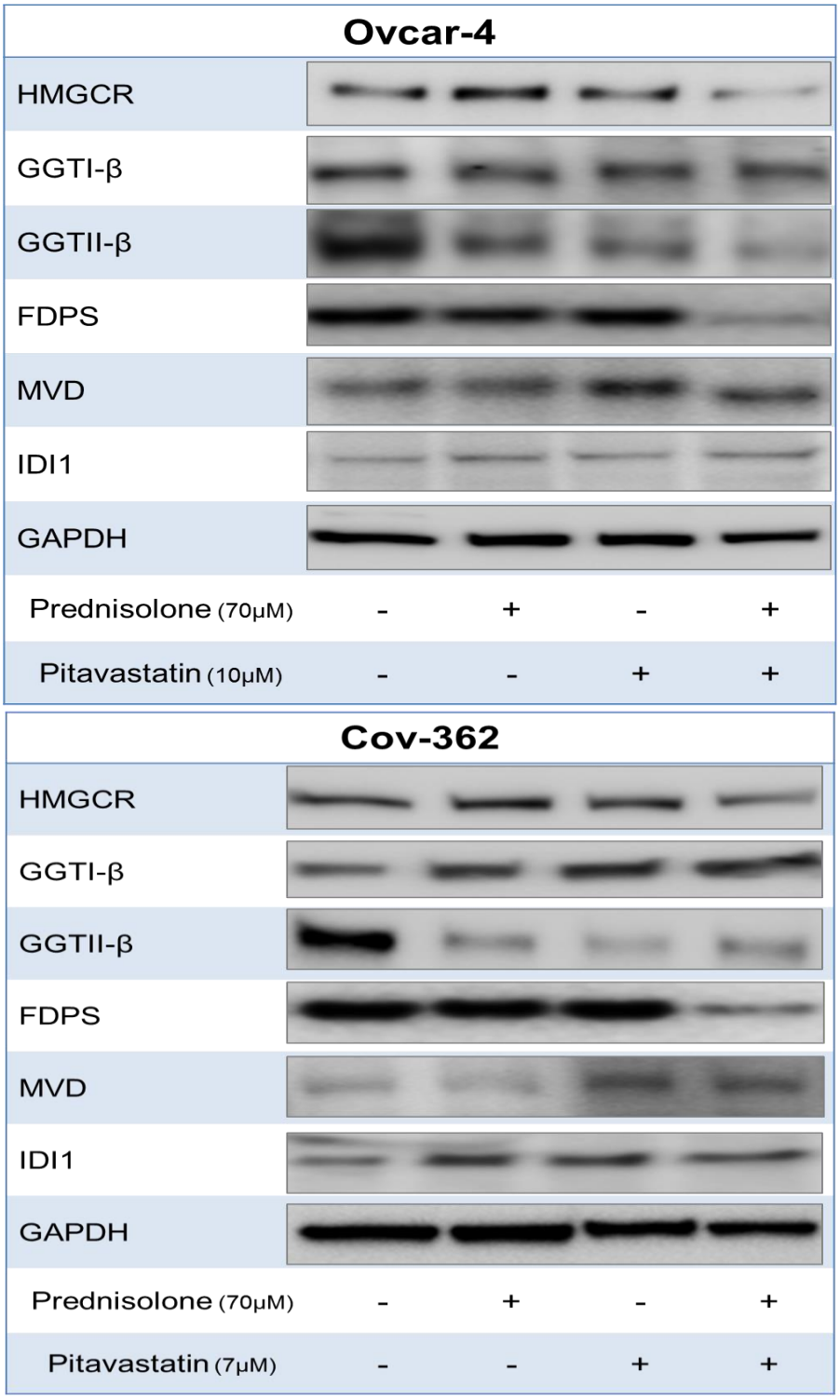


Figure 6-15 The effect of pitavastatin/ prednisolone combination on mevalonate pathway enzymes.

Ovcar-4 and Cov-362 cell line were exposed to the indicated drug concentrations for 48 hours or 72 hours, respectively. The level of MP enzymes was detected immunoblotting for HMGCR, GGTI-β, GGTII-β, FDPS, MVD, IDI1 and GAPDH, (n = 3).

6.5. Discussion

Statins in general are well tolerated when used at standard doses as anti-hypercholesterolemia agent in clinic (Ose *et al.*, 2010). However, to cause apoptosis in cancer cells, high doses are likely to be required, creating a challenge for redeployment of statins as chemotherapeutic agent. A previous study has suggested that continuous inhibition of MP *in vitro* is required to elicit the anti-cancer activities of statins (Robinson *et al.*, 2013). The use of statins at a high dose with continuous administration brings with it the substantial risk of an increase in adverse effects. The identification of appropriate drug combinations has the potential to increase the therapeutic window for statins. Thus pitavastatin was evaluated in combination with library of 100 off-patent drugs. The screen identified prednisolone, rifampicin, praziquantel, flutamide, mefenamic acid as hits which potentially potentiate the activity of pitavastatin against OC cell line. Of these, prednisolone showed the most significant synergy with pitavastatin. In addition, six compounds showed significant growth inhibitory activity against Ovcar-4 cell line when they were tested as single agents.

It is well known that steroids have the ability to induce apoptosis in lymphoid cells (Sionov *et al.*, 2008). In contrast to haematological malignancies, steroids as monotherapy show only limited activity in breast and prostate cancers but not in other cancer types (Ishiguro *et al.*, 2014; Lin and Wang, 2016). In accordance with these reports, the results showed that growth inhibitory activity of prednisolone were limited against OC cell lines. Despite the limited antitumour activity of steroids in solid tumour, it is still indicated as adjuvant therapy to reduce the adverse effects associated with cytotoxic drugs (Lin and Wang, 2016). In contrast, pitavastatin had significant growth inhibitory activity. It had been discussed previously that pitavastatin mediates cell death through reduction of MP intermediate metabolite (Casella *et al.*, 2014; Robinson *et al.*, 2014). Inhibition of the MP cause disruption of several GTPase

function which are involved in cell signalling, regulating cell cycle progression and cell survival (Swanson and Hohl, 2006; Rajalingam *et al.*, 2007). In support of this, statins induce apoptosis associated with an increase in release of mitochondrial cytochrome C to cytosol, and activation of caspases 3, 8 and 9 (Hoque, Chen and Xu, 2008; Tu *et al.*, 2011).

The synergy between the pitavastatin and prednisolone combination identified in the screen was verified in several assays including cell growth assays in monolayer and in 3D cell culture. Phase contrast microscopy shown some features of apoptosis induction (Elmore, 2007). Cells observation under microscopy revealed more significant rounding, blebbing and detachment from the plate in cells treated with the drug combination than in cells treated with the single agents. Cell death was mediated, at least in part through induction of apoptosis, because the combination synergistically increased caspase activity and PARP cleavage. The observation that the combination is synergistic in several assays gives confidence in the claim that pitavastatin and prednisolone interact synergistically.

It had been shown that MP intermediate metabolite, mevalonate and geranylgeranyl pyrophosphate reverses statin-induced apoptosis in cancer cells (Wong *et al.*, 2007). In addition, in the previous chapter, it has been observed that inhibition of both GGTI and GGTII are responsible for the potentiation of pitavastatin activity. To confirm that the mechanism of pitavastatin prednisolone combination induced apoptosis in OC cells through MP, cells were co-incubated with mevalonate, geranylgeraniol or farnesol. Only mevalonate and geranylgeranyl pyrophosphate were able to rescue cell growth, whereas farnesol could not rescue cells from the antiproliferative effect of pitavastatin prednisolone combination. This confirms that the combination works through inhibition of the mevalonate pathway and also suggests that geranylgeranyl transferases play a more critical role than farnesyl transferases in the activity of the combination.

To unveil the precise mechanism by which prednisolone was synergistic with pitavastatin, levels of MP enzymes were investigated by immunoblotting. An earlier study which aimed to characterize prednisolone-induced alterations in gene expression was considered. HMGCR, GGTI, GGTII, IDI1, MVD and FDPS showed altered expression and so were selected for analysis in these experiments (Fleuren *et al.,* 2013). The result revealed that the prednisolone-pitavastatin combination cause significant reduction in level of HMGCR and FDPS enzymes. However, a reduction of GGTII-β was observed following exposure to either drugs when used as single agents or in the combination. This suggests that prednisolone-pitavastatin combination is particularly synergistic because multiple points in the MP are affected by the drug combination. The effect of steroids on MP enzymes activity had been reported previously. Investigation of the short term effects of dexamethasone in rat hepatocytes revealed that there is reduction in cholesterol synthesis (Giudetti and Gnoni, 1998). Dexamethasone also causes down regulation of HMGCR and FTase enzymes activity in rat AR 4-2J cells. Specifically, the authors found that there is significant reduction in FT-α subunit upon treatment of the cells with dexamethasone for 48 hours. In contrast, the β-subunit of the enzyme was either unchanged or slightly reduced (Lambert and Bui, 1999). However, it was claimed that even 50 % reduction of FT activity is not sufficient to prevent Ras isoprenylation and Ras protein were even found to accumulate during dexamethasone treatment (Lambert and Bui, 1999). Therefore, it is plausible that a relatively small amount of enzyme is sufficient to maintain prenylation process, or the interplay between prenyltransferases enzymes allows one to compensate for the reduction of one of the other enzymes which maintain the cell growth and integrity. It is perhaps for this reason that relatively high concentrations of pitavastatin are required to induce apoptosis (Bell *et al.*, 2011; Cerami *et al.,* 2012; Gao *et al.,* 2013). This highlights the critical role of the mevalonate pathway in OC.

The identification of at least some of the mechanisms by which the drug combination reduces the level of MP enzymes is not trivial because the regulation of MP is extremely complex (Dimitroulakos *et al.*, 1999). The enzymes of MP, especially HMGCR, are exposed to a stringent level of feedback control mechanisms which is mediated by sterol and non-sterol products of the pathway (DeBose-Boyd, 2008). The HMGCR enzyme is regulated at several levels including regulation of its catalytic activity, its rate of degradation and synthesis (Smith and Johnson, 1989). Oxysterol, which is a natural endogenous regulator of cholesterol biosynthesis, is a product of MP participates in cholesterol homeostasis by altering enzymes stability and/or activity. It has been reported that oxysterol affects the activity of HMGCR, sequalene monooxygenase, FDPS and several enzymes in cholesterol biosynthetic pathway (Smith and Johnson, 1989; Schroepfer, 2000). For example, oxysterol accelerates the degradation of HMGCR through sterol-sensing domain in an Insig-dependent approach (Luu *et al.*, 2016). Oxysterol prevents the SREBP and SCAP complex from translocating to the Golgi complex and the subsequent proteolytic cleavage of SREBPs stimulate the transcription of genes encoding most of the enzymes in sterol biosynthetic pathway (Horton, Goldstein and Brown, 2002). Therefore, it might be speculated that steroids, which have the same ring structure as oxysterols, might bind to SREBP (Zhang, Dricu and Sjövall, 1997) leading to SREBP degradation and eventually decreasing the expression of MP enzymes. Therefore, it is possible that prednisolone triggers the reduction of HMGCR and FDPS enzymes either by decreasing transcription or increasing the degradation or both. Thus, adding prednisolone to pitavastatin may compensate for the reduction in sterols resulting from inhibition of the MP. The loss of the sterols can upregulate HMGCR as a result of the sterols no longer activating negative feedback through SREBP. Inclusion of prednisolone may prevent upregulation of HMGCR by reactivating the negative-feedback mechanism and hence synergize with pitavastatin.

In addition to sterols, another line of evidence for a negative feedback regulatory role of the pathway products is provided by the steroid hormones (Zhang, Dricu and Sjövall, 1997; Dimitroulakos *et al.*, 1999). For example, the addition of either dehydroepiandrosterone or 5-pregnen-3β-ol to diet strongly supress the HMGCR activity and cholesterol synthesis in the liver of mice and rat (Zhang, Dricu and Sjövall, 1997). Furthermore, an in vitro study has shown that dehydroepiandrosterone depleted the intracellular mevalonate pool and protein prenylation of human colonic adenocarcinoma cells exposed to growth inhibiting concentrations of the hormone (Schulz and Nyce, 1991). In contrast, there were more complicated responses to the effect of dexamethasone on a Rab protein isoforms on pancreatic AR42J cells. Dexamethasone causes downregulation of Rab3A, Rab3C and Rab3D, whereas Rab3B was upregulated at the mRNA and protein level (Klengel *et al.*, 1997).

The observed synergy of pitavastatin and prednisolone might also be explained by the modulation of glucose metabolism induced by statins. It has been reported that inhibition of glycolysis enhances the sensitivity of resistant acute lymphoblastic leukemia cells to prednisolone therapy (Hulleman *et al.*, 2009). Statins induce cell death by reduction of the MP product but the accumulation of the upstream product such as Acetyl CoA, might play a role as well. The accumulation of this metabolic precursors blocks glucose uptake through feedback inhibition of the glycolysis pathway (Jenkins *et al.*, 2011; Warita *et al.*, 2014). GLUT1, a glucose transporter protein, is highly expressed in OC and its expression was correlated with the tumour type (benign, borderline, or malignant) (Kellenberger *et al.*, 2010). In addition, it has been reported that statin cause reduction of GLUT activity in number of cells such as hepatic, adipose, muscle, or endothelial origin (Malenda *et al.*, 2012). Furthermore, the presence of glucose is essential for Akt pathway to promote mitochondrial integrity and inhibit cytochrome c release. Therefore, reduction of glucose

uptake might promote the process of programed cell death (Robey and Hay, 2006). There is considerable evidence that steroids might induce the apoptotic pathway. Although prednisolone did not induce caspase activity and PARP cleavage at the concentration tested, it was observed following exposure to the combination with pitavastatin. Steroids had the ability to induce the pro-apoptotic member of the Bcl-2 family, such as Bim, Bad and Bid and/or repress the anti-apoptotic members, such as Bcl-2, Bcl-xL and Mcl-1 (Schlossmacher, Stevens and White, 2011). In addition to the caspase-9 and -3 activation, steroids might induce the activity of caspase-8, as well (Sionov *et al.*, 2008). The activation of intrinsic and extrinsic apoptotic pathway allows procaspase-9 processing which activates the effector caspase-3 (van de Donk *et al.*, 2003).

In conclusion, drug repositioning holds a great opportunity to find new indications and new drug combinations. Pitavastatin, but not prednisolone, reduces the viability of OC cells. However, the anti-cancer activity of pitavastatin is potentiated significantly upon the combination with prednisolone and was found to be mediated through the MP by rescuing cells upon the addition of pathway intermediate metabolite. In addition, exploring the mechanism of synergy by the combination revealed that there is significant reduction in level of HMGCR and FDPS. It has been proposed that because of structural similarity of prednisolone and sterols, prednisolone might replace the sterol pool depleted by statins, and maintain the negative feedback loop in the pathway. Therefore, there is a potential therapeutic advantage of combined application of pitavastatin and prednisolone. Clinical trials of prednisolone and pitavastatin are warranted.

Chapter Seven

Conclusions and Further work

Conclusion and further work

Drug resistance is one of the main hindrances to improving a patient's prognosis and contributes significantly to the poor survival rate of OC patients (Cruz *et al.*, 2017). Therefore, new strategies for treatment of OC are a pressing requirement. Targeting metabolic pathways offers an exciting new potential therapy in the treatment of malignant disease.

Understanding the association between MP and malignancy is important for determining new therapeutic strategies (Likus *et al.*, 2016). Therefore, several enzymes in MP have been evaluated in this study and the results showed that HMGCR, a metabolic oncogene, is overexpressed in OC cell lines in comparison to normal ovarian cells and the level of GGTI and GGTII is also higher in at least a subset of OC cell line compared to normal cell line. This indicated that the MP is deregulated in OC cell lines. The deregulation of MP has been linked with carcinogenesis process and resistance to cytotoxic drugs (Mullen *et al.*, 2016). Malignant cells are more dependent on metabolites supplied by the pathway compared to their normal cellular counterparts (Siperstein, 1970). A previous study found that there is elevated level of mevalonate synthesis in breast, lymphoma, leukaemia and prostate cancer (Koyuturk *et al.*, 2007) which might be caused by aberrant activation of SREBP, a master regulator of the MP enzymes (Swinnen, Brusselmans and Verhoeven, 2006). In addition, the role of *TP53* in regulation of MP had been evaluated in OC cell lines and it was found that ectopic expression of p53 increases the level of MP enzymes and knockdown of the mutated p53 form reduces the level of MP enzymes. This highlights the importance of p53, which is almost ubiquitously altered in high grade serous OC, in the regulation of the MP. It also underscores the potential for MP inhibitors to treat OC. However, evaluation of the effect of p53 mutations in the sensitivity of OC cell line to statins therapy might reveal new avenues

for more targeted therapeutic interventions. A computational approach using gene expression has shown a number of pathways that may result in synthetic lethality if targeted in mutant *TP53* tumours (Wang and Simon, 2013). Additionally, many of the *TP53* mutant gain of function properties rely on p63 and p73 (Mantovani, Walerych and Sal, 2016). Therefore, investigation of the effects of these two family members deserves further investigation.

The preclinical studies showed that MP inhibitors are effective *in vivo* and *in vitro* (Demierre *et al.*, 2005; Kumar *et al.*, 2006; Lim *et al.*, 2009; Kidera *et al.*, 2010; Martirosyan *et al.*, 2010; Cao *et al.*, 2011; Vallianou *et al.*, 2014; Vogel *et al.*, 2017). The most important advantage observed with statins antitumour effects is that they retain their potency against OC cell line which is relatively resistant to the chemotherapy (Robinson *et al.*, 2013). Previous study found that continual blockade of HMGCR with long half-life statins was required for inducing cell death (Robinson *et al.*, 2013). In addition, there are several pharmacologically tractable targets in MP which could be evaluated to identify potential combination to increase the sensitivity of OC cells to pitavastatin. The result presented in chapter 5 showed that inhibition of the FDPS with zoledronic acid and to lesser extend risedronate potentiate the activity of pitavastatin. In contrast, inhibition of the GGTI, by GGTI-2133 or GGTI-β or GGTII-β using siRNA were unable to increase the sensitivity of OC cell lines to pitavastatin. However, the sensitivity of the OC cell lines to pitavastatin activity increase by combined inhibition of both GGTI-β and GGTII-β. Therefore, it might be argued that the redundant prenylation by the geranylgeranyl transferases enable the cells to survive. It has been reported that resistance to FTase inhibitors arise as result of redundancy (Park *et al.*, 2014) leading to geranylgeranylation in place of farnesylation. In addition, it seems likely that the activity of pitavastatin is driven through inhibition of broad range of Ras family of proteins or at least subgroups of these proteins from each transferase. This suggests that designing inhibitors that target both transferases might be superior to

molecules that inhibit either enzymes. However, this raises a concern about the possible adverse effects that might resulted from inhibition of broad range of isoprenylated proteins.

Screening a library of oral available off-patent drugs for combinatorial application with pitavastatin revealed that there are number of compounds that enhance the activity of pitavastatin. The results showed that prednisolone significantly potentiated the activity of pitavastatin. It is found also that the prednisolone-pitavastatin combination reduces the level of HMGCR and FDPS enzymes, which reinforces the importance of these two enzymes which were also inhibited by the previously assessed combination of pitavastatin and zoledronic acid. These combinations of zoledronic acid or prednisolone and pitavastatin might be a suitable option to improve the therapeutic windows of pitavastatin. Evaluation of these combinations in clinical trials will determine the usefulness of this strategy in the treatment of OC.

In addition, there are still several compounds that might be a potential candidate for combination with pitavastatin. These compounds were identified in the screen described in chapter 6 and include rifampicin, praziquantel, flutamide, mefenamic acid. Furthermore, evaluating the effect of drugs which showed significant activity against Ovcar-4 cell line when they were tested as single agents (zinc acetate, niclosamide, mebendazole, desferrioxamine mesilate, methotrexate and bortezomib) may be worthwhile.

The antitumour activity of statins has been confirmed in OC (Melichar *et al.*, 1998). However, to achieve plasma concentration that inhibit the growth, statins may need to be used close to their maximum tolerated doses which might cause adverse effects (Robinson, 2015). Although it has been stated that inhibition of prenyltransferases by statins might not be the causative of cytotoxicity in muscle (Gee *et al.*, 2015). Myopathy, and particularly rhabdomyolysis remains as one the most devastating consequences of high dose of statins

(Chaipichit *et al.*, 2015). The combination of either zoledronic acid or prednisolone with a statin might aggravate the myopathy induced by the statin. Therefore, patients should be monitored during clinical trials, and laboratory analysis including frequent routine studies which include hematological studies, liver function test, urinalyses, and determinations of creatine phosphokinase, alkaline phosphatase, serum calcium, electrolytes and fasting blood sugars (Kofman *et al.*, 1958). However, it has been suggested recently that curcumin, a natural dietary polyphenol, might supplemented as an adjunct to statin therapy in patients with muscle symptoms (Sahebkar *et al.*, 2017).

Research is underway to identify a therapeutic marker in order to select which patients might benefit from treatment with pitavastatin. In this study there were no significant correlation between HMGCR level and statin sensitivity in OC cell lines which might indicate that other molecular features influence the sensitivity to statins (Goard *et al.*, 2013). It is found that the level of GGTI-β were positively correlated with the OC cell line sensitivity to pitavastatin activity. The importance of the MP suggests that a complete screening of the expression of pathway enzymes using immunohistochemistry tissue microarray might be worthwhile. This will provide an overall picture of these enzymes in OC tissue compared to the normal tissue counterpart. Looking at this from a slightly different perspective, biomarkers may also predict which patients are resistant to pitavastatin. In contrast to GGTI, a number of Rabs are also involved in drug cytotoxic drug resistance (Recchi and Seabra, 2012). For example, Rab8 overexpression in sensitive cancer cells enhance the resistance to cisplatin (Shen and Gottesman, 2012). In addition, It was reported that Rab25 is overexpressed in OC and it is correlated with poor prognosis (Mitra, Cheng and Mills, 2012). The results showed that pitavastatin decrease the level of GGTII-β and Rab6A. Therefore, this study highlights the significance of GGTI and GGTII enzymes not only in potentiation of the activity of

pitavastatin but also as a potential marker for statin sensitivity which warrant further evaluation.

For the pitavastatin combination with zoledronic acid, a number of issues remain to be addressed. Although zoledronic acid was synergistic with pitavastatin in the majority of cell lines, the drug combination was antagonistic in Ovcar-3 cells. It is also unclear why less synergy observed when pitavastatin was combined with risedronate instead of zoledronic acid. Indeed, an antagonistic interaction as observed between risedronate and pitavastatin in Ovcar-3 cells as well as Ovcar-8 cells. It can currently only be speculated what is the cause for these observations. In the case of Ovcar-3 cells the presence of insulin in the Ovcar-3 growth medium, but not in the media for other cell lines, may contribute. The genetic background of the cells is also likely to play a key factor (Jukema *et al.*, 2012), but the identification of additional cell lines in which antagonism is observed would be necessary to assist in identifying mutations or epigenetic changes which are associated with antagonism between bisphosphonates and statins. There is also currently no clear model that links reduced protein prenylation and the induction of apoptosis. It has been observed activation of both caspase-8 and caspase-9, as well as the effector caspases3/7. This may represent separate activation of both the extrinsic and intrinsic pathways or cross-talk between these pathways, for example by cleavage of BID. Further studies are required to address these issues.

The consideration of statins selection and dosing frequency in clinical trial for treatment of cancer has been mentioned. However, a recent study from our laboratory has implemented further consideration of the patient's diet for successful use of pitavastatin in clinical trials. It is found that presence of geranylgeraniol in mouse food may reverse the cytotoxic activity of statins and feeding mice with controlled diet led to improve statin efficacy (de Wolf *et*

al., 2017). It is also had been reported that adding high fat diet reduce the activity of statins and increase renal tumour cell growth in mice (Koike *et al.*, 2011, 2012). In addition, feeding mice with a high fat diet has been found to inhibit the antitumour activity of the monoacyl glycerol lipase enzyme in xenograft study (Nomura *et al.*, 2010). Further research could determine the effects of different foodstuffs on statin activity and help to establish a guide line for suitable food consumption during clinical trials.

Most of the OC patients are diagnosed at late stage (75%) when the disease has already spread beyond the ovary at the diagnosis and those patients have very low cure rate (>20%) (Lorusso *et al.*, 2003). Successful adhesion of transformed cells is a key feature for the peritoneal spread of OC. Peritoneal metastasis is associated with resistance to chemotherapy (Ayantunde and Parsons, 2007). It has been reported that mutant *TP53* gain of function stimulates adhesion of OC cells to mesothelial cells (Lee *et al.*, 2015). For instance, several *TP53* mutant variant, such as R175H, R248Q and R273H have been reported to promote cell migration and invasion in endometrial and lung cancer cells (Pabla and Dong, 2008; Yoshikawa *et al.*, 2010; Yeudall, Wrighton and Deb, 2013). In addition, the role of MP in metastasis is well documented (Likus *et al.*, 2016). Therefore, understanding the role of different *TP53* mutations on cell adhesion, migration and invasion and exploring the role of statins might provide a new therapeutic approaches to prevent peritoneal metastasis.

In addition to prenylation, some of GTPase family proteins are subjected to phosphorylation as well. The induction of phosphorylation of Cdc42, RhoA and K-Ras by protein Kinase C mediates their relocation from plasma membrane to cytosol (Forget *et al.*, 2002; Bivona *et al.*, 2006). However, it is not known whether the translocation induced by phosphorylation leads to loss or change in the function of Ras proteins (Berndt, Hamilton and Sebti, 2011). For example, K-Ras phosphorylation by PKC mediates its removal from cell membrane to

mitochondrial membrane where is interact with $BclX_L$ and induces programed cell death (Bivona *et al.*, 2006). In addition, phosphorylation of Rab6, RhoE and RalA by aurora kinase A activated their functions (Berndt, Hamilton and Sebti, 2011). Therefore, this suggest that statins activities might synergize with PKC agonists such as bryostatin or Aurora kinase inhibitors. In addition, it is important to peruse a potential role of the unprenylated Ras proteins in driving the activity of the MP inhibitors.

The data presented here has pointed to a role for the inhibition of geranylgeranyl transferase enzymes to potentiate the activity of pitavastatin. However, this approach might be associated with several adverse effects. Therefore, another major challenge in the field of exploring the mechanism of MP inhibitors is to identify the prenyl transferase substrates which are responsible for mediating the antitumour activity in different cancer types. It has been confirmed experimentally that more than 100 proteins undergo prenylation and several hundreds of other proteins also bearing the CAXX box whose prenylation is waiting to be confirmed (Maurer-Stroh *et al.*, 2007; Berndt, Hamilton and Sebti, 2011). The importance of prenylated proteins in malignant transformation has promoted interest in developing inhibitors of the prenyl transferases enzymes as anticancer agents. However, the standard method for the following the activity are dependent on individual protein (Berndt and Sebti, 2011). Therefore, global scale analysis protein prenylation is required to understand and interpret the effects of MP inhibitors on signalling transduction pathways which might lead to identify the mechanism of action and discover a biomarker for the therapy.

Taken together, these evidence suggest that the MP is an important target for cancer treatment. In addition, these data suggest that drug combinations inhibiting multiple points in the MP may increase the therapeutic window for pitavastatin and offer a potential treatment option for management of OC.

References

Abbas, T. and Dutta, A. (2009) ‘p21 in cancer: intricate networks and multiple activities’, *Nature Reviews Cancer*, 9(6), pp. 400–414. doi: 10.1038/nrc2657.

Abe, H. *et al.* (2008) ‘High expression of Ran GTPase is associated with local invasion and metastasis of human clear cell renal cell carcinoma’, *International Journal of Cancer*. Wiley Subscription Services, Inc., A Wiley Company, 122(10), pp. 2391–2397. doi: 10.1002/ijc.23400.

Abuhaie, C.-M. *et al.* (2013) ‘Synthesis and biological evaluation of a new series of N-ylides as protein farnesyltransferase inhibitors.’, *Bioorganic & medicinal chemistry letters*. Elsevier Ltd, 23(21), pp. 5887–92. doi: 10.1016/j.bmcl.2013.08.088.

Abushahin, F. *et al.* (2008) ‘Weekly topotecan for recurrent platinum resistant ovarian cancer.’, *Gynecologic oncology*, 108(1), pp. 53–7. doi: 10.1016/j.ygyno.2007.08.062.

Achilli, T.-M., Meyer, J. and Morgan, J. R. (2012) ‘Advances in the formation, use and understanding of multi-cellular spheroids’, *Expert Opinion on Biological Therapy*, 12(10), pp. 1347–1360. doi: 10.1517/14712598.2012.707181.

Adorno, M. *et al.* (2009a) ‘A Mutant-p53/Smad Complex Opposes p63 to Empower TGFβ-Induced Metastasis’, *Cell*, 137(1), pp. 87–98. doi: 10.1016/j.cell.2009.01.039.

Adorno, M. *et al.* (2009b) ‘A Mutant-p53/Smad Complex Opposes p63 to Empower TGFβ-Induced Metastasis’, *Cell*, 137(1), pp. 87–98. doi: 10.1016/j.cell.2009.01.039.

Agarwal, R. *et al.* (2009) ‘The emerging role of the RAB25 small GTPase in cancer.’, *Traffic (Copenhagen, Denmark)*. NIH Public Access, 10(11), pp. 1561–8. doi: 10.1111/j.1600-0854.2009.00969.x.

Agarwal, R. and Kaye, S. B. (2003) ‘Ovarian cancer: strategies for overcoming resistance to chemotherapy.’, *Nature reviews. Cancer*, 3(7), pp. 502–16. doi: 10.1038/nrc1123.

Ageberg, M. *et al.* (2011) ‘Inhibition of geranylgeranylation mediates sensitivity to CHOP-induced cell death of DLBCL cell lines’, *Experimental Cell Research*, 317(8), pp. 1179–1191. doi: 10.1016/j.yexcr.2011.02.006.

Aghajanian, C. *et al.* (2012) ‘OCEANS: A Randomized, Double-Blind, Placebo-Controlled Phase III Trial of Chemotherapy With or Without Bevacizumab in Patients With Platinum-Sensitive Recurrent Epithelial Ovarian, Primary Peritoneal, or Fallopian Tube Cancer’, *Journal of Clinical Oncology*, 30(17), pp. 2039–2045. doi: 10.1200/JCO.2012.42.0505.

Di Agostino, S. *et al.* (2008) ‘The disruption of the protein complex mutantp53/p73 increases selectively the response of tumor cells to anticancer drugs’, *Cell Cycle*. Taylor & Francis, 7(21), pp. 3440–3447. doi: 10.4161/cc.7.21.6995.

Ahearn, I. M. *et al.* (2012) ‘Regulating the regulator: post-translational modification of RAS.’, *Nature reviews. Molecular cell biology*, 13(1), pp. 39–51. doi: 10.1038/nrm3255.

Ahmad, S. (2010) ‘Platinum-DNA interactions and subsequent cellular processes controlling sensitivity to anticancer platinum complexes.’, *Chemistry & biodiversity*, 7(3), pp. 543–66. doi: 10.1002/cbdv.200800340.

Ahmad, Z. (2014) ‘Statin intolerance.’, *The American journal of cardiology*. Elsevier Inc, 113(10), pp. 1765–71. doi: 10.1016/j.amjcard.2014.02.033.

Ahmed, N. and Stenvers, K. L. (2013) ‘Getting to know ovarian cancer ascites: opportunities for targeted therapy-based translational research.’, *Frontiers in oncology*, 3, p. 256. doi: 10.3389/fonc.2013.00256.

Al-Lazikani, B., Banerji, U. and Workman, P. (2012) ‘Combinatorial drug therapy for cancer in the post-genomic era.’, *Nature biotechnology*, 30(7), pp. 679–92. doi: 10.1038/nbt.2284.

Alexandrov, K. *et al.* (1994) ‘Rab escort protein-1 is a multifunctional protein that accompanies newly prenylated rab proteins to their target membranes.’, *The EMBO journal*, 13(22), pp. 5262–73. Available at: http://www.ncbi.nlm.nih.gov/pubmed/7957092 (Accessed: 5 July 2017).

Altwairgi, A. (2015) ‘Statins are potential anticancerous agentsï¿½(Review)’, *Oncology Reports*, 33(3), pp. 1019–1039. doi: 10.3892/or.2015.3741.

Appels, N. M. G. M., Beijnen, J. H. and Schellens, J. H. M. (2005) ‘Development of farnesyl transferase inhibitors: a review.’, *The oncologist*, 10(8), pp. 565–78. doi: 10.1634/theoncologist.10-8-565.

Arias-Romero, L. E. and Chernoff, J. (2013) ‘Targeting Cdc42 in cancer’, *Expert Opinion on Therapeutic Targets*, 17(11), pp. 1263–1273. doi: 10.1517/14728222.2013.828037.

Ariës, I. M. *et al.* (2015) ‘Towards personalized therapy in pediatric acute lymphoblastic leukemia: RAS mutations and prednisolone resistance.’, *Haematologica*, 100(4), pp. e132-6. doi: 10.3324/haematol.2014.112995.

Asati, V., Mahapatra, D. K. and Bharti, S. K. (2016) ‘PI3K/Akt/mTOR and Ras/Raf/MEK/ERK signaling pathways inhibitors as anticancer agents: Structural and pharmacological perspectives’, *European Journal of Medicinal Chemistry*, 109, pp. 314–341. doi: 10.1016/j.ejmech.2016.01.012.

Ashburn, T. T. and Thor, K. B. (2004) ‘Drug repositioning: identifying and developing new uses for existing drugs.’, *Nature reviews. Drug discovery*, 3(8), pp. 673–683. doi: 10.1038/nrd1468.

Asher, V., Hammond, R. and Duncan, T. J. (2010) ‘Pelvic mass associated with raised CA 125 for benign condition: a case report.’, *World journal of surgical oncology*. BioMed Central, 8, p. 28. doi: 10.1186/1477-7819-8-28.

Ayantunde, A. A. and Parsons, S. L. (2007) ‘Pattern and prognostic factors in patients with malignant ascites: a retrospective study.’, *Annals of oncology : official journal of the European Society for Medical Oncology / ESMO*, 18(5), pp. 945–9. doi: 10.1093/annonc/mdl499.

Ayroldi, E. *et al.* (2007) ‘GILZ mediates the antiproliferative activity of glucocorticoids by negative regulation of Ras signaling’, *Journal of Clinical Investigation*. American Society for Clinical Investigation, 117(6), pp. 1605–1615. doi: 10.1172/JCI30724.

Babic, A. *et al.* (2017) 'Predictors of pretreatment CA125 at ovarian cancer diagnosis: a pooled analysis in the Ovarian Cancer Association Consortium', *Cancer Causes and Control*. Springer International Publishing, 28(5), pp. 459–468. doi: 10.1007/s10552-016-0841-3.

Bafna, S., Kaur, S. and Batra, S. K. (2010) 'Membrane-bound mucins: the mechanistic basis for alterations in the growth and survival of cancer cells.', *Oncogene*, 29(20), pp. 2893–904. doi: 10.1038/onc.2010.87.

Banerjee, S., Bookman, M. A. and Gore, M. (2011) 'Systemic Therapy for Ovarian Cancer, Current Treatment, Recent Advances, and Unmet Needs', in *Emerging Therapeutic Targets in Ovarian Cancer*. New York, NY: Springer New York, pp. 1–33. doi: 10.1007/978-1-4419-7216-3_1.

Banerjee, S. and Kaye, S. B. (2013) 'New strategies in the treatment of ovarian cancer: current clinical perspectives and future potential.', *Clinical cancer research : an official journal of the American Association for Cancer Research*, epub(5), pp. 961–8. doi: 10.1158/1078-0432.CCR-12-2243.

Banks, E. (2001) 'The Epidemiology of Ovarian Cancer', in *Ovarian Cancer*. New Jersey: Humana Press, pp. 3–11. doi: 10.1385/1-59259-071-3:3.

Bartek, J. and Lukas, J. (2007) 'DNA damage checkpoints: from initiation to recovery or adaptation', *Current Opinion in Cell Biology*, pp. 238–245. doi: 10.1016/j.ceb.2007.02.009.

Bast, R. C. *et al.* (1981) 'Reactivity of a monoclonal antibody with human ovarian carcinoma.', *The Journal of clinical investigation*, 68(5), pp. 1331–7. Available at: http://www.ncbi.nlm.nih.gov/pubmed/7028788 (Accessed: 20 March 2017).

Bast, R. C., Hennessy, B. and Mills, G. B. (2009) 'The biology of ovarian cancer: new opportunities for translation.', *Nature reviews. Cancer*, 9(6), pp. 415–28. doi: 10.1038/nrc2644.

Becker, D. E. (2013) 'Basic and clinical pharmacology of glucocorticosteroids.', *Anesthesia progress*, 60(1), p. 25–31; quiz 32. doi: 10.2344/0003-3006-60.1.25.

Beckerman, R. and Prives, C. (2010) 'Transcriptional Regulation by P53', *Cold Spring Harbor Perspectives in Biology* , 2(8), p. a000935. doi: 10.1101/cshperspect.a000935.

Begg, A. C., Stewart, F. A. and Vens, C. (2011) 'Strategies to improve radiotherapy with targeted drugs', *Nature Reviews Cancer*. Nature Publishing Group, 11(4), pp. 239–253. doi: 10.1038/nrc3007.

Behrens, B. C. *et al.* (1987) 'Characterization of a cis-Diamminedichloroplatinum(II)-resistant Human Ovarian Cancer Cell Line and Its Use in Evaluation of Platinum Analogues', *Cancer Research*, 47(2), pp. 414–418.

Bell, D. *et al.* (2011) 'Integrated genomic analyses of ovarian carcinoma', *Nature*, 474(7353), pp. 609–615. doi: 10.1038/nature10166.

Bell, I. M. (2004) ‘Inhibitors of Farnesyltransferase: A Rational Approach to Cancer Chemotherapy?’, *Journal of Medicinal Chemistry*. American Chemical Society, 47(8), pp. 1869–1878. doi: 10.1021/jm0305467.

Belotte, J. *et al.* (2014) ‘The role of oxidative stress in the development of cisplatin resistance in epithelial ovarian cancer.’, *Reproductive sciences (Thousand Oaks, Calif.)*, 21(4), pp. 503–8. doi: 10.1177/1933719113503403.

Bénard, J. *et al.* (1985) ‘Characterization of a Human Ovarian Adenocarcinoma Line, IGROV1, in Tissue Culture and in Nude Mice’, *Cancer Research*, 45(10), pp. 4970–4979.

Bengtsson, E. *et al.* (2014) ‘HMG-CoA reductase expression in primary colorectal cancer correlates with favourable clinicopathological characteristics and an improved clinical outcome.’, *Diagnostic Pathology*, 9, p. 78. doi: 10.1186/1746-1596-9-78.

Bensaad, K. *et al.* (2003) ‘Change of Conformation of the DNA-binding Domain of p53 Is the Only Key Element for Binding of and Interference with p73*’. in Press. doi: 10.1074/jbc.M208233200.

Beral, V. *et al.* (2007) ‘Ovarian cancer and hormone replacement therapy in the Million Women Study.’, *Lancet*, 369(9574), pp. 1703–10. doi: 10.1016/S0140-6736(07)60534-0.

Beral, V. *et al.* (2008) ‘Ovarian cancer and oral contraceptives: collaborative reanalysis of data from 45 epidemiological studies including 23,257 women with ovarian cancer and 87,303 controls.’, *Lancet*, 371(9609), pp. 303–14. doi: 10.1016/S0140-6736(08)60167-1.

Berek, J. S. *et al.* (2017) ‘Epithelial Ovarian, Fallopian Tube, and Peritoneal Cancer’, in *Holland-Frei Cancer Medicine*. Hoboken, NJ, USA: John Wiley & Sons, Inc., pp. 1–27. doi: 10.1002/9781119000822.hfcm105.

van den Berg-Bakker, C. A. M. *et al.* (1993) ‘Establishment and characterization of 7 ovarian carcinoma cell lines and one granulosa tumor cell line: Growth features and cytogenetics’, *International Journal of Cancer*, 53(4), pp. 613–620. doi: 10.1002/ijc.2910530415.

Berg, J. M., Tymoczko, J. L. and Stryer, L. (2013) *Biochemistry*, *W H Freeman*. Available at: papers2://publication/uuid/7EB6183C-F1A3-4903-A72F-B1A659CECF68.

Berndt, N., Hamilton, A. D. and Sebti, S. M. (2011) ‘Targeting protein prenylation for cancer therapy.’, *Nature reviews. Cancer*, 11(11), pp. 775–91. doi: 10.1038/nrc3151.

Berndt, N. and Sebti, S. M. (2011) ‘Measurement of protein farnesylation and geranylgeranylation in vitro, in cultured cells and in biopsies, and the effects of prenyl transferase inhibitors’, *Nature Protocols*, 6(11), pp. 1775–1791. doi: 10.1038/nprot.2011.387.

Bertolini, F., Sukhatme, V. P. and Bouche, G. (2015) ‘Drug repurposing in oncology-patient and health systems opportunities.’, *Nature reviews. Clinical oncology*, 12(12), pp. 1–11. doi: 10.1038/nrclinonc.2015.169.

Bharwani, N., Reznek, R. H. and Rockall, A. G. (2011) ‘Ovarian Cancer Management: the

role of imaging and diagnostic challenges.', *European journal of radiology*. Elsevier Ireland Ltd, 78(1), pp. 41–51. doi: 10.1016/j.ejrad.2010.11.039.

Bieging, K. T. and Attardi, L. D. (2012) 'Deconstructing p53 transcriptional networks in tumor suppression', *Trends in Cell Biology*, pp. 97–106. doi: 10.1016/j.tcb.2011.10.006.

Bieging, K. T., Mello, S. S. and Attardi, L. D. (2014) 'Unravelling mechanisms of p53-mediated tumour suppression', *Nature Reviews Cancer*, 14(5), pp. 359–370. doi: 10.1038/nrc3711.

Bijnsdorp, I. V., Giovannetti, E. and Peters, G. J. (2011) 'Analysis of Drug Interactions', in *Methods in molecular biology (Clifton, N.J.)*, pp. 421–434. doi: 10.1007/978-1-61779-080-5_34.

Bivona, T. G. *et al.* (2006) 'PKC Regulates a Farnesyl-Electrostatic Switch on K-Ras that Promotes its Association with Bcl-Xl on Mitochondria and Induces Apoptosis', *Molecular Cell*, 21(4), pp. 481–493. doi: 10.1016/j.molcel.2006.01.012.

Blandino, G., Levine, A. J. and Oren, M. (1999) 'Mutant p53 gain of function: differential effects of different p53 mutants on resistance of cultured cells to chemotherapy.', *Oncogene*, 18(2), pp. 477–85. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9927204 (Accessed: 16 February 2017).

Blomen, V. A. *et al.* (2015) 'Gene essentiality and synthetic lethality in haploid human cells', *Science*, 350(6264).

Bommi-Reddy, A. and Kaelin, W. G. (2010) 'Slaying RAS with a synthetic lethal weapon', *Cell Research*. Nature Publishing Group, 20(2), pp. 119–121. doi: 10.1038/cr.2010.16.

Bonovas, S. *et al.* (2005) 'Use of statins and breast cancer: a meta-analysis of seven randomized clinical trials and nine observational studies.', *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*, 23(34), pp. 8606–12. doi: 10.1200/JCO.2005.02.7045.

Bookman, M. a *et al.* (2009) 'Evaluation of new platinum-based treatment regimens in advanced-stage ovarian cancer: a Phase III Trial of the Gynecologic Cancer Intergroup.', *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*, 27(9), pp. 1419–25. doi: 10.1200/JCO.2008.19.1684.

Borgquist, S. *et al.* (2008) 'Prognostic impact of tumour-specific HMG-CoA reductase expression in primary breast cancer.', *Breast cancer research : BCR*, 10(5), p. R79. doi: 10.1186/bcr2146.

Boron, W. F. and Boulpaep, E. L. (2012) *Medical Physiology, Physiology*. doi: 10.1136/pgmj.51.599.683-c.

Bos, J. L., Rehmann, H. and Wittinghofer, A. (2007) 'GEFs and GAPs: Critical Elements in the Control of Small G Proteins', *Cell*, 129(5), pp. 865–877. doi: 10.1016/j.cell.2007.05.018.

Bosch-Barrera, J. *et al.* (2011) 'Direct antitumour activity of zoledronic acid: preclinical and clinical data', *Clinical and Translational Oncology*, 13(3), pp. 148–155. doi: 10.1007/s12094-011-0634-9.

Bouaoun, L. *et al.* (2016) 'TP53 Variations in Human Cancers: New Lessons from the IARC TP53 Database and Genomics Data', *Human Mutation*, 37(9), pp. 865–876. doi: 10.1002/humu.23035.

Bowtell, D. D. *et al.* (2015) 'Rethinking ovarian cancer II: reducing mortality from high-grade serous ovarian cancer', *Nature Reviews Cancer*. Nature Publishing Group, 15(11), pp. 668–79. doi: 10.1038/nrc4019.

Brachova, P., Thiel, K. W. and Leslie, K. K. (2013) 'The consequence of oncomorphic TP53 mutations in ovarian cancer.', *International journal of molecular sciences*, 14(9), pp. 19257–75. doi: 10.3390/ijms140919257.

Brandi, J. *et al.* (2017) 'Proteomic analysis of pancreatic cancer stem cells: Functional role of fatty acid synthesis and mevalonate pathways', *Journal of Proteomics*, 150, pp. 310–322. doi: 10.1016/j.jprot.2016.10.002.

Brennan, D. J. *et al.* (2010) 'Tumour-specific HMG-CoAR is an independent predictor of recurrence free survival in epithelial ovarian cancer', *BMC Cancer*, 10(1), p. 125. doi: 10.1186/1471-2407-10-125.

Brennan, D. J. *et al.* (2011) 'Tumor-specific HMG-CoA reductase expression in primary premenopausal breast cancer predicts response to tamoxifen', *Breast Cancer Research*. BioMed Central Ltd, 13(1), p. R12. doi: 10.1186/bcr2820.

Brosh, R. and Rotter, V. (2009) 'When mutants gain new powers: news from the mutant p53 field.', *Nature reviews. Cancer*, 9(10), pp. 701–13. doi: 10.1038/nrc2693.

Brown, A. S. and Patel, C. J. (2017) 'A standard database for drug repositioning.', *Scientific data*. Nature Publishing Group, 4, p. 170029. doi: 10.1038/sdata.2017.29.

Brunner, T. B. *et al.* (2003) 'Farnesyltransferase Inhibitors: An Overview of the Results of Preclinical and Clinical Investigations', *CANCER RESEARCH*, 63, pp. 5656–5668. Available at: http://cancerres.aacrjournals.org/content/63/18/5656.full-text.pdf (Accessed: 5 April 2017).

Brusselmans, K. *et al.* (2007) 'Squalene synthase, a determinant of Raft-associated cholesterol and modulator of cancer cell proliferation.', *The Journal of biological chemistry*, 282(26), pp. 18777–85. doi: 10.1074/jbc.M611763200.

Bryan, B. A. and D'Amore, P. A. (2007) 'What tangled webs they weave: Rho-GTPase control of angiogenesis', *Cellular and Molecular Life Sciences*, pp. 2053–2065. doi: 10.1007/s00018-007-7008-z.

Brynner, R. and Stephens, T. (2001) 'Dark Remedy: The Impact of Thalidomide and its Revival as a Vital Medicine', in *An Uncertain Utopia*, pp. 1–18. doi: 10.1136/bmj.322.7302.1608.

Buchman, A. L. (2001) ‘Side effects of corticosteroid therapy.’, *Archives of general psychiatry*, 38(4), pp. 471–477. doi: 10.1001/archpsyc.1981.01780290105011.

Budanov, A. V. and Karin, M. (2008) ‘p53 Target Genes Sestrin1 and Sestrin2 Connect Genotoxic Stress and mTOR Signaling’, *Cell*, 134(3), pp. 451–460. doi: 10.1016/j.cell.2008.06.028.

Budman, D. R. and Calabro, A. (2006) ‘Zoledronic Acid (Zometa®) Enhances the Cytotoxic Effect of Gemcitabine and Fluvastatin: In vitro Isobologram Studies with Conventional and Nonconventional Cytotoxic Agents’, *Oncology*, 70(2), pp. 147–153. doi: 10.1159/000093006.

Buhaescu, I. and Izzedine, H. (2007) ‘Mevalonate pathway: a review of clinical and therapeutical implications.’, *Clinical biochemistry*, 40(9–10), pp. 575–84. doi: 10.1016/j.clinbiochem.2007.03.016.

Buick, R. N., Pullano, R. and Trent, J. M. (1985) ‘Comparative Properties of Five Human Ovarian Adenocarcinoma Cell Lines’, *Cancer Research*, 45(8), pp. 3668–3676.

Bullock, A. N. and Fersht, A. R. (2001) ‘Rescuing the function of mutant p53’, *Nature Reviews Cancer*, 1(1), pp. 68–76. doi: 10.1038/35094077.

Burg, J. S. and Espenshade, P. J. (2011) ‘Regulation of HMG-CoA reductase in mammals and yeast.’, *Progress in lipid research*. Elsevier Ltd, 50(4), pp. 403–10. doi: 10.1016/j.plipres.2011.07.002.

Burger, R. a *et al.* (2007) ‘Phase II Trial of Bevacizumab in Persistent or Recurrent Epithelial Ovarian Cancer or Primary Peritoneal Cancer: A Gynecologic Oncology Group Study’, *Journal of Clinical Oncology*, 25(33), pp. 5165–5171. doi: 10.1200/JCO.2007.11.5345.

Burger, R. A. *et al.* (2012) ‘Incorporation of Bevacizumab in the Primary Treatment of Ovarian Cancer’, *Obstetrical & Gynecological Survey*. Massachusetts Medical Society, 67(5), pp. 289–290. doi: 10.1056/NEJMoa1104390.

van der Burgh, R. *et al.* (2013) ‘Mevalonate kinase deficiency, a metabolic autoinflammatory disease.’, *Clinical immunology (Orlando, Fla.)*. Elsevier Inc., 147(3), pp. 197–206. doi: 10.1016/j.clim.2012.09.011.

Butterfield, D. A., Barone, E. and Mancuso, C. (2011) ‘Cholesterol-independent neuroprotective and neurotoxic activities of statins: perspectives for statin use in Alzheimer disease and other age-related neurodegenerative disorders.’, *Pharmacological research : the official journal of the Italian Pharmacological Society*. Elsevier Ltd, 64(3), pp. 180–6. doi: 10.1016/j.phrs.2011.04.007.

Bykov, V. J. N. *et al.* (2002) ‘Restoration of the tumor suppressor function to mutant p53 by a low-molecular-weight compound.’, *Nature medicine*, 8(3), pp. 282–288. doi: 10.1038/nm0302-282.

Bykov, V. J. N. *et al.* (2005) ‘Reactivation of mutant p53 and induction of apoptosis in human tumor cells by maleimide analogs.’, *The Journal of biological chemistry*, 280(34),

pp. 30384–30391. doi: 10.1074/jbc.M501664200.

Cafforio, P. *et al.* (2005) 'Statins activate the mitochondrial pathway of apoptosis in human lymphoblasts and myeloma cells', *Carcinogenesis*, 26(5), pp. 883–891. doi: 10.1093/carcin/bgi036.

Callaghan, R., Luk, F. and Bebawy, M. (2014) 'Inhibition of the multidrug resistance P-glycoprotein: time for a change of strategy?', *Drug metabolism and disposition: the biological fate of chemicals*. American Society for Pharmacology and Experimental Therapeutics, 42(4), pp. 623–31. doi: 10.1124/dmd.113.056176.

Camargo, M. C. *et al.* (2011) 'Review Occupational Exposure to Asbestos and Ovarian Cancer : A Meta-analysis', 119(9), pp. 1211–1217.

Cancer research UK (2017) *Ovarian cancer statistics*. Available at: http://www.cancerresearchuk.org/health-professional/cancer-statistics/statistics-by-cancer-type/ovarian-cancer#heading-One (Accessed: 1 March 2017).

Cao, Z. *et al.* (2011) 'MYC phosphorylation, activation, and tumorigenic potential in hepatocellular carcinoma are regulated by HMG-CoA reductase.', *Cancer research*. NIH Public Access, 71(6), pp. 2286–97. doi: 10.1158/0008-5472.CAN-10-3367.

Carlsson, J. and Acker, H. (1988) 'Relations between pH, oxygen partial pressure and growth in cultured cell spheroids.', *International journal of cancer. Journal international du cancer*, 42(5), pp. 715–720.

Casalou, C., Faustino, A. and Barral, D. C. (2016) 'Arf proteins in cancer cell migration', *Small GTPases*. Taylor & Francis, 7(4), pp. 270–282. doi: 10.1080/21541248.2016.1228792.

Casella, C. *et al.* (2014) 'Oxysterols synergize with statins by inhibiting SREBP-2 in ovarian cancer cells.', *Gynecologic oncology*. Elsevier Inc., 135(2), pp. 333–41. doi: 10.1016/j.ygyno.2014.08.015.

Caswell, P. T. *et al.* (2007) 'Rab25 Associates with ??5??1 Integrin to Promote Invasive Migration in 3D Microenvironments', *Developmental Cell*, 13(4), pp. 496–510. doi: 10.1016/j.devcel.2007.08.012.

Cerami, E. *et al.* (2012) 'The cBio Cancer Genomics Portal: An Open Platform for Exploring Multidimensional Cancer Genomics Data', *Cancer Discovery*, 2(5). Available at: http://cancerdiscovery.aacrjournals.org/content/2/5/401 (Accessed: 16 May 2017).

Cervantes-Gomez, F. *et al.* (2015) 'Pharmacological and Protein Profiling Suggests Venetoclax (ABT-199) as Optimal Partner with Ibrutinib in Chronic Lymphocytic Leukemia', *Clinical Cancer Research*, 21(16), pp. 3705–3715. doi: 10.1158/1078-0432.CCR-14-2809.

Chaipichit, N. *et al.* (2015) 'Statin adverse effects: patients' experiences and laboratory monitoring of muscle and liver injuries', *International Journal of Clinical Pharmacy*. Springer Netherlands, 37(2), pp. 355–364. doi: 10.1007/s11096-015-0068-5.

Chan, J. K. *et al.* (2006) 'Ovarian cancer in younger vs older women: a population-based analysis.', *British journal of cancer*, 95(10), pp. 1314–20. doi: 10.1038/sj.bjc.6603457.

Chan, K.-T. and Lung, M. L. (2004) 'Mutant p53 expression enhances drug resistance in a hepatocellular carcinoma cell line.', *Cancer chemotherapy and pharmacology*, 53(6), pp. 519–26. doi: 10.1007/s00280-004-0767-4.

Chang, F. L. and Lai, M. D. (2001) 'Various forms of mutant p53 confer sensitivity to cisplatin and doxorubicin in bladder cancer cells.', *The Journal of urology*, 166(1), pp. 304–10. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11435891 (Accessed: 13 February 2017).

Chapman-Shimshoni, D. *et al.* (2003) 'Simvastatin induces apoptosis of B-CLL cells by activation of mitochondrial caspase 9', *Experimental Hematology*, 31(9), pp. 779–783. doi: 10.1016/S0301-472X(03)00192-9.

Chen, J. (2016) 'The Cell-Cycle Arrest and Apoptotic Functions of p53 in Tumor Initiation and Progression', *Cold Spring Harbor Perspectives in Medicine*, 6(3), p. a026104. doi: 10.1101/cshperspect.a026104.

Chen, J. S. and Sambrook, P. N. (2011) 'Antiresorptive therapies for osteoporosis: a clinical overview', *Nature Reviews Endocrinology*, 8(2), pp. 81–91. doi: 10.1038/nrendo.2011.146.

Cheng, K. W. *et al.* (2004) 'The RAB25 small GTPase determines aggressiveness of ovarian and breast cancers.', *Nature medicine*, 10(11), pp. 1251–6. doi: 10.1038/nm1125.

Chiaffarino, F. *et al.* (2005) 'Hysterectomy with or without unilateral oophorectomy and risk of ovarian cancer.', *Gynecologic oncology*, 97(2), pp. 318–22. doi: 10.1016/j.ygyno.2005.01.030.

Chiaffarino, F. *et al.* (2007) 'Risk factors for ovarian cancer histotypes.', *European journal of cancer (Oxford, England : 1990)*, 43(7), pp. 1208–13. doi: 10.1016/j.ejca.2007.01.035.

Chlebowski, R. T. *et al.* (2010) 'Oral Bisphosphonate Use and Breast Cancer Incidence in Postmenopausal Women', *Journal of Clinical Oncology*, 28(22), pp. 3582–3590. doi: 10.1200/JCO.2010.28.2095.

Cho, K. R. and Shih, I.-M. (2009) 'Ovarian Cancer', *Annual Review of Pathology: Mechanisms of Disease*, 4(1), pp. 287–313. doi: 10.1146/annurev.pathol.4.110807.092246.

Chornokur, G. *et al.* (2013) 'Global ovarian cancer health disparities.', *Gynecologic oncology*. Elsevier Inc., 129(1), pp. 258–64. doi: 10.1016/j.ygyno.2012.12.016.

Chung, A. S., Lee, J. and Ferrara, N. (2010) 'Targeting the tumour vasculature: insights from physiological angiogenesis', *Nature Reviews Cancer*, 10(7), pp. 505–514. doi: 10.1038/nrc2868.

Chung, S. H. *et al.* (2013) 'Clinical efficacy of serum human epididymis protein 4 as a diagnostic biomarker of ovarian cancer: A pilot study.', *Obstetrics & gynecology science*. Korean Society of Obstetrics and Gynecology, 56(4), pp. 234–41. doi:

10.5468/ogs.2013.56.4.234.

Chushi, L. *et al.* (2016) 'HMGCR is up-regulated in gastric cancer and promotes the growth and migration of the cancer cells', *Gene*, 587(1), pp. 42–47. doi: 10.1016/j.gene.2016.04.029.

Cibula, D. *et al.* (2011) 'Tubal ligation and the risk of ovarian cancer: review and meta-analysis.', *Human reproduction update*, 17(1), pp. 55–67. doi: 10.1093/humupd/dmq030.

Clarke, A. R. *et al.* (1993) 'Thymocyte apoptosis induced by p53-dependent and independent pathways', *Nature*. Nature Publishing Group, 362(6423), pp. 849–852. doi: 10.1038/362849a0.

Clarke, P. R. and Zhang, C. (2008) 'Spatial and temporal coordination of mitosis by Ran GTPase.', *Nature reviews. Molecular cell biology*, 9(6), pp. 464–477. doi: 10.1038/nrm2410.

Clendening, J. W. *et al.* (2010) 'Dysregulation of the mevalonate pathway promotes transformation', *Proceedings of the National Academy of Sciences*, 107(34), pp. 15051–15056. doi: 10.1073/pnas.0910258107.

Clendening, J. W. and Penn, L. Z. (2012) 'Targeting tumor cell metabolism with statins.', *Oncogene*, 31(48), pp. 4967–78. doi: 10.1038/onc.2012.6.

Clézardin, P. (2011) 'Bisphosphonates' antitumor activity: an unravelled side of a multifaceted drug class.', *Bone*. Elsevier Inc., 48(1), pp. 71–9. doi: 10.1016/j.bone.2010.07.016.

Coburn, S. B. *et al.* (2017) 'International patterns and trends in ovarian cancer incidence, overall and by histologic subtype', *International Journal of Cancer*, 140(11), pp. 2451–2460. doi: 10.1002/ijc.30676.

Cole, A. J. *et al.* (2016) 'Assessing mutant p53 in primary high-grade serous ovarian cancer using immunohistochemistry and massively parallel sequencing', *Scientific Reports*. Nature Publishing Group, 6, p. 26191. doi: 10.1038/srep26191.

Coleman, M. L., Marshall, C. J. and Olson, M. F. (2004) 'RAS and RHO GTPases in G1-phase cell-cycle regulation.', *Nature reviews. Molecular cell biology*, 5(5), pp. 355–366. doi: 10.1038/nrm1365.

Coleman, R. E. (1992) 'Glucocorticoids in cancer therapy', *Biotherapy*, 4(1), pp. 37–44. doi: 10.1007/BF02171708.

Coleman, R. L. *et al.* (2015) 'A phase III randomized controlled clinical trial of carboplatin and paclitaxel alone or in combination with bevacizumab followed by bevacizumab and secondary cytoreductive surgery in platinum-sensitive, recurrent ovarian, peritoneal primary and fallopian?', *Gynecologic Oncology*. Elsevier, 137, pp. 3–4. doi: 10.1016/j.ygyno.2015.01.005.

Colicelli, J. (2004) 'Human RAS superfamily proteins and related GTPases.', *Science's STKE : signal transduction knowledge environment*, 2004(250), p. RE13. doi:

10.1126/stke.2502004re13.

Collavin, L., Lunardi, A. and Del Sal, G. (2010) 'P53-Family Proteins and Their Regulators: Hubs and Spokes in Tumor Suppression', *Cell Death Differ*, 17(6), pp. 901–911. doi: cdd201035 [pii]\r10.1038/cdd.2010.35.

Collins, R. *et al.* (2016) 'Interpretation of the evidence for the efficacy and safety of statin therapy', *The Lancet*, 388(10059), pp. 2532–2561. doi: 10.1016/S0140-6736(16)31357-5.

Colombo, N. *et al.* (2006) 'Ovarian cancer.', *Critical reviews in oncology/hematology*, 60(2), pp. 159–79. doi: 10.1016/j.critrevonc.2006.03.004.

Colombo, P.-E. *et al.* (2014) 'Sensitivity and resistance to treatment in the primary management of epithelial ovarian cancer.', *Critical reviews in oncology/hematology*. Elsevier Ireland Ltd, 89(2), pp. 207–16. doi: 10.1016/j.critrevonc.2013.08.017.

Corcos, L. and Le Jossic-Corcos, C. (2013) 'Statins: perspectives in cancer therapeutics.', *Digestive and liver disease : official journal of the Italian Society of Gastroenterology and the Italian Association for the Study of the Liver*. Editrice Gastroenterologica Italiana, 45(10), pp. 795–802. doi: 10.1016/j.dld.2013.02.002.

Corney, D. C. *et al.* (2008) 'Role of p53 and Rb in ovarian cancer.', *Advances in experimental medicine and biology*. NIH Public Access, 622, pp. 99–117. doi: 10.1007/978-0-387-68969-2_9.

Corsello, S. M. *et al.* (2017) 'The Drug Repurposing Hub: a next-generation drug library and information resource', *Nature Medicine*, 23(4), pp. 405–408. doi: 10.1038/nm.4306.

Coxon, F. P. *et al.* (2001) 'Identification of a novel phosphonocarboxylate inhibitor of Rab geranylgeranyl transferase that specifically prevents Rab prenylation in osteoclasts and macrophages.', *The Journal of biological chemistry*, 276(51), pp. 48213–22. doi: 10.1074/jbc.M106473200.

Cree, I. A. (ed.) (2011) 'Isolation and Culture of Ovarian Cancer Cells and Cell Lines - Springer', in. Humana Press (Methods in Molecular Biology).

Crown, J. and O'Leary, M. (2000) 'The taxanes: an update', *The Lancet*, 355(9210), pp. 1176–1178. doi: 10.1016/S0140-6736(00)02074-2.

Crul, M. *et al.* (2001) 'Ras biochemistry and farnesyl transferase inhibitors: a literature survey.', *Anti-cancer drugs*, 12(3), pp. 163–84. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11290863 (Accessed: 5 April 2017).

Cruz, I. N. *et al.* (2017) 'Proteomics analysis of ovarian cancer cell lines and tissues reveals drug resistance-associated proteins', *Cancer Genomics and Proteomics*, 14(1), pp. 35–52. doi: 10.21873/cgp.20017.

Cruz, P. M. R. *et al.* (2013) 'The role of cholesterol metabolism and cholesterol transport in carcinogenesis: a review of scientific findings, relevant to future cancer therapeutics', *Frontiers in Pharmacology*, 4, p. 119. doi: 10.3389/fphar.2013.00119.

Cuello F, M. *et al.* (2013) ‘Efectos de las estatinas en cáncer: ¿potencial rol en terapéutica y prevención?’, *Revista médica de Chile*, 141(2), pp. 227–236. doi: 10.4067/S0034-98872013000200013.

Cyster, J. G. *et al.* (2014) ‘25-Hydroxycholesterols in innate and adaptive immunity’, *Nature Reviews Immunology*, 14(11), pp. 731–743. doi: 10.1038/nri3755.

Dasso, M. (2002) ‘The Ran GTPase: Theme and Variations’, *Current Biology*, 12(14), pp. R502–R508. doi: 10.1016/S0960-9822(02)00970-3.

Davison, T. S. *et al.* (1999) ‘p73 and p63 Are Homotetramers Capable of Weak Heterotypic Interactions with Each Other but Not with p53*’, *The Journal of biological chemistry*, 274(26), pp. 18709–18714.

DeBose-Boyd, R. A. (2008) ‘Feedback regulation of cholesterol synthesis: sterol-accelerated ubiquitination and degradation of HMG CoA reductase.’, *Cell research*, 18(6), pp. 609–21. doi: 10.1038/cr.2008.61.

Decruze, S. B. and Kirwan, J. M. (2006) ‘Ovarian cancer’, *Current Obstetrics & Gynaecology*, 16(3), pp. 161–167. doi: 10.1016/j.curobgyn.2006.04.005.

Delarue, F. L. *et al.* (2007) ‘Farnesyltransferase and geranylgeranyltransferase I inhibitors upregulate RhoB expression by HDAC1 dissociation, HAT association and histone acetylation of the RhoB promoter.’, *Oncogene*, 26(5), pp. 633–640. doi: 10.1038/sj.onc.1209819.

Deloia, J. A. *et al.* (2012) ‘Comparison of ERCC1/XPF genetic variation, mRNA and protein levels in women with advanced stage ovarian cancer treated with intraperitoneal platinum.’, *Gynecologic oncology*. NIH Public Access, 126(3), pp. 448–54. doi: 10.1016/j.ygyno.2012.05.006.

Demierre, M.-F. *et al.* (2005) *Statins and cancer prevention.*, *Nature reviews Cancer*. doi: 10.1038/nrc1751.

Deng, C. *et al.* (1995) ‘Mice lacking p21CIP1/WAF1 undergo normal development, but are defective in G1 checkpoint control.’, *Cell*, 82(4), pp. 675–84. Available at: http://www.ncbi.nlm.nih.gov/pubmed/7664346 (Accessed: 18 January 2017).

Despierre, E. *et al.* (2010) ‘The molecular genetic basis of ovarian cancer and its roadmap towards a better treatment.’, *Gynecologic oncology*. Elsevier Inc., 117(2), pp. 358–65. doi: 10.1016/j.ygyno.2010.02.012.

Devita, V. T., Young, R. C. and Canellos, G. P. (1975) ‘Combination versus single agent chemotherapy: A review of the basis for selection of drug treatment of cancer’, *Cancer*. Wiley Subscription Services, Inc., A Wiley Company, 35(1), pp. 98–110. doi: 10.1002/1097-0142(197501)35:1<98::AID-CNCR2820350115>3.0.CO;2-B.

Dhar, M. K., Koul, A. and Kaul, S. (2013) ‘Farnesyl pyrophosphate synthase: a key enzyme in isoprenoid biosynthetic pathway and potential molecular target for drug development.’, *New biotechnology*. Elsevier B.V., 30(2), pp. 114–23. doi: 10.1016/j.nbt.2012.07.001.

Dimitroulakos, J. *et al.* (1999) 'Increased sensitivity of acute myeloid leukemias to lovastatin-induced apoptosis: A potential therapeutic approach.', *Blood*, 93(4), pp. 1308–1318.

Dittmer, D. *et al.* (1993) 'Gain of function mutations in p53', *Nature*. Available at: http://www.med.unc.edu/microimm/dittmerlab/files/publications/1990s/1993 Dittmerp53NatGen.pdf (Accessed: 12 January 2017).

Domcke, S. *et al.* (2013) 'Evaluating cell lines as tumour models by comparison of genomic profiles.', *Nature communications*. Nature Publishing Group, 4, p. 2126. doi: 10.1038/ncomms3126.

van de Donk, N. W. C. J. *et al.* (2003) 'Inhibition of protein geranylgeranylation induces apoptosis in myeloma plasma cells by reducing Mcl-1 protein levels.', *Blood*, 102(9), pp. 3354–62. doi: 10.1182/blood-2003-03-0970.

Downward, J. (2003) 'Targeting RAS signalling pathways in cancer therapy', *Nat Rev Cancer*, 3(1), pp. 11–22. doi: 10.1038/nrc969.

Dubeau, L. (2008) 'The cell of origin of ovarian epithelial tumours.', *The Lancet. Oncology*. Elsevier Ltd, 9(12), pp. 1191–7. doi: 10.1016/S1470-2045(08)70308-5.

Dudakovic, A. *et al.* (2008) 'Inhibition of geranylgeranyl diphosphate synthase induces apoptosis through multiple mechanisms and displays synergy with inhibition of other isoprenoid biosynthetic enzymes.', *The Journal of pharmacology and experimental therapeutics*, 324(3), pp. 1028–36. doi: 10.1124/jpet.107.132217.

Duncan, R. E., El-Sohemy, A. and Archer, M. C. (2004) 'Mevalonate promotes the growth of tumors derived from human cancer cells in vivo and stimulates proliferation in vitro with enhanced cyclin-dependent kinase-2 activity.', *The Journal of biological chemistry*, 279(32), pp. 33079–84. doi: 10.1074/jbc.M400732200.

Dunford, J. E. *et al.* (2006) 'Inhibition of Protein Prenylation by Bisphosphonates Causes Sustained Activation of Rac, Cdc42, and Rho GTPases', *Journal of Bone and Mineral Research*, 21(5), pp. 684–694. doi: 10.1359/jbmr.060118.

Dunton, C. J. *et al.* (2002) 'O ncologist in Advanced Ovarian Cancer', pp. 11–19. doi: 10.1634/theoncologist.7-suppl.

Edwards, C. M. *et al.* (2007) 'Apomine, an inhibitor of HMG-CoA-reductase, promotes apoptosis of myeloma cells in vitro and is associated with a modulation of myeloma in vivo.', *International journal of cancer. Journal international du cancer*, 120(8), pp. 1657–63. doi: 10.1002/ijc.22478.

El-Moselhy, M. a and El-Sheikh, A. a K. (2014) 'Protective mechanisms of atorvastatin against doxorubicin-induced hepato-renal toxicity.', *Biomedicine & pharmacotherapy = Biomédecine & pharmacothérapie*. Elsevier Masson SAS, 68(1), pp. 101–10. doi: 10.1016/j.biopha.2013.09.001.

Eldar, A. *et al.* (2013) 'Structural studies of p53 inactivation by DNA-contact mutations and its rescue by suppressor mutations via alternative protein-DNA interactions', *Nucleic*

Acids Research, 41(18), pp. 8748–8759. doi: 10.1093/nar/gkt630.

Ellis, L. M. and Hicklin, D. J. (2009) 'Resistance to Targeted Therapies: Refining Anticancer Therapy in the Era of Molecular Oncology', *Clinical Cancer Research*, 15(24), pp. 7471–7478. doi: 10.1158/1078-0432.CCR-09-1070.

Elmore, R. G. *et al.* (2008) 'Impact of statin therapy on survival in epithelial ovarian cancer', *Gynecologic Oncology*, 111(1), pp. 102–105. doi: 10.1016/j.ygyno.2008.06.007.

Elmore, S. (2007) 'Apoptosis: A Review of Programmed Cell Death', *Toxicologic Pathology*, 35(4), pp. 495–516. doi: 10.1080/01926230701320337.

Elsayed, M. *et al.* (2016) 'Synergistic Antiproliferative Effects of Zoledronic Acid and Fluvastatin on Human Pancreatic Cancer Cell Lines: An *in Vitro* Study', *Biological & Pharmaceutical Bulletin*, 39(8), pp. 1238–1246. doi: 10.1248/bpb.b15-00746.

Emmanuel, C. *et al.* (2014) 'Genomic classification of serous ovarian cancer with adjacent borderline differentiates Ras pathway and TP53-mutant tumors and identifies NRAS as an oncogenic driver', *Clinical Cancer Research*, 20(24), pp. 6618–6630. doi: 10.1158/1078-0432.CCR-14-1292.

Encinas, G. *et al.* (2015) 'Somatic mutations in breast and serous ovarian cancer young patients: a systematic review and meta-analysis', *Revista da Associa??o M?dica Brasileira*. Associação Médica Brasileira, 61(5), pp. 474–483. doi: 10.1590/1806-9282.61.05.474.

Endo, A. (2010) 'A historical perspective on the discovery of statins', *Proceedings of the Japan Academy. Series B, Physical and biological sciences*, 86(5), pp. 484–493. doi: 10.2183/pjab.86.484.

Eriksson, A. *et al.* (2015) 'Drug screen in patient cells suggests quinacrine to be repositioned for treatment of acute myeloid leukemia', *Blood Cancer Journal*, 5. doi: 10.1038/bcj.2015.31.

Farmer, H. *et al.* (2005) 'Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy', *Nature*, 434(7035), pp. 917–921. doi: 10.1038/nature03445.

Farnebo, M., Bykov, V. J. N. and Wiman, K. G. (2010) 'The p53 tumor suppressor: A master regulator of diverse cellular processes and therapeutic target in cancer', *Biochemical and Biophysical Research Communications*, 396(1), pp. 85–89. doi: 10.1016/j.bbrc.2010.02.152.

Fathalla, M. F. (1971) 'INCESSANT OVULATION—A FACTOR IN OVARIAN NEOPLASIA ?', *The Lancet*, 298(7716), p. 163. doi: 10.1016/S0140-6736(71)92335-X.

Fathalla, M. F. (2016) 'Non-hormonal interruption of incessant ovulation as a potential approach for ovarian cancer prevention', *International Journal of Gynecology and Obstetrics*, 132(3), pp. 356–358. doi: 10.1016/j.ijgo.2015.11.006.

Felder, M. *et al.* (2014) 'MUC16 (CA125): tumor biomarker to cancer therapy, a work in progress.', *Molecular cancer*. BioMed Central, 13, p. 129. doi: 10.1186/1476-4598-13-

129.

Ferris, J. S. *et al.* (2014) 'Oral contraceptive and reproductive risk factors for ovarian cancer within sisters in the breast cancer family registry.', *British journal of cancer*, 110(4), pp. 1074–80. doi: 10.1038/bjc.2013.803.

Finlay, C. A., Hinds, P. W. and Levine, A. J. (1989) 'The p53 proto-oncogene can act as a suppressor of transformation', *Cell*, 57(7), pp. 1083–1093. doi: 10.1016/0092-8674(89)90045-7.

Fiordalisi, J. J., Der, C. J. and Cox, A. D. (2006) 'Anti-Ras Strategies for Cancer Treatment', in *RAS Family GTPases*. Dordrecht: Springer Netherlands, pp. 353–380. doi: 10.1007/1-4020-4708-8_15.

Fleischer, A. C. *et al.* (2012) 'Early detection of ovarian cancer with conventional and contrast-enhanced transvaginal sonography: Recent advances and potential improvements', *Journal of Oncology*. doi: 10.1155/2012/302858.

Fleming, J. S. *et al.* (2006) 'Incessant ovulation, inflammation and epithelial ovarian carcinogenesis: revisiting old hypotheses.', *Molecular and cellular endocrinology*, 247(1–2), pp. 4–21. doi: 10.1016/j.mce.2005.09.014.

Fleuren, W. W. M. *et al.* (2013) 'Prednisolone induces the Wnt signalling pathway in 3T3-L1 adipocytes.', *Arch. Physiol. Biochem.*, 119(2), pp. 52–64. doi: 10.3109/13813455.2013.774022.

Fleury, H. *et al.* (2015) 'Novel high-grade serous epithelial ovarian cancer cell lines that reflect the molecular diversity of both the sporadic and hereditary disease.', *Genes & cancer*, 6(9–10), pp. 378–398. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4633166&tool=pmcentrez&rendertype=abstract.

Floquet, A. *et al.* (2014) 'Pegylated liposomal doxorubicin and cyclophosphamide in early recurrent ovarian carcinoma: phase I dose-finding study.', *Cancer chemotherapy and pharmacology*, 73(1), pp. 61–8. doi: 10.1007/s00280-013-2317-4.

Florea, A.-M. and Büsselberg, D. (2011) 'Cisplatin as an anti-tumor drug: cellular mechanisms of activity, drug resistance and induced side effects.', *Cancers*. Multidisciplinary Digital Publishing Institute (MDPI), 3(1), pp. 1351–71. doi: 10.3390/cancers3011351.

Flores, E. R. *et al.* (2005) 'Tumor predisposition in mice mutant for p63 and p73: Evidence for broader tumor suppressor functions for the p53 family', *Cancer Cell*, 7(4), pp. 363–373. doi: 10.1016/j.ccr.2005.02.019.

Flöter, J., Kaymak, I. and Schulze, A. (2017) 'Regulation of Metabolic Activity by p53', *Metabolites*, 7(2), p. 21. doi: 10.3390/metabo7020021.

Forget, M.-A. *et al.* (2002) 'Phosphorylation states of Cdc42 and RhoA regulate their interactions with Rho GDP dissociation inhibitor and their extraction from biological membranes.', *The Biochemical journal*, 361(Pt 2), pp. 243–54. Available at:

http://www.ncbi.nlm.nih.gov/pubmed/11772396 (Accessed: 13 July 2017).

Forman, H. J., Zhang, H. and Rinna, A. (2009) 'Glutathione: Overview of its protective roles, measurement, and biosynthesis', *Molecular Aspects of Medicine*, 30(1–2), pp. 1–12. doi: 10.1016/j.mam.2008.08.006.

Foucquier, J. and Guedj, M. (2015) 'Analysis of drug combinations: current methodological landscape', *Pharmacology Research & Perspectives*, 3(3), p. e00149. doi: 10.1002/prp2.149.

Fracasso, P. M. *et al.* (2001) 'Phase II study of paclitaxel and valspodar (PSC 833) in refractory ovarian carcinoma: A gynecologic oncology group study', *Journal of Clinical Oncology*, 19(12), pp. 2975–2982.

Freed-pastor, W. A. (2012) 'Gain-of-Function Effects of Mutant p53 Explored Using a Three- Dimensional Culture Model of Breast Cancer'.

Freed-Pastor, W. A. A. *et al.* (2012) 'Mutant p53 disrupts mammary tissue architecture via the mevalonate pathway', *Cell*. Elsevier Inc., 148(1–2), pp. 244–258. doi: 10.1016/j.cell.2011.12.017.

Freed-Pastor, W. and Prives, C. (2016) 'Targeting mutant p53 through the mevalonate pathway', *Nature Cell Biology*, 18(11), pp. 1122–1124. doi: 10.1038/ncb3435.

Frey, F. J. (1987) 'Kinetics and dynamics of prednisolone.', *Endocrine reviews*, 8(4), pp. 453–73. doi: 10.1210/edrv-8-4-453.

Fridman, J. S. and Lowe, S. W. (2003) 'Control of apoptosis by p53', *Oncogene*, 22, pp. 9030–9040. doi: 10.1038/sj.onc.1207116.

Friedrich, J. *et al.* (2009) 'Spheroid-based drug screen: considerations and practical approach', *Nat. Protocols*, 4, pp. 309–324. doi: 10.1038/nprot.2008.226.

Fruman, D. A. and Rommel, C. (2014) 'PI3K and cancer: lessons, challenges and opportunities.', *Nature reviews. Drug discovery*, 13(2), pp. 140–56. doi: 10.1038/nrd4204.

Fulda, S. (2009) 'Tumor resistance to apoptosis.', *International journal of cancer. Journal international du cancer*, 124(3), pp. 511–5. doi: 10.1002/ijc.24064.

Fumagalli, R. *et al.* (1964) 'OCCURRENCE AND SIGNIFICANCE OF STEROL PRECURSORS OF CHOLESTEROL IN HUMAN BRAIN TUMOURS', *Journal of Neurochemistry*. Blackwell Publishing Ltd, 11(8), pp. 561–565. doi: 10.1111/j.1471-4159.1964.tb11453.x.

Galli, M. C. *et al.* (1981) 'The occurrence of multiple steroid hormone receptors in disease-free and neoplastic human ovary.', *Cancer*, 47(6), pp. 1297–302. Available at: http://www.ncbi.nlm.nih.gov/pubmed/6261923 (Accessed: 20 July 2017).

Galluzzi, L. *et al.* (2012) 'Molecular mechanisms of cisplatin resistance.', *Oncogene*, 31(15), pp. 1869–83. doi: 10.1038/onc.2011.384.

Gao, J. *et al.* (2013) 'Integrative Analysis of Complex Cancer Genomics and Clinical

Profiles Using the cBioPortal', *Science Signaling*, 6(269), p. pl1-pl1. doi: 10.1126/scisignal.2004088.

Gao, J., Liao, J. and Yang, G. Y. (2009) 'CAAX-box protein, prenylation process and carcinogenesis', *American Journal of Translational Research*, 1(3), pp. 312–325.

Gatz, S. A. and Wiesmuller, L. (2006) 'p53 in recombination and repair', *Cell Death Differ*, 13(6), pp. 1003–1016. doi: 10.1038/sj.cdd.4401903.

Gayther, S. a and Pharoah, P. D. P. (2010) 'The inherited genetics of ovarian and endometrial cancer.', *Current opinion in genetics & development*. Elsevier Ltd, 20(3), pp. 231–8. doi: 10.1016/j.gde.2010.03.001.

Gazzerro, P. *et al.* (2012) 'Pharmacological actions of statins: a critical appraisal in the management of cancer.', *Pharmacological reviews*, 64(1), pp. 102–46. doi: 10.1124/pr.111.004994.

Gee, R. H. *et al.* (2015) 'Inhibition of prenyltransferase activity by statins in both liver and muscle cell lines is not causative of cytotoxicity', *Toxicology*. Elsevier Ireland Ltd, 329, pp. 40–48. doi: 10.1016/j.tox.2015.01.005.

George, S. H. L., Garcia, R. and Slomovitz, B. M. (2016) 'Ovarian Cancer: The Fallopian Tube as the Site of Origin and Opportunities for Prevention.', *Frontiers in oncology*. Frontiers Media SA, 6, p. 108. doi: 10.3389/fonc.2016.00108.

Ghavami, S. *et al.* (2017) 'Editorial (Thematic Issue: New Insights into a Classical Pathway: Key Roles of the Mevalonate Cascade in Different Diseases (Part I))', *Current Molecular Pharmacology*. Bentham Science Publishers, 10(1), pp. 3–5. doi: 10.2174/1874467209999160114145952.

Ghofrani, H. A., Osterloh, I. H. and Grimminger, F. (2006) 'Sildenafil: from angina to erectile dysfunction to pulmonary hypertension and beyond.', *Nature reviews. Drug discovery*, 5(8), pp. 689–702. doi: 10.1038/nrd2030.

Giacinti, C. and Giordano, A. (2006) 'RB and cell cycle progression', *Oncogene*, 25, pp. 5220–5227. doi: 10.1038/sj.onc.1209615.

Giudetti, A. M. and Gnoni, G. V (1998) 'Short-term effect of dexamethasone on fatty acid and cholesterol synthesis in isolated rat hepatocytes.', *Biochemistry and molecular biology international*. Informa Healthcare, 44(3), pp. 515–521. doi: 10.1080/15216549800201542.

Glaysher, S. *et al.* (2013) 'Targeting EGFR and PI3K pathways in ovarian cancer', *British Journal of Cancer*. Nature Publishing Group, 109(7), pp. 1786–1794. doi: 10.1038/bjc.2013.529.

Glynn, S. a *et al.* (2008) 'The 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors, simvastatin, lovastatin and mevastatin inhibit proliferation and invasion of melanoma cells.', *BMC cancer*, 8, p. 9. doi: 10.1186/1471-2407-8-9.

Gnant, M. and Clézardin, P. (2012) 'Direct and indirect anticancer activity of bisphosphonates: a brief review of published literature.', *Cancer treatment reviews*.

Elsevier Ltd, 38(5), pp. 407–15. doi: 10.1016/j.ctrv.2011.09.003.

Gniadecki, R. (2004) 'Depletion of membrane cholesterol causes ligand-independent activation of Fas and apoptosis', *Biochemical and Biophysical Research Communications*, 320(1), pp. 165–169. doi: 10.1016/j.bbrc.2004.05.145.

Goard, C. a. *et al.* (2013) 'Identifying molecular features that distinguish fluvastatin-sensitive breast tumor cells', *Breast Cancer Research and Treatment*, 143(2), pp. 301–312. doi: 10.1007/s10549-013-2800-y.

Göbel, A. *et al.* (2016) 'Combined inhibition of the mevalonate pathway with statins and zoledronic acid potentiates their anti-tumor effects in human breast cancer cells', *Cancer Letters*, 375(1), pp. 162–171. doi: 10.1016/j.canlet.2016.03.004.

Goc, A. *et al.* (2012) 'Simultaneous modulation of the intrinsic and extrinsic pathways by simvastatin in mediating prostate cancer cell apoptosis', *BMC Cancer*, 12(1), p. 409. doi: 10.1186/1471-2407-12-409.

Goff, B. a *et al.* (2004) 'Frequency of symptoms of ovarian cancer in women presenting to primary care clinics.', *JAMA : the journal of the American Medical Association*, 291(22), pp. 2705–12. doi: 10.1001/jama.291.22.2705.

Goff, B. a *et al.* (2007) 'Development of an ovarian cancer symptom index: possibilities for earlier detection.', *Cancer*, 109(2), pp. 221–7. doi: 10.1002/cncr.22371.

Goff, B. a (2012) 'Ovarian cancer: screening and early detection.', *Obstetrics and gynecology clinics of North America*. Elsevier, 39(2), pp. 183–94. doi: 10.1016/j.ogc.2012.02.007.

Goh, A. M., Coffill, C. R. and Lane, D. P. (2011) 'The role of mutant p53 in human cancer', *Journal of Pathology*, pp. 116–126. doi: 10.1002/path.2784.

Goldfinger, L. E. and Michael, J. V. (2017) 'Regulation of Ras signaling and function by plasma membrane microdomains', *BioScience Trends*, 11(1), pp. 23–40. doi: 10.5582/bst.2016.01220.

Goldoni, M. and Johansson, C. (2007) 'A mathematical approach to study combined effects of toxicants in vitro: Evaluation of the Bliss independence criterion and the Loewe additivity model', *Toxicology in Vitro*, 21(5), pp. 759–769. doi: 10.1016/j.tiv.2007.03.003.

Goldstein, I. *et al.* (2010) 'Understanding wild-type and mutant p53 activities in human cancer: new landmarks on the way to targeted therapies', *Cancer Gene Therapy*, 18(10), pp. 2–11. doi: 10.1038/cgt.2010.63.

Goldstein, J. L. and Brown, M. S. (1990) 'Regulation of the mevalonate pathway.', *Nature*, 343, pp. 425–430. doi: 10.1038/343425a0.

Goldstein, J. L., DeBose-Boyd, R. A. and Brown, M. S. (2006) *Protein sensors for membrane sterols*, *Cell*. doi: 10.1016/j.cell.2005.12.022.

Goncharova, N. D. (2013) 'Stress responsiveness of the hypothalamic-pituitary-adrenal

axis: age-related features of the vasopressinergic regulation.', *Frontiers in endocrinology*. Frontiers Media SA, 4, p. 26. doi: 10.3389/fendo.2013.00026.

Gottesman, M. M. and Ambudkar, S. V (2001) 'Overview: ABC transporters and human disease.', *Journal of bioenergetics and biomembranes*, 33(6), pp. 453–8. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11804186.

Graf, H. *et al.* (2008) 'Chemoembolization combined with pravastatin improves survival in patients with hepatocellular carcinoma.', *Digestion*, 78(1), pp. 34–8. doi: 10.1159/000156702.

Greenberg, A. K. *et al.* (2002) 'Glucocorticoids Inhibit Lung Cancer Cell Growth through Both the Extracellular Signal-Related Kinase Pathway and Cell Cycle Regulators', *American Journal of Respiratory Cell and Molecular Biology*. American Thoracic Society, 27(3), pp. 320–328. doi: 10.1165/rcmb.4710.

Guppy, a. E., Nathan, P. D. and Rustin, G. J. S. (2005) 'Epithelial Ovarian Cancer: A Review of Current Management', *Clinical Oncology*. Elsevier, 17(6), pp. 399–411. doi: 10.1016/j.clon.2005.05.009.

Gupta, D. and Lis, C. G. (2009) 'Role of CA125 in predicting ovarian cancer survival - a review of the epidemiological literature.', *Journal of ovarian research*. BioMed Central, 2, p. 13. doi: 10.1186/1757-2215-2-13.

Gustbée, E. *et al.* (2015) 'Tumor-specific expression of HMG-CoA reductase in a population-based cohort of breast cancer patients.', *BMC clinical pathology*. ???, 15, p. 8. doi: 10.1186/s12907-015-0008-2.

Gysin, S. *et al.* (2011) 'Therapeutic Strategies for Targeting Ras Proteins', *Genes & Cancer*, 2(3), pp. 359–372. doi: 10.1177/1947601911412376.

Haas, D. and Hoffmann, G. F. (2006) 'Mevalonate kinase deficiencies: from mevalonic aciduria to hyperimmunoglobulinemia D syndrome.', *Orphanet journal of rare diseases*, 1, p. 13. doi: 10.1186/1750-1172-1-13.

Habis, M. *et al.* (2014) 'Statin therapy is associated with improved survival in patients with non-serous-papillary epithelial ovarian cancer: a retrospective cohort analysis.', *PloS one*, 9(8), p. e104521. doi: 10.1371/journal.pone.0104521.

Hafeman, S. D., Varland, D. and Dow, S. W. (2012) 'Bisphosphonates significantly increase the activity of doxorubicin or vincristine against canine malignant histiocytosis cells', *Veterinary and Comparative Oncology*, 10(1), pp. 44–56. doi: 10.1111/j.1476-5829.2011.00274.x.

Hamilton, T. C. *et al.* (1983) 'Characterization of a Human Ovarian Carcinoma Cell Line (NIH:OVCAR-3) with Androgen and Estrogen Receptors', *Cancer Research*, 43(11), pp. 5379–5389.

Hamilton, T. C., Young, R. C. and Ozols, R. F. (1984) 'Experimental model systems of ovarian cancer: Applications to the design and evaluation of new treatment approaches', *Seminars in Oncology*, 11(3), pp. 285–298. doi: 10.5555/uri:pii:0093775484901337.

Han, K. *et al.* (2017) 'Synergistic drug combinations for cancer identified in a CRISPR screen for pairwise genetic interactions', *Nature Biotechnology*. doi: 10.1038/nbt.3834.

Han, K. H. *et al.* (2013) 'Protective Effect of Progesterone during Pregnancy against Ovarian Cancer', *Journal of Cancer Prevention*, 18(2), pp. 113–122. doi: 10.15430/JCP.2013.18.2.113.

Hanahan, D. and Weinberg, R. A. a (2011) 'Hallmarks of cancer: the next generation.', *Cell*. Elsevier Inc., 144(5), pp. 646–74. doi: 10.1016/j.cell.2011.02.013.

Hanel, W. *et al.* (2013) 'Two hot spot mutant p53 mouse models display differential gain of function in tumorigenesis', *Cell Death and Differentiation*, 20. doi: 10.1038/cdd.2013.17.

Harries, M. and Gore, M. (2002) 'Reviews Part I : Chemotherapy for epithelial ovarian cancer — treatment at first diagnosis', 3(September), pp. 529–536.

Harris, S. L. and Levine, A. J. (2005) 'The p53 pathway: positive and negative feedback loops', *Oncogene*, 24, pp. 2899–2908. doi: 10.1038/sj.onc.1208615.

Hart, T. *et al.* (2015) 'High-Resolution CRISPR Screens Reveal Fitness Genes and Genotype-Specific Cancer Liabilities', *Cell*, 163(6), pp. 1515–1526. doi: 10.1016/j.cell.2015.11.015.

Hashimoto, K. *et al.* (2007) 'Alendronate suppresses tumor angiogenesis by inhibiting Rho activation of endothelial cells.', *Biochemical and biophysical research communications*, 354(2), pp. 478–84. doi: 10.1016/j.bbrc.2007.01.014.

He, L. *et al.* (2016) 'Methods for High-Throughput Drug Combination Screening and Synergy Scoring', *bioRxiv*.

Heasman, S. J. and Ridley, A. J. (2008) 'Mammalian Rho GTPases: new insights into their functions from in vivo studies.', *Nature reviews. Molecular cell biology*, 9(9), pp. 690–701. doi: 10.1038/nrm2476.

Heintz, a P., Hacker, N. F. and Lagasse, L. D. (1985) 'Epidemiology and etiology of ovarian cancer: a review.', *Obstetrics and gynecology*, 66, pp. 127–135.

Helleman, J. *et al.* (2006) 'Mismatch repair and treatment resistance in ovarian cancer.', *BMC cancer*, 6, p. 201. doi: 10.1186/1471-2407-6-201.

Henneman, L. *et al.* (2011) 'Inhibition of the isoprenoid biosynthesis pathway; detection of intermediates by UPLC-MS/MS.', *Biochimica et biophysica acta*. Elsevier B.V., 1811(4), pp. 227–33. doi: 10.1016/j.bbalip.2011.01.002.

Hennessy, B. T., Coleman, R. L. and Markman, M. (2009) 'Ovarian cancer.', *Lancet*. Elsevier Ltd, 374(9698), pp. 1371–82. doi: 10.1016/S0140-6736(09)61338-6.

Henry, L. K. *et al.* (2015) 'Orthologs of the archaeal isopentenyl phosphate kinase regulate terpenoid production in plants.', *Proceedings of the National Academy of Sciences of the United States of America*. National Academy of Sciences, 112(32), pp. 10050–5. doi:

10.1073/pnas.1504798112.

Herman, J. P. *et al.* (2016) 'Regulation of the Hypothalamic-Pituitary-Adrenocortical Stress Response.', *Comprehensive Physiology*. NIH Public Access, 6(2), pp. 603–21. doi: 10.1002/cphy.c150015.

Herr, I. *et al.* (2003) 'Glucocorticoid Cotreatment Induces Apoptosis Resistance toward Cancer Therapy in Carcinomas', *Cancer Research*, 63(12).

Heublein and Sabine (2016) 'Some aspects of mutant p53 in ovarian cancer biology', *Translational Cancer Research*, 3(5), pp. S546–S547. doi: 10.21037/9625.

Hientz, K. *et al.* (2016) 'The role of p53 in cancer drug resistance and targeted chemotherapy'.

Hilary Russell, S. E. (2001) 'The Molecular Pathogenesis of Ovarian Cancer', in Bartlett, J. M. S. (ed.) *Ovarian Cancer: Methods and Protocols*. Totowa, NJ: Humana Press, pp. 25–36. doi: 10.1385/1-59259-071-3:25.

Hindler, K. *et al.* (2006) 'The role of statins in cancer therapy.', *The oncologist*, 11(3), pp. 306–15. doi: 10.1634/theoncologist.11-3-306.

Hodge, R. G. and Ridley, A. J. (2016) 'Regulating Rho GTPases and their regulators.', *Nature reviews. Molecular cell biology*, 17(8), pp. 496–510. doi: 10.1038/nrm.2016.67.

Holohan, C. *et al.* (2013) 'Cancer drug resistance: an evolving paradigm', *Nature Reviews Cancer*. Nature Research, 13(10), pp. 714–726. doi: 10.1038/nrc3599.

Holstein, S. A. and Hohl, R. J. (2012) 'Is there a future for prenyltransferase inhibitors in cancer therapy?', *Current opinion in pharmacology*. Elsevier Ltd, 12(6), pp. 704–9. doi: 10.1016/j.coph.2012.06.013.

Holzer, A. K., Manorek, G. H. and Howell, S. B. (2006) 'Contribution of the major copper influx transporter CTR1 to the cellular accumulation of cisplatin, carboplatin, and oxaliplatin.', *Molecular pharmacology*, 70(4), pp. 1390–1394. doi: 10.1124/mol.106.022624.

Hooff, G. P. *et al.* (2010) 'Isoprenoids, small GTPases and Alzheimer's disease.', *Biochimica et biophysica acta*. Elsevier B.V., 1801(8), pp. 896–905. doi: 10.1016/j.bbalip.2010.03.014.

Hoque, A., Chen, H. and Xu, X.-C. (2008) 'Statin induces apoptosis and cell growth arrest in prostate cancer cells.', *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology*, 17(1), pp. 88–94. doi: 10.1158/1055-9965.EPI-07-0531.

Horie, N. *et al.* (2007) 'Combined effects of a third-generation bisphosphonate, zoledronic acid with other anticancer agents against murine osteosarcoma.', *British journal of cancer*, 96(2), pp. 255–61. doi: 10.1038/sj.bjc.6603548.

Horton, J. D., Goldstein, J. L. and Brown, M. S. (2002) 'SREBPs: Activators of the

complete program of cholesterol and fatty acid synthesis in the liver', *Journal of Clinical Investigation*, pp. 1125–1131. doi: 10.1172/JCI200215593.

Housman, G. *et al.* (2014) 'Drug resistance in cancer: an overview.', *Cancers*. Multidisciplinary Digital Publishing Institute (MDPI), 6(3), pp. 1769–92. doi: 10.3390/cancers6031769.

Huang, B. Z. *et al.* (2017) 'Influence of Statins and Cholesterol on Mortality Among Patients With Pancreatic Cancer', *Journal of the National Cancer Institute*. Oxford University Press, 109(5), p. djw275. doi: 10.1093/jnci/djw275.

Huang, M. *et al.* (2014) 'Molecularly targeted cancer therapy: Some lessons from the past decade', *Trends in Pharmacological Sciences*, pp. 41–50. doi: 10.1016/j.tips.2013.11.004.

Huang, Y. *et al.* (2000) 'Nuclear factor-kappaB/IkappaB signaling pathway may contribute to the mediation of paclitaxel-induced apoptosis in solid tumor cells.', *Cancer research*, 60(16), pp. 4426–32. Available at: http://www.ncbi.nlm.nih.gov/pubmed/10969788 (Accessed: 26 September 2016).

Hulleman, E. *et al.* (2009) 'Inhibition of glycolysis modulates prednisolone resistance in acute lymphoblastic leukemia cells', *Blood*, 113(9), pp. 2014–2021. doi: 10.1182/blood-2008-05-157842.

Hung, C.-L. *et al.* (2013) 'Beyond malignancy: the role of carbohydrate antigen 125 in heart failure.', *Biomarker research*, 1(1), p. 25. doi: 10.1186/2050-7771-1-25.

Hung, M.-S. *et al.* (2017) 'Statin improves survival in patients with EGFR-TKI lung cancer: A nationwide population-based study', *PLOS ONE*. Edited by J. Souglakos. Public Library of Science, 12(2), p. e0171137. doi: 10.1371/journal.pone.0171137.

Iacopetta, D. *et al.* (2017) 'Old Drug Scaffold, New Activity: Thalidomide-Correlated Compounds Exert Different Effects on Breast Cancer Cell Growth and Progression', *ChemMedChem*, 12(5), pp. 381–389. doi: 10.1002/cmdc.201600629.

Ichim, G. and Tait, S. W. G. (2016) 'A fate worse than death: apoptosis as an oncogenic process', *Nature Reviews Cancer*, 16(8), pp. 539–548. doi: 10.1038/nrc.2016.58.

Iden, S. and Collard, J. G. (2008) 'Crosstalk between small GTPases and polarity proteins in cell polarization.', *Nature reviews. Molecular cell biology*, 9(11), pp. 846–859. doi: 10.1038/nrm2521.

Inoue, K. and Fry, E. A. (2014) 'Alterations of p63 and p73 in Human Cancers', in. Springer Netherlands, pp. 17–40. doi: 10.1007/978-94-017-9211-0_2.

Irwin, M. S. *et al.* (2003) 'Chemosensitivity linked to p73 function', *cancer cell*, 3, pp. 403–410.

Ishiguro, H. *et al.* (2014) 'Differential regulation of bladder cancer growth by various glucocorticoids: Corticosterone and prednisone inhibit cell invasion without promoting cell proliferation or reducing cisplatin cytotoxicity', *Cancer Chemotherapy and Pharmacology*, 74(2), pp. 249–255. doi: 10.1007/s00280-014-2496-7.

Issat, T. *et al.* (2007) 'Potentiated antitumor effects of the combination treatment with statins and pamidronate in vitro and in vivo.', *International journal of oncology*, 30(6), pp. 1413–25. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17487362.

Jacobs, I. *et al.* (1990) 'A risk of malignancy index incorporating CA 125, ultrasound and menopausal status for the accurate preoperative diagnosis of ovarian cancer.', *British Journal of Obstetrics and Gynaecology*, 97, pp. 922–929. doi: 10.1016/0378-5122(91)90134-C.

Jaffe, A. B. and Hall, A. (2005) 'Rho GTPases: biochemistry and biology.', *Annual review of cell and developmental biology*, 21, pp. 247–69. doi: 10.1146/annurev.cellbio.21.020604.150721.

Jagdev, S. P. *et al.* (2001) 'The bisphosphonate, zoledronic acid, induces apoptosis of breast cancer cells: evidence for synergy with paclitaxel.', *British journal of cancer*, 84(8), pp. 1126–34. doi: 10.1054/bjoc.2001.1727.

Jansen, B. A. J., Brouwer, J. and Reedijk, J. (2002) 'Glutathione induces cellular resistance against cationic dinuclear platinum anticancer drugs', *Journal of Inorganic Biochemistry*, 89(3–4), pp. 197–202. doi: 10.1016/S0162-0134(02)00381-1.

Jenkins, C. M. *et al.* (2011) 'Reversible High Affinity Inhibition of Phosphofructokinase-1 by Acyl-CoA', *Journal of Biological Chemistry*, 286(14), pp. 11937–11950. doi: 10.1074/jbc.M110.203661.

Jespersen, C. G. *et al.* (2014) 'Statin use and risk of prostate cancer: a Danish population-based case-control study, 1997-2010.', *Cancer epidemiology*. Elsevier Ltd, 38(1), pp. 42–7. doi: 10.1016/j.canep.2013.10.010.

Jessmon, P. *et al.* (2017) 'Epidemiology and treatment patterns of epithelial ovarian cancer', *Expert Review of Anticancer Therapy*. Taylor & Francis, p. 14737140.2017.1299575. doi: 10.1080/14737140.2017.1299575.

Jiang, P. *et al.* (2014) 'In vitro and in vivo anticancer effects of mevalonate pathway modulation on human cancer cells', *British Journal of Cancer*. Nature Publishing Group, 111(8), pp. 1562–1571. doi: 10.1038/bjc.2014.431.

Jiang, P. *et al.* (2014) 'Novel anti-glioblastoma agents and therapeutic combinations identified from a collection of FDA approved drugs', *Journal of Translational Medicine*, 12(1), p. 13. doi: 10.1186/1479-5876-12-13.

Joerger, A. C. *et al.* (2009) 'Structural evolution of p53, p63, and p73: implication for heterotetramer formation.', *Proceedings of the National Academy of Sciences of the United States of America*, 106(42), pp. 17705–10. doi: 10.1073/pnas.0905867106.

Joerger, A. and Fersht, A. (2007) 'Structure–function–rescue: the diverse nature of common p53 cancer mutants', *Oncogene*, 26, pp. 2226–2242. doi: 10.1038/sj.onc.1210291.

Jones, L. H. and Bunnage, M. E. (2017) 'Applications of chemogenomic library screening in drug discovery', *Nature Reviews Drug Discovery*, 16(4), pp. 285–296. doi: 10.1038/nrd.2016.244.

Jones, P. M. and Drapkin, R. (2013) 'Modeling High-Grade Serous Carcinoma: How Converging Insights into Pathogenesis and Genetics are Driving Better Experimental Platforms', *Frontiers in Oncology*. Frontiers, 3, p. 217. doi: 10.3389/fonc.2013.00217.

Jordan, S. J. *et al.* (2006) 'Does smoking increase risk of ovarian cancer? A systematic review.', *Gynecologic oncology*, 103(3), pp. 1122–9. doi: 10.1016/j.ygyno.2006.08.012.

Jordan, S. J. *et al.* (2010) 'Breastfeeding and risk of epithelial ovarian cancer.', *Cancer causes & control : CCC*, 21(1), pp. 109–16. doi: 10.1007/s10552-009-9440-x.

Jordan, S. J. *et al.* (2012) 'Breast-feeding and risk of epithelial ovarian cancer', *Cancer Causes and Control*, 23(6), pp. 919–927. doi: 10.1007/s10552-012-9963-4.

Jukema, J. W. *et al.* (2012) 'The controversies of statin therapy: Weighing the evidence', *Journal of the American College of Cardiology*, pp. 875–881. doi: 10.1016/j.jacc.2012.07.007.

Kadmiel, M. and Cidlowski, J. A. (2013) 'Glucocorticoid receptor signaling in health and disease', *Trends in Pharmacological Sciences*, pp. 518–530. doi: 10.1016/j.tips.2013.07.003.

Kahn, R. A. (2003) *ARF family GTPases*. Kluwer Academic Publishers. Available at: https://books.google.co.uk/books?hl=en&lr=&id=bOTlBwAAQBAJ&oi=fnd&pg=PR7&dq=The+Arf+Family&ots=3J99P75W6_&sig=iVxkV4d3gDb2LfSH2XpBbyc14hU#v=onepage&q=The Arf Family&f=false (Accessed: 3 April 2017).

Kajiyama, Y. *et al.* (2010) 'Prednisolone causes anxiety- and depression-like behaviors and altered expression of apoptotic genes in mice hippocampus', *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, 34(1), pp. 159–165. doi: 10.1016/j.pnpbp.2009.10.018.

Kaku, T. *et al.* (2003) 'Histological classification of ovarian cancer.', *Medical electron microscopy : official journal of the Clinical Electron Microscopy Society of Japan*, 36(1), pp. 9–17. doi: 10.1007/s007950300002.

Kamai, T. *et al.* (2004) 'Overexpression of RhoA, Rac1, and Cdc42 GTPases Is Associated with Progression in Testicular Cancer', *Clinical Cancer Research*, 10(14). Available at: http://clincancerres.aacrjournals.org/content/10/14/4799.short (Accessed: 3 April 2017).

Kamisuki, S. *et al.* (2009) 'A small molecule that blocks fat synthesis by inhibiting the activation of SREBP.', *Chemistry & biology*. Elsevier Ltd, 16(8), pp. 882–92. doi: 10.1016/j.chembiol.2009.07.007.

Kandoth, C. *et al.* (2013) 'Mutational landscape and significance across 12 major cancer types', *Nature*, 503(7471), pp. 333–339. doi: 10.1007/s13398-014-0173-7.2.

Kang, H. J. *et al.* (2013) 'Clinical Relevance of Gain-Of-Function Mutations of p53 in High-Grade Serous Ovarian Carcinoma', *PLoS ONE*. Edited by E. Asselin. Public Library of Science, 8(8), p. e72609. doi: 10.1371/journal.pone.0072609.

Karlsson, R. *et al.* (2009) *Rho GTPase function in tumorigenesis*, *Biochimica et Biophysica*

Acta - Reviews on Cancer. doi: 10.1016/j.bbcan.2009.03.003.

Karnezis, A. N. *et al.* (2017) 'The disparate origins of ovarian cancers: pathogenesis and prevention strategies', *Nature Reviews Cancer*. Nature Research, 17(1), pp. 65–74. doi: 10.1038/nrc.2016.113.

Karni-Schmidt, O., Lokshin, M. and Prives, C. (2016) 'The Roles of MDM2 and MDMX in Cancer', *Annual Review of Pathology: Mechanisms of Disease*, 11(1), pp. 617–644. doi: 10.1146/annurev-pathol-012414-040349.

Karnoub, A. E. and Weinberg, R. A. (2008) 'Ras oncogenes: split personalities.', *Nature reviews. Molecular cell biology*, 9(7), pp. 517–531. doi: 10.1038/nrm2438.

Kastan, M. B. and Bartek, J. (2004) 'Cell-cycle checkpoints and cancer', *Nature*, 432(7015), pp. 316–323. doi: 10.1038/nature03097.

Katz, M. S. (2005) 'Therapy Insight: potential of statins for cancer chemoprevention and therapy', *Nature Clinical Practice Oncology*, 2(2), pp. 82–89. doi: 10.1038/ncponc0097.

Katzung, B. G., Masters, S. B., & Trevor, A. J. (2012) 'Adrenocorticosteroids & Adrenocortical Antagonists', in *Basic & clinical pharmacology*, pp. 697–713.

Katzung, B. G., Masters, S. B. and Trevor, A. J. (2012) *Basic & clinical pharmacology, A Lange medical book.*

Kau, T. R., Way, J. C. and Silver, P. A. (2004) 'Nuclear transport and cancer: from mechanism to intervention', *Nature Reviews Cancer*, 4(2), pp. 106–117. doi: 10.1038/nrc1274.

Kawata, S. *et al.* (2001) 'Effect of pravastatin on survival in patients with advanced hepatocellular carcinoma. A randomized controlled trial.', *British journal of cancer*, 84(7), pp. 886–91. doi: 10.1054/bjoc.2000.1716.

Keith, C. T., Borisy, A. A. and Stockwell, B. R. (2005) 'Innovation: Multicomponent therapeutics for networked systems', *Nature Reviews Drug Discovery*, 4(1), pp. 71–78. doi: 10.1038/nrd1609.

Kellenberger, L. D. *et al.* (2010) 'The role of dysregulated glucose metabolism in epithelial ovarian cancer.', *Journal of oncology*. Hindawi, 2010, p. 514310. doi: 10.1155/2010/514310.

Kerr, J. F. R. (2002) 'History of the events leading to the formulation of the apoptosis concept', *Toxicology*, pp. 471–474. doi: 10.1016/S0300-483X(02)00457-2.

Khanim, F. L. *et al.* (2011) 'Redeployment-based drug screening identifies the anti-helminthic niclosamide as anti-myeloma therapy that also reduces free light chain production.', *Blood cancer journal*. Nature Publishing Group, 1(10), p. e39. doi: 10.1038/bcj.2011.38.

Kidera, Y. *et al.* (2010) 'Reduction of lung metastasis, cell invasion, and adhesion in mouse melanoma by statin-induced blockade of the Rho/Rho-associated coiled-coil-

containing protein kinase pathway.', *Journal of experimental & clinical cancer research : CR*. BioMed Central, 29(1), p. 127. doi: 10.1186/1756-9966-29-127.

Kigawa, J. (2013) 'New strategy for overcoming resistance to chemotherapy of ovarian cancer.', *Yonago acta medica*, 56(2), pp. 43–50. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3771207&tool=pmcentrez&rendertype=abstract.

Kilari, D., Guancial, E. and Kim, E. S. (2016) 'Role of copper transporters in platinum resistance.', *World journal of clinical oncology*. Baishideng Publishing Group Inc, 7(1), pp. 106–13. doi: 10.5306/wjco.v7.i1.106.

Klengel, R. *et al.* (1997) 'Differential Expression of Rab3 Isoforms during Differentiation of Pancreatic Acinar Cell Line AR42J', *Biochemical and Biophysical Research Communications*, 236(3), pp. 719–722. doi: 10.1006/bbrc.1997.7039.

Kobayashi, H. *et al.* (1993) 'Acquired multicellular-mediated resistance to alkylating agents in cancer.', *Proceedings of the National Academy of Sciences*, 90(8), pp. 3294–3298. doi: 10.1073/pnas.90.8.3294.

Kobayashi, Y. *et al.* (2015) 'Mevalonate Pathway Antagonist Suppresses Formation of Serous Tubal Intraepithelial Carcinoma and Ovarian Carcinoma in Mouse Models.', *Clinical cancer research : an official journal of the American Association for Cancer Research*. NIH Public Access, 21(20), pp. 4652–62. doi: 10.1158/1078-0432.CCR-14-3368.

Kofman, S. *et al.* (1958) 'The use of prednisolone in the treatment of disseminated breast carcinoma', *Cancer*, 11(1), pp. 226–232. doi: 10.1002/1097-0142(195801/02)11:1<226::AID-CNCR2820110135>3.0.CO;2-S.

Koike, H. *et al.* (2011) '237 HIGH-FAT DIET INCREASED RENAL CANCER GROWTH, AND THE ANTITUMOR SENSITIZATION EFFECT DUE TO SURVIVIN INHIBITION IN SIMVASTATIN TREATMENT FOR RENAL CANCER CELLS', *The Journal of Urology*, 185(4), p. e96. doi: 10.1016/j.juro.2011.02.306.

Koike, H. *et al.* (2012) '198 High-fat diet increased Leptin, and the antitumor sensitization effect due to survivin inhibition in simvastatin treatment for renal cancer', *European Urology Supplements*, 11(1), p. e198–e198a. doi: 10.1016/S1569-9056(12)60195-X.

Kojima, K. *et al.* (2006) 'Concomitant inhibition of MDM2 and Bcl-2 protein function synergistically induce mitochondrial apoptosis in AML', *Cell cycle*. Available at: http://www.tandfonline.com/doi/abs/10.4161/cc.5.23.3520 (Accessed: 23 January 2017).

Konstantinopoulos, P. A. and Matulonis, U. A. (2013) 'Current Status and Evolution of Preclinical Drug Development Models of Epithelial Ovarian Cancer', *Frontiers in Oncology*. Frontiers, 3, p. 296. doi: 10.3389/fonc.2013.00296.

Konstantinopoulos, P. a, Karamouzis, M. V and Papavassiliou, A. G. (2007) 'Post-translational modifications and regulation of the RAS superfamily of GTPases as anticancer targets.', *Nature reviews. Drug discovery*, 6(7), pp. 541–55. doi: 10.1038/nrd2221.

Koyuturk, M. *et al.* (2007) ‘Simvastatin induces apoptosis in human breast cancer cells: p53 and estrogen receptor independent pathway requiring signalling through JNK.’, *Cancer letters*. Elsevier, 250(2), pp. 220–8. doi: 10.1016/j.canlet.2006.10.009.

Kroeger, P. T. and Drapkin, R. (2017) ‘Pathogenesis and heterogeneity of ovarian cancer’, *Current Opinion in Obstetrics and Gynecology*, 29(1), pp. 26–34. doi: 10.1097/GCO.0000000000000340.

Krycer, J., Phan, L. and Brown, A. (2012) ‘A key regulator of cholesterol homoeostasis, SREBP-2, can be targeted in prostate cancer cells with natural products’, *Biochemical Journal*, 446(2), pp. 191–201. doi: 10.1042/BJ20120545.

Kumar, A. S. *et al.* (2006) ‘A call for clinical trials: lipophilic statins may prove effective in treatment and prevention of particular breast cancer subtypes.’, *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. American Society of Clinical Oncology, 24(13), p. 2127; author reply 2127-8. doi: 10.1200/JCO.2005.04.9882.

Kumar, R. and Thompson, E. B. (2005) ‘Gene regulation by the glucocorticoid receptor: Structure:function relationship’, *The Journal of Steroid Biochemistry and Molecular Biology*, 94(5), pp. 383–394. doi: 10.1016/j.jsbmb.2004.12.046.

Kuoppala, J., Lamminpää, A. and Pukkala, E. (2008) ‘Statins and cancer: A systematic review and meta-analysis.’, *European journal of cancer (Oxford, England : 1990)*, 44(15), pp. 2122–32. doi: 10.1016/j.ejca.2008.06.025.

Kurisetty, V. V *et al.* (2008) ‘RAN GTPase is an effector of the invasive/metastatic phenotype induced by osteopontin’, *Oncogene*. Nature Publishing Group, 27(57), pp. 7139–7149. doi: 10.1038/onc.2008.325.

Kurman, R. J. and Shih, I.-M. (2010) ‘The Origin and Pathogenesis of Epithelial Ovarian Cancer: A Proposed Unifying Theory’, *The American Journal of Surgical Pathology*, 34(3), pp. 433–443. doi: 10.1097/PAS.0b013e3181cf3d79.

Kusama, T. *et al.* (2003) ‘Selective inhibition of cancer cell invasion by a geranylgeranyltransferase-I inhibitor.’, *Clinical & experimental metastasis*, 20(6), pp. 561–7.

Lackner, M. R. *et al.* (2005) ‘Chemical genetics identifies Rab geranylgeranyl transferase as an apoptotic target of farnesyl transferase inhibitors’, *Cancer Cell*, 7(4), pp. 325–336. doi: 10.1016/j.ccr.2005.03.024.

Laezza, C. *et al.* (2015) ‘P53 Regulates the Mevalonate Pathway in Human Glioblastoma Multiforme.’, *Cell death & disease*, 6, p. e1909. doi: 10.1038/cddis.2015.279.

Lambert, J. M. R. *et al.* (2009) ‘PRIMA-1 Reactivates Mutant p53 by Covalent Binding to the Core Domain’, *Cancer Cell*, 15(5), pp. 376–388. doi: 10.1016/j.ccr.2009.03.003.

Lambert, M. and Bui, N. D. (1999) ‘Dexamethasone-induced decrease in HMG-CoA reductase and protein farnesyl transferase activities does not impair ras processing in AR 4-2 J cells’, *Molecular and Cellular Biochemistry*, 202(1–2), pp. 101–108. Available at:

http://www.scopus.com/inward/record.url?eid=2-s2.0-0033379416&partnerID=40&md5=d53a33e7184d5125f1e5874c95acd6e0.

Lanceley, A. *et al.* (2011) 'Ovarian cancer: symptoms, treatment and long-term patient management', *Primary Health Care*, 21(7), pp. 31–38. doi: 10.7748/phc2011.09.21.7.31.c8689.

Landen, C. N., Birrer, M. J. and Sood, A. K. (2008) 'Early events in the pathogenesis of epithelial ovarian cancer.', *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*, 26(6), pp. 995–1005. doi: 10.1200/JCO.2006.07.9970.

Lane, D. P., Cheok, C. F. and Lain, S. (2010) 'p53-based cancer therapy.', *Cold Spring Harbor perspectives in biology*. doi: 10.1101/cshperspect.a001222.

LANE, D. P. and CRAWFORD, L. V. (1979) 'T antigen is bound to a host protein in SY40-transformed cells', *Nature*, 278(5701), pp. 261–263. doi: 10.1038/278261a0.

Langdon, S. P. (ed.) (2004) 'Isolation and Culture of Ovarian Cancer Cell Lines - Springer', in. Humana Press (Methods in Molecular Medicine^{TM}).

Langedijk, J. *et al.* (2015) 'Drug repositioning and repurposing: terminology and definitions in literature', *Drug Discovery Today*, 20(8), pp. 1027–1034. doi: 10.1016/j.drudis.2015.05.001.

Laptenko, O. and Prives, C. (2006) 'Transcriptional regulation by p53: one protein, many possibilities.', *Cell death and differentiation*, 13(6), pp. 951–961. doi: 10.1038/sj.cdd.4401916.

Lavie, O. *et al.* (2013) 'The effect of statins on risk and survival of gynecological malignancies', *Gynecologic Oncology*, 130(3), pp. 615–619. doi: 10.1016/j.ygyno.2013.05.025.

Lavin, M. and Gueven, N. (2006) 'The complexity of p53 stabilization and activation', *Cell Death and Differentiation*, 13, pp. 941–950. doi: 10.1038/sj.cdd.4401925.

Lavrik, I. (2014) 'Systems biology of death receptor networks: live and let die', *Cell Death and Disease*, 5. doi: 10.1038/cddis.2014.160.

Le, D.-C. *et al.* (2012) 'Reproductive factors in relation to ovarian cancer: a case-control study in Northern Vietnam.', *Contraception*. Elsevier Inc., 86(5), pp. 494–9. doi: 10.1016/j.contraception.2012.02.019.

Lécuru, F. *et al.* (2017) 'Debulking Surgery: Interval Debulking Surgery Versus Primary: Pros and Cons on How to Evaluate Quality', in *Ovarian Cancers*. Cham: Springer International Publishing, pp. 33–42. doi: 10.1007/978-3-319-32110-3_3.

Ledermann, J. *et al.* (2012) 'Olaparib Maintenance Therapy in Platinum-Sensitive Relapsed Ovarian Cancer', *New England Journal of Medicine*, 366(15), pp. 1382–1392. doi: 10.1056/NEJMoa1105535.

Lee, J.-G. *et al.* (2015) 'Mutant p53 promotes ovarian cancer cell adhesion to mesothelial

cells via integrin β4 and Akt signals.', *Scientific reports*, 5(February), p. 12642. doi: 10.1038/srep12642.

Lee, P. C. W., Nguyen, A. D. and Debose-Boyd, R. A. (2007) 'Mutations within the membrane domain of HMG-CoA reductase confer resistance to sterol-accelerated degradation.', *Journal of lipid research*. American Society for Biochemistry and Molecular Biology, 48(2), pp. 318–27. doi: 10.1194/jlr.M600476-JLR200.

Lee, Y. *et al.* (2007) 'A candidate precursor to serous carcinoma that originates in the distal fallopian tube', *The Journal of Pathology*. John Wiley & Sons, Ltd., 211(1), pp. 26–35. doi: 10.1002/path.2091.

Leo, J. C. L. *et al.* (2004) 'Glucocorticoid and Mineralocorticoid Cross-Talk with Progesterone Receptor to Induce Focal Adhesion and Growth Inhibition in Breast Cancer Cells', *Endocrinology*, 145(3), pp. 1314–1321. doi: 10.1210/en.2003-0732.

Levine, A. J. and Oren, M. (2009) 'The first 30 years of p53: growing ever more complex.', *Nature reviews. Cancer*, 9(10), pp. 749–58. doi: 10.1038/nrc2723.

Levy-Lahad, E. and Friedman, E. (2007) 'Cancer risks among BRCA1 and BRCA2 mutation carriers.', *British journal of cancer*, 96(1), pp. 11–5. doi: 10.1038/sj.bjc.6603535.

Lewis, a D., Hayes, J. D. and Wolf, C. R. (1988) 'Glutathione and glutathione-dependent enzymes in ovarian adenocarcinoma cell lines derived from a patient before and after the onset of drug resistance: intrinsic differences and cell cycle effects.', *Carcinogenesis*, 9(7), pp. 1283–7. Available at: http://www.ncbi.nlm.nih.gov/pubmed/2898306.

Li, A. J. *et al.* (2010) 'Serum low-density lipoprotein levels correlate with survival in advanced stage epithelial ovarian cancers', *Gynecologic Oncology*. Elsevier Inc., 116(1), pp. 78–81. doi: 10.1016/j.ygyno.2009.09.027.

Li, J. *et al.* (2009) 'HE4 as a biomarker for ovarian and endometrial cancer management.', *Expert review of molecular diagnostics*. NIH Public Access, 9(6), pp. 555–66. doi: 10.1586/erm.09.39.

Li, L. *et al.* (2006) 'Simvastatin enhances learning and memory independent of amyloid load in mice', *Annals of Neurology*, 60(6), pp. 729–739. doi: 10.1002/ana.21053.

Li, S. and De Souza, P. (2011) 'Ras Isoprenylation and pAkt Inhibition by Zoledronic Acid and Fluvastatin Enhances Paclitaxel Activity in T24 Bladder Cancer Cells.', *Cancers*, 3(1), pp. 662–74. doi: 10.3390/cancers3010662.

Li, X., Wu, X. B. and Chen, Q. (2014) 'Statin use is not associated with reduced risk of skin cancer: a meta-analysis', *British Journal of Cancer*. Nature Publishing Group, 110(3), pp. 802–807. doi: 10.1038/bjc.2013.762.

Li, Y. and Prives, C. (2007) 'Are interactions with p63 and p73 involved in mutant p53 gain of oncogenic function?', *Oncogene*, 26, pp. 2220–2225. doi: 10.1038/sj.onc.1210311.

Lien, H.-C. *et al.* (2008) 'Differential expression of glucocorticoid receptor in carcinomas of the human digestive system', *Histopathology*. Blackwell Publishing Ltd, 52(3), pp. 314–

324. doi: 10.1111/j.1365-2559.2007.02953.x.

Likus, W. *et al.* (2016) 'Could drugs inhibiting the mevalonate pathway also target cancer stem cells?', *Drug Resistance Updates*, 25, pp. 13–25. doi: 10.1016/j.drup.2016.02.001.

Lim, L. Y. *et al.* (2009) 'Mutant p53 mediates survival of breast cancer cells', *British Journal of Cancer*. Nature Publishing Group, 101(9), pp. 1606–1612. doi: 10.1038/sj.bjc.6605335.

Lin, K. T. and Wang, L.-H. H. (2016) 'New dimension of glucocorticoids in cancer treatment', *Steroids*, 111, pp. 84–88. doi: 10.1016/j.steroids.2016.02.019.

Ling, K.-S. *et al.* (2005) 'Mechanisms Involved in Chemoresistance in Ovarian Cancer', *Taiwanese Journal of Obstetrics and Gynecology*. Taiwan Association of Obstetric & Gynecology, 44(3), pp. 209–217. doi: 10.1016/S1028-4559(09)60143-1.

Linzer, D. I. H., Maltzman, W. and Levine, A. J. (1979) 'The SV40 a gene product is required for the production of a 54,000 MW cellular tumor antigen', *Virology*, 98(2), pp. 308–318. doi: 10.1016/0042-6822(79)90554-3.

Liu, H. *et al.* (2009) 'Statins induce apoptosis in ovarian cancer cells through activation of JNK and enhancement of Bim expression.', *Cancer chemotherapy and pharmacology*, 63(6), pp. 997–1005. doi: 10.1007/s00280-008-0830-7.

Liu, Y. *et al.* (2014) 'Effect of statin on risk of gynecologic cancers: a meta-analysis of observational studies and randomized controlled trials.', *Gynecologic oncology*. Elsevier Inc., 133(3), pp. 647–55. doi: 10.1016/j.ygyno.2014.04.007.

Lobell, R. B. *et al.* (2002) 'Preclinical and Clinical Pharmacodynamic Assessment of L-778 , 123 , a Dual Inhibitor of Farnesyl : Protein Transferase and Geranylgeranyl : Protein Transferase Type-I Preclinical and Clinical Pharmacodynamic Assessment of L- 778 , 123 , a Dual Inhibitor ', pp. 747–758.

Lorusso, D. *et al.* (2003) 'Phase III multicenter randomized trial of amifostine as cytoprotectant in first-line chemotherapy in ovarian cancer patients', *Ann Oncol*, 14(7), pp. 1086–1093. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12853351.

Lorusso, D. *et al.* (2010) 'Review role of topotecan in gynaecological cancers: current indications and perspectives.', *Critical reviews in oncology/hematology*. Elsevier Ireland Ltd, 74(3), pp. 163–74. doi: 10.1016/j.critrevonc.2009.08.001.

Louie, K. G. *et al.* (1985) 'Radiation Survival Parameters of Antineoplastic Drug-sensitive and -resistant Human Ovarian Cancer Cell Lines and Their Modification by Buthionine Sulfoximine', *Cancer Research*, 45(5), pp. 2110–2115.

Lowe, K. a *et al.* (2013) 'An international assessment of ovarian cancer incidence and mortality.', *Gynecologic oncology*. Elsevier Inc., 130(1), pp. 107–14. doi: 10.1016/j.ygyno.2013.03.026.

Löwenberg, M. *et al.* (2008) 'Novel insights into mechanisms of glucocorticoid action and the development of new glucocorticoid receptor ligands', *Steroids*, 73(9), pp. 1025–1029.

doi: 10.1016/j.steroids.2007.12.002.

Lu, L. *et al.* (2016) 'Rap1A promotes ovarian cancer metastasis via activation of ERK/p38 and notch signaling', *Cancer Medicine*, 5(12), pp. 3544–3554. doi: 10.1002/cam4.946.

Lu, Y.-S. *et al.* (2005) 'Effects of glucocorticoids on the growth and chemosensitivity of carcinoma cells are heterogeneous and require high concentration of functional glucocorticoid receptors.', *World journal of gastroenterology*. Baishideng Publishing Group Inc, 11(40), pp. 6373–80. doi: 10.3748/wjg.v11.i40.6373.

Lu, Y.-S. *et al.* (2006) 'Glucocorticoid receptor expression in advanced non-small cell lung cancer: clinicopathological correlation and in vitro effect of glucocorticoid on cell growth and chemosensitivity', *Lung Cancer*. Elsevier, 53(3), pp. 303–310. doi: 10.1016/j.lungcan.2006.05.005.

Lutz, A. M. *et al.* (2011) 'Early Diagnosis of Ovarian Carcinoma: Is a Solution in Sight?', *Radiology*. Radiological Society of North America, Inc., 259(2), pp. 329–345. doi: 10.1148/radiol.11090563.

Luu, W. *et al.* (2016) 'Oxysterols: Old Tale, New Twists', *Annual Review of Pharmacology and Toxicology*, 56(1), pp. 447–467. doi: 10.1146/annurev-pharmtox-010715-103233.

Luu, W., Gelissen, I. C. and Brown, A. J. (2017) 'Manipulating Cholesterol Status Within Cells', in, pp. 41–52. doi: 10.1007/978-1-4939-6875-6_4.

Ly, T. K. *et al.* (2010) 'Activation of the Ran GTPase is subject to growth factor regulation and can give rise to cellular transformation.', *The Journal of biological chemistry*. American Society for Biochemistry and Molecular Biology, 285(8), pp. 5815–26. doi: 10.1074/jbc.M109.071886.

Maas, H. a a M. *et al.* (2005) 'The influence of age and co-morbidity on treatment and prognosis of ovarian cancer: a population-based study.', *Gynecologic oncology*, 97(1), pp. 104–9. doi: 10.1016/j.ygyno.2004.12.026.

Mahtani, R. and Jahanzeb, M. (2010) 'Bisphosphonates as anticancer therapy for early breast cancer.', *Clinical breast cancer*. Elsevier Inc., 10(5), pp. 359–66. doi: 10.3816/CBC.2010.n.047.

Malenda, A. *et al.* (2012) 'Statins impair glucose uptake in tumor cells.', *Neoplasia (New York, N.Y.)*. Neoplasia Press, 14(4), pp. 311–23. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22577346 (Accessed: 24 July 2017).

Manoukian, G. E. *et al.* (2011) 'Pilot trial of bone-targeted therapy combining zoledronate with fluvastatin or atorvastatin for patients with metastatic renal cell carcinoma.', *Clinical genitourinary cancer*, 9(2), pp. 81–8. doi: 10.1016/j.clgc.2011.07.001.

Mantovani, F., Walerych, D. and Sal, G. Del (2016) 'Targeting mutant p53 in cancer: a long road to precision therapy', *The FEBS Journal*. doi: 10.1111/febs.13948.

Manzoni, M. and Rollini, M. (2002) 'Biosynthesis and biotechnological production of

statins by filamentous fungi and application of these cholesterol-lowering drugs.', *Applied microbiology and biotechnology*, 58(5), pp. 555–64. doi: 10.1007/s00253-002-0932-9.

Marczak, A., Bukowska, B. and Rogalska, A. (2014) 'WP 631 and Epo B synergize in SKOV-3 human ovarian cancer cells', *Environmental Toxicology and Pharmacology*. Elsevier B.V., 37(1), pp. 256–266. doi: 10.1016/j.etap.2013.12.002.

Martin, L. P., Hamilton, T. C. and Schilder, R. J. (2008) 'Platinum resistance: the role of DNA repair pathways.', *Clinical cancer research : an official journal of the American Association for Cancer Research*, 14(5), pp. 1291–5. doi: 10.1158/1078-0432.CCR-07-2238.

Martirosyan, A. *et al.* (2010) 'Lovastatin induces apoptosis of ovarian cancer cells and synergizes with doxorubicin: potential therapeutic relevance.', *BMC cancer*, 10, p. 103. doi: 10.1186/1471-2407-10-103.

Mathews Griner, L. A. *et al.* (2014) 'High-throughput combinatorial screening identifies drugs that cooperate with ibrutinib to kill activated B-cell-like diffuse large B-cell lymphoma cells.', *Proceedings of the National Academy of Sciences of the United States of America*. National Academy of Sciences, 111(6), pp. 2349–54. doi: 10.1073/pnas.1311846111.

Matusewicz, L. *et al.* (2015) 'The effect of statins on cancer cells—review', *Tumor Biology*, 36(7), pp. 4889–4904. doi: 10.1007/s13277-015-3551-7.

Maurer-Stroh, S. *et al.* (2007) 'Towards complete sets of farnesylated and geranylgeranylated proteins.', *PLoS computational biology*, 3(4), p. e66. doi: 10.1371/journal.pcbi.0030066.

Maynor, M. *et al.* (2008) *Synthesis and evaluation of 3- and 7-substituted geranylgeranyl pyrophosphate analogs*, *Bioorganic & Medicinal Chemistry Letters*. doi: 10.1016/j.bmcl.2008.02.014.

McCluggage, W. G. (2011) 'Morphological subtypes of ovarian carcinoma: a review with emphasis on new developments and pathogenesis.', *Pathology*, 43(5), pp. 420–32. doi: 10.1097/PAT.0b013e328348a6e7.

McGuire, W. P. and Markman, M. (2003) 'Primary ovarian cancer chemotherapy: current standards of care.', *British journal of cancer*, 89 Suppl 3, pp. S3-8. doi: 10.1038/sj.bjc.6601494.

McKay, L. and Cidlowski, J. (2003) 'Physiologic and pharmacologic effects of corticosteroids'. Available at: http://www.ncbi.nlm.nih.gov/books/NBK13780 (Accessed: 9 November 2016).

McTaggart, S. J. (2006) 'Isoprenylated proteins', *Cellular and Molecular Life Sciences*, 63(3), pp. 255–267. doi: 10.1007/s00018-005-5298-6.

Meehan, R. S. and Chen, A. P. (2016) 'New treatment option for ovarian cancer: PARP inhibitors', *Gynecologic Oncology Research and Practice*, 3(1), p. 3. doi: 10.1186/s40661-016-0024-7.

Meier, W. *et al.* (2012) ‘Randomized phase II trial of carboplatin and paclitaxel with or without lonafarnib in first-line treatment of epithelial ovarian cancer stage IIB-IV.’, *Gynecologic oncology*. Elsevier Inc., 126(2), pp. 236–40. doi: 10.1016/j.ygyno.2012.04.050.

Melichar, B. *et al.* (1998) ‘Growth inhibitory effects of aromatic fatty acids on ovarian tumor cell lines.’, *Clinical cancer research : an official journal of the American Association for Cancer Research*, 4(12), pp. 3069–76. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9865922.

Mello, S. S. and Attardi, L. D. (2013) ‘Not all p53 gain-of-function mutants are created equal.’, *Cell death and differentiation*. Nature Publishing Group, 20(7), pp. 855–7. doi: 10.1038/cdd.2013.53.

Mertens-Walker, I., Baxter, R. C. and Marsh, D. J. (2012) ‘Gonadotropin signalling in epithelial ovarian cancer.’, *Cancer letters*. Elsevier Ireland Ltd, 324(2), pp. 152–9. doi: 10.1016/j.canlet.2012.05.017.

Minton, M. J. *et al.* (1981) ‘Corticosteroids for elderly patients with breast cancer’, *Cancer*. Wiley Subscription Services, Inc., A Wiley Company, 48(4), pp. 883–887. doi: 10.1002/1097-0142(19810815)48:4<883::AID-CNCR2820480404>3.0.CO;2-V.

Mitra, S., Cheng, K. W. and Mills, G. B. (2012) ‘Rab25 in cancer: a brief update.’, *Biochemical Society transactions*. NIH Public Access, 40(6), pp. 1404–8. doi: 10.1042/BST20120249.

Mitry, R. R. and Hughes, R. D. (eds) (2012) ‘Introduction to Cell Culture - Springer’, in. Humana Press (Methods in Molecular Biology).

Mo, H. and Elson, C. E. (2004) ‘Studies of the isoprenoid-mediated inhibition of mevalonate synthesis applied to cancer chemotherapy and chemoprevention.’, *Experimental biology and medicine (Maywood, N.J.)*, 229(7), pp. 567–85. doi: 229/7/567.

Mohamed, A., Smith, K. and de Chaves, E. P. (2015) ‘The Mevalonate Pathway in Alzheimer’s Disease — Cholesterol and Non-Sterol Isoprenoids’, in *Alzheimer’s Disease - Challenges for the Future*. InTech, pp. 167–222. doi: 10.5772/59904.

Mok, S. C. *et al.* (2007) ‘Etiology and pathogenesis of epithelial ovarian cancer.’, *Disease markers*, 23(5–6), pp. 367–76. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3851194&tool=pmcentrez&rendertype=abstract.

Montagnana, M. *et al.* (2009) ‘The utility of serum human epididymis protein 4 (HE4) in patients with a pelvic mass’, *Journal of Clinical Laboratory Analysis*, 23(5), pp. 331–335. doi: 10.1002/jcla.20340.

Moon, H. *et al.* (2014) ‘Statins: protectors or pretenders in prostate cancer?’, *Trends in Endocrinology & Metabolism*. Elsevier Ltd, 25(4), pp. 188–196. doi: 10.1016/j.tem.2013.12.007.

Moore, R. G. *et al.* (2009) ‘A novel multiple marker bioassay utilizing HE4 and CA125 for

the prediction of ovarian cancer in patients with a pelvic mass', *Gynecologic Oncology*, 112(1), pp. 40–46. doi: 10.1016/j.ygyno.2008.08.031.

Morgan, M. a *et al.* (2005) 'Combining prenylation inhibitors causes synergistic cytotoxicity, apoptosis and disruption of RAS-to-MAP kinase signalling in multiple myeloma cells.', *British journal of haematology*, 130(6), pp. 912–25. doi: 10.1111/j.1365-2141.2005.05696.x.

Moriceau, G. *et al.* (2010) 'Synergistic effect of Apomine and Lovastatin on osteosarcoma cell growth in vitro and in vivo', *Bone*. Elsevier B.V., 46(2010), p. S38. doi: 10.1016/j.bone.2010.01.082.

Moriceau, G. *et al.* (2012) 'Synergistic inhibitory effect of apomine and lovastatin on osteosarcoma cell growth', *Cancer*, 118(3), pp. 750–760. doi: 10.1002/cncr.26336.

Morris, R. *et al.* (2008) 'Topotecan weekly bolus chemotherapy for relapsed platinum-sensitive ovarian and peritoneal cancers', *Gynecologic Oncology*, 109(3), pp. 346–352. doi: 10.1016/j.ygyno.2008.02.028.

Moutsatsou, P. and Papavassiliou, A. G. (2008) 'The glucocorticoid receptor signalling in breast cancer', *Journal of Cellular and Molecular Medicine*. Wiley-Blackwell, 12(1), p. 145. doi: 10.1111/j.1582-4934.2007.00177.x.

Mukhtar, R. Y. A., Reid, J. and Reckless, J. P. D. (2005) 'Pitavastatin', *International Journal of Clinical Practice*, 59(2), pp. 239–252. doi: 10.1111/j.1742-1241.2005.00461.x.

Mullany, L. K. *et al.* (2015) 'Mullany, L.K. et al., 2015. Specific TP53 Mutants Overrepresented in Ovarian Cancer Impact CNV, TP53 Activity, Responses to Nutlin-3a, and Cell Survival. Neoplasia (United States), 17(10), pp.789–803.Specific TP53 Mutants Overrepresented in Ovarian Cancer', *Neoplasia (United States)*, 17(10), pp. 789–803. doi: 10.1016/j.neo.2015.10.003.

Mullen, P. J. *et al.* (2016) 'The interplay between cell signalling and the mevalonate pathway in cancer', *Nature Reviews Cancer*. Nature Research, 16(11), pp. 718–731. doi: 10.1038/nrc.2016.76.

Muller, P. A. J. *et al.* (2009) 'Mutant p53 Drives Invasion by Promoting Integrin Recycling', *Cell*, 139, pp. 1327–1341. doi: 10.1016/j.cell.2009.11.026.

Murdoch, W. J. (2005) 'Carcinogenic potential of ovulatory genotoxicity.', *Biology of reproduction*, 73(4), pp. 586–90. doi: 10.1095/biolreprod.105.042622.

Murphy, M. A. *et al.* (2012) 'Non-steroidal anti-inflammatory drug use and ovarian cancer risk: findings from the NIH-AARP Diet and Health Study and systematic review.', *Cancer causes & control : CCC*, 23(11), pp. 1839–52. doi: 10.1007/s10552-012-0063-2.

Napoli, M. and Flores, E. R. (2017) 'The p53 family orchestrates the regulation of metabolism: physiological regulation and implications for cancer therapy', *British Journal of Cancer*. Nature Publishing Group, 116(2), pp. 149–155. doi: 10.1038/bjc.2016.384.

Nayan, M. *et al.* (2017) 'Statin use and kidney cancer survival outcomes: A systematic

review and meta-analysis', *Cancer Treatment Reviews*, 52, pp. 105–116. doi: 10.1016/j.ctrv.2016.11.009.

Nelson, D. L. and Cox, M. M. (2013) *Lehninger Principles of Biochemistry 6th ed.*, *Book*. doi: 10.1016/j.jse.2011.03.016.

Nemunaitis, J. *et al.* (2000) 'Adenovirus-mediated p53 gene transfer in sequence with cisplatin to tumors of patients with non-small-cell lung cancer', *J Clin Oncol*, 18(3), pp. 609–622.

Ness, R. B. *et al.* (2011) 'Contraception methods, beyond oral contraceptives and tubal ligation, and risk of ovarian cancer.', *Annals of epidemiology*, 21(3), pp. 188–96. doi: 10.1016/j.annepidem.2010.10.002.

Neville-Webbe, H. L. *et al.* (2005) 'Sequence- and schedule-dependent enhancement of zoledronic acid induced apoptosis by doxorubicin in breast and prostate cancer cells', *International Journal of Cancer*, 113(3), pp. 364–371. doi: 10.1002/ijc.20602.

Ng, J. S., Low, J. J. H. and Ilancheran, a (2012) 'Epithelial ovarian cancer.', *Best practice & research. Clinical obstetrics & gynaecology*. Elsevier Ltd, 26(3), pp. 337–45. doi: 10.1016/j.bpobgyn.2011.12.005.

Nguyen, U. T. T. *et al.* (2009) 'Analysis of the eukaryotic prenylome by isoprenoid affinity tagging', *Nature Chemical Biology*, 5(4), pp. 227–235. doi: 10.1038/nchembio.149.

Nilsson, S., Huelsenbeck, J. and Fritz, G. (2011) 'Mevalonate pathway inhibitors affect anticancer drug-induced cell death and DNA damage response of human sarcoma cells.', *Cancer letters*. Elsevier Ireland Ltd, 304(1), pp. 60–9. doi: 10.1016/j.canlet.2010.12.022.

Nomura, D. K. *et al.* (2010) 'Monoacylglycerol Lipase Regulates a Fatty Acid Network that Promotes Cancer Pathogenesis', *Cell*, 140(1), pp. 49–61. doi: 10.1016/j.cell.2009.11.027.

Normand, S. *et al.* (2009) 'Specific increase in caspase-1 activity and secretion of IL-1 family cytokines: a putative link between mevalonate kinase deficiency and inflammation.', *European cytokine network*, 20(3), pp. 101–7. doi: 10.1684/ecn.2009.0163.

O'Connor, R. (2007) 'The pharmacology of cancer resistance.', *Anticancer research*, 27(3A), pp. 1267–72. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18079276.

O'Neill, P. R. *et al.* (2012) 'G-protein signaling leverages subunit-dependent membrane affinity to differentially control βγ translocation to intracellular membranes.', *Proceedings of the National Academy of Sciences of the United States of America*. National Academy of Sciences, 109(51), pp. E3568-77. doi: 10.1073/pnas.1205345109.

Ohashi, K. *et al.* (2003) 'Early embryonic lethality caused by targeted disruption of the 3-hydroxy-3-methylglutaryl-CoA reductase gene.', *The Journal of biological chemistry*, 278(44), pp. 42936–41. doi: 10.1074/jbc.M307228200.

Okamoto, S. *et al.* (2014) 'Zoledronic acid induces apoptosis and S-phase arrest in mesothelioma through inhibiting Rab family proteins and topoisomerase II actions.', *Cell*

death & disease. Nature Publishing Group, 5(11), p. e1517. doi: 10.1038/cddis.2014.475.

Olive, K. P. *et al.* (2004) 'Mutant p53 gain of function in two mouse models of Li-Fraumeni syndrome', *Cell*, 119(6), pp. 847–860. doi: 10.1016/j.cell.2004.11.004.

Olivier, M., Hollstein, M. and Hainaut, P. (2010) 'TP53 Mutations in Human Cancers: Origins, Consequences, and Clinical Use', *Cold Spring Harbor Perspectives in Biology*, 2(1), pp. a001008–a001008. doi: 10.1101/cshperspect.a001008.

Olsen, C. M. *et al.* (2007) 'Obesity and the risk of epithelial ovarian cancer: a systematic review and meta-analysis.', *European journal of cancer (Oxford, England : 1990)*, 43(4), pp. 690–709. doi: 10.1016/j.ejca.2006.11.010.

Oren, M. and Kotler, E. (2016) 'p53 mutations promote proteasomal activity', *Nature Cell Biology*. Nature Research, 18(8), pp. 833–835. doi: 10.1038/ncb3392.

Oren, M. and Rotter, V. (2010) 'Mutant p53 gain-of-function in cancer.', *Cold Spring Harbor perspectives in biology*. Cold Spring Harbor Laboratory Press, 2(2), p. a001107. doi: 10.1101/cshperspect.a001107.

Ose, L. *et al.* (2010) 'Long-term treatment with pitavastatin is effective and well tolerated by patients with primary hypercholesterolemia or combined dyslipidemia', *Atherosclerosis*. Elsevier Ireland Ltd, 210(1), pp. 202–208. doi: 10.1016/j.atherosclerosis.2009.12.009.

Oskay-Oezcelik, G. *et al.* (2008) 'Biweekly pegylated liposomal doxorubicin as second-line treatment in patients with relapsed ovarian cancer after failure of platinum and paclitaxel: results from a multi-center phase II study of the NOGGO.', *Anticancer research*, 28(2B), pp. 1329–34. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18505074.

Osmak, M. (2012) 'Statins and cancer: current and future prospects.', *Cancer letters*. Elsevier Ireland Ltd, 324(1), pp. 1–12. doi: 10.1016/j.canlet.2012.04.011.

Ovarian cancer research UK (2014) *Cancer mortality for common cancers | Cancer Research UK*. Available at: http://www.cancerresearchuk.org/health-professional/cancer-statistics/mortality/common-cancers-compared#heading-Zero (Accessed: 14 March 2017).

Pabla, N. and Dong, Z. (2008) 'Cisplatin nephrotoxicity: mechanisms and renoprotective strategies.', *Kidney international*, 73(9), pp. 994–1007. doi: 10.1038/sj.ki.5002786.

Pallottini, V. *et al.* (2008) 'Regulation of HMG-CoA reductase expression by hypoxia', *Journal of Cellular Biochemistry*, 104(3), pp. 701–709. doi: 10.1002/jcb.21757.

Palmiere, D. *et al.* (2005) 'Medroxyprogesterone acetate elevation of Nm23-H1 metastasis suppressor expression in hormone receptor-negative breast cancer', *Journal of the National Cancer Institute*, 97(9), pp. 632–642. doi: 10.1093/jnci/dji111.

Pantziarka, P., Bouche, G., Meheus, L., Sukhatme, V. and Sukhatme, V. P. (2014) 'Repurposing Drugs in Oncology (ReDO)-mebendazole as an anti-cancer agent.', *Ecancermedicalscience*, 8, p. 443. doi: 10.3332/ecancer.2014.443.

Pantziarka, P., Bouche, G., Meheus, L., Sukhatme, V., Sukhatme, V. P., *et al.* (2014) 'The Repurposing Drugs in Oncology (ReDO) Project.', *Ecancermedicalscience*. Cancer Intelligence, 8, p. 442. doi: 10.3332/ecancer.2014.442.

Papadopoulos, A. S. and Cleare, A. J. (2011) 'Hypothalamic–pituitary–adrenal axis dysfunction in chronic fatigue syndrome', *Nature Reviews Endocrinology*, 8(1), pp. 22–32. doi: 10.1038/nrendo.2011.153.

Park, J. *et al.* (2014) 'Human isoprenoid synthase enzymes as therapeutic targets.', *Frontiers in chemistry*. Frontiers Media SA, 2(July), p. 50. doi: 10.3389/fchem.2014.00050.

Parker, K. A. (2011) 'Storage of cell lines.', *Methods in molecular biology (Clifton, N.J.)*, 731, pp. 27–34. doi: 10.1007/978-1-61779-080-5_3.

Parrales, A. and Iwakuma, T. (2016) 'p53 as a regulator of lipid metabolism in cancer', *International Journal of Molecular Sciences*. doi: 10.3390/ijms17122074.

Parrales A *et al.* (2016) 'DNAJA1 controls the fate of misfolded mutant p53 through the mevalonate pathway', *Nature Cell Biology*, 18(11), pp. 1233–1243. doi: 10.1038/ncb3427.

Pasalich, M. *et al.* (2013) 'Reproductive factors for ovarian cancer in southern Chinese women.', *Journal of gynecologic oncology*, 24(2), pp. 135–40. doi: 10.3802/jgo.2013.24.2.135.

Perren, T. J. *et al.* (2011) 'A Phase 3 Trial of Bevacizumab in Ovarian Cancer', *New England Journal of Medicine*. Massachusetts Medical Society, 365(26), pp. 2484–2496. doi: 10.1056/NEJMoa1103799.

Petitjean, A. *et al.* (2007) 'Impact of mutant p53 functional properties on TP53 mutation patterns and tumor phenotype: lessons from recent developments in the IARC TP53 database', *Human Mutation*, 28(6), pp. 622–629. doi: 10.1002/humu.20495.

Petrik, J. (2013) 'Challenges in Experimental Modeling of Ovarian Cancerogenesis', in Malek, A. and Tchernitsa, O. (eds) *Ovarian Cancer*. Humana Press (Methods in Molecular Biology), pp. 371–376.

Pieretti, M. and Turker, M. S. (1997) 'Mutation , ageing , and ovarian cancer Reduced acute rejection after liver transplantation with Neoral-based triple immunosuppression', 349, pp. 700–701.

Pinheiro, S. P. *et al.* (2009) 'Use of nonsteroidal antiinflammatory agents and incidence of ovarian cancer in 2 large prospective cohorts.', *American journal of epidemiology*, 169(11), pp. 1378–87. doi: 10.1093/aje/kwp062.

Pufall, M. A. (2015) 'Glucocorticoids and Cancer.', *Advances in experimental medicine and biology*, 872, pp. 315–33. doi: 10.1007/978-1-4939-2895-8_14.

Pujade-Lauraine, E. *et al.* (2014) 'Bevacizumab Combined With Chemotherapy for Platinum-Resistant Recurrent Ovarian Cancer: The AURELIA Open-Label Randomized Phase III Trial', *Journal of Clinical Oncology*. American Society of Clinical Oncology,

32(13), pp. 1302–1308. doi: 10.1200/JCO.2013.51.4489.

Pyragius, C. E. *et al.* (2013) 'Aberrant lipid metabolism: an emerging diagnostic and therapeutic target in ovarian cancer.', *International journal of molecular sciences*, 14(4), pp. 7742–56. doi: 10.3390/ijms14047742.

Räikkönen, J. *et al.* (2009) 'Zoledronic acid induces formation of a pro-apoptotic ATP analogue and isopentenyl pyrophosphate in osteoclasts in vivo and in MCF-7 cells in vitro', *British Journal of Pharmacology*, 157(3), pp. 427–435. doi: 10.1111/j.1476-5381.2009.00160.x.

Rajalingam, K. *et al.* (2007) 'Ras oncogenes and their downstream targets', *Biochimica et Biophysica Acta - Molecular Cell Research*, pp. 1177–1195. doi: 10.1016/j.bbamcr.2007.01.012.

Raje, N. and Anderson, K. (1999) *Thalidomide--revival story*, *N*.

Ramsay, E. E. and Dilda, P. J. (2014) 'Glutathione S-conjugates as prodrugs to target drug-resistant tumors.', *Frontiers in pharmacology*. Frontiers Media SA, 5, p. 181. doi: 10.3389/fphar.2014.00181.

Ratman, D. *et al.* (2013) 'How glucocorticoid receptors modulate the activity of other transcription factors: A scope beyond tethering', *Molecular and Cellular Endocrinology*, 380(1), pp. 41–54. doi: 10.1016/j.mce.2012.12.014.

Recchi, C. and Seabra, M. C. C. (2012) 'Novel functions for Rab GTPases in multiple aspects of tumour progression.', *Biochemical Society transactions*. Portland Press Ltd, 40(6), pp. 1398–403. doi: 10.1042/BST20120199.

Reedijk, J. (1987) 'The mechanism of action of platinum antitumor drugs', *Pure and Applied Chemistry*, 59(2), pp. 181–192. doi: 10.1351/pac198759020181.

Reigard, S. a. *et al.* (2005) 'Interplay of isoprenoid and peptide substrate specificity in protein farnesyltransferase', *Biochemistry*, 44, pp. 11214–11223. doi: 10.1021/bi050725l.

Rennert, G. *et al.* (2014) 'The effect of bisphosphonates on the risk of endometrial and ovarian malignancies.', *Gynecologic oncology*. Elsevier Inc., 133(2), pp. 309–13. doi: 10.1016/j.ygyno.2014.02.014.

Rennert, G., Pinchev, M. and Rennert, H. S. (2010) 'Use of Bisphosphonates and Risk of Postmenopausal Breast Cancer', *Journal of Clinical Oncology*, 28(22), pp. 3577–3581. doi: 10.1200/JCO.2010.28.1113.

Reuther, G. W. and Der, C. J. (2000) 'The Ras branch of small GTPases: Ras family members don't fall far from the tree', *Current Opinion in Cell Biology*, pp. 157–165. doi: 10.1016/S0955-0674(99)00071-X.

Ricciardelli, C. *et al.* (2013) 'Chemotherapy-induced hyaluronan production: a novel chemoresistance mechanism in ovarian cancer.', *BMC cancer*. BMC Cancer, 13(1), p. 476. doi: 10.1186/1471-2407-13-476.

Riley, T. *et al.* (2008) 'Transcriptional control of human p53-regulated genes', *Nature Reviews Molecular Cell Biology*, 9(5), pp. 402–412. doi: 10.1038/nrm2395.

Rivlin, N. *et al.* (2011) 'Mutations in the p53 Tumor Suppressor Gene: Important Milestones at the Various Steps of Tumorigenesis.', *Genes & cancer*. Impact Journals, LLC, 2(4), pp. 466–74. doi: 10.1177/1947601911408889.

Robey, R. B. and Hay, N. (2006) 'Mitochondrial hexokinases, novel mediators of the antiapoptotic effects of growth factors and Akt.', *Oncogene*, 25, pp. 4683–4696. doi: 10.1038/sj.onc.1209595.

Robinson, E. *et al.* (2013) 'Preclinical evaluation of statins as a treatment for ovarian cancer.', *Gynecologic oncology*. Elsevier Inc., 129(2), pp. 417–24. doi: 10.1016/j.ygyno.2013.02.003.

Robinson, E. *et al.* (2014) 'New strategies for the treatment of ovarian cancer: Figure 1', *Biochemical Society Transactions*, 42(1), pp. 125–129. doi: 10.1042/BST20130203.

Robinson, E. (2015) *The preclinical evaluation of simvastatin and pitavastatin for the treatment of ovarian cancer*. Keele university.

Rodon, J., Perez, J. and Kurzrock, R. (2010) 'Combining Targeted Therapies: Practical Issues to Consider at the Bench and Bedside', *The Oncologist*, 15(1), pp. 37–50. doi: 10.1634/theoncologist.2009-0117.

Roelofs, A. J. *et al.* (2007) 'Apomine enhances the antitumor effects of lovastatin on myeloma cells by down-regulating 3-hydroxy-3-methylglutaryl-coenzyme A reductase.', *The Journal of pharmacology and experimental therapeutics*, 322(1), pp. 228–35. doi: 10.1124/jpet.106.116467.

Rogers, M. *et al.* (2015) 'Synergistic growth inhibition of PC3 prostate cancer cells with low-dose combinations of simvastatin and alendronate', *Anticancer Research*, 35(4), pp. 1851–1860. Available at: http://www.ncbi.nlm.nih.gov/pubmed/25862838.

Rogers, M. J. *et al.* (2011) 'Biochemical and molecular mechanisms of action of bisphosphonates', *Bone*. Elsevier Inc., 49(1), pp. 34–41. doi: 10.1016/j.bone.2010.11.008.

Rojas, A. M. *et al.* (2012) 'The Ras protein superfamily: Evolutionary tree and role of conserved amino acids', *Journal of Cell Biology*, pp. 189–201. doi: 10.1083/jcb.201103008.

Rooth, C. (2013) 'treatment and management', 22(17), pp. 23–31.

Rosenberg, B. (1985) 'Fundamental studies with cisplatin.', *Cancer*, 55(10), pp. 2303-l6. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12737842.

Rothbarth, J., Vahrmeijer, A. L. and Mulder, G. J. (2002) 'Modulation of cytostatic efficacy of melphalan by glutathione: mechanisms and efficacy.', *Chemico-biological interactions*, 140(2), pp. 93–107. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12076518.

Rotter, V. (1983) ‘p53, a transformation-related cellular-encoded protein, can be used as a biochemical marker for the detection of primary mouse tumor cells’, *Proc Natl Acad Sci U S A*, 80(9), pp. 2613–2617.

Rotter, V., Abutbul, H. and Ben-Ze’ev, A. (1983) ‘P53 transformation-related protein accumulates in the nucleus of transformed fibroblasts in association with the chromatin and is found in the cytoplasm of non-transformed fibroblasts.’, *The EMBO journal*, 2(7), pp. 1041–7.

Rubatt, J. M. *et al.* (2012) ‘Pre-treatment tumor expression of ERCC1 in women with advanced stage epithelial ovarian cancer is not predictive of clinical outcomes: a Gynecologic Oncology Group study.’, *Gynecologic oncology*. NIH Public Access, 125(2), pp. 421–6. doi: 10.1016/j.ygyno.2012.01.008.

Russell, P. *et al.* (1999) ‘Nuclear localization of Cdc25 is regulated by DNA damage and a 14-3-3 protein’, *Nature*. Nature Publishing Group, 397(6715), pp. 172–175. doi: 10.1038/16488.

Sacchettini, J. C. and Poulter, C. D. (1997) ‘Creating isoprenoid diversity’, *Science*, 2(September), pp. 1788–1789. doi: 10.1126/science.277.5333.1788.

Sahebkar, A. *et al.* (2017) ‘Curcumin: An effective adjunct in patients with statin-associated muscle symptoms?’, *Journal of cachexia, sarcopenia and muscle*. Wiley-Blackwell, 8(1), pp. 19–24. doi: 10.1002/jcsm.12140.

Sain, N. *et al.* (2006) ‘Potentiation of paclitaxel activity by the HSP90 inhibitor 17-allylamino-17-demethoxygeldanamycin in human ovarian carcinoma cell lines with high levels of activated AKT’, *Mol Cancer Ther*, 5(5), pp. 1197–208. doi: 10.1158/1535-7163.MCT-05-0445.

Saito, Y. (2011) ‘Pitavastatin: an overview.’, *Atherosclerosis. Supplements*. Elsevier Ireland Ltd, 12(3), pp. 271–276. doi: 10.1016/S1567-5688(11)70886-8.

Samatar, A. a. and Poulikakos, P. I. (2014) ‘Targeting RAS–ERK signalling in cancer: promises and challenges’, *Nature Reviews Drug Discovery*, 13(12), pp. 928–942. doi: 10.1038/nrd4281.

Samimi, G. *et al.* (2003) ‘Increase in expression of the copper transporter ATP7A during platinum drug-based treatment is associated with poor survival in ovarian cancer patients.’, *Clinical cancer research : an official journal of the American Association for Cancer Research*, 9(16 Pt 1), pp. 5853–9. Available at: http://www.ncbi.nlm.nih.gov/pubmed/14676106 (Accessed: 21 March 2017).

Samimi, G. *et al.* (2004) ‘Modulation of the Cellular Pharmacology of Cisplatin and Its Analogs by the Copper Exporters ATP7A and ATP7B’, *Molecular Pharmacology*, 66(1), pp. 25–32. doi: 10.1124/mol.66.1.25.

Sánchez CA *et al.* (2008) ‘Statin-induced inhibition of MCF-7 breast cancer cell proliferation is related to cell cycle arrest and apoptotic and necrotic cell death mediated by an enhanced oxidative stress.’, *Cancer Invest*, 26(7), pp. 698–707. doi: 10.1080/07357900701874658.

Sandberg, R. and Ernberg, I. (2005) 'Assessment of tumor characteristic gene expression in cell lines using a tissue similarity index (TSI)', *Proceedings of the National Academy of Sciences*, 102(6), pp. 2052–2057. doi: 10.1073/pnas.0408105102.

Sane, K. M. *et al.* (2010) 'A novel geranylgeranyl transferase inhibitor in combination with lovastatin inhibits proliferation and induces autophagy in STS-26T MPNST cells.', *The Journal of pharmacology and experimental therapeutics*, 333(1), pp. 23–33. doi: 10.1124/jpet.109.160192.

Sapiezynski, J. *et al.* (2016) 'Precision targeted therapy of ovarian cancer', *Journal of Controlled Release*, 243, pp. 250–268. doi: 10.1016/j.jconrel.2016.10.014.

Sarrabayrouse, G. *et al.* (2017) 'Regulatory properties of statins and rho gtpases prenylation inhibitiors to stimulate melanoma immunogenicity and promote anti-melanoma immune response', *International Journal of Cancer*, 140(4), pp. 747–755. doi: 10.1002/ijc.30422.

Sasieni, P. D. *et al.* (2011) 'What is the lifetime risk of developing cancer?: the effect of adjusting for multiple primaries', *British Journal of Cancer*, 105(3), pp. 460–465. doi: 10.1038/bjc.2011.250.

Sato, R. (2010) 'Sterol metabolism and SREBP activation.', *Archives of biochemistry and biophysics*. Elsevier Inc., 501(2), pp. 177–81. doi: 10.1016/j.abb.2010.06.004.

Scarlett, U. K. *et al.* (2012) 'Ovarian cancer progression is controlled by phenotypic changes in dendritic cells.', *The Journal of experimental medicine*, 209(3), pp. 495–506. doi: 10.1084/jem.20111413.

Schafer, W. R. and Rine, J. (1992) 'Protein Prenylation ':, *Interactions*, pp. 4–7.

Schlienger, S. *et al.* (2016) 'ADP-ribosylation factor 1 expression regulates epithelial-mesenchymal transition and predicts poor clinical outcome in triple-negative breast cancer.', *Oncotarget*. Impact Journals, LLC, 7(13), pp. 15811–27. doi: 10.18632/oncotarget.7515.

Schlossmacher, G., Stevens, A. and White, A. (2011) 'Glucocorticoid receptor-mediated apoptosis: Mechanisms of resistance in cancer cells', *Journal of Endocrinology*, pp. 17–25. doi: 10.1530/JOE-11-0135.

Schmidt, S. *et al.* (2004) 'Glucocorticoid-induced apoptosis and glucocorticoid resistance: molecular mechanisms and clinical relevance', *Cell Death and Differentiation*. Nature Publishing Group, 11, pp. S45–S55. doi: 10.1038/sj.cdd.4401456.

Schoneveld, O. J. L. M., Gaemers, I. C. and Lamers, W. H. (2004) 'Mechanisms of glucocorticoid signalling', *Biochimica et Biophysica Acta (BBA) - Gene Structure and Expression*, 1680(2), pp. 114–128. doi: 10.1016/j.bbaexp.2004.09.004.

Schorge, J. O. *et al.* (2010) 'SGO White Paper on ovarian cancer: etiology, screening and surveillance.', *Gynecologic oncology*. Elsevier Inc., 119(1), pp. 7–17. doi: 10.1016/j.ygyno.2010.06.003.

Schroepfer, G. J. (2000) ‘Oxysterols: modulators of cholesterol metabolism and other processes.’, *Physiological reviews*, 80(1), pp. 361–554.

Schubbert, S., Shannon, K. and Bollag, G. (2007) ‘Hyperactive Ras in developmental disorders and cancer.’, *Nature reviews. Cancer*, 7(4), pp. 295–308. doi: 10.1038/nrc2109.

Schüler, S. *et al.* (2013) ‘Ovarian epithelial tumors and reproductive factors: a systematic review.’, *Archives of gynecology and obstetrics*, 287(6), pp. 1187–204. doi: 10.1007/s00404-013-2784-1.

Schulz, S. and Nyce, J. W. (1991) ‘Inhibition of protein isoprenylation and p21ras membrane association by dehydroepiandrosterone in human colonic adenocarcinoma cells in vitro.’, *Cancer research*, 51(24), pp. 6563–7.

Scoles, D. R. *et al.* (2010) ‘Liver X receptor agonist inhibits proliferation of ovarian carcinoma cells stimulated by oxidized low density lipoprotein.’, *Gynecologic oncology*. Elsevier Inc., 116(1), pp. 109–16. doi: 10.1016/j.ygyno.2009.09.034.

Sebti, S. M. and Hamilton, A. D. (2000) ‘Farnesyltransferase and geranylgeranyltransferase I inhibitors and cancer therapy: Lessons from mechanism and bench-to-bedside translational studies’, *Oncogene*. Nature Publishing Group, 19(56), pp. 6584–6593. doi: 10.1038/sj.onc.1204146.

Selivanova, G. and Wiman, K. G. (2007) ‘Reactivation of mutant p53: molecular mechanisms and therapeutic potential’, *Oncogene*, 26(15), pp. 2243–2254. doi: 10.1038/sj.onc.1210295.

Sequist, L. V. *et al.* (2009) ‘Phase 1-2a Multicenter Dose-Ranging Study of Canfosfamide in Combination with Carboplatin and Paclitaxel as First-Line Therapy for Patients with Advanced Non-small Cell Lung Cancer’, *Journal of Thoracic Oncology*, 4(11), pp. 1389–1396. doi: 10.1097/JTO.0b013e3181b6b84b.

Shah, S. R. *et al.* (2015) ‘Novel applications of statins for bone regeneration’, *National Science Review*, 2(1), pp. 85–99. doi: 10.1093/nsr/nwu028.

Shai, A. *et al.* (2014) ‘Statins, aspirin and risk of thromboembolic events in ovarian cancer patients.’, *Gynecologic oncology*. Elsevier Inc., 133(2), pp. 304–8. doi: 10.1016/j.ygyno.2014.03.006.

Shao, W. and Espenshade, P. J. (2012) ‘Expanding roles for SREBP in metabolism’, *Cell Metabolism*, pp. 414–419. doi: 10.1016/j.cmet.2012.09.002.

Sharpe, L. J. and Brown, A. J. (2013) ‘Controlling cholesterol synthesis beyond 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR).’, *The Journal of biological chemistry*, 288(26), pp. 18707–15. doi: 10.1074/jbc.R113.479808.

Shaulsky, G., Goldfinger, N. and Rotter2, V. (1991) ‘Alterations in Tumor Development in Vivo Mediated by Expression of Wild Type or Mutant p53 Proteins1’, *CANCER RESEARCH*, 51(1), pp. 5232–5237.

Shaw, P. *et al.* (1992) ‘Induction of apoptosis by wild-type p53 in a human colon tumor-

derived cell line', *Biochemistry*, 89, pp. 4495–4499.

Shen, D. W. and Gottesman, M. M. (2012) 'RAB8 enhances TMEM205-mediated cisplatin resistance', *Pharmaceutical Research*, 29(3), pp. 643–650. doi: 10.1007/s11095-011-0562-y.

Shen, K. *et al.* (2010) 'Pegylated liposomal doxorubicin as a single agent or as combination therapy with carboplatin in patients with recurrent or refractory epithelial ovarian cancer', *Clinical Oncology and Cancer Research*, 6(6), pp. 387–393. doi: 10.1007/s11805-009-0387-1.

Shi, T. *et al.* (2017) '*BRCA1* and *BRCA2* mutations in ovarian cancer patients from China: ethnic-related mutations in *BRCA1* associated with an increased risk of ovarian cancer', *International Journal of Cancer*, 140(9), pp. 2051–2059. doi: 10.1002/ijc.30633.

Shih, I.-M. and Kurman, R. J. (2004) 'Ovarian Tumorigenesis', *The American Journal of Pathology*, 164(5), pp. 1511–1518. doi: 10.1016/S0002-9440(10)63708-X.

Shimoyama, S. (2011) 'Statins are logical candidates for overcoming limitations of targeting therapies on malignancy: their potential application to gastrointestinal cancers.', *Cancer chemotherapy and pharmacology*, 67(4), pp. 729–39. doi: 10.1007/s00280-011-1583-2.

Siddiqui, R. a *et al.* (2014) 'Characterization of lovastatin–docosahexaenoate anticancer properties against breast cancer cells', *Bioorganic & Medicinal Chemistry*. Elsevier Ltd, 22(6), pp. 1899–1908. doi: 10.1016/j.bmc.2014.01.051.

Siegel, R. L., Miller, K. D. and Jemal, A. (2015) 'Cancer statistics, 2015', *CA: A Cancer Journal for Clinicians*, 65(1), pp. 5–29. doi: 10.3322/caac.21254.

Siegel, R. L., Miller, K. D. and Jemal, A. (2016) 'Cancer statistics, 2016', *CA: A Cancer Journal for Clinicians*, 66(1), pp. 7–30. doi: 10.3322/caac.21332.

Siegel, R., Naishadham, D. and Jemal, A. (2013) 'Cancer statistics, 2013.', *CA: a cancer journal for clinicians*, 63(1), pp. 11–30. doi: 10.3322/caac.21166.

da Silva, R. D. *et al.* (2013) 'Impact of Statin Use on Oncologic Outcomes in Patients with Urothelial Carcinoma of the Bladder Treated with Radical Cystectomy', *The Journal of Urology*. Springer, New York, 190(2), pp. 487–492. doi: 10.1016/j.juro.2013.02.003.

Silwal-Pandit, L., Langerød, A. and Børresen-Dale, A.-L. (2017) '*TP53* Mutations in Breast and Ovarian Cancer', *Cold Spring Harbor Perspectives in Medicine*, 7(1), p. a026252. doi: 10.1101/cshperspect.a026252.

Singh, B. *et al.* (2002) 'p53 regulates cell survival by inhibiting PIK3CA in squamous cell carcinomas.', *Genes & development*. Cold Spring Harbor Laboratory Press, 16(8), pp. 984–93. doi: 10.1101/gad.973602.

Sionov, R. V. *et al.* (2008) 'Mechanisms regulating the susceptibility of hematopoietic malignancies to glucocorticoid-induced apoptosis.', *Advances in cancer research*, 101, pp. 127–248. doi: 10.1016/S0065-230X(08)00406-5.

Sionov, R. V., Hayon, I. L. and Haupt, Y. (2013) 'The Regulation of p53 Growth Suppression'. Landes Bioscience.

Siperstein, M. D. (1970) 'Cholesterol and cancer.', *Transactions of the American Clinical and Climatological Association*, 81, pp. 107–18. Available at: http://www.ncbi.nlm.nih.gov/pubmed/4330034 (Accessed: 8 February 2017).

Siperstein, M. D. and Fagan, V. M. (1964) 'Deletion of the Cholesterol-negative Feedback System in Liver Tumors', *Cancer Research*, 24(7). Available at: http://cancerres.aacrjournals.org/content/24/7/1108 (Accessed: 11 April 2017).

Sławińska-Brych, A., Zdzisińska, B. and Kandefer-Szerszeń, M. (2014) 'Fluvastatin inhibits growth and alters the malignant phenotype of the C6 glioma cell line.', *Pharmacological reports : PR*, 66(1), pp. 121–9. doi: 10.1016/j.pharep.2014.01.002.

Sleijfer, S. *et al.* (2005) 'The potential of statins as part of anti-cancer treatment.', *European journal of cancer (Oxford, England : 1990)*, 41(4), pp. 516–22. doi: 10.1016/j.ejca.2004.12.009.

Smith, E. R. and Xu, X.-X. (2003) 'Etiology of epithelial ovarian cancer: a cellular mechanism for the role of gonadotropins', *Gynecologic Oncology*, 91(1), pp. 1–2. doi: 10.1016/S0090-8258(03)00463-3.

Smith, E. R. and Xu, X.-X. (2008) 'Ovarian ageing, follicle depletion, and cancer: a hypothesis for the aetiology of epithelial ovarian cancer involving follicle depletion.', *The Lancet. Oncology*. Elsevier Ltd, 9(11), pp. 1108–11. doi: 10.1016/S1470-2045(08)70281-X.

Smith, L. L. and Johnson, B. H. (1989) 'Biological activities of oxysterols', *Free Radical Biology and Medicine*, pp. 285–332. doi: 10.1016/0891-5849(89)90136-6.

Smittenaar, C. *et al.* (2016) 'Cancer incidence and mortality projections in the UK until 2035', *British Journal of Cancer*, 115(9), pp. 1147–1155. doi: 10.1038/bjc.2016.304.

Sonnenblick, A. *et al.* (2014) 'An update on PARP inhibitors—moving to the adjuvant setting', *Nature Reviews Clinical Oncology*, 12(1), pp. 27–41. doi: 10.1038/nrclinonc.2014.163.

Sorrell, A. D. *et al.* (2013) 'Tumor protein p53 (TP53) testing and Li-Fraumeni syndrome : current status of clinical applications and future directions.', *Molecular diagnosis & therapy*. NIH Public Access, 17(1), pp. 31–47. doi: 10.1007/s40291-013-0020-0.

Sorrentino, G. *et al.* (2014) 'Metabolic control of YAP and TAZ by the mevalonate pathway', *Nature Cell Biology*, 16(4), pp. 357–366. doi: 10.1038/ncb2936.

Soslow, R. a (2008) 'Histologic subtypes of ovarian carcinoma: an overview.', *International journal of gynecological pathology : official journal of the International Society of Gynecological Pathologists*, 27(2), pp. 161–74. doi: 10.1097/PGP.0b013e31815ea812.

Soussi, T. and Béroud, C. (2001) 'Assessing TP53 status in human tumours to evaluate

clinical outcome', *Nature Reviews Cancer*. Nature Publishing Group, 1(3), pp. 233–239. doi: 10.1038/35106009.

Spann, N. J. and Glass, C. K. (2013) 'Sterols and oxysterols in immune cell function.', *Nature immunology*, 14(9), pp. 893–900. doi: 10.1038/ni.2681.

van der Spek, E. *et al.* (2007) 'High dose simvastatin does not reverse resistance to vincristine, adriamycin, and dexamethasone (VAD) in myeloma.', *Haematologica*, 92(12). doi: 10.3324/haematol.12071.

Van Der Spek, E. *et al.* (2006) 'Dose-finding study of high-dose simvastatin combined with standard chemotherapy in patients with relapsed or refractory myeloma or lymphoma', *Haematologica*, 91(4), pp. 542–545.

Srikanth, S., Woo, J. S. and Gwack, Y. (2017) 'A large Rab GTPase family in a small GTPase world', *Small GTPases*. Taylor & Francis, 8(1), pp. 43–48. doi: 10.1080/21541248.2016.1192921.

Steffensen, K. *et al.* (2016) 'Identification of high-risk patients by human epididymis protein 4 levels during follow-up of ovarian cancer', *Oncology Letters*. doi: 10.3892/ol.2016.4533.

Steffensen, K. D. *et al.* (2011) 'Prognostic Impact of Prechemotherapy Serum Levels of HER2, CA125, and HE4 in Ovarian Cancer Patients.', *International journal of gynecological cancer official journal of the International Gynecological Cancer Society*, 21(6), pp. 1040–1047. doi: 10.1097/IGC.0b013e31821e052e.

Stenmark, H. (2009) 'Rab GTPases as coordinators of vesicle traffic.', *Nature reviews. Molecular cell biology*. Nature Publishing Group, 10(8), pp. 513–25. doi: 10.1038/nrm2728.

Stewart, L. M. *et al.* (2013) 'In vitro fertilization, endometriosis, nulliparity and ovarian cancer risk.', *Gynecologic oncology*. Elsevier Inc., 128(2), pp. 260–4. doi: 10.1016/j.ygyno.2012.10.023.

Strano, S. *et al.* (2007) 'Mutant p53: an oncogenic transcription factor', *Oncogene*, 26(15), pp. 2212–2219. doi: 10.1038/sj.onc.1210296.

Stresing, V. *et al.* (2007) 'Bisphosphonates in cancer therapy.', *Cancer letters*, 257(1), pp. 16–35. doi: 10.1016/j.canlet.2007.07.007.

Sun, J. *et al.* (1999) 'Antitumor efficacy of a novel class of non-thiol-containing peptidomimetic inhibitors of farnesyltransferase and geranylgeranyltransferase I: Combination therapy with the cytotoxic agents cisplatin, taxol, and gemcitabine', *Cancer Research*, 59(19), pp. 4919–4926. doi: 10.1016/s0163-7258(97)00014-4.

Sun, J. *et al.* (2003) 'Geranylgeranyltransferase I Inhibitor GGTI-2154 Induces Breast Carcinoma Apoptosis and Tumor Regression in H-Ras Transgenic Mice', *Cancer Research*, 63(24). Available at: http://cancerres.aacrjournals.org/content/63/24/8922 (Accessed: 7 April 2017).

Sundahl, N. *et al.* (2016) 'Selective glucocorticoid receptor-activating adjuvant therapy in cancer treatments', *Oncoscience*, 3(7–8), pp. 22–30. doi: 10.18632/oncoscience.315.

Swanson, K. and Hohl, R. (2006) 'Anti-Cancer Therapy: Targeting the Mevalonate Pathway', *Current Cancer Drug Targets*, 6(1), pp. 15–37. doi: 10.2174/156800906775471743.

Swinnen, J. V, Brusselmans, K. and Verhoeven, G. (2006) 'Increased lipogenesis in cancer cells: new players, novel targets', *Current Opinion in Clinical Nutrition and Metabolic Care*, 9(4), pp. 358–365. doi: 10.1097/01.mco.0000232894.28674.30.

Swisher, S. G. *et al.* (2003) 'Induction of p53-regulated genes and tumor regression in lung cancer patients after intratumoral delivery of adenoviral p53 (INGN 201) and radiation therapy', *Clinical Cancer Research*, 9(1 I), pp. 93–101.

Syed, V. and Ho, S.-M. (2003) 'Progesterone-induced apoptosis in immortalized normal and malignant human ovarian surface epithelial cells involves enhanced expression of FasL', *Oncogene*. Nature Publishing Group, 22(44), pp. 6883–6890. doi: 10.1038/sj.onc.1206828.

Tait, S. W. G. and Green, D. R. (2010) 'Mitochondria and cell death: outer membrane permeabilization and beyond', *Nature Reviews Molecular Cell Biology*, 11(9), pp. 621–632. doi: 10.1038/nrm2952.

Takai, Y., Sasaki, T. and Matozaki, T. (2001) 'Small GTP-binding proteins.', *Physiological reviews*, 81(1), pp. 153–208. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11152757.

Tamura, T. *et al.* (2011) 'Zoledronic acid, a third-generation bisphosphonate, inhibits cellular growth and induces apoptosis in oral carcinoma cell lines', *Oncology Reports*, 25(4), pp. 1139–1143. doi: 10.3892/or.2011.1152.

Tang, F.-H. *et al.* (2017) 'KRAS mutation coupled with p53 loss is sufficient to induce ovarian carcinosarcomas in mice', *International Journal of Cancer*, 140(8), pp. 1860–1869. doi: 10.1002/ijc.30591.

Tapia, G. and Diaz-Padill, I. (2013) 'Molecular Mechanisms of Platinum Resistance in Ovarian Cancer', in *Ovarian Cancer - A Clinical and Translational Update*. InTech. doi: 10.5772/55562.

Taylor-Harding, B. *et al.* (2010) 'Fluvastatin and cisplatin demonstrate synergistic cytotoxicity in epithelial ovarian cancer cells.', *Gynecologic oncology*. Elsevier Inc., 119(3), pp. 549–56. doi: 10.1016/j.ygyno.2010.08.017.

Teplinsky, E. and Muggia, F. (2015) 'EGFR and HER2: is there a role in ovarian cancer?', *Translational Cancer Research*, 4(1), pp. 107–117. doi: 10.21037/3934.

Testa, J. R. *et al.* (1994) 'Spontaneous Transformation of Rat Ovarian Surface Epithelial Cells Results in Well to Poorly Differentiated Tumors with a Parallel Range of Cytogenetic Complexity Spontaneous Transformation of Rat Ovarian Surface Epithelial Cells Results in Well to Poorly', pp. 2778–2784.

Thibault, A. *et al.* (1996) ‘Phase I study of lovastatin , an inhibitor of the mevalonate pathway , in patients with cancer . of the Mevalonate in Patients’, pp. 483–491.

Thigpen, J. T. *et al.* (2005) ‘Role of pegylated liposomal doxorubicin in ovarian cancer.’, *Gynecologic oncology*, 96(1), pp. 10–8. doi: 10.1016/j.ygyno.2004.09.046.

Thurnher, M., Gruenbacher, G. and Nussbaumer, O. (2013) ‘Regulation of mevalonate metabolism in cancer and immune cells.’, *Biochimica et biophysica acta*. Elsevier B.V., 1831(6), pp. 1009–15. doi: 10.1016/j.bbalip.2013.03.003.

Thurnher, M., Nussbaumer, O. and Gruenbacher, G. (2012) ‘Novel aspects of mevalonate pathway inhibitors as antitumor agents.’, *Clinical cancer research : an official journal of the American Association for Cancer Research*, 18(13), pp. 3524–31. doi: 10.1158/1078-0432.CCR-12-0489.

Torgovnick, A. and Schumacher, B. (2015) ‘DNA repair mechanisms in cancer development and therapy.’, *Frontiers in genetics*. Frontiers Media SA, 6, p. 157. doi: 10.3389/fgene.2015.00157.

Traverso, N. *et al.* (2013) ‘Role of glutathione in cancer progression and chemoresistance.’, *Oxidative medicine and cellular longevity*, 2013, p. 972913. doi: 10.1155/2013/972913.

Trimbos, J. B. *et al.* (2003) ‘Impact of adjuvant chemotherapy and surgical staging in early-stage ovarian carcinoma: European Organisation for Research and Treatment of Cancer-Adjuvant ChemoTherapy in Ovarian Neoplasm trial.’, *Journal of the National Cancer Institute*, 95(2), pp. 113–125. doi: 10.1093/jnci/95.2.113.

Tsao, S.-W. *et al.* (1995) ‘Characterization of Human Ovarian Surface Epithelial Cells Immortalized by Human Papilloma Viral Oncogenes (HPV-E6E7 ORFs)’, *Experimental Cell Research*, 218(2), pp. 499–507. doi: 10.1006/excr.1995.1184.

Tsilidis, K. K. *et al.* (2011) ‘Oral contraceptive use and reproductive factors and risk of ovarian cancer in the European Prospective Investigation into Cancer and Nutrition.’, *British journal of cancer*, 105(9), pp. 1436–42. doi: 10.1038/bjc.2011.371.

Tsubaki, M. *et al.* (2015) ‘Statins improve survival by inhibiting spontaneous metastasis and tumor growth in a mouse melanoma model.’, *American journal of cancer research*. e-Century Publishing Corporation, 5(10), pp. 3186–97. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC4656740.

Tu, Y. S. *et al.* (2011) ‘Involvement of Chk1-Cdc25A-cyclin A/CDk2 pathway in simvastatin induced S-phase cell cycle arrest and apoptosis in multiple myeloma cells’, *European Journal of Pharmacology*, 670(2–3), pp. 356–364. doi: 10.1016/j.ejphar.2011.09.031.

Tworoger, S. S. *et al.* (2007) ‘Association of oral contraceptive use, other contraceptive methods, and infertility with ovarian cancer risk.’, *American journal of epidemiology*, 166(8), pp. 894–901. doi: 10.1093/aje/kwm157.

Tworoger, S. S. *et al.* (2008) ‘Caffeine, alcohol, smoking, and the risk of incident epithelial

ovarian cancer.', *Cancer*, 112(5), pp. 1169–77. doi: 10.1002/cncr.23275.

Valencia, A. *et al.* (1991) 'The ras Protein Family: Evolutionaryy Tree and Role of Conserved Amino Acids', *Biochemistry*, 30(19), pp. 4637–4648. doi: 10.1021/bi00473a001.

Vallianou, N. G. *et al.* (2014) 'Statins and cancer.', *Anti-cancer agents in medicinal chemistry*, 14(5), pp. 706–12. Available at: http://www.ncbi.nlm.nih.gov/pubmed/24295174 (Accessed: 7 March 2017).

Vasey, P. a (2003) 'Resistance to chemotherapy in advanced ovarian cancer: mechanisms and current strategies.', *British journal of cancer*, 89 Suppl 3, pp. S23-8. doi: 10.1038/sj.bjc.6601497.

Vasudevan, A. *et al.* (1999) 'Potent, highly selective, and non-thiol inhibitors of protein geranylgeranyltransferase-I', *Journal of Medicinal Chemistry*, 42(8), pp. 1333–1340. doi: 10.1021/jm9900873.

Vaughan, S. *et al.* (2011) 'Rethinking ovarian cancer: recommendations for improving outcomes.', *Nature reviews. Cancer*. Nature Publishing Group, 11(10), pp. 719–25. doi: 10.1038/nrc3144.

Vega, F. M. and Ridley, A. J. (2008) 'Rho GTPases in cancer cell biology', *FEBS Letters*, 582(14), pp. 2093–2101. doi: 10.1016/j.febslet.2008.04.039.

Veneris, J. T. *et al.* (2017) 'High glucocorticoid receptor expression predicts short progression-free survival in ovarian cancer', *Gynecologic Oncology*, 146(1), pp. 153–160. doi: 10.1016/j.ygyno.2017.04.012.

Vergote, I. *et al.* (2010) 'Randomized phase III study of canfosfamide in combination with pegylated liposomal doxorubicin compared with pegylated liposomal doxorubicin alone in platinum-resistant ovarian cancer', *International Journal of Gynecological Cancer*, 20(5), pp. 772–780. doi: 10.1111/IGC.0b013e3181daaf59.

Vigil, D. *et al.* (2010) 'Ras superfamily GEFs and GAPs: validated and tractable targets for cancer therapy?', *Nature reviews. Cancer*, 10(12), pp. 842–57. doi: 10.1038/nrc2960.

Vine, M. F. *et al.* (2003) 'Characterization of prediagnostic symptoms among primary epithelial ovarian cancer cases and controls', *Gynecologic Oncology*, 90(1), pp. 75–82. doi: 10.1016/S0090-8258(03)00175-6.

Vinet, L. and Zhedanov, A. (2010) 'A "missing" family of classical orthogonal polynomials', *Antimicrobial agents and chemotherapy*. Edited by Intergovernmental Panel on Climate Change. Cambridge: Cambridge University Press, 58(12), pp. 7250–7. doi: 10.1088/1751-8113/44/8/085201.

Vogel, T. J. *et al.* (2017) 'Statin treatment is associated with survival in a nationally representative population of elderly women with epithelial ovarian cancer.', *Gynecologic oncology*. National Cancer Institute Surveillance, Epidemiology, and End Results Program, 135(0), pp. 741–756. doi: 10.1016/j.ygyno.2017.05.009.

Volden, P. A. and Conzen, S. D. (2013) 'The influence of glucocorticoid signaling on tumor progression', *Brain, Behavior, and Immunity*, 30, pp. S26–S31. doi: 10.1016/j.bbi.2012.10.022.

Volpe, A. *et al.* (2013) 'Advanced bladder cancer: new agents and new approaches. A review.', *Urologic oncology*. Elsevier Inc., 31(1), pp. 9–16. doi: 10.1016/j.urolonc.2010.03.022.

Vousden, K. H. and Ryan, K. M. (2009) 'P53 and Metabolism.', *Nature reviews. Cancer*, 9(10), pp. 691–700. doi: 10.1038/nrc2715.

Wade, M., Wang, Y. V. and Wahl, G. M. (2010) 'The p53 orchestra: Mdm2 and Mdmx set the tone', *Trends in Cell Biology*, 20(5), pp. 299–309. doi: 10.1016/j.tcb.2010.01.009.

Wang, H. *et al.* (2004) 'Pretreatment with Dexamethasone Increases Antitumor Activity of Carboplatin and Gemcitabine in Mice Bearing Human Cancer Xenografts: In Vivo Activity, Pharmacokinetics, and Clinical Implications for Cancer Chemotherapy', *Clinical Cancer Research*, 10(5), pp. 1633–1644. doi: 10.1158/1078-0432.CCR-0829-3.

Wang, H. *et al.* (2007) 'Dexamethasone as a chemosensitizer for breast cancer chemotherapy: Potentiation of the antitumor activity of adriamycin, modulation of cytokine expression, and pharmacokinetics', *International Journal of Oncology*, 30(4), pp. 947–953. doi: 10.3892/ijo.30.4.947.

Wang, J., Yao, X. and Huang, J. (2017) 'New tricks for human farnesyltransferase inhibitor: cancer and beyond', *Med. Chem. Commun.* Royal Society of Chemistry. doi: 10.1039/C7MD00030H.

Wang, M. and Casey, P. J. (2016) 'Protein prenylation: unique fats make their mark on biology', *Nature Reviews Molecular Cell Biology*. Nature Research, 17(2), pp. 110–122. doi: 10.1038/nrm.2015.11.

Wang, S. *et al.* (2013) 'Clinical analysis of ovarian epithelial carcinoma with coexisting pelvic endometriosis.', *American journal of obstetrics and gynecology*, 208(5), p. 413.e1-5. doi: 10.1016/j.ajog.2012.12.004.

Wang, T. *et al.* (2015) 'Identification and characterization of essential genes in the human genome', *Science*, 350(6264).

Wang, V. *et al.* (2005) 'Ovarian cancer is a heterogeneous disease', *Cancer Genetics and Cytogenetics*, 161(2), pp. 170–173. doi: 10.1016/j.cancergencyto.2004.12.014.

Wang, X. and Simon, R. (2013) 'Identification of potential synthetic lethal genes to p53 using a computational biology approach', *BMC Medical Genomics*, 6(1), p. 30. doi: 10.1186/1755-8794-6-30.

Wang, Z. *et al.* (2003) 'Microarray analysis uncovers the induction of the proapoptotic BH3-only protein Bim in multiple models of glucocorticoid-induced apoptosis', *Journal of Biological Chemistry*, 278(26), pp. 23861–23867. doi: 10.1074/jbc.M301843200.

Wang, Z. and Sun, Y. (2010) 'Targeting p53 for Novel Anticancer Therapy.',

Translational oncology, 3(1), pp. 1–12. doi: 10.1593/tlo.09250.

Wang And, X. and Guo, Z. (2007) 'The role of sulfur in platinum anticancer chemotherapy.', *Anti-cancer agents in medicinal chemistry*, 7(1), pp. 19–34. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17266503.

Warita, K. *et al.* (2014) 'Statin-induced mevalonate pathway inhibition attenuates the growth of mesenchymal-like cancer cells that lack functional E-cadherin mediated cell cohesion.', *Scientific reports*, 4, p. 7593. doi: 10.1038/srep07593.

Wasko, B. M., Dudakovic, A. and Hohl, R. J. (2011) 'Bisphosphonates induce autophagy by depleting geranylgeranyl diphosphate.', *The Journal of pharmacology and experimental therapeutics*, 337(2), pp. 540–6. doi: 10.1124/jpet.110.175521.

Watanabe, M. *et al.* (2008) 'Inhibitors of protein geranylgeranyltransferase I and Rab geranylgeranyltransferase identified from a library of allenoate-derived compounds.', *The Journal of biological chemistry*. American Society for Biochemistry and Molecular Biology, 283(15), pp. 9571–9. doi: 10.1074/jbc.M706229200.

Weber, M. S. *et al.* (2006) 'Statins in the treatment of central nervous system autoimmune disease.', *Journal of neuroimmunology*, 178(1–2), pp. 140–8. doi: 10.1016/j.jneuroim.2006.06.006.

Weis, S. M. and Cheresh, D. A. (2011) 'Tumor angiogenesis: molecular pathways and therapeutic targets', *Nature Medicine*, 17(11), pp. 1359–1370. doi: 10.1038/nm.2537.

Welsh, C. *et al.* (2004) 'Reduced levels of XPA, ERCC1 and XPF DNA repair proteins in testis tumor cell lines.', *International journal of cancer. Journal international du cancer*, 110(3), pp. 352–61. doi: 10.1002/ijc.20134.

Wennerberg, K. (2005) 'The Ras superfamily at a glance', *Journal of Cell Science*, 118(5), pp. 843–846. doi: 10.1242/jcs.01660.

Wiggans, A. J. *et al.* (2015) 'Poly(ADP-ribose) polymerase (PARP) inhibitors for the treatment of ovarian cancer', in Morrison, J. (ed.) *Cochrane Database of Systematic Reviews*. Chichester, UK: John Wiley & Sons, Ltd, p. CD007929. doi: 10.1002/14651858.CD007929.pub3.

Williams, J. *et al.* (2005) 'Expression of Bcl-xL in ovarian carcinoma is associated with chemoresistance and recurrent disease', *Gynecologic Oncology*, 96(2), pp. 287–295. doi: 10.1016/j.ygyno.2004.10.026.

Wiman, K. (2006) 'News and Commentary Strategies for therapeutic targeting of the p53 pathway in cancer', *Cell Death and Differentiation*, 13, pp. 921–926. doi: 10.1038/sj.cdd.4401921.

Wiman, K. G. (2007) 'Restoration of Wild-Type p53 Function in Human Tumors: Strategies for Efficient Cancer Therapy', *Advances in Cancer Research*, pp. 321–338. doi: 10.1016/S0065-230X(06)97014-6.

Winter-Vann, A. M. and Casey, P. J. (2005) 'Post-prenylation-processing enzymes as new

targets in oncogenesis.', *Nature reviews. Cancer*, 5(May), pp. 405–412. doi: 10.1038/nrc1612.

Winter, M. C. and Coleman, R. E. (2013) 'Bisphosphonates in the adjuvant treatment of breast cancer.', *Clinical oncology (Royal College of Radiologists (Great Britain))*. Elsevier Ltd, 25(2), pp. 135–45. doi: 10.1016/j.clon.2012.10.010.

Witham, J. *et al.* (2007) 'The Bcl-2/Bcl-XL family inhibitor ABT-737 sensitizes ovarian cancer cells to carboplatin.', *Clinical cancer research : an official journal of the American Association for Cancer Research*. Clinical Cancer Research, 13(23), pp. 7191–8. doi: 10.1158/1078-0432.CCR-07-0362.

Wolf, D., Harris, N. and Rotter, V. (1984) 'Reconstitution of p53 expression in a nonproducer Ab-MuLV-transformed cell line by transfection of a functional p53 gene', *Cell*, 38(1), pp. 119–126. doi: 10.1016/0092-8674(84)90532-4.

De Wolf, E. *et al.* (2017) 'Dietary geranylgeraniol can limit the activity of pitavastatin as a potential treatment for drug-resistant ovarian cancer', *Scientific Reports*, 7(1), p. 5410. doi: 10.1038/s41598-017-05595-4.

Wong, A. S. *et al.* (1999) 'Constitutive and conditional cadherin expression in cultured human ovarian surface epithelium: influence of family history of ovarian cancer.', *International journal of cancer. Journal international du cancer*, 81(2), pp. 180–8. doi: 10.1002/(SICI)1097-0215(19990412)81:2<180::AID-IJC3>3.0.CO;2-7.

Wong, K. B. *et al.* (1999) 'Hot-spot mutants of p53 core domain evince characteristic local structural changes.', *Proceedings of the National Academy of Sciences of the United States of America*, 96(15), pp. 8438–42. doi: 10.1073/pnas.96.15.8438.

Wong, W.-L., Dimitroulakos and Penn, L. (2002) 'REVIEW HMG-CoA reductase inhibitors and the malignant cell: the statin family of drugs as triggers of tumor-specific apoptosis', (September 2001), pp. 508–519. doi: 10.1038/sj/leu/2402476.

Wong, W. W. *et al.* (2001) 'Cerivastatin Triggers Tumor-specific Apoptosis with Higher Efficacy than Lovastatin Cerivastatin Triggers Tumor-specific Apoptosis with Higher Efficacy than Lovastatin 1', pp. 2067–2075.

Wong, W. W.-L. *et al.* (2007) 'Determinants of sensitivity to lovastatin-induced apoptosis in multiple myeloma.', *Molecular cancer therapeutics*, 6(6), pp. 1886–97. doi: 10.1158/1535-7163.MCT-06-0745.

Wright, A. A., Bohlke, K. and Edelson, M. I. (2016) 'Neoadjuvant Chemotherapy for Newly Diagnosed Advanced Ovarian Cancer: Society of Gynecologic Oncology and ASCO Clinical Practice Guideline Summary.', *Journal of oncology practice*. American Society of Clinical OncologyAlexandria, VA, 12(12), pp. 1254–1257. doi: 10.1200/JOP.2016.016873.

Wu, Q. *et al.* (2017) *MiR-221-3p targets ARF4 and inhibits the proliferation and migration of epithelial ovarian cancer cells, Biochemical and Biophysical Research Communications*. doi: 10.1016/j.bbrc.2017.01.002.

Wu, W. *et al.* (2004) 'Microarray Analysis Reveals Glucocorticoid-Regulated Survival Genes That Are Associated With Inhibition of Apoptosis in Breast Epithelial Cells', *Cancer Research*, 64(5).

Wu, Y. S. *et al.* (2017) 'Bevacizumab combined with chemotherapy for ovarian cancer: an updated systematic review and meta-analysis of randomized controlled trials.', *Oncotarget*. Impact Journals, 8(6), pp. 10703–10713. doi: 10.18632/oncotarget.12926.

Xia, F., Lee, C. W. and Altieri, D. C. (2008) 'Tumor Cell Dependence on Ran-GTP–Directed Mitosis', *Cancer Research*, 68(6). Available at: http://cancerres.aacrjournals.org/content/68/6/1826 (Accessed: 4 April 2017).

Xu, J. *et al.* (2008) 'Assessment of the impact of dosing time on the pharmacokinetics/pharmacodynamics of prednisolone.', *The AAPS journal*, 10(2), pp. 331–41. doi: 10.1208/s12248-008-9038-3.

Yakirevich, E. *et al.* (2006) 'Multidrug resistance-related phenotype and apoptosis-related protein expression in ovarian serous carcinomas', *Gynecologic Oncology*, 100(1), pp. 152–159. doi: 10.1016/j.ygyno.2005.08.050.

Yang, D. *et al.* (2017) 'High Expression of Cell Division Cycle 42 Promotes Pancreatic Cancer Growth and Predicts Poor Outcome of Pancreatic Cancer Patients', *Digestive Diseases and Sciences*. Springer US, 62(4), pp. 958–967. doi: 10.1007/s10620-017-4451-z.

Yang, X. *et al.* (2004) 'Resistance to chemotherapy-induced apoptosis via decreased caspase-3 activity and overexpression of antiapoptotic proteins in ovarian cancer.', *Journal of cancer research and clinical oncology*, 130(7), pp. 423–8. doi: 10.1007/s00432-004-0556-9.

Yang, X. *et al.* (2005) 'Regulation of HtrA2/Omi by X-linked inhibitor of apoptosis protein in chemoresistance in human ovarian cancer cells', *Gynecologic Oncology*, 97(2), pp. 413–421. doi: 10.1016/j.ygyno.2004.12.055.

Yang, X. *et al.* (2017) 'Overexpression of Rab1B and MMP9 predicts poor survival and good response to chemotherapy in patients with colorectal cancer', *Aging*. doi: 10.18632/aging.101200.

Yeganeh, B. *et al.* (2014) 'Targeting the mevalonate cascade as a new therapeutic approach in heart disease, cancer and pulmonary disease.', *Pharmacology & therapeutics*. Elsevier B.V., 143(1), pp. 87–110. doi: 10.1016/j.pharmthera.2014.02.007.

Yemelyanov, A. *et al.* (2007) 'Tumor suppressor activity of glucocorticoid receptor in the prostate', *Oncogene*. Nature Publishing Group, 26(13), pp. 1885–1896. doi: 10.1038/sj.onc.1209991.

Yemelyanova, A. *et al.* (2011) 'Immunohistochemical staining patterns of p53 can serve as a surrogate marker for TP53 mutations in ovarian carcinoma: an immunohistochemical and nucleotide sequencing analysis', *Modern Pathology*, 24(9), pp. 1248–1253. doi: 10.1038/modpathol.2011.85.

Yeudall, W. A., Wrighton, K. H. and Deb, S. (2013) 'Mutant p53 in cell adhesion and

motility', *Methods in Molecular Biology*, 962, pp. 135–146. doi: 10.1007/978-1-62703-236-0-11.

Yonish-Rouach, E. *et al.* (1991) 'Wild-type p53 induces apoptosis of myeloid leukaemic cells that is inhibited by interleukin-6.', *Nature*, pp. 345–7. doi: 10.1038/352345a0.

Yoshikawa, K. *et al.* (2010) 'Mutant p53 R248Q but not R248W enhances in vitro invasiveness of human lung cancer NCI-H1299 cells.', *Biomedical research (Tokyo, Japan)*, 31(6), pp. 401–11. doi: 10.2220/biomedres.31.401.

Yu, O. *et al.* (2014) 'Use of Statins and the Risk of Death in Patients With Prostate Cancer', *Journal of Clinical Oncology*, 32(1), pp. 5–11. doi: 10.1200/JCO.2013.49.4757.

Yuen, H.-F. *et al.* (2012) 'Ran Is a Potential Therapeutic Target for Cancer Cells with Molecular Changes Associated with Activation of the PI3K/Akt/mTORC1 and Ras/MEK/ERK Pathways', *Clinical Cancer Research*, 18(2). Available at: http://clincancerres.aacrjournals.org/content/18/2/380 (Accessed: 4 April 2017).

Zeimet, A. G. and Marth, C. (2003) 'Why did p53 gene therapy fail in ovarian cancer?', *The Lancet Oncology*, 4(7), pp. 415–422. doi: 10.1016/S1470-2045(03)01139-2.

Zekri, J., Mansour, M. and Karim, S. M. (2014) 'The anti-tumour effects of zoledronic acid', *Journal of Bone Oncology*. Elsevier, 3(1), pp. 25–35. doi: 10.1016/j.jbo.2013.12.001.

Zhang, C. *et al.* (2007) 'Clinical and mechanistic aspects of glucocorticoid-induced chemotherapy resistance in the majority of solid tumors', *Cancer Biology and Therapy*, 6(2), pp. 278–287. doi: 10.4161/cbt.6.2.3652.

Zhang, J. *et al.* (2013) 'Statins, autophagy and cancer metastasis.', *The international journal of biochemistry & cell biology*, 45(3), pp. 745–52. doi: 10.1016/j.biocel.2012.11.001.

Zhang, J., Dricu, A. and Sjövall, J. (1997) 'Studies on the relationships between 7??-hydroxylation and the ability of 25- and 27-hydroxycholesterol to suppress the activity of HMG-CoA reductase', *Biochimica et Biophysica Acta - Lipids and Lipid Metabolism*, 1344(3), pp. 241–249. doi: 10.1016/S0005-2760(96)00148-8.

Zhang, Q. *et al.* (2002) 'Cell-specific abnormal prenylation of Rab proteins in platelets and melanocytes of the gunmetal mouse', *British Journal of Haematology*. Blackwell Science Ltd, 117(2), pp. 414–423. doi: 10.1046/j.1365-2141.2002.03444.x.

Zhang, W. *et al.* (1994) 'A temperature-sensitive mutant of human p53.', *The EMBO journal*, 13(11), pp. 2535–44.

Zhang, W.-J. *et al.* (2016) 'Pitavastatin suppressed liver cancer cells in vitro and in vivo', *OncoTargets and Therapy*, Volume 9, pp. 5383–5388. doi: 10.2147/OTT.S106906.

Zhang, Y. *et al.* (2009) 'Lipophilic bisphosphonates as dual farnesyl/geranylgeranyl diphosphate synthase inhibitors: an X-ray and NMR investigation.', *Journal of the American Chemical Society*, 131(14), pp. 5153–62. doi: 10.1021/ja808285e.

Zhang, Y. *et al.* (2016) 'TP53 mutations in epithelial ovarian cancer', *Translational Cancer Research*, 5(6), pp. 650–663. doi: 10.21037/9452.

Zhao, W. *et al.* (2014) 'A new Bliss independence model to analyze drug combination data', *J Biomol Screen*, 19(5), pp. 817–821. doi: 10.1177/1087057114521867.

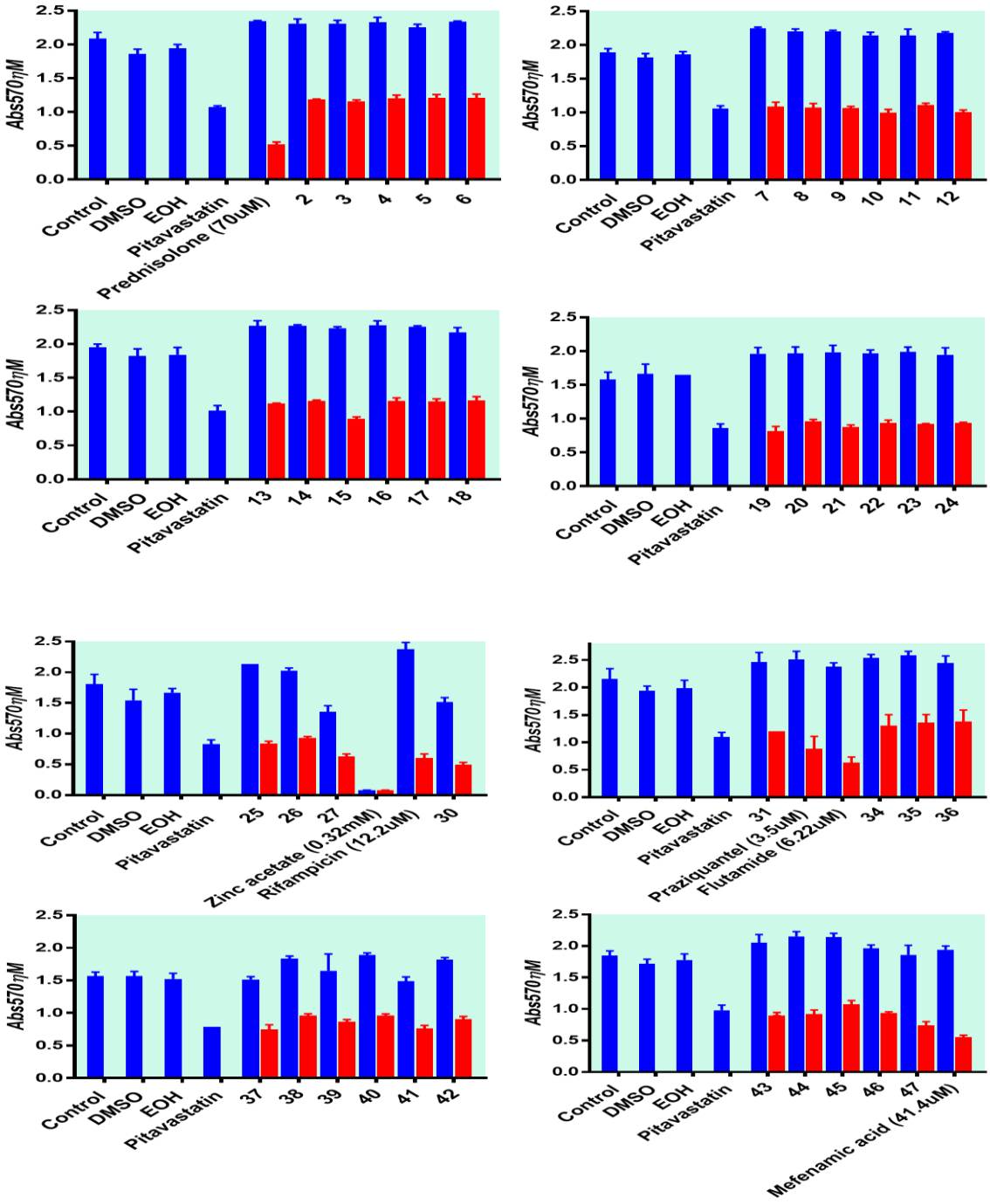
Zhong, W.-B. *et al.* (2003) 'Lovastatin Induces Apoptosis of Anaplastic Thyroid Cancer Cells via Inhibition of Protein Geranylgeranylation and de Novo Protein Synthesis', *Endocrinology*, 144(9), pp. 3852–3859. doi: 10.1210/en.2003-0098.

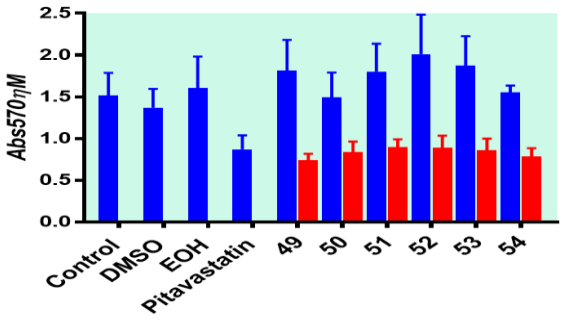
Zhou, B. *et al.* (2008) 'Hormone replacement therapy and ovarian cancer risk: a meta-analysis.', *Gynecologic oncology*, 108(3), pp. 641–51. doi: 10.1016/j.ygyno.2007.12.003.

Zietarska, M. *et al.* (2007) 'Molecular description of a 3D in vitro model for the study of epithelial ovarian cancer (EOC)', *Molecular Carcinogenesis*, 46(10), pp. 872–885. doi: 10.1002/mc.20315.

Appendix (I)

Ovcar-4 cell line (5000 cells/well/96 well plate) were exposed to the vehicle, pitavastatin (10µM), library compounds and to a combination of individual compound with pitavastatin for 72 hours. A triplicate of each drug addition was made and drugs effect measured using SRB assay (Mean±SD; n=2).

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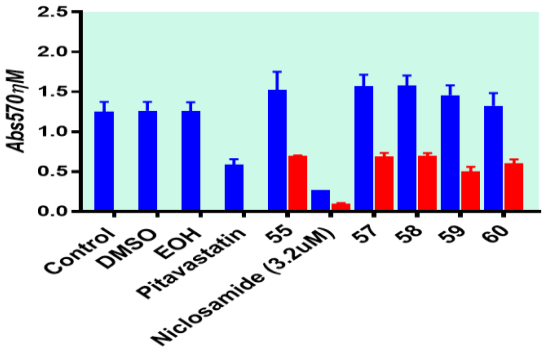
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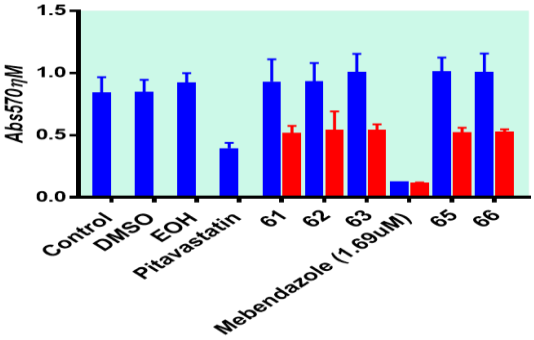
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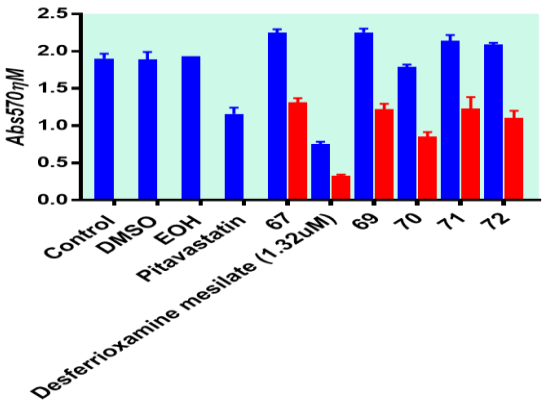
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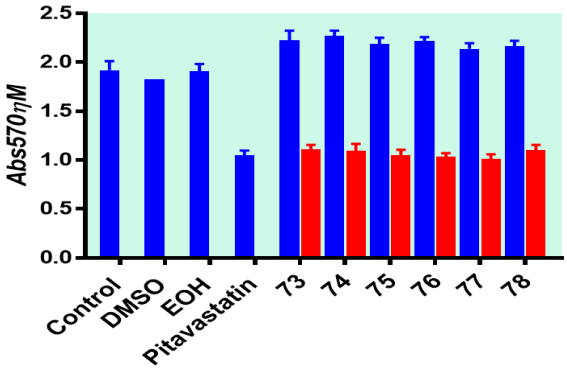
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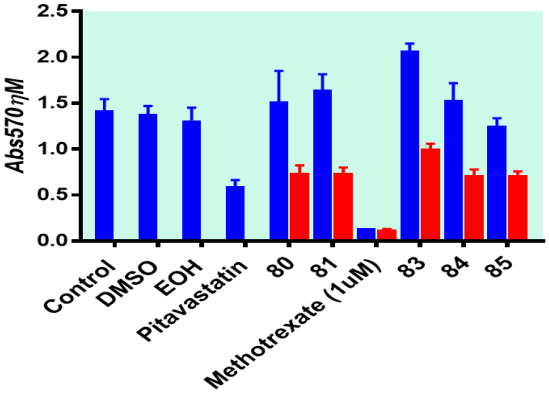
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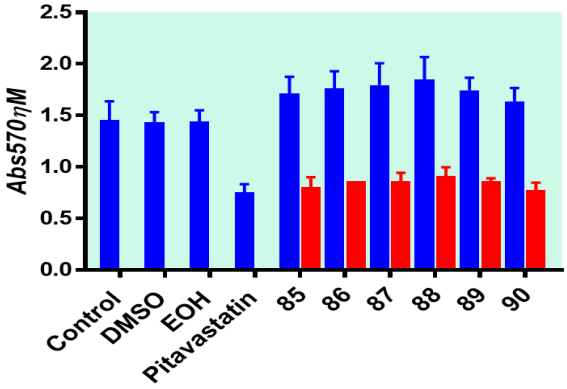
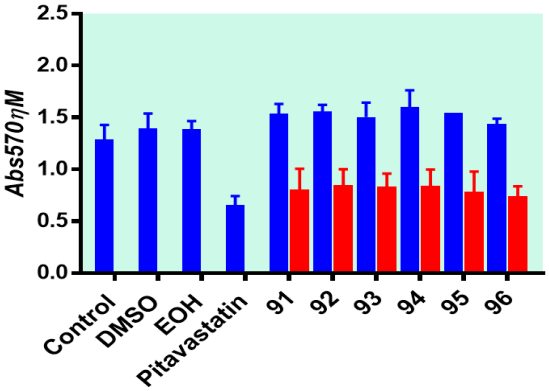
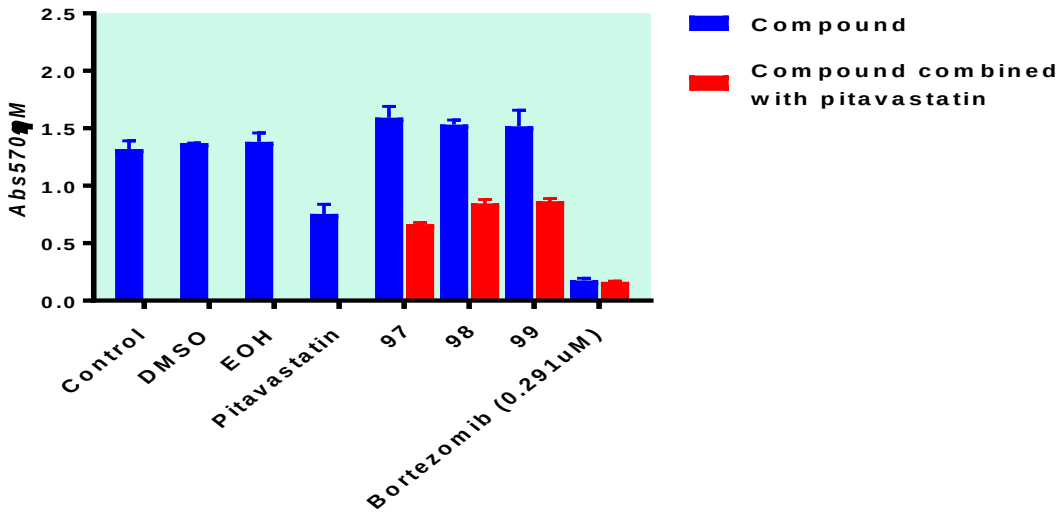
2.5
2.0
1.5
1.0
0.5
0.0
Control
DMSO
EOH
Pitavastatin
80
81
Methotrexate (1uM)
83
84
85


Abs570ηM
2.5
2.0
1.5
1.0
0.5
0.0
Control
DMSO
EOH
Pitavastatin
85
86
87
88
89
90


Abs570ηM
2.5
2.0
1.5
1.0
0.5
0.0
Control
DMSO
EOH
Pitavastatin
91
92
93
94
95
96


2.5
2.0
1.5
1.0
0.5
0.0
Abs570nM
Control
DMSO
EOH
Pitavastatin
97
98
99
Bortezomib (0.291uM)
Compound
Compound combined with pitavastatin