# RNA Polymerase I Inhibition in Ovarian Cancer

John Robert Cornelison

Lynchburg,Virginia

Masters of Science, Biotechnology, Johns Hopkins University, 2000

Bachelors of Science, Biology, George Mason University, 1998

A Dissertation presented to the Graduate Faculty of the University of Virginia School of Medicine in Candidacy for the Degree of Doctor of Philosophy

Department of Experimental Pathology

12/1/2020

This work is dedicated to all the researchers who showed me the way: Juha Kononen MD PhD, Paul Meltzer MD PhD, and Soe Than MD PhD.

Also to Chip Landen, MD,  Janet Cross, PhD, and Amy Bouton, PhD who gave me a chance.

But most importantly it is dedicated to those who made it possible; my wife Danielle Llaneza and my parents who tolerated graduate school for too long.

**LIST OF ABBREVIATIONS**

| | |
|---|---|
| **ADP** | **Adenosine diphosphate** |
| **ATP** | **Adenosine triphosphate** |
| **CCC** | **Clear Cell Carcinoma** |
| **cGAMP** | **Cyclic guanine monophosphate–adenosine monophosphate** |
| **cGAS** | **Cyclic cGAMP synthetase** |
| **chIP** | **Chromatin immunoprecipitation** |
| **CRISPR** | **Clustered Regularly Interspaced Short Palindromic Repeats** |
| **DNA** | **Deoxyribonucleic Acid** |
| **EMT** | **Epithelial to Mesenchymal Transition** |
| **EOC** | **Endometrioid Ovarian Cancer** |
| **GSH** | **Glutathione S** |
| **HR** | **Homologous recombination** |
| **HRD** | **Homologous recombination deficient** |
| **HGSOC** | **High Grade Serous Ovarian Cancer** |
| **IC50** | **50% or half maximal Inhibitory Concentration** |
| **LGSOC** | **Low Grade Serous Ovarian Cancer** |
| **MDR** | **Multi-Drug Resistance** |
| **MOC** | **Mucinous Ovarian Cancer** |
| **mRNA** | **Messenger Ribonucleic Acid** |
| **nCas9** | **nickase Cas9** |
| **NER** | **Nucleotide Excision Repair** |
| **NHEJ** | **Non-Homologous End Joining** |
| **OVAC** | **Ovarian Cancer** |
| **PARP** | **Poly ADP Ribose Polymerase** |
| **PCR** | **Polymerase Chain Reaction** |
| **Pol I** | **RNA polymerase I** |
| **POL1RB** | **RNA polymerase I subunit** |
| **Pt** | **Platinum chemotherapy** |
| **RNA** | **Ribonucleic Acid** |
| **RNA-seq** | **RNA high-throughput sequencing/next generation sequence** |
| **ROS** | **Reactive Oxygen Species** |
| **RRN3** | **RRN3 homologue** |
| **sgRNA** | **Single guide Ribonucleic Acid** |
| **STING** | **Stimulator of inflammatory genes** |
| **TCGA** | **The Cancer Genome Atlas** |
| **UBTF** | **Upstream Binding Transcription Factor** |

# RNA polymerase I inhibition in ovarian cancer

**Abstract**:

Ovarian cancer is the fifth leading cancer killer of women and little progress has been made in achieving better outcomes. A primary reason for its lethality is acquired chemotherapy resistance. We sought to model chemoresistance in patient derived xenografts (PDX) in mice that are treated with standard of care (SOC) chemotherapy. After transcriptomic profiling of treated PDX we identified the ribosome biogenesis system as upregulated in tumors surviving treatment. Our primary hypothesis is that chemoresistant ovarian cancer requires enhanced ribosome RNA transcription to survive chemotherapy, and pharmacologic inhibition of ribogenesis will kill chemoresistant disease. Our results indicated that ovarian cancer is sensitive to RNA polymerase I inhibition with CX-5461, that paclitaxel resistant cells may be hypersensitive to it, and that CX-5461 induces the DNA damage response. During the course of these experiments we observed an unreported effect of CX-5461 treatment. Cytosolic DNA began accumulating ~1 hour after treatment. The cytosolic DNA sensing system activated and led to a type I interferon response. Agonists to the cytosolic DNA sensing system are widely sought after as a possible means of increasing response rates to checkpoint inhibitors.

# 1 Introduction

## 1.1 Pathology

### 1.1.1 Ovarian cancer overview

Ovarian cancer is a complex array of disparate disease states categorized by cell type of origin and histologic subtype. Overall, ovarian cancer remains a rare occurrence, but it takes a devastating toll on women worldwide. Even with its relative rarity, it remains the 5$^{th}$ leading cancer killer of women (Jelovac & Armstrong, 2011). The American Cancer Society estimates that ~21,000 women in America will be diagnosed with ovarian cancer and ~13,000 will die of the disease in 2020 alone (Siegel et al., 2020). Worldwide those numbers are 10 fold higher with ~220,000 diagnoses and ~130,000 deaths (Bowtell et al., 2015; Jayson et al., 2014). The reasons for this lethality are multi-faceted, but generally can be summarized into 2 primary mechanisms: ovarian cancer is caught late in its tumor evolution due to an overall non-specific set of symptoms, and chemotherapy resistance is common (Agarwal & Kaye, 2003b; Herzog & Monk, 2017; J. Liu et al., 2020).

The three primary types of ovarian cancer are epithelial, germ cell, and sex cord stromal with each having distinct sub lineages (Berek et al., 2012). Epithelial is by far the most common and deadliest form of ovarian cancer and

therefore this thesis primarily focuses here (Cannistra, 2004). However, I will briefly introduce the other types.

### 1.1.2 Germ cell tumors

Germ cell tumors arise from the reproductive cell, usually in teenage or young adult females, and generally only on one ovary (Pectasides et al., 2008). Ovarian germ cell tumor, while sounding specific, actually covers several different types of cancer with dysgerminoma being the most prevalent (Low et al., 2012). While 30% of ovarian cancers are germ cell tumors, *malignant* germ cell tumors account for only 5% of ovarian cancers in developed countries (Low et al., 2012) .

There are two major classifications of germ cell tumors: germinomatous or seminomatous germ-cell tumors (GGCT, SGCT), and non-germinomatous or non-seminomatous germ-cell tumors (NGGCT, NSGCT) (FF et al., 2014). The classification underlies a significant clinical distinction: the non-germinomatous tumors tend to occur earlier in life, proliferate faster and have poorer prognosis (Maoz et al., 2020). In the case of benign disease, germ-cell tumors can be cured by ovary-sparing cystectomy or oophorectomy (Boussios et al., 2020).

Malignant germ cell tumors require aggressive interventions, with treatment availability greatly affecting prognosis. Optimum cytoreductive surgery with salpingo-oophorectomy/hysterectomy is often followed with a combination cytotoxic chemotherapy regiment. Bleomycin, etoposide and carboplatin

combinations are commonly used and tumors usually respond well to platinum-based chemotherapies (Low et al., 2012).

### 1.1.3 Sex cord stromal tumors

Sex cord stromal tumors are rare malignancies representing approximately 7% of the overall ovarian cancer burden (Horta & Cunha, 2015). Generally they are non-aggressive, develop slowly and present in a younger demographic than the most common epithelial ovarian tumors (Horta & Cunha, 2015). The stromal originating cells are involved in steroid hormone production. Therefore, sex cord stromal tumors are associated with various hormonal diseases or hormone-mediated syndromes (Horta & Cunha, 2015; Schultz et al., 2016; R. H. Young, 2018).

Tissue origin defines subtypes of sex cord stromal tumors as granulosa cell tumors for the adult granulosa cells inside the ovary, and thecoma for the thecal cells in the ovarian follicle (Dridi et al., 2018). Sertoli cell tumors are a much rarer variant that can effect both men and women (Q. Xu et al., 2018; R. H. Young, 2005). Germ cell tumors are often well-differentiated, slow-growing masses that don't respond to chemotherapies (Boussios et al., 2020). Treatment is surgical resection with some evidence suggesting that younger patients may benefit from a course of platinum-based chemotherapy after surgery (Boussios et al., 2020).

### 1.1.4 Epithelial ovarian cancers

Epithelial ovarian cancer (EOC) accounts for ~90% of new diagnoses, and is generally what people are referring to when talking about ovarian cancer (Kurman & Shih, 2008; Lengyel, 2010). It is thought to arise from either the ovarian surface epithelium or the distal fallopian tube, with ~80% of serous carcinomas being the latter (Crum et al., 2011; George et al., 2016; Kurman & Shih, 2008; Y. Lee et al., 2007). EOC is broadly broken down into 5 subtypes: serous, mucinous, clear cell, endometrioid and Brenner urothelial transitional cell tumors (Berek et al., 2012). Each histologic subtype has its own molecular and morphologic characteristics (L Hollis et al., 2016). Figure 1.1 shows a concise map of the various histologies and molecular abnormalities that make up the serous subtype of epithelial ovarian cancer as an example.

| | HGS | Endometrioid | Clear cell | Mucinous | LGS |
|---|---|---|---|---|---|
| Approximate proportion of OC cases | 70% | 10% | 10% | <5% | <5% |
| Overall prognosis | Poor | Favourable | Intermediate | Intermediate | Intermediate |
| Tissue of origin / precursor lesion | Distal fallopian epithelium | Endometriosis | Endometriosis | Poorly defined | Serous borderline tumor |
| Intrinsic chemosensitivity | High | High | Low | Low | Low |
| Associated hereditary syndromes | Germline *BRCA1/2* | Lynch syndrome | Lynch syndrome | | |
| Typical stage at diagnosis | 80% advanced stage | 50% early stage | 60% early stage | 80% early stage | Typically advanced stage |
| Frequent molecular abnormalities | Chromosome instability *BRCA1, BRCA2 TP53, NF1, RB1 CCNE1 amp.* | *PTEN, PIK3CA, ARID1A, CTNNB1* | *PTEN, PIK3CA, ARID1A,* chr20q13.2, *amp* | *KRAS, HER2 amp* | *KRAS, BRAF* |

Early stage: FIGO stage I or II; advanced stage: FIGO stage III-IV; amp: amplification

**Figure 1.1 Histologic subtypes of EOC summarized. Taken from** (L Hollis et al., 2016)

All epithelial ovarian cancers are divided into 2 subtypes characterizing their response and clinical course (Cannistra, 2004). Type 1 includes mucinous,

endometrioid, low grade serous, malignant Brenner tumors, and clear cell carcinoma. Type II tumors are high grade serous, carcinosarcoma, and undifferentiated carcinomas (Babaier & Ghatage, 2020; Prat, 2012; Ramalingam, 2016).

#### 1.1.4.1 Serous Ovarian Cancers

Serous carcinomas make up the majority of epithelial ovarian cancer diagnoses (Lengyel, 2010). They are divided into two distinct tumor types that not only respond very differentially to treatment, but also follow very different disease progressions. Type I (low grade) is a less aggressive, rare subtype and type II (high grade) is the more common and deadly variant (Bell et al., 2011).

#### 1.1.4.2 Low grade

Low grade serous ovarian cancer (LGSOC) is a slower growing malignancy that is generally resistant to chemotherapy (Ricciardi et al., 2018). Despite this, patients have a far better prognosis than the high grade serous ovarian cancer (HGSOC) that is most common (Gadducci & Cosio, 2020). LGSOC represents a distinct molecular lineage compared to HGSOC. It lacks the dominant *TP53* initiating mutation that essentially defines HGSOC, and contains *KRAS* and *BRAF* mutations. The RAS system can be targeted with a potent specific MEK inhibitor, selumetinib, that has shown efficacy in clinical trials in LGSOC (Takekuma et al., 2016). LGSOC represents only ~1-3% of ovarian cancers and less than 5% of serous ovarian cancer (Gadducci & Cosio, 2020; Ramalingam, 2016; Ricciardi et al., 2018).

#### 1.1.4.3 High grade

High grade serous ovarian cancer (HGSOC) is thought to have a specific genetic evolution, first occurring in the distal fallopian tube secretory epithelium (Erickson et al., 2013; George et al., 2016; Labidi-Galy et al., 2017; Reade et al., 2014). It is believed that an initial mutation in *TP53* seen in >95% of cases may increase the cells' chances of neoplastic transformation over a period of several years. The *P53* originating hit leads to a large number of genetic abnormalities with the tumor being dominated by chromosomal amplifications and deletions (Bell et al., 2011).

One of the difficulties of early detection of HGSOC is that biomarker discovery is impaired by the overall diversity of genetic alterations. The initial *TP53* alteration increases mutation and chromosome instability rates, but leads to few common abnormalities across individual tumors (Bell et al., 2011). Amplifications involving *MYC* at 8q24 and *MECOM* at 3q26 are found in approximately 30% of patients in the TCGA data set, representing the most common gains. Many of these amplifications have been shown to be double minutes with hundreds of copies of the *MYC* and the *MECOM/EV1* proto-oncogenes (Tanner et al., 2000). The next most common alteration lies in BReast CAncer1 *(BRCA1*) and BReast Cancer2 *(BRCA2*), with mutation rates for each at around 10-25% depending upon the cohort studied (Ashworth, 2008b; Edwards et al., 2008; Herzog & Monk, 2017). (Figure 1.2)

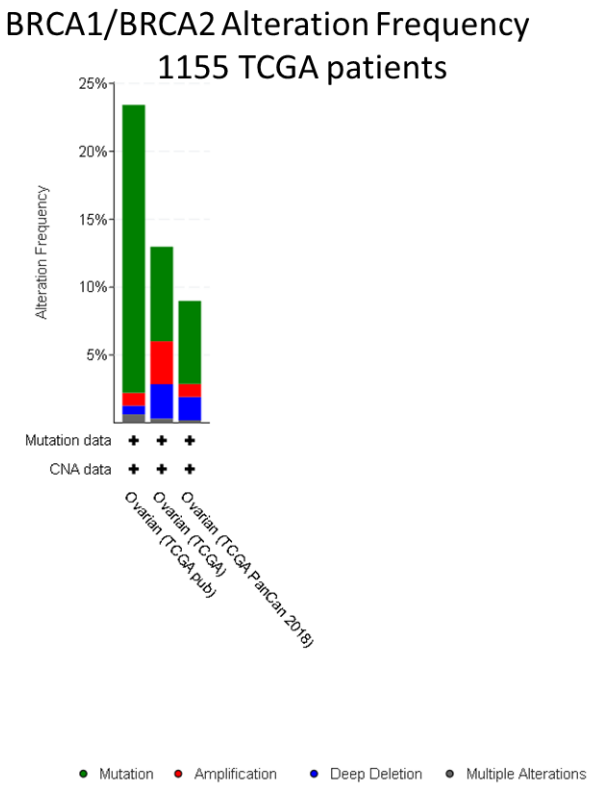



**Figure 1.2 Our analysis of online TCGA BRCA statuses for 1155 patients showing the relative rarity of BRCA mutations in HGSOC** (Cerami et al., 2012; Gao et al., 2013)**.**

HGSOC is now thought to have a precursor lesion that arises in the distal fallopian tube (Labidi-Galy et al., 2017). This serous tubal intraepithelial carcinoma represents the earliest stage of the disease making HGSOC really a primary fallopian tube carcinoma that in later stages disseminates to the ovary and peritoneal cavity (Crum et al., 2011; Y. Lee et al., 2007).

Although the genetic makeup varies substantially, current SOC treatment is optimal cytoreduction followed by carboplatin/paclitaxel chemotherapy. Alternatives are based upon homologous recombination deficiencies (HRDs) that

identify candidates for poly(ADP-ribose) Polymerase (PARP) inhibitor treatment, or markers for sensitivity to anti-angiogenic therapy such as bevicizumab (De Picciotto et al., 2016).

### 1.1.5 Mucinous

Mucinous ovarian cancer (MOC) represents a unique group of tumors compared to the other EOC subtypes. In most aspects it is unlike the other epithelial subtypes: better prognosis, differences in chemosensitivity, distinct molecular evolution and different risk factors associated with the disease (Babaier & Ghatage, 2020; Kelemen & Köbel, 2011). MOC overall has a better prognosis than HGSOC, but this changes in its later stages (Ricci et al., 2018). Early stage is commonly identified and surgical intervention often is curative (Gorringe et al., 2020). Late stage MOC does not respond well to platinum chemotherapy and so its prognosis is dismal after progression (Babaier & Ghatage, 2020; Groen et al., 2015; Ricci et al., 2018). Early stage disease has a 90% 5 year survival rate whereas patients with disseminated disease survive for ~12-18 months (Babaier & Ghatage, 2020).

It is thought that *KRAS* is a frequent early mutation that arises as the mucinous epithelial transforms in a stepwise fashion from normal to borderline then to full carcinoma (Gorringe et al., 2020). Evidence for this evolution remains in some metastatic disease in the form of benign cell clusters within the tumor that have the same *KRAS* mutation as the transformed cells (Kelemen & Köbel, 2011; Ricci et al., 2018). Later stage molecular alterations can include *TP53*,

*MSI-H*, and also *HER2* amplification, the commonly found chromosomal gain seen in invasive ductal carcinoma of the breast (Babaier & Ghatage, 2020). These alterations give therapeutic opportunities that are not present in other EOC subtypes.

The lack of a BRCA-like phenotype from HRDs generally means that MOC does not respond well to platinums or PARP inhibitors. Around 10% of MOC are estrogen receptor (ER) positive and hormone-based options have shown some efficacy in that setting (Gorringe et al., 2020). KRAS pathway involvement gives physicians opportunities for several small molecule inhibitor-based clinical trials targeting various members (BRAF, MEK1, MEK2). Overall MOC represents another heterogeneous set of tumor subtypes that, due to their variation, require therapeutic interventions tailored to the molecular makeup rather than the tissue of origin (Kelemen & Köbel, 2011).

### 1.1.6 Clear cell

Clear cell carcinoma (CCC) is another rare variant of EOC that is associated with endometriosis (Jin et al., 2014). It has a worse prognosis than HGSOC, especially in later stages (del Carmen et al., 2012). This comes from the lack of sensitivity to platinum-based therapies. Other cytotoxic agents have shown some promise, such as the topoisomerase inhibitor irinotecan, but superior response rates to the current standard of care have not been seen (del Carmen et al., 2012). In the recurrent setting less than 1% of patients respond to

chemotherapy after first line treatment failure (del Carmen et al., 2012; Mabuchi et al., 2016).

Common genetic alterations seen in CCC consist of *ARID1A*, *PIK3CA*, and the ATM pathway member *PPP2R1A* (Jin et al., 2014; Mabuchi et al., 2016). Pathway alterations seen in transcriptomic profiling studies have shown deregulation of mTOR/AKT, HIF1a/VEGF, and the STAT/MET systems and future targeted therapies are attacking these alterations (Friedlander et al., 2016; Tang et al., 2018).

### 1.1.7 Endometrioid

Endometrioid ovarian cancer (EVOC) is another endometriosis related rare EOC variant more commonly seen in younger patients (Groen et al., 2015).They are also more often caught in early stages leading to better outcomes than HGSOC (Paik et al., 2018; Pierson et al., 2020).

Molecular underpinnings of EVOC are unique in terms of EOC with *CTNNB1* and *PTEN* found in combination with the typical EOC drivers *TP53*, *ARID1A*, and *PIK3CA* (Paik et al., 2018; Pierson et al., 2020). They also possess a higher degree of microsatellite instability compared to other histotypes (Pierson et al., 2020). Detailed whole exome sequencing has identified therapeutic options currently under investigation such as targeting patient subsets that have high mutational loads (such as POLε) or HRD groups with PARP inhibitors (Cheaib et al., 2015; Heckl et al., 2018; Pierson et al., 2020).

### 1.1.8 Malignant Brenner tumors

Malignant Brenner tumors account for less than 0.05% of EOC (Lang et al., 2017a; Yingao Zhang et al., 2019). Brenner tumors are generally benign with malignant variants accounting for <5% of all diagnoses. Due to their rarity a consensus therapeutic intervention is little studied. Surgery followed by a conservative adjuvant chemotherapy regimen is recommended, but others have suggested surgery alone or, in the case of stage 1a disease, “watchful waiting” (Lang et al., 2017b; Yamamoto et al., 1999; Yingao Zhang et al., 2019).

## 1.2 Ovarian cancer therapies

Considering the overall diversity of cell types, mutational backgrounds, and disease progression between the various subtypes of ovarian cancer, it is surprising there is a common strategy for treatment. Optimal cytoreduction surgery followed by cisplatin/paclitaxel was the SOC until carboplatin was found to be better tolerated making carboplatin/paclitaxel the norm today (Harries & Kaye, 2001).

Platinums are strong crosslinking agents creating DNA adducts. Cisplatin was the most used until carboplatin was found to be more tolerable with comparable efficacy. Taxanes such as paclitaxel are considered to be microtubule stabilizing drugs (Perez, 2009). Microtubules exist as monomers that self-assemble and disassemble in a process known as treadmilling (Rodionov & Borisy, 1997). Taxanes generally lower the energy requirements in such a way that microtubules can no longer remove the attached monomer at

one end while continuing to assemble monomers on the opposite end (Mukhtar et al., 2014; Orr et al., 2003; Yvon et al., 1999). During mitosis the spindle uses an almost random linear firing of assembled microtubules to grab onto chromosomes (Yamada & Gorbsky, 2006). With taxanes in place, the microtubules cannot disassemble properly and fail to attach correctly to aligned chromosomes, leading to cell death (Rodionov & Borisy, 1997; Yvon et al., 1999).

The platinum taxane combination therapy has been in use for almost 30 years. After relapse there are several cytotoxic agents with varying levels of activity in use. The topoisomerase inhibitors or poisons topotecan and etoposide are still widely used (Y.-C. Lee et al., 2015). Cyclophosphamide is a nitrogen mustard alkylating agent that is a variation of the first ever chemotherapy and can show efficacy in the recurrent setting (Wong et al., 2017). Other agents can include but are not limited to liposomal doxorubicin, camptothecin, and gemcitabine (Gibson et al., 2013; Harries & Kaye, 2001; Olive et al., 2009). None of these therapies show durable curative power once in the recurrent setting however.

With the current treatment landscape filling with dozens of targeted therapies and immunotherapies, individualized treatments are required to identify optimal interventions. Due to the relative rarity of the other subtypes we will focus on HGSOC and emerging therapies for primary treatment as well as dealing with recurrent disease.

### 1.2.1 PARP inhibition

Using poly(ADP-ribose) polymerase (PARP) inhibitors for treating BRCA or HRD HGSOC is a highly active area of research with several already approved therapies on the market (McLachlan et al., 2016). PARP is an enzyme responsible for the correct repair of DNA damage and it does this through directly interacting with the repair machinery on the damaged site (Evans & Matulonis, 2017; Herzog & Monk, 2017). PARP has a strong affinity for single strand DNA breaks and autoactivates, forming chains of PAR attached to the DNA adjacent to the break (A. Chen, 2011). These polymers act as a scaffolding system to recruit and assemble the various repair system effector molecules (Javle & Curtin, 2011). If PARP is functionally inhibited, these single strand DNA breaks with attached PARP polymers will evolve into double strand breaks (DSBs) due to replication fork collision (Dockery et al., 2017). In cancer cells with deficient HR repair systems these DSB's will build up over time whereas normal cells have the capacity to repair them (Patel et al., 2011).

By using small molecules to 'trap' PARP on the DNA, cancers deficient in DNA repair will be preferentially targeted in a synthetic lethal fashion due to being unable to clear the polymer chains (Evans & Matulonis, 2017). Several molecules have been investigated with rucaparib showing significant promise in HGSOC (Cortez et al., 2018). The use of PARP inhibitors is not limited to patients with BRCA mutations (Neijenhuis et al., 2013). It is thought that up to 50% of all HGSOC have some form of deficiency in their HR/DNA damage repair

system and some of these tumors have been shown to benefit from PARP inhibitor therapy (Javle & Curtin, 2011).

Resistance to PARP inhibitors can occur in surprising ways, with reversion of mutated DNA repair genes back to function normally being one method commonly seen (Ashworth, 2008b). Upregulation of the multi-drug resistance (MDR)1 efflux pumps has also been shown to induce resistance to PARP inhibitors (Rottenberg et al., 2008) making drugs that can inhibit the efflux pumps a major area of research (Issaeva et al., 2010).

### 1.2.2 Immunotherapies

The most commonly used therapeutic strategy in HGSOC today is combined platinum/taxane treatment after surgery. Several other options are becoming more clinically viable. The discovery of immune system checkpoint inhibition, like antibodies targeting PD-1, has revolutionized the treatment of several types of cancers and is being actively explored in HGSOC (Huang et al., 2017; Zhu & Lang, 2017).

The immune system has a dedicated surveillance network for identifying and destroying cells displaying neoplastic changes, neoantigens or other signals of dysfunction. Tumors as they form generally set off various checkpoint alarms and inflammatory processes that signal to the immune effector cells. For any growing lesion to form a viable tumor it has to evade immune system detection. Tumors are generally thought to use PD-L1 to identify themselves to the immune system as “self” and stop immune effector cell recognition and attack (Zhu &

Lang, 2017). Many types of tumors have used this as a mechanism to hide from immune system detection, and blocking this escape tactic has shown efficacy in a diverse set of cancers. In HGSOC, trials have shown positive results using both anti-PD-1 antibody therapies as well as antibodies directed at PD-L1, its normal ligand (Hamanishi et al., 2015; Weiss et al., 2016). HGSOC has been identified as having high expression of the PD-1 receptor and PD-1 inhibitors such as pembrolizumab and nivolumab are in FDA trials (Weiss et al., 2016). CTLA-4 is another checkpoint inhibitor that is in trials and ipilimumab is currently under investigation in HGSOC (Buchbinder & Desai, 2016).

Resistance to checkpoint inhibitors is both intrinsic and acquired with intense research underway deterring sensitivities in many tumor types. The recognition system appears to have multiple compensatory factors interacting and blockade of a single receptor has been shown to compensate with upregulation of parallel pathways (Huang et al., 2017). Several examples have been identified of tumors making dramatic changes to their cell surface markers to evade the immune system entirely. Deletion of MHC class II has been seen and presents a difficult resistance mechanism to overcome (Aust et al., 2017).

### 1.2.3 Bevacizumab

HGSOC has few common mutations which lead to fewer novel therapeutic opportunities. Currently the major targeted therapy in use is bevacizumab, a monoclonal antibody to VEGF (Marchetti et al., 2019; Rossi et al., 2017). This inhibition leads to a reduction of neovascularization, and increases permeability

of the vascular walls (Tomao et al., 2013). Cancer generally has to acquire an enhanced blood supply to increase nutrient availability and deal with waste buildup as a consequence of its sustained high proliferation rates. The neovascularization that tumors undergo is imperfect and leads to problems with vascular leakage (Hanahan & Weinberg, 2011). Successful targeting of the vascularization machinery is thought to enhance these issues causing a significant increase in the concentration of cytotoxic chemotherapies that can be delivered to the tumor bed (Musella et al., 2017; Reinthaller, 2016).

## 1.3 Chemoresistance (Modified from (Cornelison, Llaneza, et al., 2017))

### 1.3.1 Introduction

First-line therapeutic interventions in ovarian cancer have evolved over the last few decades from a single nitrogen mustard alkylating agent to the current SOC: cytoreductive surgery followed by combination taxane-platinum treatment (Agarwal & Kaye, 2003a; Bowtell et al., 2015; Harries & Kaye, 2001). Currently it is thought that 60-80% of EOC patients receiving this combination after surgery will achieve complete remission, with ~80% of these having a chemoresistant recurrence (Cannistra, 2004; Lengyel, 2010; X.-Y. Zhang & Zhang, 2016). Acquired platinum resistance remains a largely incurable condition and the need for novel targeted therapeutics, new combination therapies, and innovative therapeutic strategies to specifically address the chemoresistant phenotype, are desperately needed (Abubaker et al., 2013; Bagnoli et al., 2016; Coward et al.,

2015). Studies have elucidated many of the mechanisms that underlie the development of chemotherapy resistance in HGSOC (for reviews see (Abdullah & Chow, 2013; Dobbin et al., 2014; Jayson et al., 2014; Krzystyniak et al., 2016; X. Liu et al., 2012)) and successfully targeting these systems in the clinic is critical in extending patient survival. We will focus on HGSOC chemoresistance and emerging therapies that may show promise in mitigating and possibly defeating it.

### 1.3.2 Specific chemoresistance mechanisms

Historically, the first major studies that encountered acquired resistance to therapy were the clinical trials in 1965 in pediatric hematopoietic malignancies by Frie *et al* (Frei et al., 1965). In using combinations of several cytotoxic agents, they saw the first real progress in extending the lives of children with leukemia. This success was cut short by recurrences where the leukemic cells had acquired the ability to resist treatment by hiding in a reservoir on the other side of the blood-brain barrier. The therapeutic agents used were unable to efficiently pass through the blood-brain barrier, leading to the patients eventually succumbing to overwhelming, multi-drug resistant disease. This gave the first clear evidence of dormant cancers taking advantage of our own defenses to resist treatment (Zahreddine & Borden, 2013).

Broadly speaking, resistance to therapies is categorized into intrinsic or acquired resistance. Intrinsic resistance is the innate ability of the cancer cells to maintain and persist through their first exposure to treatment. Acquired

resistance is the evolution of the cancer cells, following treatment exposure, to an unaffected and persistent state whereby cells maintain and expand in the presence of subsequent therapies (Abdullah & Chow, 2013; Ling et al., 2005; Tapia & Diaz-padilla, 2013). Acquired resistance can simply be thought of as microevolution: any survival advantage, whether geographic or molecular, will be clonally selected for (Cooke & Brenton, 2011; Salomon-Perzyński et al., 2017). In terms of bacteria, this has been seen since the invention of antibiotics. For cancer cells, the overall story is similar but more complicated. One current hypothesis for chemoresistance is that a small percentage of a tumor consists of cancer stem cells or tumor initiating cells that are capable of self-renewal and recreating the tumor *en mass* (Abdullah & Chow, 2013). The presence of these cells creates unique challenges in addressing intrinsic and acquired resistance in chemoresistant tumors.

#### 1.3.2.1 Intrinsic versus acquired resistance

The inherent ability of cells to survive chemotherapy can be mediated through several distinct mechanisms. Drug efflux pumps (ATP binding cassette (ABC) transporters) lower drug concentrations within cells (reviewed in (Gottesman et al., 2002) with follow-up (Gottesman & Pastan, 2015)), and therapeutic agents can be biochemically altered through detoxifying enzymes such as cytochrome p450 and glutathione transferases (reviewed in (Beard & Connor, 2003; Rodriguez-Antona & Ingelman-Sundberg, 2006). Intrinsic

mechanisms of resistance are critical in determining initial response to therapies and may influence subsequent outcomes that lead to acquired resistance.

Acquired resistance is developed by the step-wise, molecular evolution of tumor cells through natural selection of changes giving a survival advantage (Lin & Lu, 2001). These can include mutations in genes that drive increased anti-apoptotic signaling (i.e. xIAP/cIAP, BCL-2, BCL-$X_L$ , and MCL-1), DNA repair capacity, and the tolerability of genetic damage. Other alterations can decrease sensitivity to DNA damage checkpoint signaling, or change extracellular matrix (ECM)-collagen VI surface proteins, and the genes responsible for interacting with the ECM and stromal cells surrounding the tumor (Coward et al., 2015; Fu et al., 2011; Harries & Kaye, 2001; X. Liu et al., 2012; Patch et al., 2015; Szakács et al., 2006; Yap et al., 2009). Cells can also acquire changes to drug targets that are mediated through multiple mechanisms (Groenendijk & Bernards, 2014; Orr et al., 2003), or in the case of some targeted therapies, by simply bypassing the target through an alternate pathway (Cree & Charlton, 2017; Kavallaris et al., 1997). The simplest example of this is seen in androgen therapy for prostate cancer. After androgen deprivation therapy, the cells amplify the level of androgen receptor to the point that the levels are too high to stoichiometrically defeat (C. D. Chen et al., 2004). Thus, acquired resistance can dramatically alter tumor cells to a point that they can be resistant to one or several types of therapy, but may also become uniquely sensitive to other therapies that have no effect on treatment-naïve cancer cells.

Overall, cancer is a disease of heterogeneity. The inherent genetic instability that gave rise to the tumor cells gives each the ability to quickly respond to changes in its local molecular microenvironment. This gives tumors the ability to use multiple combinations of these adaptive intrinsic and acquired mechanisms of resistance to defeat most modern chemotherapies and targeted therapeutics. For HGSOC, studies have focused on drug efflux and enhanced DNA damage repair capacity as being the critical, and druggable, mediators of resistance.

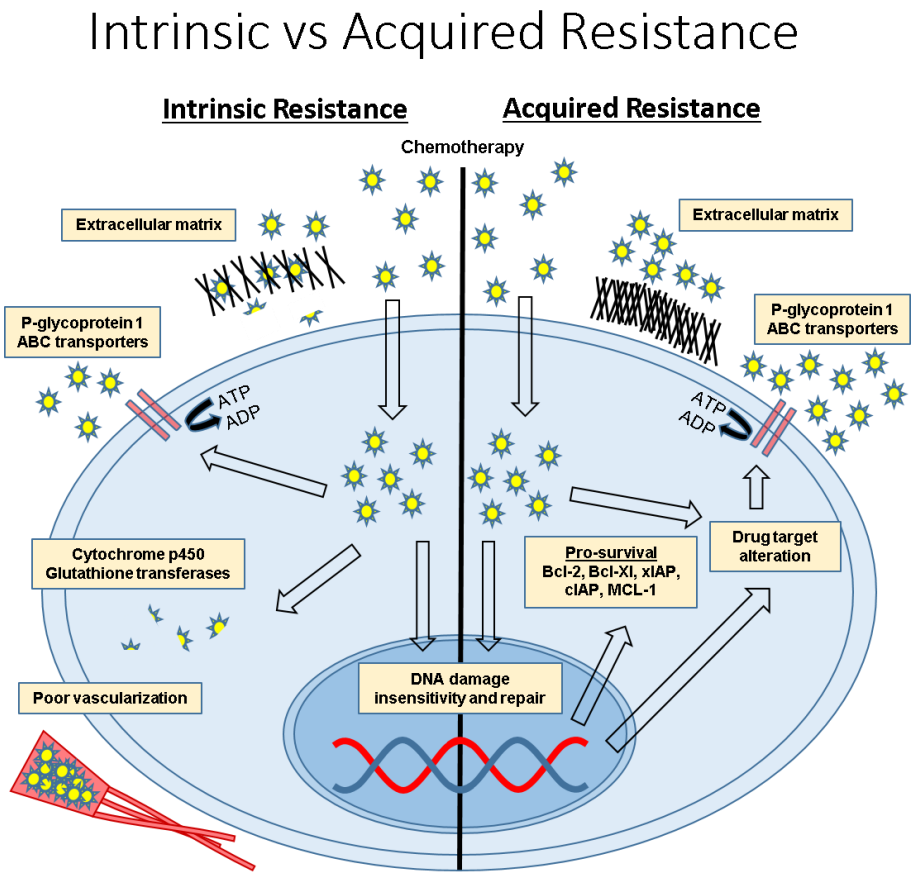



**Figure 1.3 Intrinsic versus extrinsic resistance.** Intrinsic resistance is generally thought to be the overall tumor heterogeneity. Changes caused by genetic and epigenetic alterations due to the tumor's genetic instability for example. This can also include changes to stem cell versus differentiated cell hierarchies within the tumor itself compared to the surrounding tissue. Extrinsic factors can include physical

changes to the local molecular microenvironment such as hypoxic conditions due to poor vascularity or signaling differences between the tumor and the “tumor associated” stromal tissue.

#### 1.3.2.2 Efflux pumps and MDR1

The ability of cancer cells to lower the intracellular concentration of drugs is well documented and the ATP binding cassette (ABC) transporters have been extensively studied in cancer chemoresistance. They are a diverse group of ATP-dependent efflux pumps containing 48 different family members with a common structural theme: a variable transmembrane domain and a conserved nucleotide binding domain (Szakács et al., 2006; Xia & Lee, 2010). In action, the transmembrane domain binds its target which induces a conformation change, removing the bound substrate from the cell using the hydrolysis of ATP for energy (Gottesman et al., 2002; Gottesman & Pastan, 2015; Vaidyanathan et al., 2016). In ovarian cancer chemoresistance, the most commonly targeted of these transporters are the ABCB subfamily, with MDR1 (also known as p-glycoprotein and ABCB1) being the most heavily studied. The other ABC transporters appear to be limited in their ability to use paclitaxel as a substrate, with MDR1 being the primary p-glycoprotein responsible for reducing its intracellular concentrations (Agarwal & Kaye, 2003a; Hedditch et al., 2014; Orr et al., 2003).

MDR1 is responsible for the efflux of a truly staggering number of toxic compounds with diverse properties (Schinkel, 1999). Several large studies have been undertaken and identified single nucleotide polymorphisms in MDR1 that are predictive of response to therapy, (S. E. Johnatty et al., 2008; Sharon E. Johnatty et al., 2013) with several promising drugs emerging to reduce their effects (Vaidyanathan et al., 2016; Yang, Iyer, Singh, Choy, et al., 2015). Over the last 15 years, intense research has explored strategies to clinically inhibit MDR1 with small molecules. Resistance to taxane therapy in ovarian cancer appears to occur through multiple mechanisms depending on the genetic background of the cells, but MDR1 efflux is thought to be the primary mechanism (Kavallaris et al., 1997; Ling et al., 2005). Eliminating MDR1-mediated paclitaxel/cisplatin efflux specifically has been shown to reverse resistance in the laboratory, but translating this promising outcome to the clinic has been incredibly challenging (Planting et al., 2005; B. Wang et al., 2015; Wu et al., 2016). Drugs that inhibit MDR1 can also inhibit some cytochrome p450 isoenzymes, which are necessary to detoxify the typically used cytotoxic chemotherapies in the non-cancerous cells. Targeting proteins responsible for protecting cells from toxic molecules is problematic, and combining MDR1 inhibition with cytotoxic chemotherapy can cause unpredictable and dangerous side effects (El-Awady et al., 2016; Wandel et al., 1999).

First generation small molecule inhibitors of MDR pumps had limited success due to these unforeseen consequences and overall toxicity problems, with clinical trials being marred by poor tolerability (Kapse-Mistry et al., 2014;

Seiden et al., 2002; Shukla et al., 2011). Second generation inhibitors with minimal cytochrome p450 inhibition showed promise but generally targeted multiple ABC transporters, resulting in negative effects in a diverse set of tissues. Using this information, combined with advances in structural chemistry and drug design, third generation p-glycoprotein inhibitors appear to be more specific and better tolerated, with several currently being clinically investigated (Binkhathlan & Lavasanifar, 2013; Weidner et al., 2016). Apatinib has been found to reverse paclitaxel resistance in both *in vitro* and *in vivo* PDX model systems (Mi et al., 2010). These third generation agents, as well as Tariquidar, have shown significant improvement in potency, specificity and tolerability with clinical trials ongoing (Chung et al., 2016). Ovarian cancer groups are investigating these latest agents using novel encapsulation methods to deliver them, with some success (Y. Zhang et al., 2016). Overall pharmacologic inhibition of the efflux pumps remains challenging due to limits in balancing toxicity risks against therapeutic gains.

Targeting the MDR1 mRNA for degradation with siRNA/ASO is also being investigated as a method to ensure target specificity, and combining this with nanoliposomes that target cancer cells has been attempted in an effort to limit toxicity (Kapse-Mistry et al., 2014; Yang, Iyer, Singh, Choy, et al., 2015). Yang *et al* published findings on self-assembling nanoparticles that target CD44 to deliver MDR1 siRNA specifically to cancer cells in a mouse PDX model of ovarian cancer. When used in combination with paclitaxel, the siRNA delivery was specific to the cancer cells as increased sensitivity to paclitaxel was

demonstrated (Yang, Iyer, Singh, Milane, et al., 2015). While these modifications in drug delivery and specificity may alleviate some toxicity hurdles observed in the treatment of chemoresistant disease, it is also important to consider therapies that do not directly overlap with primary mechanisms of chemoresistance.

The development of novel tubule stabilizers that are not substrates for MDR1 efflux, such as the epothilone family, has shown efficacy in cells and PDX previously resistant to paclitaxel (Diaz-Padilla & Oza, 2011; Rivera & Gomez, 2010; Vishnu et al., 2012; Zagouri et al., 2015). Ixabepilone has been shown to have IC50s an order of magnitude lower than typical concentrations seen with paclitaxel, and also has less neuropathies and other side effects in patients (Rivera & Gomez, 2010). Positive clinical outcomes have been seen using Ixabepilone in recurrent ovarian cancer in combination with bevacizumab, and studies are ongoing to determine optimum patient enrollment criteria (Roque et al., 2015). These results may highlight the utility of combination therapies that are not directly targeting major mechanisms of chemoresistance in ovarian cancer.

While some success has been observed in targeting MDR1 to overcome resistance, any real clinical gain has remained elusive. A recent whole genome sequencing study of a cohort of chemoresistant ovarian cancer has shown MDR1 to be rearranged in some chemoresistant populations, with promoter fusion driving upregulation. The promiscuous nature of the ABC transporters combined with the diverse side effects and toxicity problems has prompted some investigators to call for a change in tactics. Moving upstream of MDR1 to the

epithelial-mesenchymal transition (EMT) pathway ties together multiple mechanisms associated with the acquisition of resistance and EMT has a special position in ovarian cancer progression.

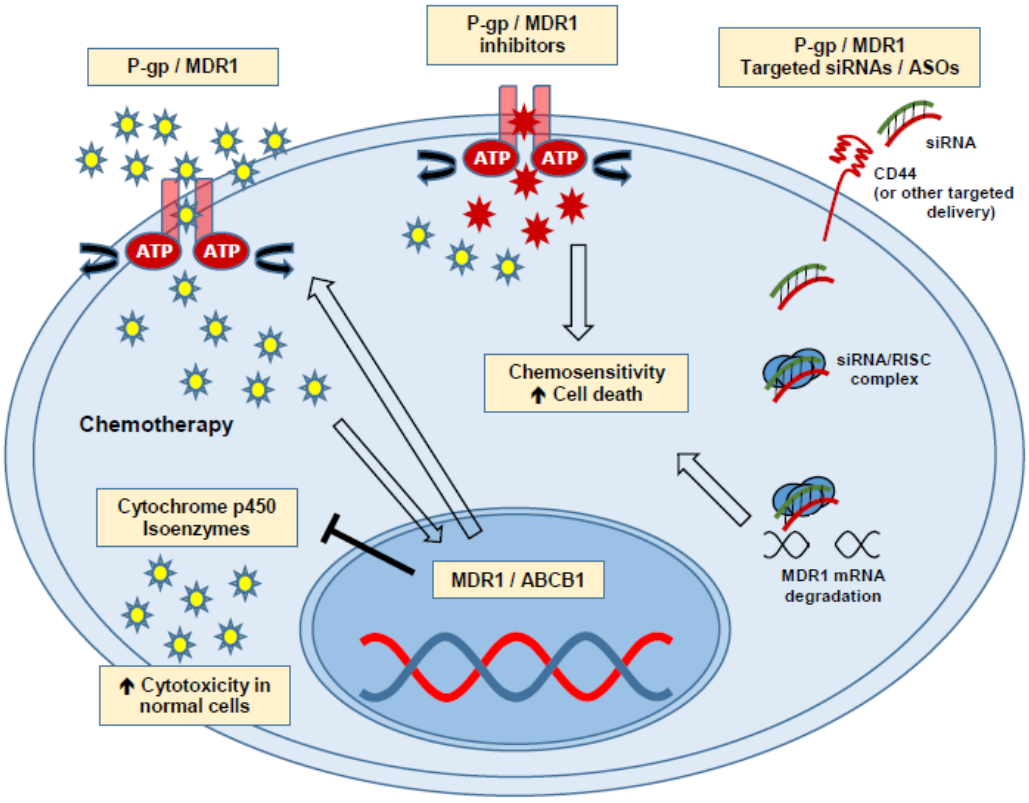



**Figure 1.4 Chemoresistance by multi-drug resistant transporter export.** The ATP binding cassette family of drug efflux pumps represent a substantial problem for getting required drug concentrations to the tumor cells. By overexpressing these genes tumor cells can survive a variety of cytotoxic insults that allow them to rapidly build resistance to many chemotherapeutics.

#### 1.3.2.3 Epithelial-Mesenchymal Transition

EMT is the process of epithelial cells losing their apical-basal polarization, detaching through loss of adhesion, and becoming mesenchymal in both appearance and invasiveness (Davidson et al., 2012; Rosano et al., 2011). There are myriad genetic changes involved in the process with cells typically losing expression of the adhesion protein E-cadherin, along with several of the tight junction proteins (Davidson et al., 2012; Thiery et al., 2009). During EMT, cells begin expressing mesenchymal markers like vimentin and N-cadherin, as well as markers associated with stem cell populations such as ALDH1 and endothelin-A (Abdullah & Chow, 2013; Bagnato & Rosanò, 2012; M.-J. Young et al., 2015). These changes are driven by the transcription factors snail, slug, zeb1 and twist, and many of the key pathways involved in ovarian cancer regulate their expression (Gupta et al., 2013; Haslehurst et al., 2012; Kajiyama et al., 2007; Sakamoto et al., 2016). EMT is seen after chemotherapy in surviving ovarian cancer cells and it's been shown that snail and slug EMT transcription factors are upregulated (Haslehurst et al., 2012; Latifi et al., 2011; Rosano et al., 2011; Shah et al., 2017). Thus, factors altered in EMT are expressed in ovarian cancer and may be associated with chemoresistance.

Chemoresistant phenotypic changes in ovarian cancer appear strongly correlated with EMT and the presence of subpopulations of cancer stem cells (Abubaker et al., 2013; Bagnato & Rosanò, 2012; Haslehurst et al., 2012; Rosano et al., 2011, 2014). The pro-survival, anti-apoptotic signaling, efflux

pump overexpression, and resistance to DNA damage seen in chemoresistant populations can all come from sustained EMT (Gupta et al., 2013; Miow et al., 2015; Thiery et al., 2009). One relatively unique property of ovarian cancer is its fondness for metastasizing to the omentum, and interactions with this fatty compartment may play a critical role in the development of chemoresistance (Lengyel, 2010; Pradeep et al., 2014). HGSOC does not require a hematologic route of metastasis as the entire peritoneal cavity is accessible from its site of origin, and HGSOC appears to readily shed and can establish colonies throughout the chest cavity (Pradeep et al., 2014). This shedding has been shown to involve EMT and the formation of tumor spheroids, which are protected by cancer-associated fibroblasts (CAFs) and hijacked immune system effector cells (Lengyel, 2010). Ovarian cancer has been shown to be capable of responding to chemotherapy, with an acquired EMT phenotype giving rise to chemoresistance (Davidson et al., 2012; Marchini et al., 2013). The endothelin receptor has been identified as a major source of EMT changes in response to chemotherapy, and blocking it has shown some reversal of chemoresistance (Rosano et al., 2011). Zibotentan is an antagonist of endothelin receptor A (ETA) (Tomkinson et al., 2011). After failing phase III trials, this drug is still being investigated, and its utility in chemoresistant disease in ovarian cancer is still promising. Targeting EMT in ovarian cancer may both stop the acquisition of chemoresistance and inhibit tumor progression and metastasis; however, cells that continue to evade death may have greater abilities to endure in the face of severe DNA damage.

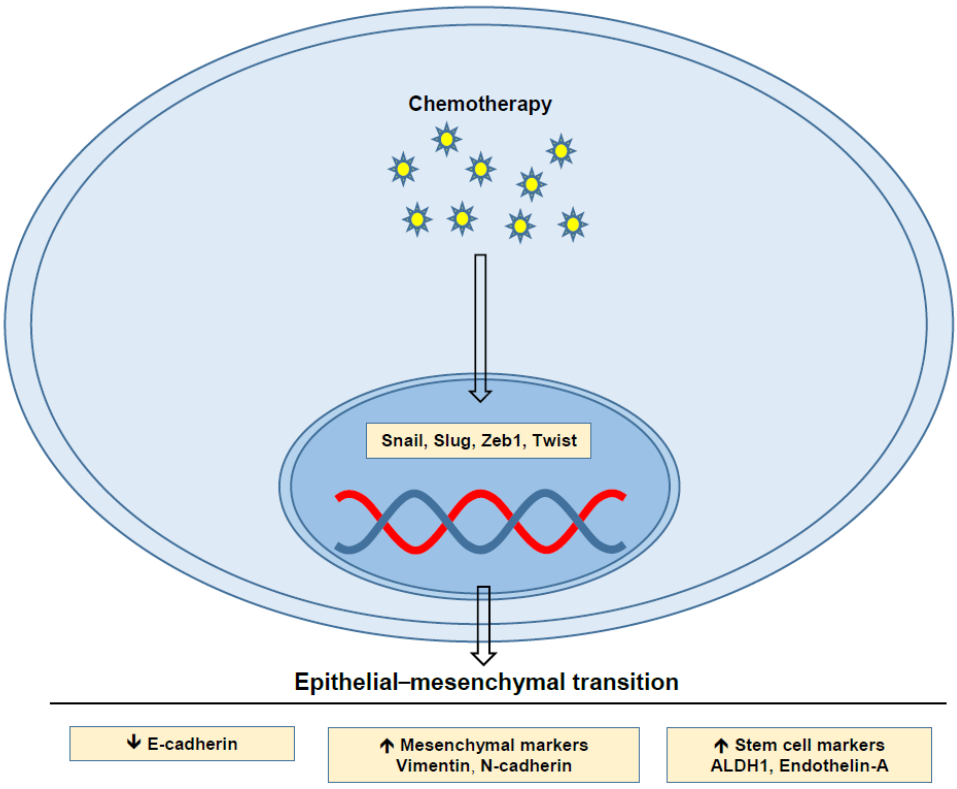



**Figure 1.5 Resistance dependent on epithelial to mesenchymal transition.** EMT plays a role in metastasis where the epithelial cells transition to the more motile mesenchymal cell state. The transition in cancer is thought to be reversible, or exist in a hybrid state, depending upon the needs of the cell. After colonizing distant metastatic sites many cells have been shown to reverse their EMT using MET. The transition to the mesenchymal state enhances tumor cell survival, can induce overexpression of genes related to drug resistance, as well as give cells characteristics associated with cancer stem cells**.**

#### 1.3.2.4 DNA damage tolerance and repair capacity

DNA damage response pathways are very well characterized and generally signal through checkpoint kinase 1 and checkpoint kinase 2 (CHK1/CHK2) to determine the amount of damage present, amplify the repair signal to enhance repair efficiency, and delay the cell cycle to allow time for repair (Smith et al., 2010). Damage is picked up by MRE11/RAD50/NBS1 (MRN complex), which signals through the phosphotidylinositol 3-kinase (PI3) family

members ataxia telangiectasia mutated (ATM), Rad-3-related (ATR) and DNA-dependent protein kinase (DNA-pk) by phosphorylation of H2AX on serine 139. This in turn amplifies the repair signal, and enhances the buildup of DNA repair pathway members at the site of damage (J.-H. Lee & Paull, 2005; Rogakou et al., 1998). In order to allow sufficient time for repair, the cell cycle must be halted. The G2-> M boundary is maintained through phosphorylation of CDC2 on threonine 14 and tyrosine 15 by Wee1 and MYT1 kinases, which sequesters the CDC2/cyclin B complex in the cytoplasm (Fattaey & Booher, 1997; McGowan & Russell, 1993; Pines & Hunter, 1994; Wells et al., 1999). For mitosis to proceed, CDC2 must be activated by CDC25-mediated dephosphorylation and translocation to the nucleus (Atherton-Fessler et al., 1994; Hunter, 1995). The delay is induced by ATM and ATR activation of the CHK2/CHK1 kinases, respectively, as CHK2/CHK1 phosphorylate CDC25B, inhibiting its phosphatase activity on CDC2 and leaving the inactive complex in the cytoplasm (Smith et al., 2010). Thus, the cell cycle is tightly regulated by several mechanisms, and sensitivity or resistance of cancer to treatments may involve alterations in these processes that promote imminent death or allow for recovery and repair.

Platinums are the drug of choice for treating many aggressive cancers, and most are quite sensitive to its toxicity (Ho et al., 2016). The mechanisms of platinum toxicity have yet to be fully elucidated, but it’s thought to act as a severe DNA damaging agent, creating inter- and intra-strand cross links from the formation of platinum:DNA adducts (D. Wang & Lippard, 2005). Ovarian cancers presenting with some form of HRD generally respond better to DNA damaging

therapies like cisplatin, and also may benefit from PARP I inhibitor therapy (Nojima et al., 2005; Reinbolt & Hays, 2013). PARP inhibitors exploit the broken DNA repair pathway to act as a synthetic lethal (Ashworth, 2008a). The deficient repair system also increases the efficacy of DNA damaging agents, depending on the specific gene mutation, and improves overall survival. Ovarian cancers where there are no HR deficiencies are less defined and may represent an early step in tumor initiation or chemoresistance (Reinbolt & Hays, 2013).

TP53 mutant cells are thought to have a deficient G1 arrest ability with an enhanced G2 delay, allowing them to cope with genetic damage (Bache et al., 2001; Matheson et al., 2016). Over time, cancer cells can become resistant to platinum-induced DNA damage and apoptosis through G2 delay and enhanced repair (Selvakumaran et al., 2003; Venezia et al., 2004). The HGSOC combination of TP53 loss and HR repair deficiency has led many groups to ask the question: can we force cells with severe DNA damage through the cell cycle by attacking the delay mechanisms, and lead cells to death by apoptosis or mitotic catastrophe? CDC2 phosphorylation by Wee1 kinase is an attractive drug target for this, with Wee1 kinase specific inhibitors, such as MK-1775 (low nanomolar IC50), showing promising results (Leijen et al., 2010; Osman et al., 2015). PD0166285 is a newer wee1 inhibitor and G2 checkpoint abrogator (Hashimoto et al., 2006; PosthumaDeBoer et al., 2011). With HGSOC being almost entirely TP53 mutant, removing the G2 arrest has been shown to reverse platinum resistance, and targeting this may help alleviate chemoresistance in some subpopulations of ovarian cancer (Heijink et al., 2015).

Another mechanism of chemoresistance that may be required for the upregulation of any and all processes related to DNA damage repair, EMT, drug efflux, and the capacity of the cell to utilize intrinsic mechanisms of resistance, as well as to develop acquired mechanisms of resistance, is ribosomal biogenesis.

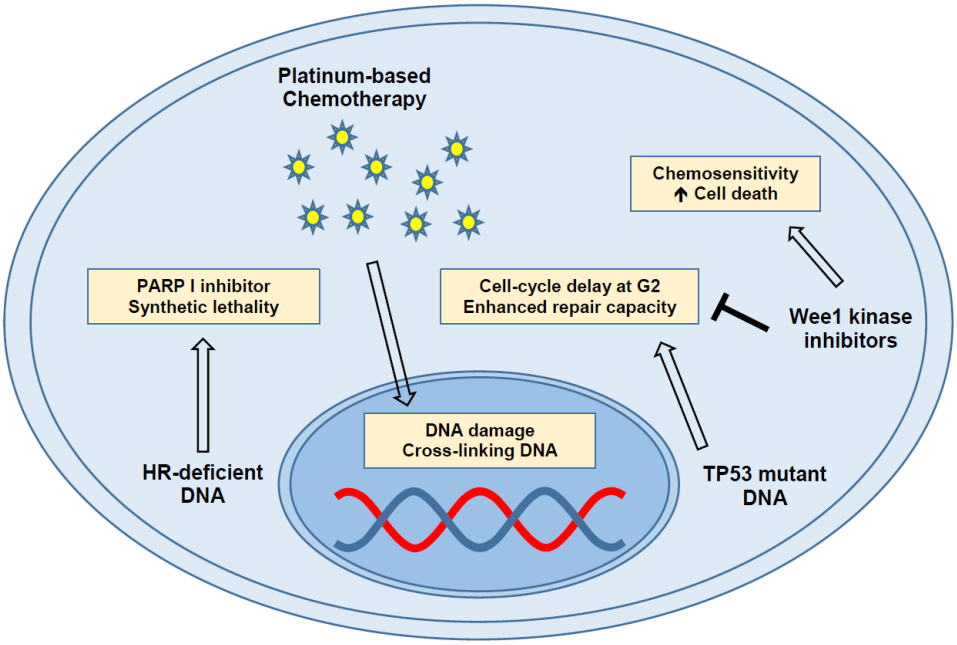



**Figure 1.6 Chemoresistance by enhanced DNA damage repair ability, tolerance to abnormally high levels of DNA damage, or decreased detection of damage present.** High grade serous ovarian cancer cells have >95% rate of mutant TP53. These cells have damaged G to S phase transition control and rely upon pausing at G2 to mitosis to repair damage. This allows them to evade cell death due to DNA damage by raising the threshold of damage required to induce apoptosis.

### 1.3.2.5 Ribosomal Biogenesis

One of the first identified hallmarks of cancer, dating back to the late 1800's, was enlarged and/or pronounced nucleoli (Derenzini et al., 2009). The size of the nucleoli is generally thought to reflect the amount of ribosomal biogenesis happening in the nucleolus, and proliferating cells typically require a

large amount of ribosomes to progress through the cell cycle (Sirri et al., 2008). Ribosomes are critical molecular machines common to all life that allow the conversion of RNA messages to nascent proteins. Ribosomal synthesis and processing is one of the most energetically demanding activities cells undergo, and due to this, ribosome synthesis is also one of the most regulated systems in eukaryotic life (Deisenroth & Zhang, 2011). Altering the rate of ribosomal biogenesis can lead to p53 stabilization, representing a phenotype known as nucleolar stress (Boulon et al., 2010; J. E. Quin et al., 2014; Woods et al., 2015). Given that ribosomal biogenesis is central to life and appears to play a prominent role in cancer, it's important to consider if and how this system can be targeted in cancer cells specifically.

Recently the idea of targeting ribosomal biogenesis has come forward as a viable strategy for treating various forms of cancer (Karita Peltonen et al., 2014; Pickard & Bierbach, 2013) and thoroughly reviewed for HGSOC in (Yan et al., 2017). Ribosomal synthesis begins with the formation of the RNA polymerase I pre-initiation complex, followed by transcription of the 47s pre-ribosomal RNA. The preinitiation complex is setup first by binding of the high mobility group protein UBTF within the rDNA promoter region, ejecting the H1 histone. This interaction is then stabilized by the binding of the selectivity complex SL1, which commits the promoter for transcription by multiple rounds of RNA polymerase I. After transcription, a complex series of modifications spanning several subcellular compartments leads to the assembly of functional ribosomes (Ray et al., 2013; Russell & Zomerdijk, 2006). Although there are several key players in

ribosomal biogenesis, there may be mechanisms that are specific and can be targeted in cancer cells without negatively impacting the majority of normal cells.

RNA polymerase I is only responsible for the transcription of the pre-ribosomal RNA, so targeting it with small molecule inhibitors appears to be the most appealing strategy for specifically shutting down ribosomal synthesis (Karita Peltonen et al., 2014; Poortinga et al., 2014). Two specific pol I inhibitors, CX-5461 and BMH-21, have come out recently with slightly different targets. CX-5461 is thought to inhibit the binding of SL1 to the rDNA promoter, leading to preinitiation complex failure and possibly an abnormal chromatin structure detected as a form of damage (Haddach et al., 2012a; H. Xu et al., n.d.; Ye et al., 2017). BMH-21, on the other hand, is a GC-rich DNA intercalating agent that inhibits formation of the pre-initiation complex leading to degradation of RPA194, the major subunit of the RNA pol I holoenzyme (Colis et al., 2014; K. Peltonen et al., 2014). These inhibitors may begin to shed light on the possibility and efficacy of targeting ribosomal biogenesis in cancer.

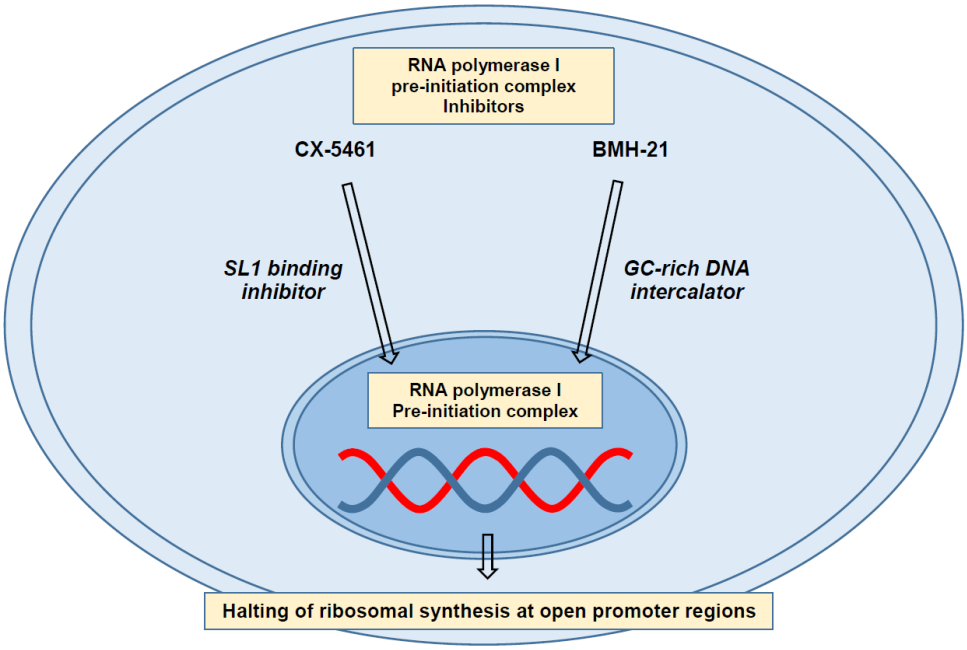



**Figure 1.7 Ribosome biogenesis inhibition through RNA polymerase I promoter targeting.** The role of ribosome biogenesis in chemotherapy resistance is poorly understood. The most simple explanation is enhanced ribosome production allows the cells to translate the necessary machinery to protect against cytotoxic drugs. Specialized ribosomes, DNA damage induced senescence as an apoptotic escape, and ribosome biogenesis being coupled to cell fate are all theories being explored (Gabut et al., 2020; Larsen & Stucki, 2016; Lindström et al., 2018; Xue & Barna, 2012). Why normal cells can tolerate ribosome biogenesis inhibition compared to transformed cells is a major unanswered question.

### 1.3.3 Summary

In summary, while the understanding of mechanisms underlying intrinsic and acquired chemoresistance in ovarian cancer is expanding rapidly, overall therapeutic options remain limited. Promising results generated *in vitro* showing the therapeutic potential of targeting MDR1 have fallen short in practical application in the clinic due to toxicity. Alterations in EMT targets has had minimal success given there are several mechanisms remaining active that allow

the tumor to adapt. As well, agents that try to exploit high levels of DNA damage may only be clinically applicable in contexts where tumors are also HR deficient.

The pitfalls and shortcomings of translating these novel targets and associated drugs to the clinic further highlight the essential need for approaches to treatment that go beyond targeting single mechanisms. The mechanisms targeted previously only represent a handful of players in a complex and heterogeneous system that comprises chemoresistance in ovarian cancer. The novel approach of targeting ribosomal biogenesis may prove more useful than previous approaches in improving outcomes for a larger proportion of patients with chemoresistant ovarian cancer in the future; however, previous research indicates that it is essential to seek out the next therapy for chemoresistance, while exploring the therapeutic potentials of current novel targets, so a broader population of patients with chemoresistant ovarian cancer will experience positive outcomes.

# 2 Transcriptomic analysis of chemoresistant HGSOC

HGSOC ovarian cancer at diagnosis is generally hypersensitive to platinum chemotherapy with almost 80% of patients in remission after surgery and treatment (Abubaker et al., 2013). It is known now that left over cell populations that survive the initial chemotherapy have the capacity to become chemoresistant (Abubaker et al., 2013; Armstrong, 2002; Dobbin et al., 2014). In the recurrent setting, chemoresistance is fatal, so identifying and studying these populations is a critical goal in the field. To better understand this acquired

resistance, cell lines have been exposed to chemotherapies to create highly resistant cell line variants after repeated exposures. These cell lines, and cell lines in general, are poor substitutes for human tumors (Goodspeed et al., 2016). They have been in use for many years and, due to their inherent genetic instability, most have evolved into something with vast genetic differences from their originating cells (Goodspeed et al., 2016; E. A. White et al., 2014). Tumors in humans also have a large degree of heterogeneity, with the cancer altering the local molecular microenvironment to better suit its needs (Hanahan & Weinberg, 2011). Changes to vasculature, variations in oxygenation, nutrient availability variations and host immune responses to the growing lesion create a very complex “pseudo-organ” that really cannot be modeled in a monoclonal cell line (Kerbel et al., 1994; E. A. White et al., 2014).

To address this issue human tumors are implanted in mice (as patient derived xenografts (PDX)), allowed to grow, and then characterized to see how well they replicate the characteristics of the tumor seen in the patient. Previously in the Landen lab, multiple ovarian cancer PDX models were developed and characterized (Dobbin et al., 2014). We sought to use these models to see how these tumors change after combined carboplatin/paclitaxel chemotherapy. Mice were treated with weekly doses of carboplatin (90 mg/kg) and paclitaxel (20 mg/kg) for 4 weeks. These tumors were then harvested with the expectation that tumors surviving treatment would be enriched for chemoresistant cell populations. We could then use various –omic profiling techniques to identify mechanisms that allowed them to survive combined cytotoxic chemotherapy.

Tumor samples were collected from the original PDX established and the treated PDX and sequenced for transcriptomic changes by RNA-seq.

## 2.1 Illumina Trueseq

RNA-seq is a robust technique for profiling transcriptome changes between samples. Briefly, RNA is collected and scored for quality, depleted of rRNA, converted to cDNA, fragmented, blunt ended and 5' phosphorylated, adaptors are ligated, and then sent for PCR amplification producing a barcoded sample (Stark et al., 2019; Z. Wang et al., 2009).

Illumina Trueseq flow cells are carpeted with DNA strands complementary to the sequencing adaptors ligated on before PCR (Kumar et al., 2012). Amplified sample fragments hybridize in random locations and are then exposed to bridge amplification for second strand synthesis. After completion the DNA end not bound to the carpet is free to fold in place and will anneal to an adjacent sequence adaptor. This stabilized structure can then be amplified at each spot.

During the actual sequencing run a polymerase will add fluorescently labeled nucleotides one at a time and a camera images at the exact rate the polymerase adds the nucleotides. The images then can be processed to show which nucleotide (A,C,T, or G) was added at each step, thus reading out the sequence. The sequencing end product is a massive file with finalized sequences a few hundred base pairs long. Downstream processing is the alignment to host genome, producing counts and doing statistical tests for

differential expression between two states (Kumar et al., 2012; Stark et al., 2019).

## 2.2 RNA-seq data processing and analysis

Our PDX cohort consists of 21 samples representing 10 separate patient models from chemotherapy naïve tumor samples (Figure 2.1). We have conditions representing both pre and post chemotherapy in mice, and have characterized the chemosensitivity of each based upon their response in mice taking into account whether the original patient ever showed a response to chemotherapy after surgery. Twelve original samples were single end sequenced while the newer 9 samples were paired end and we can compensate for this by using sequence method as a batch effect.

| sample | chemotherapy | chemosensitivity | batch.id | patient.id |
|---|---|---|---|---|
| *sample106post* | tx | sens | z | a |
| *sample106pre* | untx | sens | z | a |
| *sample108post* | tx | res | z | b |
| *sample108pre* | untx | res | z | b |
| *sample115post* | tx | sens | z | c |
| *sample115pre* | untx | sens | z | c |
| *sample116post* | tx | sens | z | d |
| *sample116pre* | untx | sens | z | d |
| *sample121post* | tx | sens | z | e |
| *sample121pre* | untx | sens | z | e |
| *sample136post* | tx | sens | z | f |
| *sample136pre* | untx | sens | z | f |
| *sample152_4w* | tx | res | y | g |
| *sample152_r* | tx | res | y | g |
| *sample152_untx* | untx | res | y | g |
| *sample155_4w* | tx | res | y | h |
| *sample155_r* | tx | res | y | h |
| *sample155_untx* | untx | res | y | h |
| *sample208_4w* | tx | res | y | i |
| *sample208_r* | tx | res | y | i |
| *sample208_untx* | untx | sens | y | i |

**Figure 2.1 PDX cohort 1.**

### 2.2.1 FastQC/multiQC

In a sequencing run the output file has a quality estimate for every nucleotide as well as information on where the sequence was obtained geographically on the flow cell. This allows for problems with the chip itself or artifacts in general to be identified and removed in a process called trimming.

Best practices used to consider trimming based on a sliding window quality scale analysis to eliminate low quality reads as the most efficient way to run analyses. The algorithms to do this also trim away adaptors left over and other sequencing artifacts that are not a part of your experimental data. FASTQC

is the most commonly used software to characterize the quality of your sequence and illustrate any problems in the data (Adams, n.d.; Leggett et al., 2013).

After running FastQC on all of our samples we had no dramatic problems indicated and so I chose not to use trimming software other than to trim adaptors. Throwing away information is now debated in the literature as new aligning software takes into account quality scores (Figure 2.2: example FastQC output).

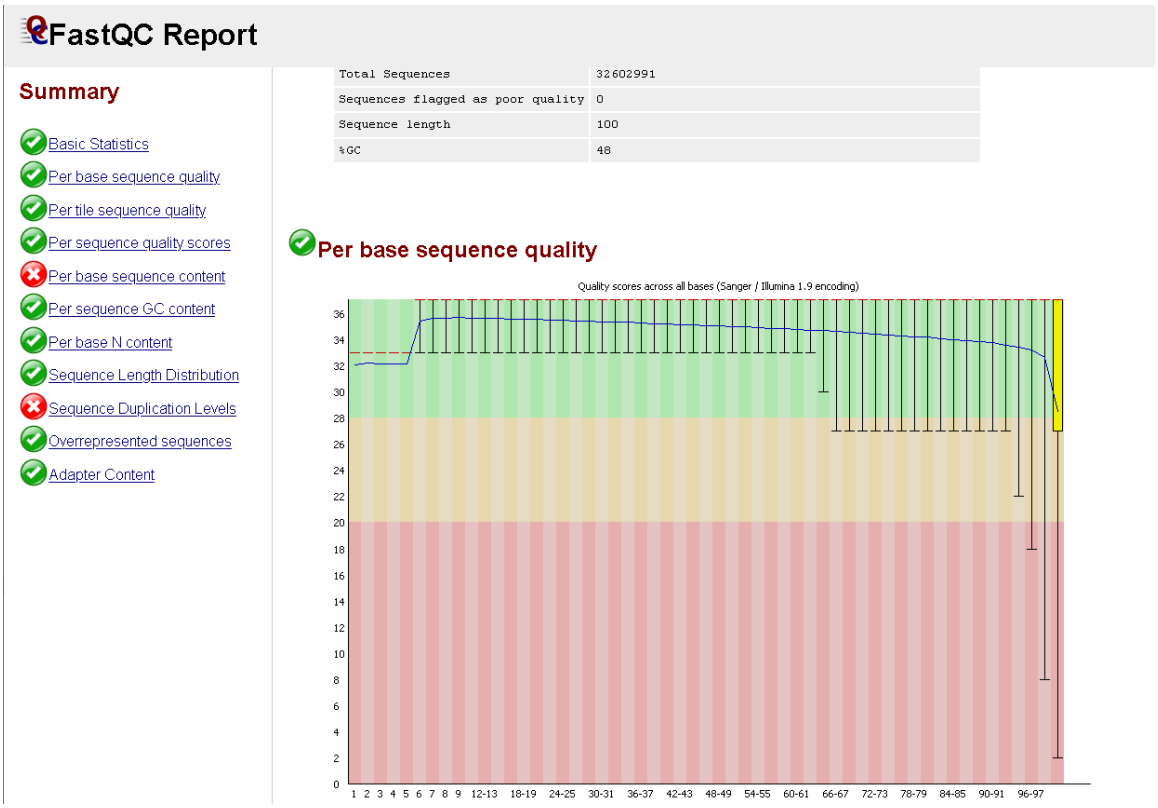



**Figure 2.2 FastQC example from our cohort.** FastQC analyzes sequencing output files looking for various problems such as damage to the chip surface and low quality reads.

### 2.2.2 Kallisto Alignment

To make sense of the sequence fragments they must be assembled to a build of the human genome. There are many different aligners available and each has its own sets of benefits and problems. Overall the older aligning software is slow and extremely resource intensive. High performance computing was required and moving multi-gigabyte files back and forth from a cluster is time consuming and mistakes can be made. Recent advances in the mathematics behind the alignment process have led to the development of very efficient pseudo aligners that can do in an hour what used to take a week.

Early aligning software only gave gene level information based on sequence abundances. We have characterized the function of many genes in various cell types. While it is common to talk about genes as being the functional unit in molecular biology, the reality is much more complicated. Each gene can have >10 transcripts, and these transcripts can play different functional roles depending upon many different variables. Some transcripts have exact opposite effects making gene level counts an oversimplification at best and misleading at worst. Aligners capable of identifying specific transcripts as being differentially expressed are now common and should be used to get the best picture of what is occurring in your system.

Kallisto is one of the most popular transcript-aware aligning software packages (Bray et al., 2016). It can run on windows and requires little in computer hardware to align even large datasets. It aligns fragments to a

reference genome and creates probabilistic map of the exons to identify the most likely transcript being sequenced. These de Brujins graphs (Pevzner et al., 2001) act on kmers (small nucleotide fragments) but can loosely be described as identifying the most likely path to assemble the transcripts (Figure 2.3). Since we give Kallisto the reference transcriptome to align to, individually transcripts are read using the ENSEMBL database. Read abundances are called to specific transcripts, not their parent gene. This allows us to do a transcript based analysis downstream called Sleuth, as well as collapse the transcript counts to gene level to get the usual gene expression estimates for differential expression profiling in Deseq2 or EdgeR (Bray et al., 2016).

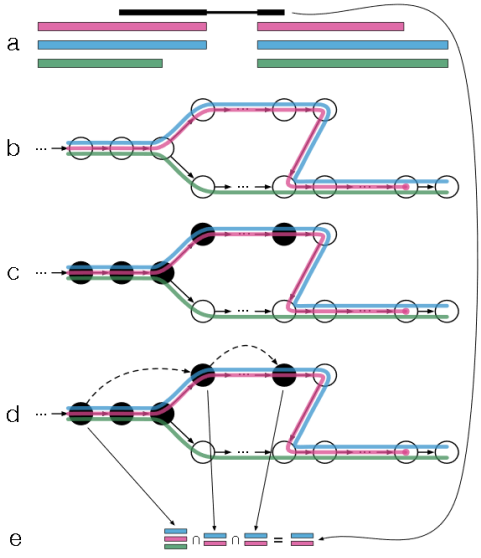



**Figure 2.3 An example of a "de Brujin graph" (from (Bray et al., 2016).**
The 3 colors loosely represent paths through individual transcript assemblies with the final alignment counts being able identify transcripts specifically.

Our reads are aligned to the Hg38 build transcript index using Kallisto with 100 bootstraps for Sleuth analysis, as well as gene level estimates for Deseq2. The alignment call is:

```
kallisto quant -i transcripts.idx -t 12 --bias -o outputname
 -b 100 seq1.fastq.gz seq 2.fastq.gz
```

### 2.2.3 Gene level differential expression profiling using Deseq2

Kallisto pseudocounts must be collapsed and converted into a non-normalized data set appropriate for the Deseq2 algorithm (Love et al., 2014) Tximport was created to import kallisto abundances directly into Deseq2. After compiling the files the tximport and deseq2 object are used:

```
txim.kallisto <- tximport(files, type = "kallisto",
tx2gene = transcript2gene, ignoreTxVersion = TRUE)
```

Our contrasts for the analysis are based on the sample parameters (Figure 2.4).

| sample | tx | batch.id |
|---|---|---|
| ***sample106post*** | sens | z |
| ***sample106pre*** | sens | z |
| ***sample108post*** | res | z |
| ***sample108pre*** | res | z |
| ***sample115post*** | sens | z |
| ***sample115pre*** | sens | z |
| ***sample116post*** | sens | z |
| ***sample116pre*** | sens | z |
| ***sample121post*** | sens | z |
| ***sample121pre*** | sens | z |
| ***sample136post*** | sens | z |
| ***sample136pre*** | sens | z |
| ***sample152_4w*** | res | y |
| ***sample152_r*** | res | y |
| ***sample152_untx*** | res | y |
| ***sample155_4w*** | res | y |
| ***sample155_r*** | res | y |
| ***sample155_untx*** | res | y |
| ***sample208_4w*** | res | y |
| ***sample208_r*** | res | y |
| **sample208_untx** | sens | y |

**Figure 2.4 Contrasts for Deseq2 PDX DE analysis.**

Creating the deseq2 data set with the appropriate parameters:

```
dds <- DESeqDataSetFromTximport(txim.kallisto,
        sampleTable, ~batch.id + tx)
```

To ensure our variables in the correct positions for comparison we can relevel to double check:

```
dds$tx <- relevel( dds$tx, "sens" )
```

We now have our Deseq2 data ready for analysis where we are taking into account the expected batch effect since half of our samples were single end

sequenced and the other half paired, end and attempting to identify changes associated with chemotherapy resistance status (tx):

```
dds <- DESeq(dds)
res <- results( dds )
summary(res)
```

Now that we have our differentially expressed (DE) gene list we can move on to downstream pathway analysis. Our samples by principle component analysis separated well into their distinct categories (Figure 2.5). This level of separation is quite good and somewhat surprising due to the level of variation typically seen between patients with HGSOC.

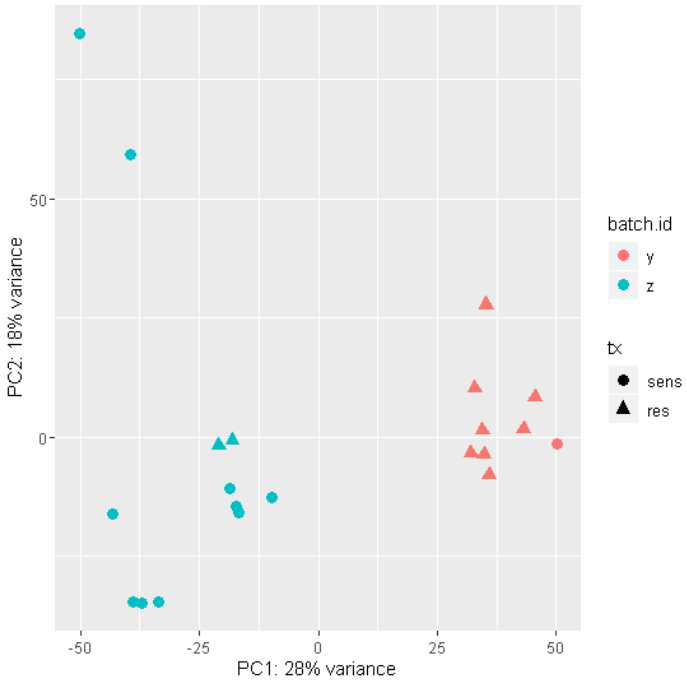



**Figure 2.5 Principle component analysis of our samples.**

Pathway analysis is a common way to assay changes that are occurring in broadly categorized phenotypic systems. In this case we piped our data directly to the pathview package from Deseq2 output. The first KEGG pathway hit is the overall ribosome system appearing upregulated (Figure 2.6).

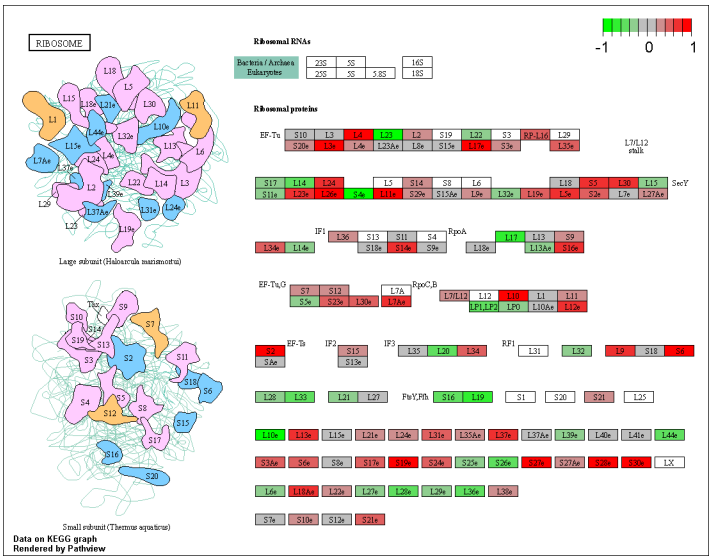



**Figure 2.6 Top KEGG pathway hit in our resistant PDX models is the ribosome.**

Secondary hits are also interesting (Figure 2.7). The olfactory system has been suggested to be involved in cancer (Sanz et al., 2014).

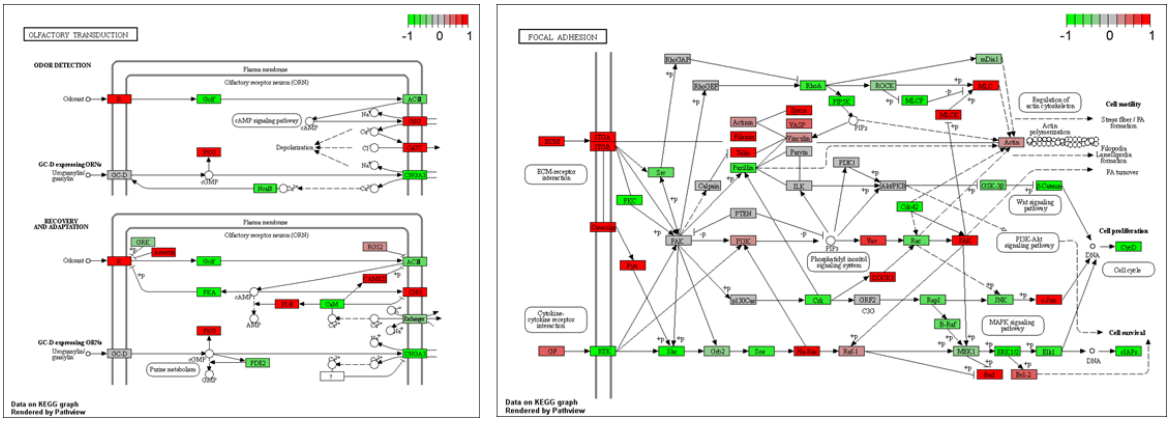



**Figure 2.7 Secondary KEGG pathway hits in resistant HGSOC PDX, the olfactory system and the focal adhesion pathway.**

Functional enrichment programs are also available that attempt to model the differentially expressed gene lists and how they may show functional phenotypes. FunRich is a program designed to assist in functional enrichment analysis on multiple public databases containing 1.5 million annotations (Pathan et al., 2015, 2017). It showed a more specific phenotype involving ribosome biogenesis, the actual promoter opening, transcription and elongation steps of Pol I (Figure 2.8).

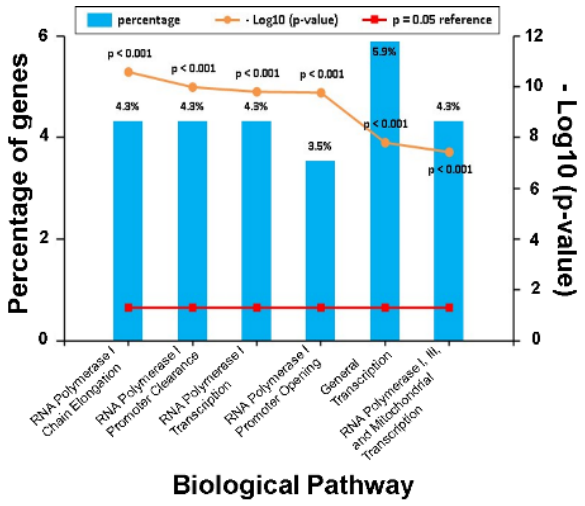



**Figure 2.8 FunRICH analysis of differentially expressed genes from chemoresistant PDX.** Functional enrichment combined with network analysis using the FunRICH software allows for multiple databases to be searched and aggregated in one place.

Online analysis programs are offered from the various bioinformatic groups that create the commonly used tools. EnrichR is a suite of downstream analysis tools that can check differentially expressed gene lists across multiple pathway mapping databases, transcription factor enrichment tools, and even against RO1 fundings that mention genes in your list (E. Y. Chen et al., 2013; Kuleshov et al., 2016). Running our lists on their mapping gives us similar findings confirming that the ribosome system appears upregulated in resistant tumors (Figure 2.9) along with ribosome biogenesis specifically (Figure 2.10).

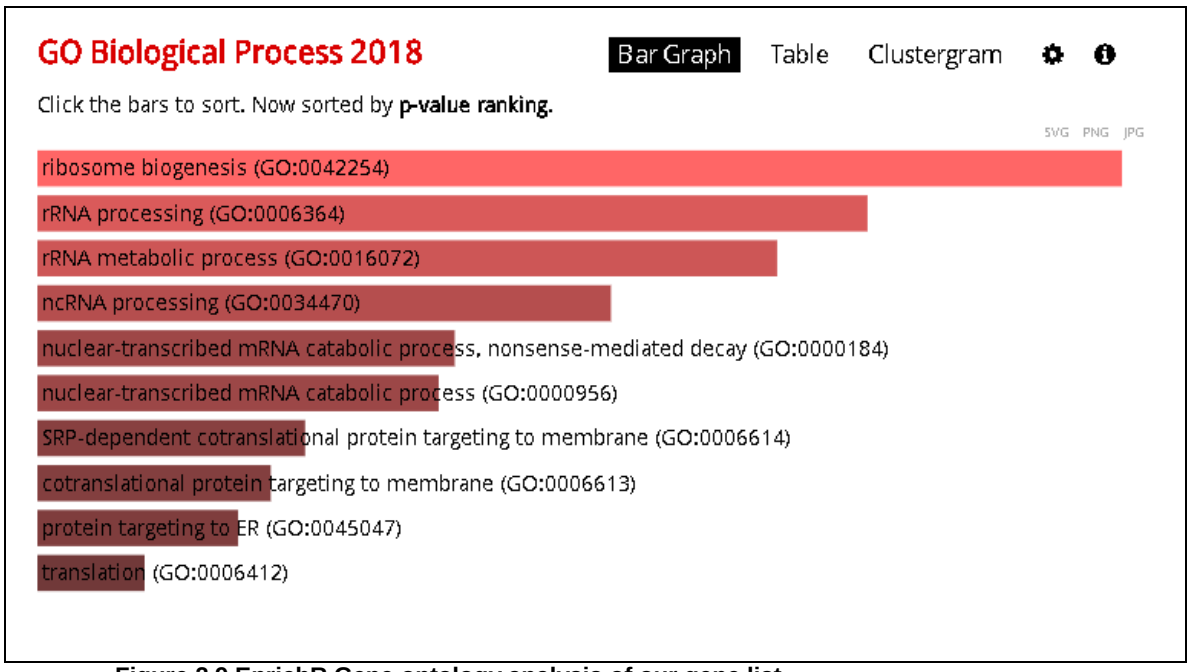



**Figure 2.9 EnrichR Gene ontology analysis of our gene list.**

KEGG 2019 Human

Bar Graph | Table | Clustergram

Hover each row to see the overlapping genes.

10 entries per page

Search:

| Index | Name | P-value | Adjusted p-value | Odds Ratio | Combined score |
|---|---|---|---|---|---|
| 1 | Ribosome | 0.000001507 | 0.0004642 | 4.18 | 56.07 |
| 2 | Ribosome biogenesis in eukaryotes | 0.0002191 | 0.03374 | 3.96 | 33.37 |
| 3 | Spliceosome | 0.0005534 | 0.05682 | 3.28 | 24.62 |
| 4 | Progesterone-mediated oocyte maturation | 0.003387 | 0.2086 | 3.23 | 18.39 |
| 5 | Cell cycle | 0.003984 | 0.2045 | 2.90 | 16.04 |
| 6 | RNA transport | 0.002977 | 0.2292 | 2.67 | 15.51 |
| 7 | Thyroid hormone signaling pathway | 0.008724 | 0.3839 | 2.76 | 13.08 |
| 8 | RNA degradation | 0.01417 | 0.5457 | 3.04 | 12.93 |
| 9 | Nitrogen metabolism | 0.06624 | 1.000 | 4.71 | 12.77 |
| 10 | RNA polymerase | 0.04161 | 1.000 | 3.87 | 12.31 |

**Figure 2.10 EnrichR version of KEGG also confirming our upregulation of ribosomes in resistant models.**

## 2.3 Confirmation of PDX findings in a second cohort of human patient tumors treated with neoadjuvant chemotherapy.

Before leaving University of Alabama, Dr. Landen began a clinical trial looking at neoadjuvant treatment effects in patients with HGSOC. Through a continuing collaboration we obtained RNA-seq raw output from Rebecca Arend MD from that clinical trial, and ran our pipeline on her patient data. There were 18 patients with obtained tumor samples from pre and post neoadjuvant chemotherapy (NACT), and that also had follow up clinical data in their response state after surgery and adjuvant treatment (Figure 2.11).

| SampleID | Patient | Age | Stage | PrePost | PlatinumResistance | BRCAstatus |
|---|---|---|---|---|---|---|
| *1post_20* | 1 | 64 | IV | Post | R | |
| *1pre_19* | 1 | 64 | IV | Pre | R | |
| *3post_22* | 3 | 77 | IV | Post | S | NEG |
| *3pre_21* | 3 | 77 | IV | Pre | S | NEG |
| *11post2_24* | 11 | 68 | IIIC | Post | S | NEG |
| *11pre_23* | 11 | 68 | IIIC | Pre | S | NEG |
| *12post_26* | 12 | 53 | IIIC | Post | R | |
| *12pre_25* | 12 | 53 | IIIC | Pre | R | |
| *14post2_28* | 14 | 61 | IIIC | Post | R | NEG |
| *14pre_27* | 14 | 61 | IIIC | Pre | R | NEG |
| *14pre_alicia_37* | 14 | 61 | IIIC | Pre | R | NEG |
| *22B22post* | 22 | 66 | IIIC | Post | S | NEG |
| *14B22pre* | 22 | 66 | IIIC | Pre | S | NEG |
| *24postA_30* | 24 | 74 | IIIC | Post | S | NEG |
| *24pre_29* | 24 | 74 | IIIC | Pre | S | NEG |
| *27post_32* | 27 | 73 | IV | Post | S | NEG |
| *27pre_31* | 27 | 73 | IV | Pre | S | NEG |
| *22B36post* | 36 | 65 | IIIC | Post | S | |
| *12B36pre* | 36 | 65 | IIIC | Pre | S | |
| *100post* | 100 | 76 | IIIC | Post | NA | |
| *100pre* | 100 | 76 | IIIC | Pre | NA | |
| *101post_4* | 101 | 61 | IVB | Post | S | |
| *101pre_3* | 101 | 61 | IVB | Pre | S | |
| *102post* | 102 | 72 | IIIC | Post | S | |
| *102pre* | 102 | 72 | IIIC | Pre | S | |
| *103post_8* | 103 | 67 | IV | Post | S | |
| *103pre_7* | 103 | 67 | IV | Pre | S | |
| *105post_10* | 105 | 67 | IIIC | Post | R | |
| *105pre_9* | 105 | 67 | IIIC | Pre | R | |
| *107post_12* | 107 | 70 | IV | Post | S | |
| *107pre_11* | 107 | 70 | IV | Pre | S | |
| *108post* | 108 | 67 | IIIC | Post | NA | |
| *108pre_13* | 108 | 67 | IIIC | Pre | NA | |
| *109post_16* | 109 | 59 | IIIC | Post | NA | |
| *109pre_15* | 109 | 59 | IIIC | Pre | NA | |
| *110post_18* | 110 | 82 | IIIC | Post | NA | |
| *110pre_17* | 110 | 82 | IIIC | Pre | NA | |

**Figure 2.11 Patient cohort for secondary analysis.**

We hypothesized that the ribosome biogenesis system and/or ribosomes themselves would be upregulated after NACT and in resistant patients. Running our pipeline with contrasts set to take into account acute effects of chemotherapy gave results backing our hypothesis that the ribosome system would be upregulated. Pathway analysis showed the ribosome system activating as well as fatty acid degradation systems (Figure 2.12). The overall ribosome phenotype appeared stronger than in our PDX with less of the overall ribosome proteins showing any decrease in expression.

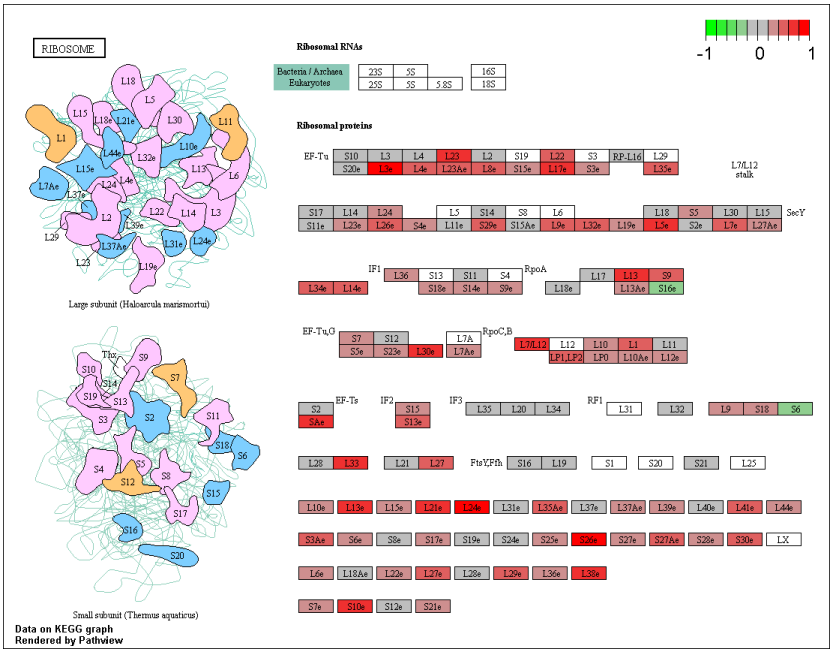



**Figure 2.12 Patient data confirming the ribosome system upregulating.**

Interestingly enough, fatty acid degradation is considered to be a critical player in drug tolerance, cancer as a whole and considered an important marker of the overall malignant phenotype (Kuo & Ann, 2018). Another player that came up was the renin-angiotensin system (Figure 2.13). This pathway has long been considered important in immune responses not only to the growing tumor, but also to how the extracellular microenvironment interacts with infiltrating immune effector cells as it is directly involved in the tumor microenvironment (Lyu et al., 2015; Pinter & Jain, 2017).

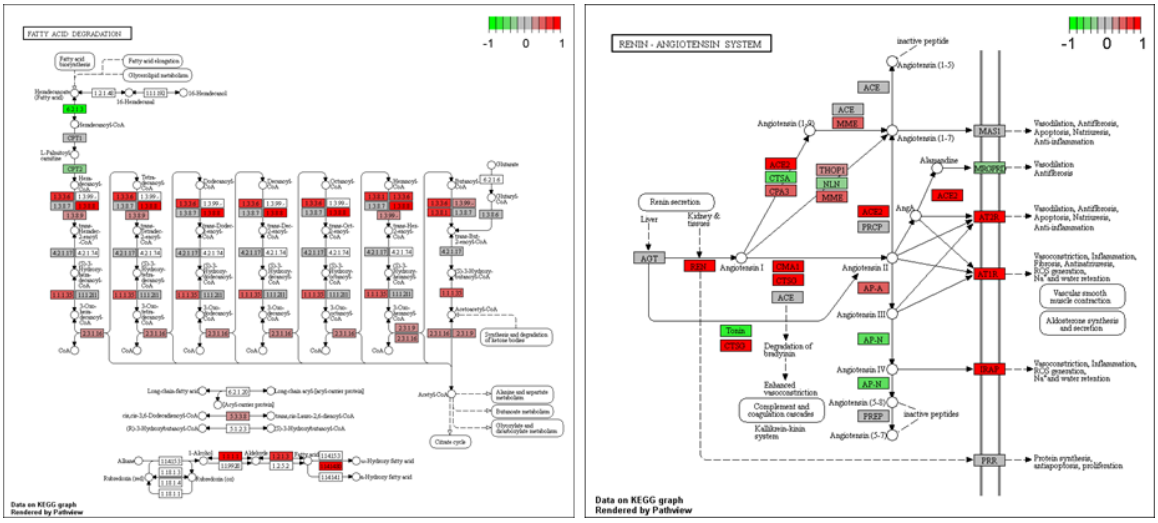



**Figure 2.13 Fatty acid and renin-angiotensin system upregulating in resistant patients.**

***Through both of these analyses we made the hypothesis that targeting ribosome biogenesis in ovarian cancer could be an effective therapeutic strategy and will target chemoresistant disease***. Targeting the initiation of the pathway may be the most specific to tumor cells due to their upregulation.

# 3 Targeting ribosome biogenesis

## 3.1 Ribosome overview

Ribosomes are a massive multicomponent molecular machine required to translate proteins from RNA (Thomson et al., 2013). Approximately 82 ribosomal proteins (this is thought to be changeable leading to the idea of specialized ribosomes (Xue & Barna, 2012)) are joined together on 4 structural RNA molecules called the ribosomal RNAs (rRNA) (Shiue et al., 2010). These

RNAs are encoded by the ribosomal RNA gene cassette located on the short arms of the acrocentric chromosomes 13,14,15, 21, and 22 (Leslie, 2014; Montanaro et al., 2008). Instead of there being 2 copies per genome like most other genes there are ~200-500 per genome with a variable number active at any given time (Shaw & McKeown, 2011; Thomson et al., 2013). There is a constant transcription of rRNA off the repeats in every cell at all times with cancer cells being able to synthesize as many as 3000 new ribosomes every 60 seconds (Pelletier et al., 2018). The repeats are mitotically bookmarked to allow them to begin transcribing before most other genes towards the end of mitosis (Sarge & Park-Sarge, 2009; Zaidi et al., 2010). The repeats themselves are mostly head to tail repeats with a spacer region protecting them from aberrant read through transcription (Grummt, 2003). Their transcription is done primarily by RNA polymerase I (Pol I) for the 28s, 15s and 5.8s rRNAs with RNA polymerase III transcribing the 5s (Russell & Zomerdijk, 2005; Schneider, 2012). Safely transcribing this cassette, thus initiating ribosome synthesis, is the primary function of the phase transition organelle known as the nucleolus (Hein et al., 2013). It is thought that due to the close proximity of 10 chromosome short arms the nucleolus evolved as a way to keep DNA damage repair systems away from the transcribing repeats since, outside of S-phase, DNA repair is via non homologous end joining (NHEJ), is error prone, and can lead to acrocentric chromosome fusions (Larsen & Stucki, 2016; Shav-Tal, 2005; van Sluis & McStay, 2017).

It takes >1000 proteins interacting in a complex web to synthesize ribosomes, and their biogenesis is the most energy demanding effort that a cell undertakes (Thomson et al., 2013). For that reason cells have evolved a multifaceted system of regulatory elements at each step of the process to maintain a coordinated system that is not wasting resources or producing faulty proteins. One such major component is that ribosome biogenesis is directly coupled to the mTOR/AKT system (Gentilella et al., 2015; Hsieh et al., 2011; Kusnadi et al., 2015). This serves to maintain a synthesis rate that is compatible with existing resource availability, as mTOR/AKT essentially acts as a nutrient availability sensor (Kusnadi et al., 2015).

### 3.1.1 rDNA repair

Due to the problems with NHEJ, specific mechanisms are in place to repair damaged rDNA (Edenberg et al., 2014; Korsholm et al., 2019; van Sluis & McStay, 2015). After damage is detected, the nucleolus appears to re-organize with damaged components on the edge of nucleus/nucleolar boundary where a type of homologous repair can occur without risking chromosomal fusions (Shav-Tal, 2005). Some repeats are inverted and there's evidence that these inverted copies can be used as a template for repair of damaged rDNA that's error free (Shav-Tal, 2005).

### 3.1.2 Nucleolar stress detection by *TP53*

Another system that appears tied to the ribosome biogenesis network is *TP53.* Of the 82 ribosomal proteins that makeup the complete ribosome, several

have been shown to compete with *TP53* for *MDM2* ubuiquitylation (Denis Drygin et al., 2014; Goudarzi et al., 2014). *MDM2* is an E3 ubiquitin ligase that, by adding ubiquitin to specific proteins, marks them for proteosomal degradation (Crane et al., 2015; James et al., 2014). *TP53* has evolved as a so-called dead man's switch: it is always translated without needing a specific activation and must be actively degraded or else the cell will activate DNA repair systems, senesce, turn on autophagy or die by mitochondrial-dependent apoptosis driven by *TP53* transcriptional targets (Golomb et al., 2014). This allows a cell to rapidly respond to various cellular insults, balancing death with being able to repair specific types of damage and recover from stress-inducing events.

If something damages rDNA, its transcription rate will drop and the stoichiometry between the amount of rRNAs available and the number of ribosomal proteins waiting for assembly (Vlatković et al., 2014). As excess ribosomal proteins buildup in the cytoplasm, MDM2 begins marking them for degradation. This indirectly induces stabilization of *TP53* since there is less MDM2 available for its degradation (Boulon et al., 2010; Hein et al., 2013). As *TP53* levels rise, systems activate to assess damage and decide between repair or death. This system is robust enough that any alteration in ribosome synthesis rates outside of specific constraints, whether up or down, can induce death (Hein et al., 2013). It was found early in cancer research that simply turning on a single strong proto-oncogene could lead a normal cell to die in a process named oncogenic stress (Ramos et al., 2017). The nucleolar *TP53*-ribosomal protein

stoichiometry appears to be a major source of that phenotype (Matos-Perdomo & Machín, 2019).

### 3.1.3 rDNA transcription and the rDNA pre-initiation complex

The ribosomal RNA pre-initiation complex (rPIC vs. PIC for non-ribosomal) is unlike all other transcriptional systems. The ribosomal PIC has evolved to maintain a stabilized open promoter structure (Grummt, 2003; Leary & Huang, 2001). Instead of the DNA being tightly wrapped around the 8 core nucleosome like all other DNA, the rDNA is loosely associated at best with the upstream binding factor (UBTF) actively competing with histone 1 for DNA binding (Prieto & McStay, 2005; Sanij et al., 2015; Woolnough et al., 2016). The rDNA is so loosely associated with nucleosomes that DAPI staining of metaphase spread will show a blank spot at the acrocentric chromosome positions where the rDNA repeats are (Shaw & McKeown, 2011). This loose structure allows for very fast transcription rates as well as rapid changing of the overall rDNA transcription rate itself in response to varying levels of resource availability or stress.

Active repeats contain distinct epigenetic markings that confer an open status. There appears to be 3 states the rDNA chromatin can be maintained in: open, closed, or a hybrid state between the two (Shiue et al., 2010). The closed state is associated with heterochromatin, contains the typical hypermethylated CpG sites, and has histone H4 hypoacetylation, methylation of H3K9 and H4K20, as well as H3K27me3 (Grummt & Längst, 2013; F. Yu et al., 2015). Open sites associated with transcription are usually hypomethylated, and have acetylation of

H4 and H3K4me2. Histone H4 hypoacetylation, along with methylation of H3K9 and H4K20 correlate with transcriptional silencing (Grummt, 2007; Srivastava et al., 2016). It is also thought that cells use only the male or female rDNA repeats with the chosen one available and the other silenced by a process known as nucleolar dominance.

The open rDNA repeat is essentially peppered with UBTF in a fast on and off configuration change (Woolnough et al., 2016). The selectivity complex (SL1), comprised of various transcription factor molecules, stabilizes the UBTF rDNA interaction with the stabilized structure forming an enhancesome configuration. The final shape of this transcription enhancing structure is unknown but current models suggest either a barrel with UBTF/SL1 in the center or a bend that brings upstream elements and enhancers within range of the active promoter (Goodfellow & Zomerdijk, 2013; Santoro et al., 2002). Once stabilized RRN3 identifies and binds to transcriptionally competent Pol I and brings the polymerase to the promoter surface and, after binding RRN3, is recycled back out of the nucleolus (Prieto & McStay, 2005). After positioning, there is a secondary structure change in the conformation of the rDNA induced by a G-quadruplex:protein interaction that allows polymerase promoter escape to occur with the next RRN3/Pol I complex positioning soon after (Panov et al., 2006). A specific rDNA repeat can contain tens of Pol I enzymes actively transcribing simultaneously, with the elongation rate being fine-tuned to allow for rapid transcription without polymerase collisions (Russell & Zomerdijk, 2006).

As the rRNA is pulled away from the rDNA and processed, it undergoes a constant stream of modifications to assemble the mature ribosome with various assembly parts being done in the nucleolus, the nucleoplasm and cytosol. (Calo et al., 2014)

### 3.1.4 Ribosomal requirements of cancer

The overall rate of ribosome biogenesis has been thought of as a proxy measurement for the rate of cell proliferation (Ruggero, 2012). Since the ribosomes are required for translation the oncogenic protein demands of a growing tumor are dependent on a large supply of ribosomes to meet these proliferation demands. Deregulating ribosome synthesis is thought to be both necessary and sufficient to induce transformation (Montanaro et al., 2008).

Many of the major players in oncogenic transformation can interact directly on the rDNA or the with rDNA transcriptional machinery (Hein et al., 2013). MYC's primary role may in fact be to maintain the unusually high rate of rDNA transcription required (Potapova et al., 2019; R. J. White, 2008). Cyclins and cyclin-dependent kinases have been shown to directly alter rDNA chromatin allowing for fine-tuned transcription rates during mitosis (Klein & Grummt, 1999). In cancer, aberrant cyclin availability is a way for a cancer to increase its ribosome levels. Rb and TP53 both can directly interact with the rDNA promoter with hyperphosphorylated Rb being integral to rDNA control during mitosis (R. J. White, 2008).

### 3.1.5 rDNA transcription as a drug target

With all of these direct and indirect interactions between rDNA and oncogenes there has long been an interest in targeting the ribosomal system for cancer intervention (Denis Drygin et al., 2010; Ruggero, 2012). The complex regulation of ribosome biogenesis was thought to make this a problematic system to attempt to target pharmacologically. Ribosomes are required for life and normal cells can have high translational demands depending on their functions (Nguyen et al., 2015). Toxicity was thought to be a major barrier to ever actually targeting the system. There have been several findings counter to this line of thinking though. Most cytotoxic chemotherapies are cross linking agents or DNA damage-inducing agents. As many as 36 of the 48 chemotherapies in use today directly target the rDNA promoter (Burger et al., 2010). Drugs like actinomycin D are thought to get their primary mechanism of efficacy through this interaction and, while being toxic agents, they are useable in the clinical setting (Scala et al., 2016).

Two groups began investigating inhibitors of Pol I as a means to target ribosome biogenesis for cancer therapy (Figure 3.1). Laiho et al. created a series of compounds that are DNA intercalators with specificity for the rDNA promoter (K. Peltonen et al., 2014). The most promising candidate (BMH-21) is very toxic at nanomolar concentrations and with some specificity to dividing cells, if not certain cancers (Colis et al., 2014). Surprisingly enough, BMH-21 does not

appear to induce DNA repair machinery or marking of DNA damage by phosphorylation of H2AX on serine 139 (Colis et al., 2014).

The other group was Drygin et al. with a supposed “direct” inhibitor of pol I transcription (Haddach et al., 2012b). After a high throughput screen for compounds decreasing rDNA transcription they discovered a rather interesting small molecule named CX-5461. It appeared in cell-free rDNA transcription assays that CX-5461 inhibited SL1 binding to the rDNA. This led both *in vitro* and *in vivo* to a rapid and robust decrease in rDNA transcription to ~10% of baseline levels (Haddach et al., 2012b).

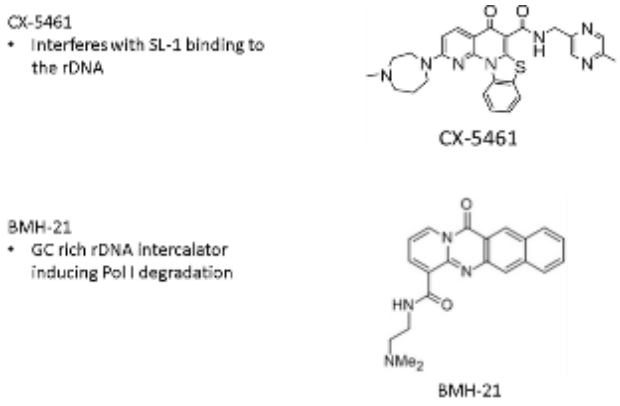



**Figure 3.1 Drugs specifically targeting RNA polymerase I.**

### 3.1.6 Our hypothesis

We showed evidence that ribosome biogenesis is deregulated in chemoresistant ovarian cancer cells. ***From this we hypothesize that treatment with DNA damaging agents requires cancer cells to rapidly transcribe rRNA to help survive genotoxic insult, thus making RNA polymerase I a possible***

***drug target for attacking chemoresistant ovarian cancer cell populations.***

***We chose BMH-21 and CX-5461 as a means to investigate this hypothesis.***

# 4 Targeting RNA polymerase I in ovarian cancer

## (modified from (Cornelison, Dobbin, et al., 2017))

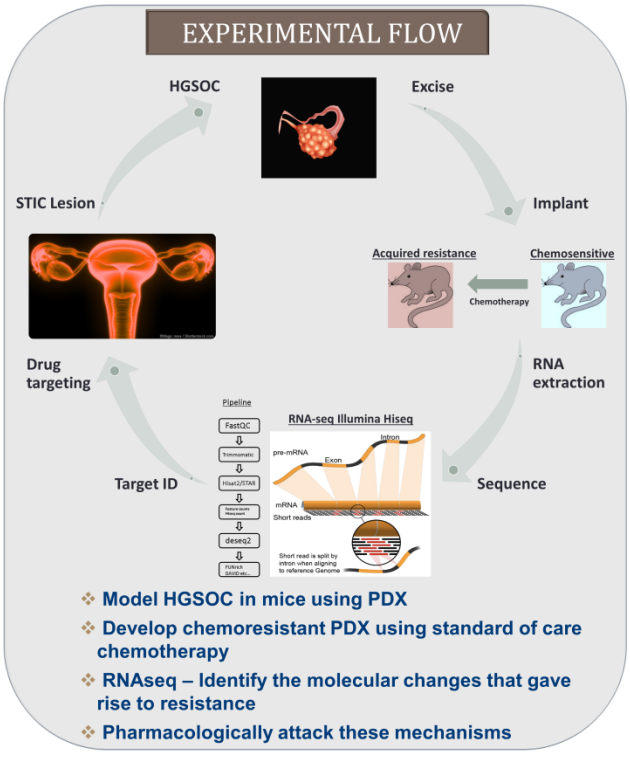



**Figure 4.1 Graphical overview of the experimental setup to investigate transcriptomic changes after standard of care chemotherapy in PDX models.**

## 4.1 INTRODUCTION

Although ovarian cancer patients usually present with advanced disease, most will have a positive response to initial therapy consisting of surgical debulking and combined platinum/taxane chemotherapy (Berek et al., 2012). Nonetheless, as many as 80% of patients will ultimately develop recurrence with chemoresistant disease, suggesting that a small chemoresistant population must be identified and targeted in order to achieve durable cures (Bowtell et al., 2015). Although in recent years evaluation of multiple chemotherapeutic and targeted agents, alone or in combination, has yielded a modest improvement in survival, drug resistance remains the major cause of death of ovarian cancer patients (Armstrong, 2002; Borst, 2012; Rubin et al., 1999).

An increase in size and number of nucleoli was one of the earliest hallmarks of cancer identified and is still a useful prognostic indicator today (Shiue et al., 2010). The nucleolus is the primary site of ribosomal biogenesis, and enlarged nucleoli correlate with accelerated ribosomal RNA (rRNA) synthesis by RNA polymerases (Shiue et al., 2010; Thomson et al., 2013). Synthesis of ribosomes is one of the most complex and energy demanding processes in cells. RNA polymerase I (Pol I) transcribes the ribosomal DNA (rDNA) to produce the pre-rRNA precursor that is processed into 18S, 5.8S and 28S rRNAs through a number of nucleolytic steps (R. J. White, 2008). RNA polymerase III (Pol III) synthesizes the 5S rRNA, which is also a structural component of the ribosome. Together, Pol I and Pol III account for ~80 % of total

nuclear transcription (R. J. White, 2008). An increase in rRNA synthesis in the nucleolus by Pol I has been correlated with an adverse prognosis in cancer (Williamson et al., 2006).

A number of clinically approved chemotherapeutic drugs act, at least in part, through inhibition of ribosomal RNA synthesis (Burger et al., 2010). However, none of these drugs directly target Pol I (Burger et al., 2010; Denis Drygin et al., 2010; K. M. Hannan et al., 2013). Recently, small molecule inhibitors have been developed, such as CX-5461 and CX-3543, which have apparent specificity for RNA Pol I (D. Drygin et al., 2009; Denis Drygin et al., 2011; Haddach et al., 2012a; K. Peltonen et al., 2014). CX-5461 does not inhibit mRNA synthesis by RNA polymerase II, does not inhibit DNA replication or protein synthesis, and has preferential activity for cancer cells over non-transformed cells (Haddach et al., 2012a). Bywater *et al*. showed that CX-5461 exhibits inhibition of rRNA transcription dependent on TP53 mutational status and induces TP53-dependent apoptotic cell death of hematologic malignancies, while maintaining normal cells (Bywater et al., 2012).

Our interest in CX-5461 came from the discovery that, in heterogeneous PDX models of ovarian cancer treated with chemotherapy, high-throughput analysis suggested that ribosomal machinery was upregulated in the surviving population. Other investigators have suggested that ribosomal biogenesis is increased in gynecologic cancers (Artero-Castro et al., 2011) and might be a key component in the efficacy of some targeted therapies, such as RUNX factors

(Deltcheva & Nimmo, 2017) and c-MYC's role in both cancer and global gene expression. However, an association of this process with chemoresistance and a mechanism to directly target ribosomal biogenesis in ovarian cancer has not been examined. In this study, we further investigate the activity of RNA Pol-I in treated tumors and the effects and mechanisms of CX-5461 on the viability of both chemosensitive and chemoresistant ovarian cancer, in cell lines and PDX models. We show that CX-5461 has activity alone on ovarian cancer cells and demonstrates increased efficacy in taxane-resistant ovarian cancer cells, independent of TP53 mutational status. Chemo-responsive ovarian PDX tumors show an increase in Pol I activity after chemotherapy, and have a variable response to Pol I inhibition, achieving a complete response in one model. These findings suggest that targeting Pol I inhibition is an exciting opportunity as a therapeutic strategy in treating ovarian cancer, particularly in targeting the chemoresistant population.

## 4.2 Results

### 4.2.1 Increase in expression of ribosomal machinery by chemotherapy

As previously reported (Dobbin et al., 2014), six PDX models were established immediately after resection from advanced high-grade serous ovarian cancer patients, with 10 mice per model. When tumors were 0.75cm in at least one dimension, mice were treated with either vehicle or combined carboplatin/paclitaxel weekly for 4 weeks. Seven days after the final dose (to

allow acute effects of chemotherapy to dissipate), tumors were collected and preserved in multiple formats. RNA was extracted and subjected to RNA-Seq analysis. IPA pathway analysis comparing matched treated and untreated PDX, described more thoroughly in our previous report, found that ribosomal synthesis machinery was significantly different in all pairs, and was the most commonly upregulated pathway after treatment in 4 of the 6 pairs. This analysis was confirmed using a larger cohort with the KEGG finding shown in Figure 2.12. Our first priority after this preliminary global analysis was to confirm whether findings related to increases in ribosomal machinery with treatment could be verified. To confirm these high-throughput data, qPCR was conducted on the matched treated-untreated ovarian cancer PDX for *UBTF, POLR1B,* and *RRN3*. These genes encode transcription factors for rRNA synthesis (*UBTF* and *RRN3*) as well as the second largest subunit of Pol I (*POLR1B*), and can influence the rate of rRNA synthesis when differentially expressed (Denis Drygin et al., 2011; Schneider, 2012). In all 6 pairs, either *UBTF, POLR1B,* or both were significantly upregulated in the post-therapy samples compared to their untreated tumor, with *RRN3* upregulated in two (Figure 4.2A, B, C). The degree of increase was, however, highly variable in the 6 models. Additionally, the amount of 18S rRNA and 28S rRNA was determined, as a measure of overall ribosomal content. There was a surprising increase in the relative expression of the rRNA transcripts after chemotherapy treatment. 18S levels increased 6.59-fold ($p$=0.010) and 28S levels increased 5.53-fold ($p$=0.019) (Figure 4.2D). This was especially surprising, given our previous finding that immunohistochemical staining of these

same samples for Ki-67 showed that proliferation was significantly reduced, on average from 67% in untreated and 12% in treated samples. Dogma would suggest that ribosomal production should correlate with proliferation rates, but clearly does not in the setting of chemotherapy. The significant upregulation of ribosomal activity and total ribosomes in surviving cells led us to hypothesize that inhibiting ribosomal synthesis may be a method for targeting ovarian cancer and, specifically, the cell populations surviving primary chemotherapy. We previously presented a principle component analysis of 6 pairs of untreated/treated PDX tumors, showing that although tumors had a different baseline expression profile, as would be expected from 6 different patients, the *changes* when comparing matched treated and untreated tumors were similar. This relationship suggests that although tumors are different initially, globally they are having very similar responses to chemotherapy.

The increase in ribosomal synthesis activity led us to pathologically examine nucleoli of untreated and treated tumors. We found that nucleoli were much more prominent in PDX tumors after treatment (Figure 4.2F). The examining pathologist noted that this was a common finding seen in patient samples collected after neoadjuvant chemotherapy, and has been previously published in breast and ovarian cancer (Y. Wang et al., 2013). In a blinded manner, the pathologist scored the percentage of malignant cells that would be considered to have prominent “macronucleoli”. In 3 of the 4 paired sections in which enough tumor was available to examine, there were significantly more macronucleoli after chemotherapy treatment (Figure 4.2G).

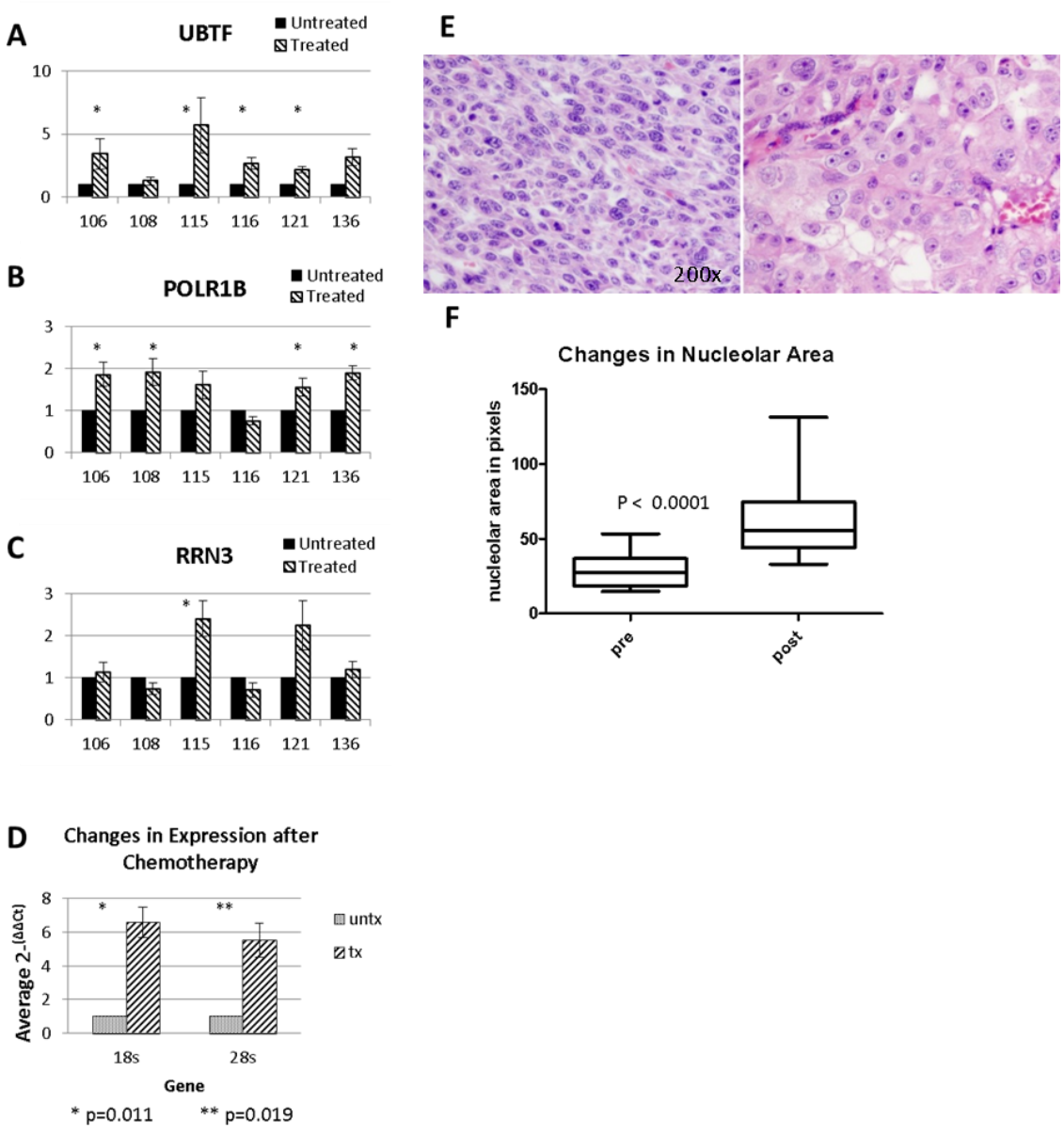



**Figure 4.2 Expression of RNA Polymerase I initiation factors in ovarian cancer PDX models.** Comparison of 6 pairs of untreated/treated (Carbo/taxol) PDX tumors showed similar changes in expression profiles. One common pathway was ribosomal synthesis. (A,B,C) qPCR was conducted on 6 pairs of ovarian cancer PDX treated with carboplatin and paclitaxel or control for UBTF, POLR1B, and RRN3 and gene expression was compared to the untreated matched PDX. The tumor cell population surviving initial chemotherapy generally had a greater expression of UBTF, POLR1B, and RRN3. (* = $p < 0.05$) (D) Total level of ribosomal subunits 18S and 28S was determined. Treated ovarian cancer PDXs had a 37.9-fold increase of 18S (p=0.010) and a 39.0-fold increase of 28S (p=0.019). (E) Comparison of H&E sections with

and without treatment, demonstrating marked macronucleoli by (F) Quantification of macronucleoli before and after chemotherapy treatment in PDX models.

### 4.2.2 RNA Pol-I inhibition diminishes cell viability in ovarian cancer cell lines

CX-5461 has recently been reported as a selective inhibitor of Pol I with the ability to inhibit solid tumor growth (Denis Drygin et al., 2011), although in some settings it has been reported to be more effective in cells with wild-type *TP53.* In high-grade serous ovarian cancer, *TP53* mutations have been identified in 96% of tumors (Cancer Genome Atlas Research Network et al., 2011). Nonetheless, based on the RNA-seq and follow up qPCR data, we examined the response of multiple ovarian cancer cell lines, and in 3 instances had matched chemoresistant lines: A2780ip/A2780cp20, SKOV3ip1/SKOV3TR, and HeyA8/HeyA8MDR. Viability as measured by the MTT assay demonstrated an IC50 range of 32 nM-5.5 μM (Figure 4.3).

| Matched Pairs Chemosensitive/chemoresistant | P53 state | CX-5461 IC50 (mM) |
|---|---|---|
| A2780ip2 | WT | 0.032 |
| A2780cp20 | MT | 0.146 |
| | | |
| SKOV3ip1 | MT | 0.595 |
| SKOV3TRip2 | MT | 0.109 |
| | | |
| HeyA8 | WT | 2.05 |
| HeyA8MDR | WT | 0.191 |
| Other Cancer Cell lines | | |
| PEO1 | MT | 0.525 |
| PEO4 | MT | 1.20 |
| OV90 | MT | 1.06 |
| OVCAR3 | MT | 2.00 |
| OAW42 | WT | 0.300 |
| OVSAHO | MT | 0.636 |
| CAOV3 | MT | 0.955 |
| COV362 | MT | 0.645 |
| ES2 | MT | 0.294 |
| Normal immortalized cells | | |
| HIO-180 | WT | 5.49 |

**Figure 4.3 CX-5461 IC50 of various 15 OVCA and 1 normal cell line.**

Interestingly, in the matched chemosensitive/chemoresistant pairs, the two taxane-resistant lines SKOV3TRip2 and HeyA8MDR were more sensitive to CX-5461 than their matched chemosensitive SKOV3ip1 and HeyA8 lines, by 5.5 and 10.5-fold, respectively (Figure 4.3A, B). Interestingly, A2780ip2 was more sensitive to CX-5461 than the platinum-resistant A2780cp20, with an IC50 of 32 nM compared to 146 nM (Figure 4.3C). TP53 status was examined in these lines, and it was discovered that A2780cp20 at some point in its evolution acquired a

TP53 mutation (missense previously published (Skilling et al., 1996) and confirmed in our cells). Together, these differential effects suggest that the mechanism of action of CX-5461 clearly has some correlation with TP53 status, but more data is needed to clarify what sensitizes cells to CX-5461 treatment. Additionally, the increased sensitivity in taxane-resistant cells suggests that this might be an outstanding therapy in the setting of resistant disease, consistent with the *in vivo* observation that treated tumors had increased ribosomal synthesis.

### 4.2.3 CX-5461 causes cell cycle arrest in G2/M

In order to examine the biologic effects of CX-5461, we first examined cell cycle composition with treatment, employing Propidium Iodide (PI) incorporation and flow cytometric analysis. The six chemosensitive/chemoresistant paired cell lines were treated with either vehicle control, the IC50 of CX-5461, or the IC90 of CX-5461 for 48 hours. Interestingly, there was pronounced G2/M arrest in both SKOV3 cell lines with the IC90 dose, from 24.48% to 37.89% in SKOV3ip1, and 36.45% to 56.16% in SKOV3TRip2 (Figure 4.4E). This was also observed with the IC90 dose in A2780cp20 (38.86% to 69.61%), HeyA8MDR (31.75% to 51.49%) and the IC50 dose in A2780ip2 (26.31% to 42.71%) (Figure 4.4D,F). An arrest in G2/M is consistent with prior observations that Pol I activity is the highest in the G2/M phase (Klein & Grummt, 1999).

To confirm arrest at G2/M, cyclin B was measured by immunofluorescence. The G2/M transition is tightly regulated by the interplay of cyclin B and CDC2, with the dephosphorylation of CDC2 being required for movement into mitosis by the phosphatase CDC25. G2-arrested cells should show a buildup of cyclin B and pCDC2 (tyr15) in arrested cells. Immunofluorescence after treatment with CX-5461 showed intense perinuclear staining of cyclin B after treatment (Figure 4.4G). Furthermore, immunoblotting indicated an increase in pCDC2 (tyr15) and cyclin B after treatment in all cell lines (Figure 4.4H), verifying significant arrest in late G2.

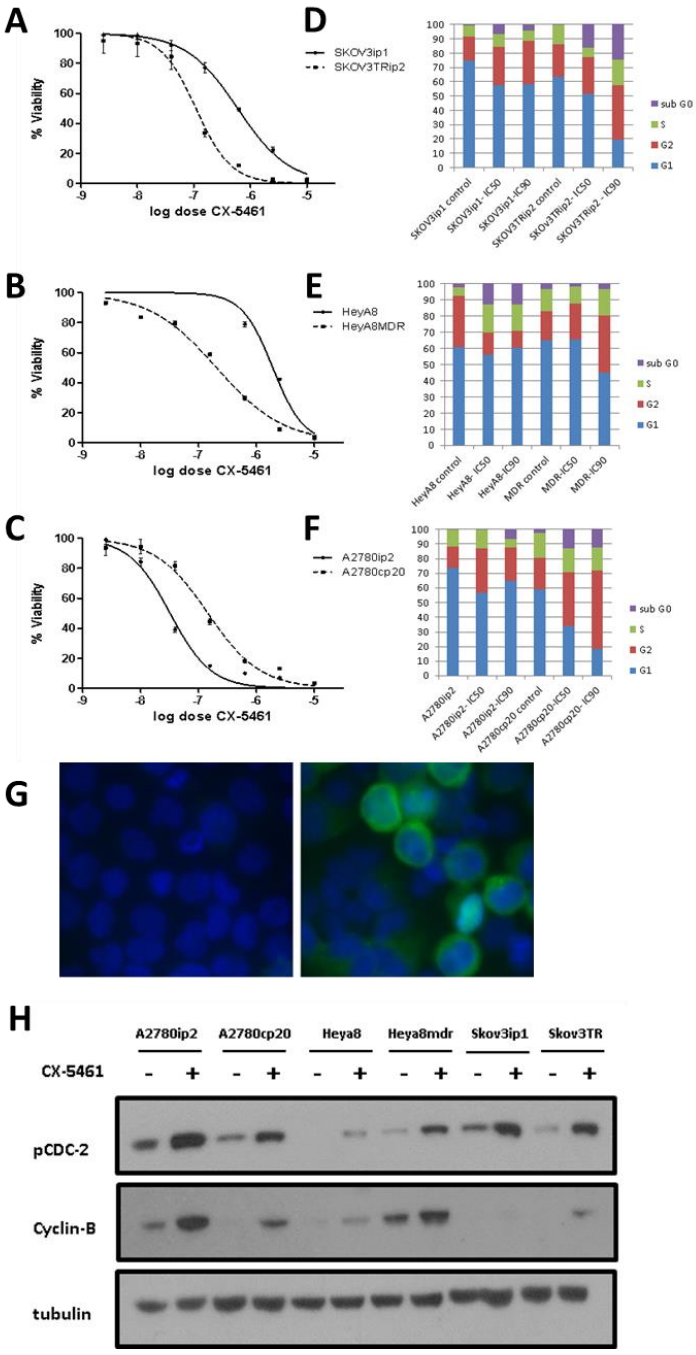



**Figure 4.4 Response of ovarian cancer cell lines to CX-5461.** Cell viability and cell cycle analysis was conducted on 3 pairs of chemosensitive and chemoresistant ovarian cancer cell lines. (A-C) Dose response curves from MTT assay of each pair. Results show both SKOV3TRip2 and HeyA8MDR resistant lines being more sensitive to CX-5461 treatment than their chemosensitive parental line. (D-F) Cell cycle analysis of SKOV3ip1, SKOV3TRip2, HeyA8, HeyA8MDR, A2780ip2, and A2780cp20 using Propidium Iodide after 48 hr treatment with the control, IC50, or IC90 dose of CX-5461. In general, treatment with CX-5461 resulted in an increase in sub-$G_0$ fraction and a portion of cells in the S/G2 phase. (G)

Immunofluorescence assay example verifying G2/M phase cell cycle arrest in A2780cp20 treated cells with 1μM CX-5461 versus vehicle control. Results show an increase in Cyclin-B perinuclear staining in arrested cells compared to untreated control. (H) Western blot analysis verifying G2/M phase arrest in 6 cell lines. Treatment with IC50 dosages of CX-5461 showed an increase in expression of pCDC2(tyr15) and Cyclin-B versus untreated controls at 72 hours.

### 4.2.4 Transcription of rDNA is increased in chemoresistant cell lines despite lower overall growth rate

Since protein synthesis rate is directly proportional to cell growth rate, ribosomal production is traditionally thought to mirror proliferation rates. However this paradigm is not consistent with our *in vivo* observation that treated tumors had upregulation of Pol I machinery despite low proliferation rates, and that the SKOV3TRip2 and HeyA8MDR cell lines were more sensitive to CX-5461 despite their lower doubling times. To identify the potential mechanism by which chemoresistant tumor cells are more sensitive to inhibition of rRNA synthesis, we characterized rRNA synthesis in SKOV3ip1 and SKOV3TRip2 cells. Based on isotopic labeling (Figure 4.5A) we observed a dramatic increase in rRNA synthesis in the chemoresistant line compared to the control cells. To confirm our isotopic labeling results, we measured pre-rRNA abundance using RT-qPCR, probing for the 5'-external transcribed sequence (5'-ETS; Figure 4.5B). Since this RNA species is short-lived, its abundance is used as a measure of rRNA synthesis rate. We observed a significant increase in HeyA8MDR compared to parental HeyA8; in SKOV3TRip2 compared to SKOV3ip1; but no significant change in A2780cp20 compared to A2780ip2. Therefore relative sensitivity to CX-5461 does follow with rRNA synthesis rate, at least when comparing these

pairs. It does not follow with sensitivity when comparing different cell lines, and therefore cannot be used as an absolute marker of response alone. However, it does confirm that rRNA production does not follow precisely with proliferation rate, and is increased in the taxane-resistant lines when compared to parental controls.

Increased total rRNA can be achieved by increasing the rate of transcription by Pol I, by inhibition of rRNA decay, or by a combination of these mechanisms. To test whether transcription by Pol I was induced, we measured polymerase occupancy on the rDNA repeat using chromatin immunoprecipitation (ChIP). We observed a two- to three-fold increase in polymerase occupancy of the gene in the SKOV3TRip2 cells compared to the parental line (Figure 4.5C). Together, these data suggest that synthesis of rRNA by Pol I is increased in the taxane-resistant line, despite its slower growth rate.

Enhanced production of rRNA could suggest that more ribosomes are present and functional in the taxane-resistant cells, or may represent an overproduction in response to faulty ribosomal functionality. In order to semi-quantitatively examine engagement of ribosomes onto mRNA, we performed sucrose gradient fractionation of cytoplasmic fractions from cycloheximide-treated cells. As shown in figure 4.5D, ribosome profiles from equal cell numbers reveal competent ability of ribosomes to bind to mRNA in both cell lines. Each peak following the 40S, 60S, and 80S peaks represents an mRNA strand with at least 2 ribosomes attached. The first peak is composed of an mRNA with 2

ribosomes attached, the next with 3 ribosomes, and so on. There is a suggestion that there are actually more actively-translating ribosomes/mRNA units, though this method is semi-quantitative and that conclusion is not definitive. However the prominent polysome peaks do confirm that there is not a decrease in the number of translating ribosome/mRNA units in the SKOV3TRip2 line, and therefore increased production of ribosomes is not due to defective ribosomal functionality. It does suggest that the increased ribosome production leads to increased translation. Therefore chemoresistant cells appear to be using increasing translation as a mechanism of chemoresistance.

Together, all of these data suggest that rRNA synthesis by Pol I is dramatically increased in the taxane-resistant cells without a complementary increase in ribosome concentration, or a deficiency in ribosomal functionality, despite its slower growth rate. This is contrary to the dogma that proliferation and ribosomal production/activity are universally directly correlated.

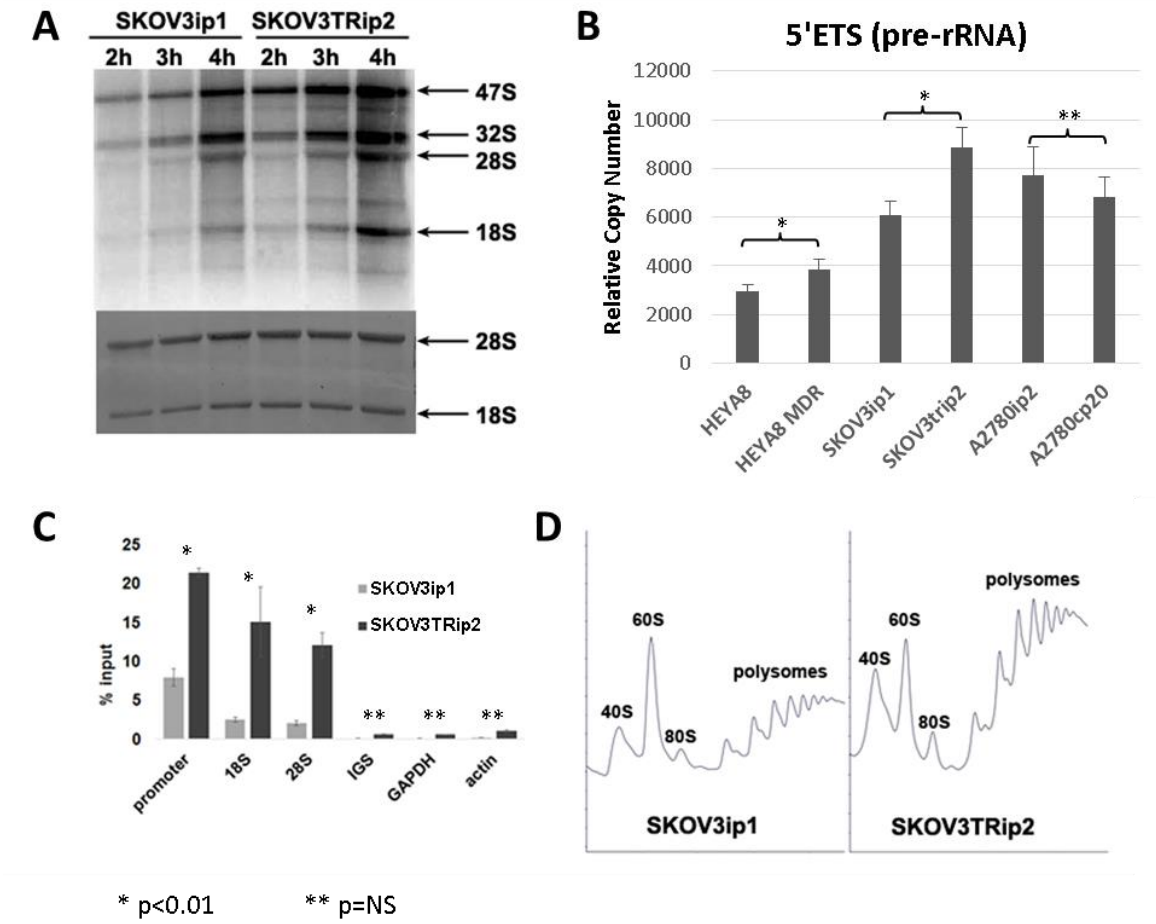



**Figure 4.5 rRNA synthesis and polymerase occupancy is increased in SKOV3TRip2 cells compared to SKOV3ip1 cells.** (A) Cells were pulse labeled with 32P-orthophosphate as a function of time. Total RNA was purified by Trizol, run on a formaldehyde denaturing agarose gel, dried, and visualized by autoradiography. The rate of 32P incorporation into rRNA in SKOV3TRip2 cells was 2.1 fold (+/- 0.3) higher than in SKOV3ip1 cells. (B) rDNA transcription was analyzed by RT-qPCR by targeting short-lived 5'ETS, and the rRNA signal was normalized to the control GAPDH mRNA. (C) CHIP analysis of RPA194 association with rDNA. Pol I molecules were immunoprecipitated by anti-A194 monoclonal antibody. The mean and SD are shown. (D) Polysome profiles were performed by sucrose gradient centrifugation.

### 4.2.5 Treatment with CX-5461 increases DNA damage markers in ovarian cancer cell lines

G2/M checkpoint arrest is a frequent consequence of genotoxic insult and is used to prevent entry into mitosis after DNA damage has occurred by a variety of mechanisms (Liang et al., 2009). Early sensing of DNA damage is done by the kinases ATM and ATR and further activation of the checkpoint is mediated through a variety of signals, both TP53-dependent and independent (Burma et al., 2001; Rogakou et al., 1998; Yuan et al., 2010). An indicator of multiple forms of DNA damage is marked via phosphorylation of the histone γH2AX at Ser139 by the kinases ATM and ATR (Huen & Chen, 2008). After treatment with CX-5461, ovarian cancer cell lines showed a significant increase in γH2AX(Ser139) foci in the nucleus (Figure 4.6A). After DNA damage is detected, the ATM/ATR kinases also activate their downstream effectors CHK1 and CHK2, beginning a broad range of signaling, from inactivation of CDC25 leading to G2 arrest to TP53-mediated apoptosis. Immunoblotting after CX-5461 treatment showed an increase in the phosphorylated forms of CHK1(Ser317) and CHK2(Tyr68) as well as a dramatic increase in the TP53-mediated tumor suppressor p21 in the TP53 wild type cell line A2780ip2 (Figure 4.6B).

### 4.2.6 CX-5461 induces mitotic catastrophe

In performing immunofluorescent stains, it was noted that there was a significant increase in the size and presence of multinucleated cells with CX-

5461 treatment. During the final stages of functional mitosis, a cleavage furrow is formed and a contractile ring pinches off the nascent daughter cells. Thus cells were stained with F-actin to identify cell membranes, and multinucleated cells were quantified (Figure 4.6C). Surprisingly, these images are collected at equal power, which can be appreciated if one focuses on the size of the nuclei, which are not significantly different, whereas the total body size is increased dramatically. Characterization and quantitation of binuclei, using both manual and image analysis based methods (Figure 4.8), showed a dramatic increase in cell volume after treatment with CX-5461, as well as an increase in the number of multinucleated cells. We also performed immunofluorescent staining for Aurora-B kinase, as correct localization of this protein is required for cytokinesis. Normally Aurora-B is localized at the condensing contractile ring in between the two daughter cells. In CX-5461-treated cells, however, Aurora-B kinase showed mislocalization during cytokinesis, lateralized relative to the nucleus, suggesting mitotic catastrophe (Figure 4.6D).

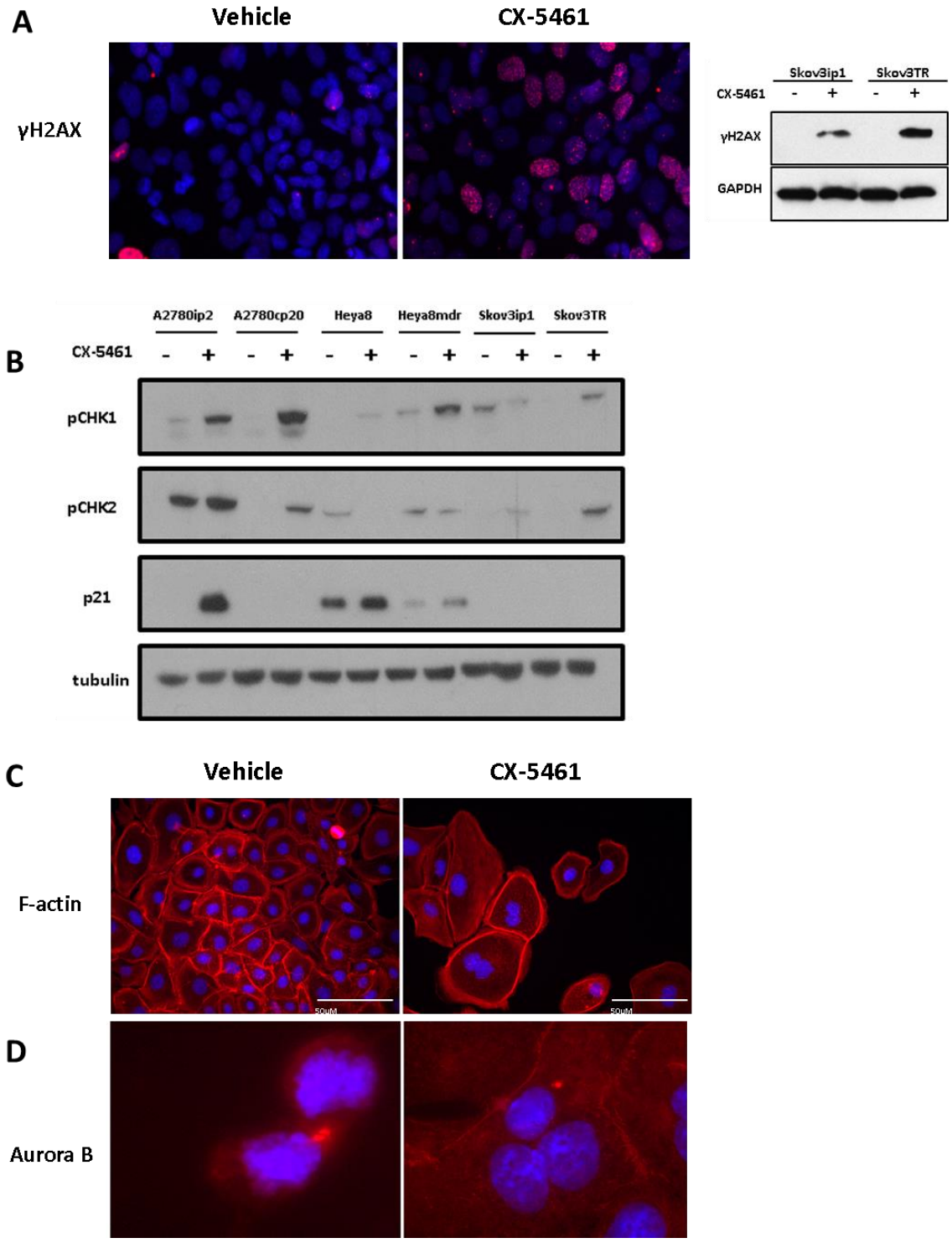



**Figure 4.6 CX-5461 induces DNA damage response via ATM/ATR in ovarian cancer cell lines.** (A) Immunofluorescent detection of the pH2AX in control SKOV3ip1 cells (red) and cells exposed to 500nM of CX-5461. The punctate nuclear staining illustrated an accumulation of DNA damage foci. Pairs of chemosensitive/chemoresistant ovarian cancer cell lines were treated with their respective IC50 dosages of CX-5461. (B) Western blot analysis shows activation of the pCHK1(ser317) and pCHK2(T68), downstream substrates of ATM and ATR kinases. TP53 wild type cell lines showed a subsequent activation of p21 but

drug response was independent of TP53 status. (C) DAPI (blue) labeling showed failed cytokinesis and an accumulation of multinucleated cells after CX-5461 treatment, with F-actin (red) indicating shared cytoplasm. (D) Aurora-B kinase (red) detection showing normal localization to the cleavage furrow during cytokinesis in control SKOV3ip1 cells. After treatment with CX-5461 Aurora-B mislocalization was apparent in an accumulation of multinucleated cells.

### 4.2.7 Ovarian Cancer PDXs have variable response to Pol I inhibition

Based on the activity of CX-5461 *in vitro* even in the presence of *TP53* mutations, five different ovarian cancer PDX models (from 5 advanced-stage papillary serous ovarian cancer patients) were treated with single-agent CX-5461 at 50mg/kg every three days for 45 days. Treatment was initiated when tumors were approximately 1.0 cm in at least one dimension. Response was varied among the 5 models, with PDX 208 and 182 growing on treatment, PDX 127 having stable disease, and PDX 153 and 225 showing response, with PDX 225 having a complete regression of tumor (Figure 4.7A). As a measure of health of the mice during treatment, weight was collected with each treatment, and behaviors carefully monitored. There were no behavioral changes, or functional abnormalities such as diarrhea. There was an appropriate weight gain throughout the treatment course (Figure 4.7B), as has been demonstrated at this dose level in prior studies (Bywater et al., 2012). Tissue sections of liver, kidney, heart, and lung were examined pathologically and there was no gross toxicity to these major organs.

In order to determine if baseline expression of Pol I-associated genes could represent a potential biomarker for treatment response, previously-

collected untreated tumors from each PDX model were analyzed using qPCR for *POLR1B, RRN3,* and *UBTF* (Figure 4.7C). There was no significant association between expression of these factors in the tumors prior to treatment and the response to treatment.

Next, to determine the effects of CX-5461 on expression of these genes, qPCR was performed on the treated PDXs 208, 182, 127, and 153 for *POLR1B, RRN3,* and *UBTF;* additionally, *ITS1* was examined as a quantification of pre-rRNA. Since PDX 225 had a complete response to CX-5461, there was no tumor to analyze and compare to the untreated sample. Of these genes, the only correlative marker for model 153, which had a positive response, was a decrease in *POLR1B,* which was not noted in the other 3 models (data not shown). Interestingly, *ITS1* decreased with treatment of the 208 and 182 models. These data suggests that while CX-5461 may be targeting and inhibiting ribosome synthesis, the lack of effect in some models may be due to other unknown mechanisms. Additional models and more thorough interrogation of the full expression profiles of these tumors will be required to fully understand the variability in response and potentially identify biomarkers predictive of response.

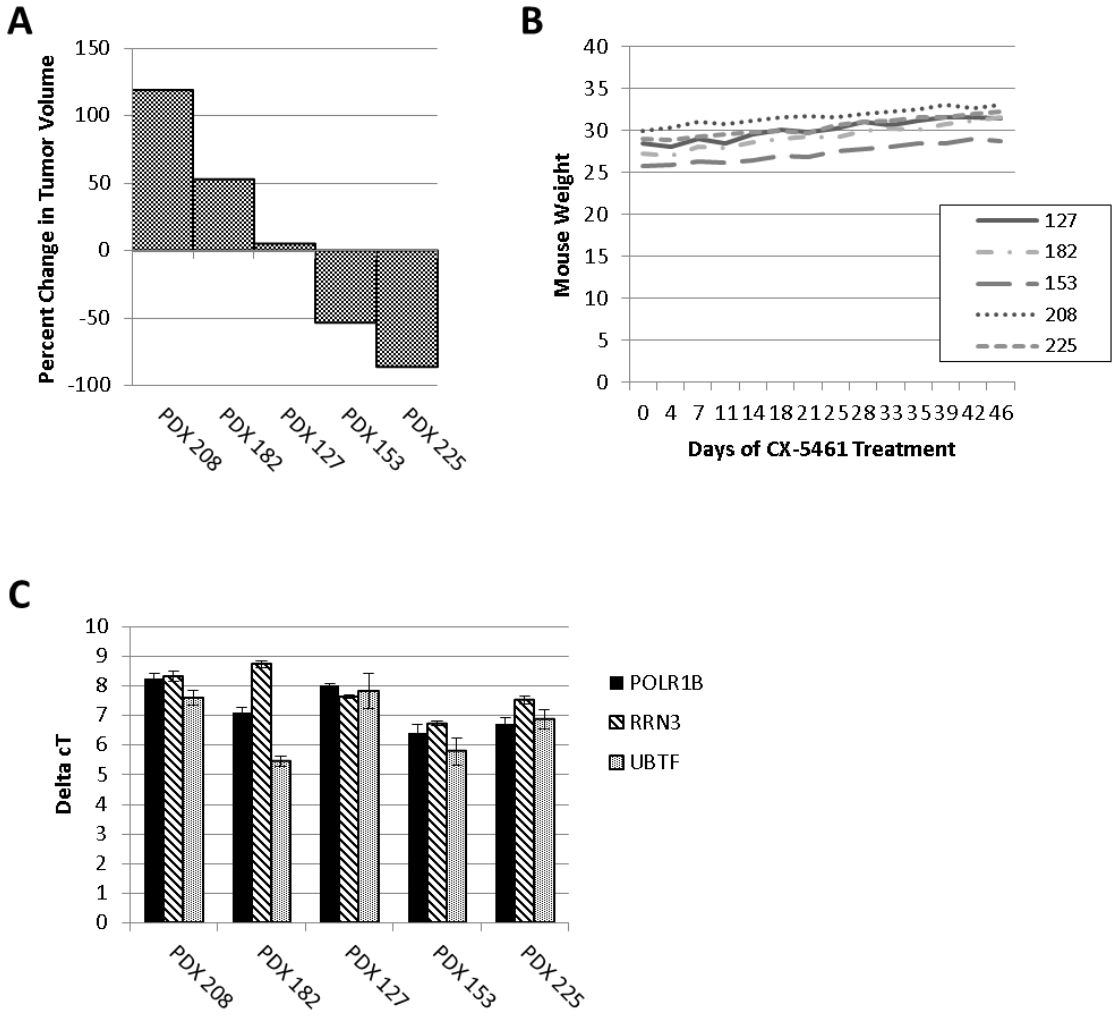


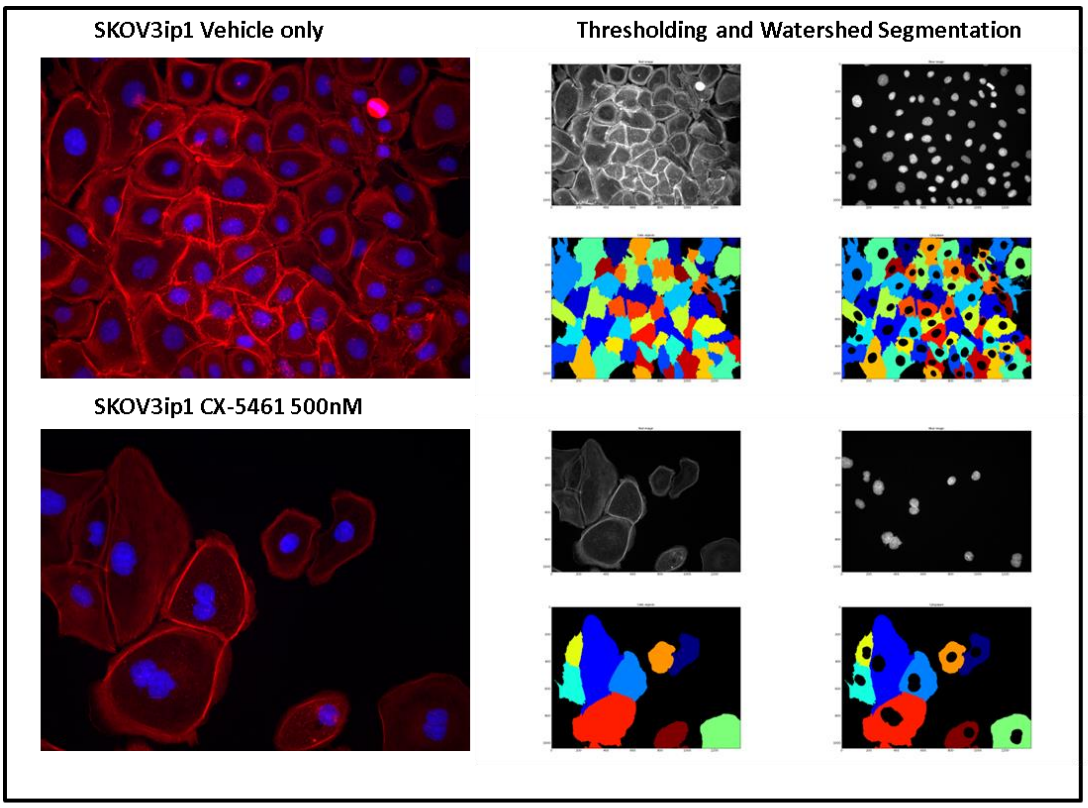

**Figure 4.7 Response of ribosomal translation factors after CX-5461 treatment.** (A) Waterfall plot displaying percent change of tumor volume in PDXs 208, 182, 127, 153, and 225 after 45 days of treatment with 50mg/kg CX-5461 q3D. (B) Weight of mice through duration of treatment with CX-5461. (C) qPCR of Pol I initiation factors *RRN3, POLR1B,* and *UBTF*. $\Delta C_T$ was calculated. There was not an association between baseline expression of these genes and response to CX-5461.

### 4.2.8 Levels of DNA damage quantitated after CX-5461 treatment.

Previous publications have focused on the lack of genotoxic damage with CX-5461 treatment. (Ferreira et al., 2020; Haddach et al., 2012a) We showed the exact opposite of these findings and confirmed them previously by western. The

dichotomy between our reported findings appears explainable as differences in time frames. The previous reports only looked a few hours after treatment. We showed pH2AX staining at 24-72 hours following CX-5461 treatment. Quantifying the levels of damage using pH2AX treatment can be challenging so I created a simple pipeline in CellProfiler to accomplish this.

Images were taken at random at 200x magnification. All image segmentation (Figure 4.8A) and quantitation was performed by CellProfiler (Figure 4.8B). Summary quantitation in representative images of changes to segmented cells' parameters before and after treatment.



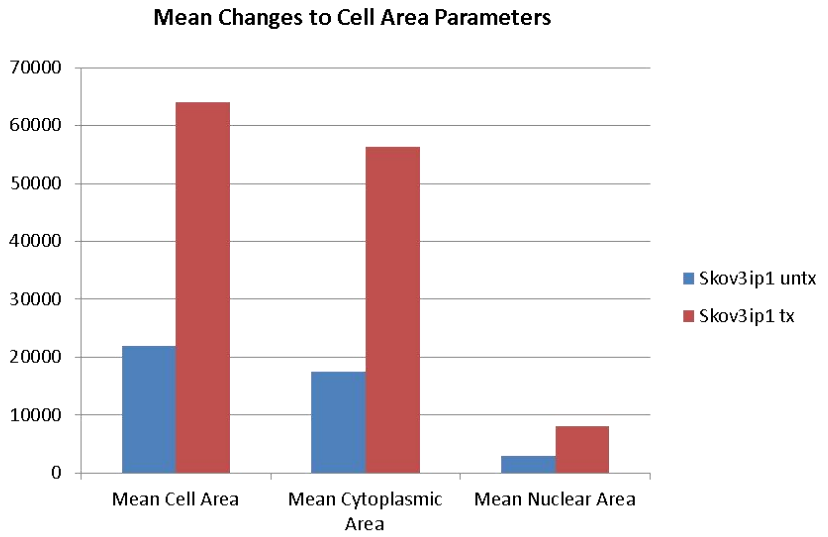




**Figure 4.8 Quantitation of pH2AX foci using cell profiler.** (A) Sample images of immunofluorescence of F-actin (red) and DAPI (blue) before and after IC50treatment with CX-5461. Images were taken at random at 200x magnification. Thresholding was performed to identify DAPI stained nuclei using the Mixture of Gaussian (MoG) technique to bin pixels into foreground and background classes. Cytoplasm, marked by F-actin, was segmented using the watershed technique after Sobel transformation followed by Otsu thresholding. All image segmentation and quantitation was performed by CellProfiler. (B) Summary quantitation in representative images of changes to segmented cells' parameters before and after treatment.

## 4.3 Discussion of CX-5461 results in chemoresistant ovarian cancer

Ovarian cancer unfortunately has limited chemotherapeutic options after patients develop resistance to the standard regimen of platinum- and taxane-based therapy. Therefore, it is imperative to identify novel targets that could be used in conjunction with standard therapy to hopefully improve patient outcomes. Previously, our group has developed and characterized an ovarian cancer PDX model that recapitulates the complexity of a patient's tumor in terms of heterogeneity and biological activity (Dobbin et al., 2014). When we conducted RNA-seq analysis comparing matched chemotherapy treated and untreated PDXs, we found that genes related to ribosomal synthesis were significantly up-regulated in the tumor cells surviving a platinum and taxane based therapy. In this study, we confirm that ribosomal machinery, particularly basal ribosomal RNA transcription components, are increased in surviving tumors, and that targeting Pol I is effective in cell lines and PDX models *in vivo*. We show that efficacy is not entirely dependent on TP53 status or proliferation rates, and is more effective in taxane-resistant cell lines. The mechanism of CX-5461 toxicity is in part through induction of arrest in G2/M of the cell cycle with frequent mitotic catastrophe, and significant DNA damage.

Transcription of rRNA genes by Pol I and subsequent processing of the rRNA are fundamental control steps in the synthesis of functional ribosomes (K. M. Hannan et al., 2013; Shiue et al., 2010; Thomson et al., 2013).

Chemotherapeutic agents have been known for years to cause an increase in nucleolar size and reorganization of nucleolar morphology (McCluggage et al., 2002). Although it is generally thought that increases in nucleolar size are associated with increased ribosomal biogenesis, it is a matter of debate as to what the increase means after chemotherapy. He *et al*. have presented evidence suggesting a ribosomal autophagy (ribophagy) pathway can be activated in cancer cells after chemotherapy as part of the survival process (He et al., 2015). This pathway is distinct from the general autophagy pathway, and it is thought that this can be both a mechanism the cell can use to lower energy demands in times of nutrient deprivation, and also a way to globally shut down translation rapidly by consuming mature 60s and 40s ribosomal subunits. If the latter is active then the generation of nascent ribosomes to replace the degraded mature subunits could account for the overall increase in nucleolar size (Klein & Grummt, 1999). This could account for the overall increased sensitivity of the chemoresistant population to CX-5461 treatment. If rRNA transcription in the nucleolus is inhibited, cells undergo cell cycle arrest associated with apoptosis, senescence or autophagy (R. D. Hannan et al., 2013). Aberrant regulation of rRNA synthesis by Pol I and ribosome biogenesis (the complex and highly coordinated cellular process leading to the production of ribosomes) is associated with the etiology of a broad range of human diseases and is especially pervasive in cancer (Bywater et al., 2013; Narla & Ebert, 2011; Williamson et al., 2006).

Admittedly our discovered association between ribosomal biogenesis and survival after chemotherapy treatment does not delineate whether we are seeing changes that are induced by exposure to chemotherapy, or if treatment with chemotherapy selects cells with inherently different ribosomal biogenesis processes ongoing that allow for their survival, and thus are revealed by our selection method. Furthermore our analysis does not distinguish between different types of ribosomes destined to mediate transcription of particular genes. Further studies are needed to distinguish between these possibilities, and discover how cells respond to chemotherapy that may allow survival.

There have been numerous chemotherapeutic drugs developed that have an impact on ribosomal biogenesis, even if the effects are nonspecific, including cisplatin, actinomycin D, camptothecin (irinotecan/topotecan), mitomycin C, 5-fluorouracil, and doxorubicin (Burger et al., 2010; Denis Drygin et al., 2010; K. M. Hannan et al., 2013). However, none of these chemotherapeutic drugs selectively targets Pol I to allow definitive conclusions on what degree their therapeutic effect is mediated through ribosome biogenesis (Bywater et al., 2013). In that respect evidence has been accumulating that CX-5461 is a G-quadruplex stabilizer that targets many areas of the genome and can damage telomeres (G. Li et al., 2018; H. Xu et al., n.d., 2017). CX-5461 was recently developed by Cylene Pharmaceuticals and is an orally available small molecule that targets transcription by Pol I selectively (Denis Drygin et al., 2011; Haddach et al., 2012a). CX-5461 is thought to impair initiation of Pol I transcription by disrupting the binding of the Pol I transcription initiation factor SL-1 to the

ribosomal DNA promoter (Denis Drygin et al., 2011; Haddach et al., 2012a). The marking of double strand breaks by γH2AX is a well- characterized phenomenon (Bewersdorf et al., 2006; Kinner et al., 2008; Liang et al., 2009; Rogakou et al., 1998; Yuan et al., 2010). Double strand breaks do induce γH2AX but this phosphorylation is not limited only to DNA strand breakage (Turinetto & Giachino, 2015). The consistent, late (48-72hr) marking of γH2AX after CX-5461 treatment in ovarian cancer cells could indicate a senescent state after an overwhelming amount of nucleolar stress. Hannan *et al*. have found a similar non-canonical ATM/ATR response phenotype, and our results agree with theirs (J. Quin et al., 2016). Panov *et al*. put forth evidence that the stabilization of g-quadruplex formation may play a role in CX-5461's mechanism of action at the rDNA promoter, and our results show DNA damage response machinery as the most probable route of efficacy, with TP53 activation being a later event that is dependent on DNA damage response machinery as opposed to nucleolar stress (Andrews et al., 2013).

Bywater *et al.* demonstrated that human hematologic cancer cells (leukemia and lymphoma) with wild type TP53 are more sensitive to CX-5461 than TP53 mutant cells *in vitro* and *in vivo*. As a result, they suggested that CX-5461 has therapeutic effect for hematologic malignancies by TP53 mutational status-dependent apoptosis (Bywater et al., 2012). But, in solid tumor cell lines, CX-5461 induces both senescence and autophagy by a TP53-independent process (Denis Drygin et al., 2011; L. Li et al., 2016). These differential responses between the nucleolar stress and cell death according to different

types of cancer may mean that hematological malignancies have unique nucleolar biology susceptible to activation of TP53-dependent apoptosis following acute perturbations of ribosome biogenesis. We have seen that CX-5461 is more potent against A2780ip2 cells with wild type *TP53* than against A2780cp20 with a *TP53* mutation. However, we found that the taxane-resistant lines SKOV3TRip2 and HeyA8MDR were more sensitive to Pol I inhibition than their parental lines, even though SKOV3 has a mutation in *TP53* in both lines, while HeyA8 is wild-type for *TP53* in both lines. These data demonstrate that there are also important TP53-independent mechanisms induced by CX-5461 that still need to be elucidated. Interestingly, another inhibitor of RNA Pol I has been developed, BMH-21. This molecule binds preferentially to rRNA-encoding DNA, and reportedly behaves in a TP53-independent manner (K. Peltonen et al., 2014). This could indicate the promising aspects of CX-5461 are due to off-target effects that have not been fully elucidated yet. Due to the differences in activity it may be that the effectiveness of CX-5461 is something other than rDNA transcriptional silencing.

Based on our RNAseq data and the *in vitro* cell line data, we moved forward with treating 5 PDX lines with CX-5461 in a single agent setting. If up-regulation of ribosomal synthesis is a hallmark of aggressive cancer and is required for cellular proliferation, inhibition may lead to a novel treatment approach. In a single agent setting, we found a 60% clinically significant benefit rate (complete or partial response, or stable disease). An appropriate concern is whether inhibition of such an important process will be tolerable in patients. We

and others have not seen gross organ damage at the pathologic level, and no weight loss in treatment mice. It may be that, similar to cytotoxic agents, cancer cells have higher susceptibility to inhibition of ribosomal biogenesis, and a therapeutic window can be found which is safe in patients. Many more extensive studies are required in this arena, and phase I trials with this medication are underway in Canada (https://clinicaltrials.gov/ct2/show/NCT02719977). Regarding a predictive biomarker, in our investigation, the only unifying feature is that higher basal levels of Pol I initiation factors *RRN3* and *POLR1B* correlated with a greater response to therapy, and that was only in one model This has been confirmed in recent publications where the rDNA activity was correlated with CX-5461 sensitivity (Son et al., 2020). More data will be required in a larger cohort to confirm where these are effective biomarkers for response. However, overall these data demonstrate the potential of CX-5461, and potentially other inhibitors of ribosomal synthesis, as a new approach to ovarian cancer, both in chemosensitive and chemoresistant tumors.

# 5 CX-5461 induced DNA damage leads to cytosolic DNA presentation

## 5.1 Abstract

While investigating the downstream effects of CX-5461 treatment we noticed a previously unreported phenotype. Treatment with CX-5461 induces a rapid accumulation of cytosolic DNA approximately 1 hour after treatment. This

accumulation leads to transcriptional upregulation of ‘STimulator of Interferon Genes’ (STING) in the same time frame, phosphorylation of IRF3, and activation of type I interferon response. CX-5461 treatment-mediated immune activation may be a powerful mechanism of action to exploit, leading to novel drug combinations with a chance of increasing immunotherapy efficacy, possibly with some cancer specificity limiting deleterious toxicities.

## 5.2 Graphical Summary

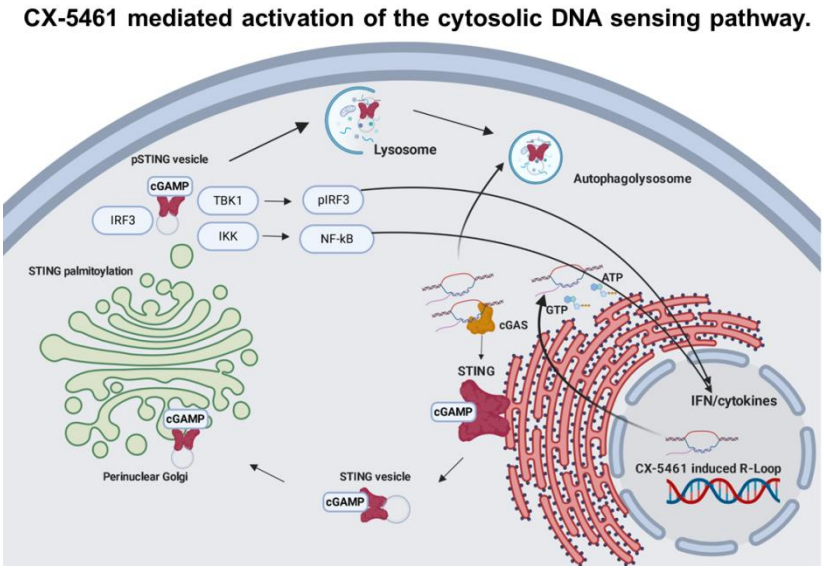



**Figure 5.1 CX-5461 induced R-loop formation accumulates in the cytoplasm.** cGAS binding to the R-loop DNA activates the secondary messenger 2,3-cGAMP using cGAS as a catalyst. Activated STING buds off the ER moving to the perinuclear golgi where it is palmitoylated. STING brings together TBK1, IRF3, and IKK. TBK1 phosphorylates STING which leads to phosphorylation of IRF3 and activation of NFκB signaling through RelA. Both pathways lead to type I interferon activation, senescence associated secretory phenotype (SASP), and interferon stimulated gene (ISG) transcription.

## 5.3 Introduction

Ovarian cancer remains the deadliest gynecologic malignancy and 40 years of research has seen little change in overall survival rates, with the exception of PARP inhibition in BRCA-mutated tumors.(Berek et al., 2012; Bowtell et al., 2015) The primary reason for this is chemotherapy resistance in the recurrent setting. Novel therapeutic options capable of attacking chemoresistant disease are a major goal in the field. In previous studies, both in our lab and by others, cell populations resistant to cytotoxic chemotherapy have shown an increase in ribosome biogenesis, making RNA polymerase I a possible drug target for chemoresistant EOC.(Cornelison, Dobbin, et al., 2017; Kim et al., 2016) There are currently two known inhibitors of RNA polymerase I, the polymerase responsible for transcribing the ribosomal DNA repeat region in mammalian cells: CX-5461 from Drygin et al. and BMH-21 from the Laiho lab.(Haddach et al., 2012b; K. Peltonen et al., 2014) Treatment with CX-5461 has also been shown to cause G-quadruplex stabilization, DNA damage, ATM/ATR mediated DNA damage response, senescence, and autophagy.(Sanij et al., 2020; H. Xu et al., 2017)

In this study we characterize a previously unreported consequence of CX-5461 treatment-induced DNA damage: a rapid and robust buildup of cytoplasmic nucleic acid, visible with Sir-Hoechst in live cells. We hypothesized that this is cytosolic DNA building up from CX-5461-induced DNA damage, and that this buildup will lead to downstream activation of the cytosolic DNA detection system

and response pathways. Therapies that can activate STING, especially ones with some cancer selectivity, can be a powerful mechanism to exploit in order to increase the efficacy of immunotherapies that lead to durable cures.

## 5.4 Results

### 5.4.1 CX-5461 induces cytoplasmic DNA accumulation

While investigating the effects of CX-5461 treatment on nucleolar proteins in live cells we noticed an unanticipated phenotype. Treated cells appeared to fill with cytoplasmic nucleic acid approximately 1 hour after treatment. Detecting cytosolic DNA can be challenging as common dyes like DAPI do not detect DNA in the cytoplasm.(Johansen et al., 1995) Sir-Hoechst is a newer, live-cell dye that allows imaging in the near-infrared range of the spectrum to avoid UV damage.(Lukinavičius et al., 2015)

To investigate whether CX-5461 treatment was inducing cytosolic DNA accumulation, COV362 serous ovarian cancer cells were treated with IC50 (1μM) CX-5461 for 5 hours then stained with 1μM Sir-Hoechst and imaged live. Dramatic buildup of cytosolic staining was seen after 1 hour of treatment (Figure 5.2A). Next, we reasoned that by labeling with Sir-Hoechst first, allowing nuclear dye accumulation, and then washing away excess dye we could have a “pre-labeled” nucleus to monitor for changes after CX-5461 treatment. COV362 cells were labeled with Sir-Hoechst for 2 hours, visually verified for nuclear positivity, washed, and then media was added with IC50 CX-5461(no additional Sir-Hoechst). After ~1 hour cells began displaying discreet, microsomal appearing

cytoplasmic granules of Sir-Hoechst labeled DNA (Figure 5.2B). Cells were imaged for 17 hours at 15 minute intervals. Staining for picogreen, anti-dsDNA, and anti-RNA:DNA hybrid (R-loop) immunofluorescence were used to further elucidate the nature of cytosolic buildup. R-loop staining in the cytosol appeared to increase after treatment which coincides with the recent publication showing CX-5461 inducing R-loop formation(Sanij et al., 2020) (Figure 5.2C).

Other DNA-damaging agents have been shown to induce the cytoplasmic DNA detection system like etoposide.(Hong et al., 2019) To examine staining levels compared to CX-5461, we treated cells with IC50 dosages of BMH-21, carboplatin, paclitaxel, etoposide and temozolomide. Example images showed barely visible cytoplasmic DNA after etoposide treatment but much less than CX-5461 treatment induced. (Figure 5.2D) After quantitation of cytoplasmic positive cells etoposide, carboplatin and paclitaxel showed a small amount of cytosolic DNA accumulation but significantly lower than CX-5461 (Figure 5.2D lower panel).

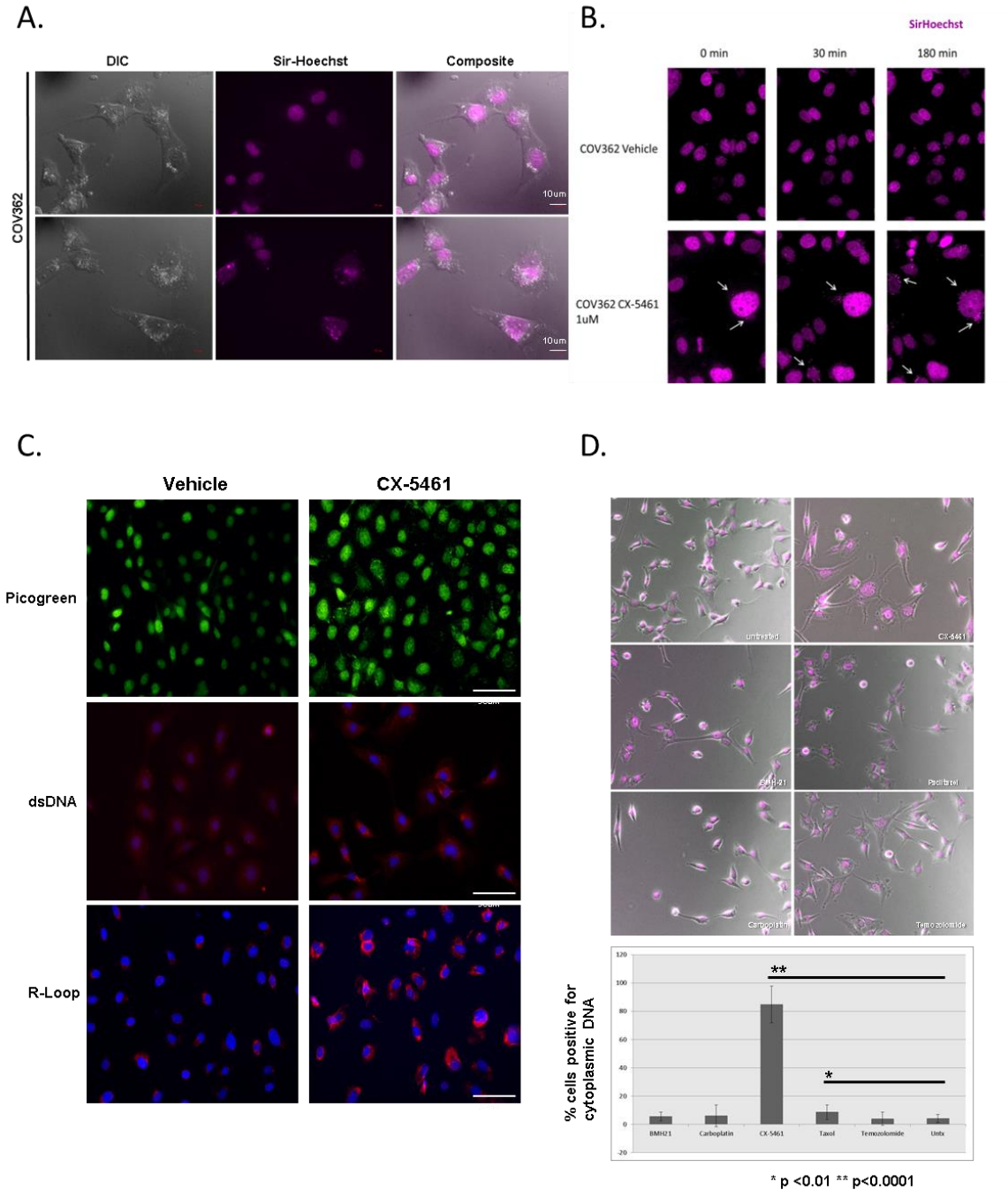



**Figure 5.2 CX-5461 induces cytoplasmic DNA accumulation.** (A) Live cell imaging of vehicle only versus 1uM CX-5461 treated COV262 cells stained with Sir-Hoechst. (B) Sir-Hoechst pre-labeled nuclei were treated with vehicle only and 1uM CX-5461. (C) Vehicle only and 1uM CX-5461 treated cells were stained with Picogreen, anti-dsDNA, and anti-RNA:DNA (R-loop) antibodies. (D) Example images of IC50 dosages across multiple drugs in COV362 cells labeled with Sir-Hoechst. Quantitation of cytoplasmic positive cells after treatment with vehicle, 1uM CX-5461, 100nM BMH-21, 5nM paclitaxel, 50uM carboplatin, 100uM etoposide, and 250uM temozolomide.

### 5.4.2 CX-5461-mediated cytosolic DNA induces cGAS/STING system

Cytosolic DNA from genotoxic damage and pathogen infection is detected by a complex system leading to a type I interferon response.(Dou et al., 2017; Pépin et al., 2017; Song et al., 2019) To examine transcriptomic evidence of activation of the STING system, RNA-seq analysis of HeyA8 cells treated with CX-5461 for 24 hours was performed. Downstream analysis using gene set association analysis of sequence count data (GSAAseqSP(Xiong et al., 2015)) showed the expected loss of ribosome signaling from CX-5461 treatment, an upregulation of the cytosolic DNA detection system, as well as activation of IFNA signaling.(Figure 5.3A)

STING upregulation was monitored in HeyA8 ovarian cancer cells treated with IC50 dosages of CX-5461 (2μM) over time. Cells were lysed and immunoblotting confirmed upregulation of STING at 60 minutes, coinciding with the presentation of cytoplasmic DNA seen by live cell imaging (Figure 5.3B). Other cell lines showed a variety of responses dependent on the original level of STING prior to treatment. This may be based on TP53 status since all cells showed cytoplasmic DNA after treatment.

STING leads to phosphorylation of IRF3 and type I interferon activation.(Vanpouille-Box et al., 2018) Immunoblotting of IRF3, pIRF3(S396) and loading control showed phosphorylation of IRF3 after 1 hour of CX-5461 treatment in COV362 and HeyA8 cells (Figure 5.3C). pIRF3 transcriptional

targets in response to cytoplasmic dsDNA include the cytokines: CXCL10, MIP1a and RANTES, as well as a variety of interleukins depending upon the cell type.(Kondo et al., 2013; Konno et al., 2013) In order to assay type I interferon induction, COV362 cells were treated with IC50 doses of CX-5461 (1μM) and profiled using the human innate immune panel from Luminex. COV362 cells were treated with CX-5461 over a time course. IL-6, CXCL10, CCL2 and CCL3 showed moderate activation after treatment (Figure 5.3D).

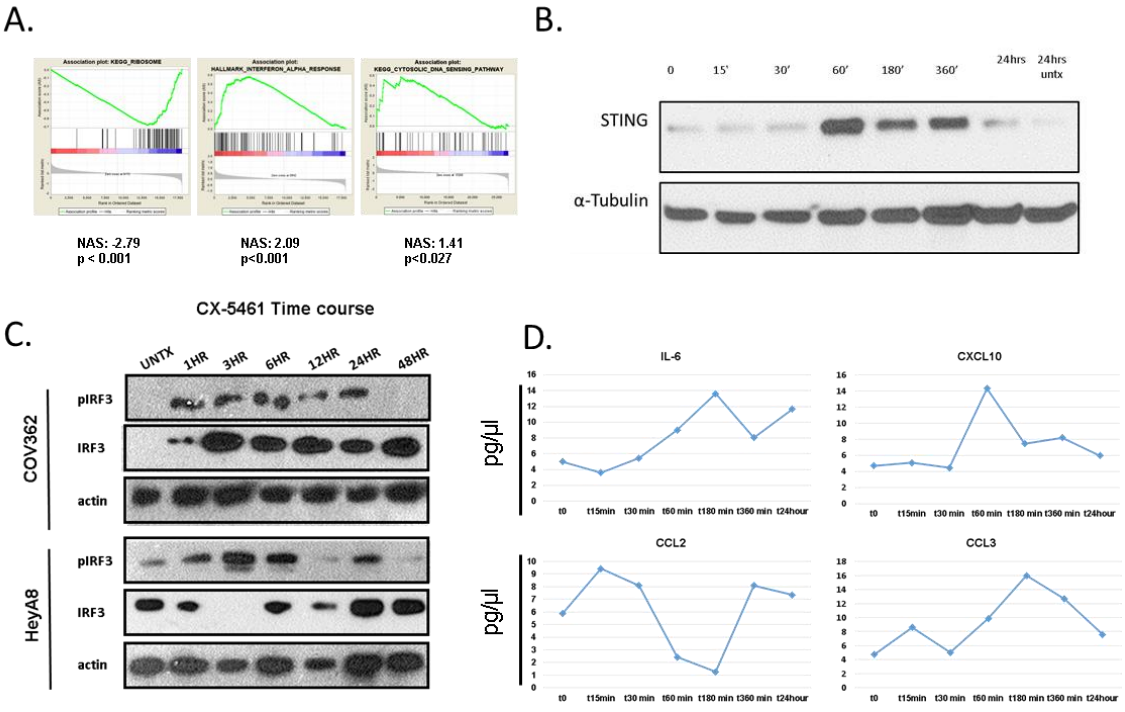



**Figure 5.3 CX-5461 mediated cytosolic DNA induces cGAS/STING system. (A)** GSAAseqSP analysis of RNA-seq from 2uM CX-5461 treated HeyA8 cells. **(B)** Immunoblotting of STING transcriptional upregulation over a time course of 2uM CX-5461 treated HeyA8 cells. **(C)** Immunoblotting of pIRF3(S396), IRF3, and Actin over time in COV362 and HeyA8 cells after 1uM CX-5461 treatment. **(E)** Luminex of cytokines IL-6, CXCL10, CCL2, and CCL3 after 1uM CX-5461 treatment in COV362 cells over time.

### 5.4.3 In vivo CX-5461 treatment induces type I interferons

To examine whether the same changes occur *in vivo,* we implanted 4 groups of 5 nude mice SQ with 1 million cells per injection of COV362 HGSOC cells or HeyA8 endometrioid cells. After tumors grew to a size of at least 0.5cm in one dimension, mice were treated with 50mg/kg CX-5461 by oral gavage weekly or vehicle only (Na2PO4 pH-4.5). Tumors were serially measured and after 3 weeks, were harvested and protein extracted for Luminex profiling. Overall tumor burden was slightly reduced by CX-5461 monotherapy even after only 3 treatments (Figure 5.4A). Luminex innate immune profiling showed an increase in Eotaxin, MIP1a, CXCL10, and Il-1b in COV362 SQ (Figure 5.4B). Pathologist review of IHC of FFPE-collected tumors showed the same activation *in vivo* that was seen *in vitro*, in that HeyA8 cells showed a moderate cytoplasmic increase in STING staining after treatment whereas COV362 showed a moderate decrease after treatment (Figure 5.4C).

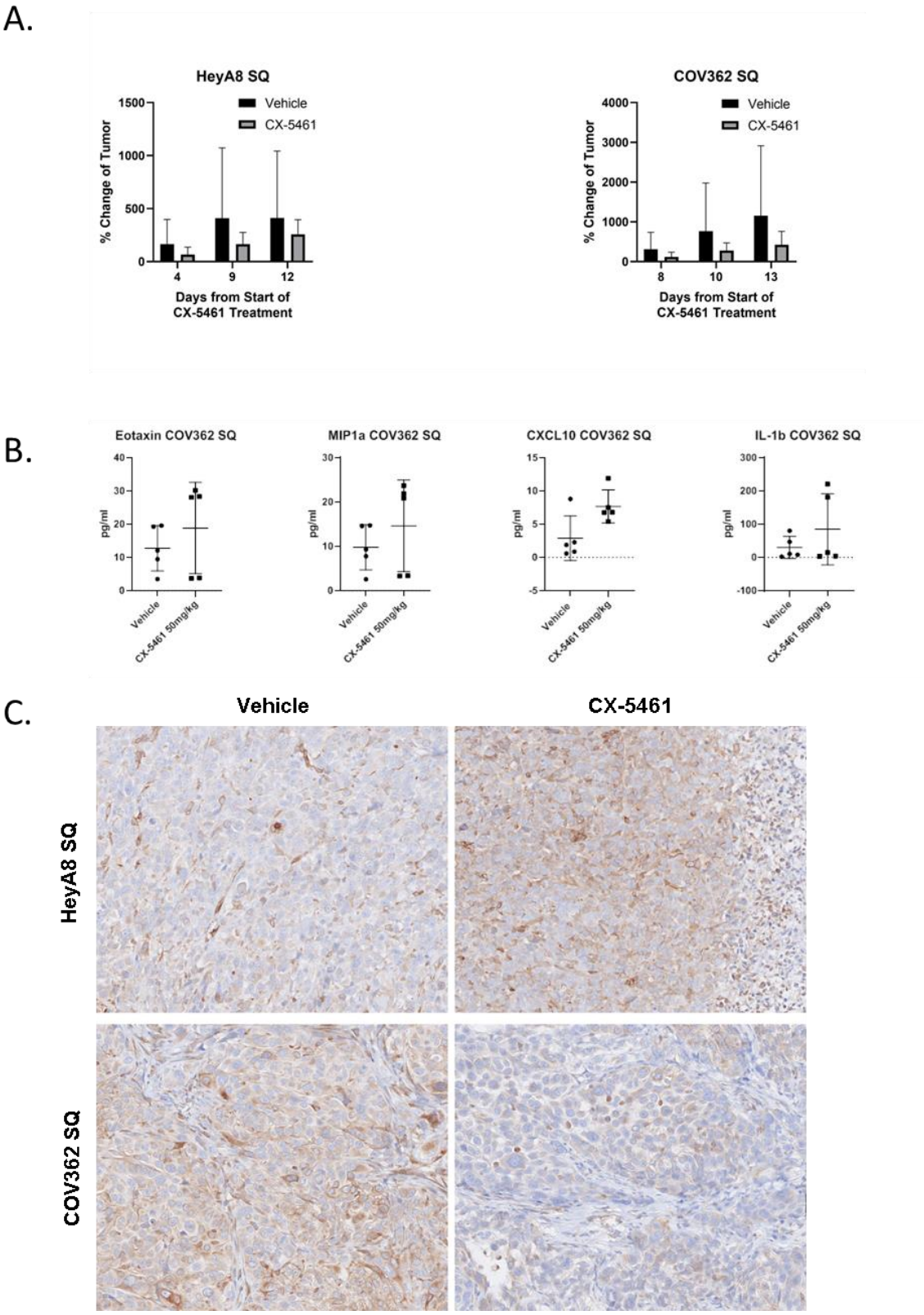



**Figure 5.4** ***In vivo*** **CX-5461 mediated cytosolic DNA induces cGAS/STING system. (A)** Percent

change in tumor volume in HeyA8 SQ and COV362 SQ xenografts after 50mg/kg CX-5461 3 week treatment in groups of 5 nude mice. **(B)** Luminex of selected cytokines Eotaxin, MIP1a, CXCL10, and Il-1b after 50mg/kg CX-5461 treatment for 3 weeks in COV362 SQ and HeyA8 SQ xenografts. **(C)** Immunohistochemistry of total STING in HeyA8 and COV362 SQ tumors before and after 3 week treatment with 50mg/kg CX-5461 or vehicle control.

## 5.5 Discussion

Our preliminary data has shown that the pol I inhibitor CX-5461 induces a significant accumulation of cytosolic DNA, transcriptionally activates STING in selected cell lines, and induces phosphorylation of STING and IRF3. We immediately ruled out contamination by testing for 200+ species of mycoplasma by touchdown-PCR. We also repeated with CX-5461 obtained from different suppliers and solubilized in different organic solvents such as DMF and DMSO, with no change being seen. In order to rule out an autophagy mediated buildup of vacuolar sequestered nucleic acid dye, cells were pre-treated with 10nM rapamycin to induce autophagy as well as 10μM chloroquine to block degradation of autophagolysosomes, alone and combined. No build up cytosolic staining was seen in the absence of CX-5461 treatment.

Identifying cytosolic DNA from DNA damaging agents has been seen previously, but not to the extent induced with CX-5461, and its activation of the STING system is entirely novel. Identification of novel STING activators is an exciting field currently under study. CX-5461 treatment also induces secretion of the type I interferon associated cytokines: IL-1b, IL-6, CXCL10, CCL2. The buildup of cytoplasmic DNA is dramatic and appears to be relatively stable in the treated cells.

Critical questions remain requiring further study. We believe the autophagy induced by CX-5461 may be a cytosolic DNA dependent phenomenon. The inflammatory phenotype may be a STING dependent activation, but it also could be partly associated with a SASP as CX-5461 induces senescence in many cell types. Most importantly, does the cancer specificity attributed to CX-5461 apply to this STING activation? As we saw variability in response in different cell lines, a comprehensive analysis of multiple cell subtype and a biomarker for response are needed. A cancer-specific STING activator would be a monumental discovery in terms of increasing the number of patients responding to immunotherapy, and moving immunotherapies from temporary efficacy to durable cures.

### 5.5.1 Chapter acknowledgements

We would like to thank Jim Mandell MD PhD for review of pathology and IHC staining, as well as, due to rapid impact format limitations, all the labs we could not reference, for their continuing work in CX-5461 and pol I inhibition. Thanks to the UVA CCSG for core services support, the Flow Cytometry Core Facility for Luminex runs, and the BTRF and UVA Pathology Core for embedding, staining, and slide scanning of FFPE.

# 6 Conclusions and future directions

## 6.1 Continuing in better models

While we have put forth evidence suggesting targeting Pol I in HGSOC is an effective therapeutic strategy worth further exploration, there are still many unanswered questions. Future directions will focus on determining the efficacy of targeting the ribosomal biogenesis system with pol I inhibitors to combat chemoresistance in models specific to HGSOC. We will expand this work into a larger number of PDX *in vivo* models as well as develop new pairs of sensitive/resistant cell lines using lines that have been found to be more representative of high-grade serous ovarian cancer. We initially characterized chemoresistance in our models using combined carboplatin/paclitaxel chemotherapy, the standard of care. We will further this work through testing currently used therapeutics independently and in combination to identify specific resistant populations sensitive to pol I inhibitor therapy. This will test the hypotheses that pol I inhibitors represent an effective treatment in both targeting HGSOC and combating its acquisition of chemoresistance.

## 6.2 Finding a biomarker to predict CX-5461 response

Within HGSOC patient populations we have seen a significant variation in pol I efficacy, and our working hypothesis is that the state of the rDNA promoters is critical in determining the cell's sensitivity to pol I inhibition. Fully elucidating

the epigenetic status, pol I occupancy, elongation rate and total number of transcribing rDNA genes in development of chemoresistance will be a major step forward in understanding pol I inhibitor cancer specificity, and it may identify new attack surfaces to combat the development of resistance. Our preliminary data also showed that shutting down ribogenesis using rapamycin to simulate starvation greatly reduces sensitivity to pol I inhibitors in HGSOC (Figure 6.1). Due to these data we hypothesize that the rate of ribosome biosynthesis determines the sensitivity to pol I inhibitors, and that characterizing the various states of ribosome biogenesis rate control will elucidate the cell populations most vulnerable to pol I inhibition.

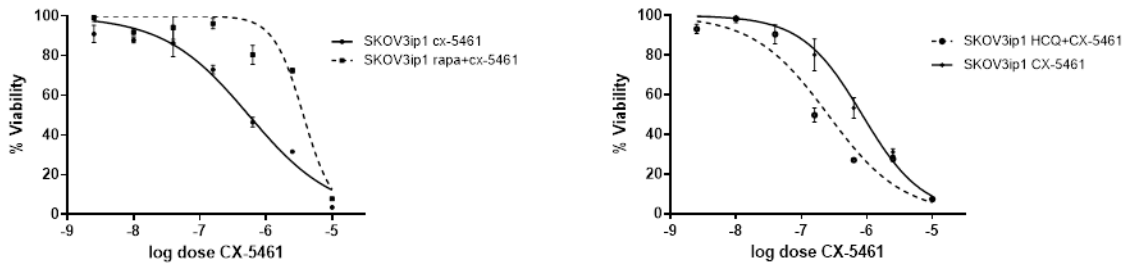



**Figure 6.1 Autophagy may be coupled to CX-5461 efficacy.** Pretreatment of SKOV3ip1 and SKOV3tr cells with 10nM rapamycin reduces CX-5461 IC50 from 500nM to 3.4uM. Pretreatment with 10mM chloroquine shows additive effects with IC50 decreasing from 500nM to 200nM. Rapamycin induces autophagy by mimicking nutrient depravation. Nutrient sensing controls rDNA transcription rate in most cell types (Matos-Perdomo & Machín, 2019).

The primary factors controlling the rate of ribosome synthesis are: the total number of transcribing rDNA loci, polymerase occupancy on transcribing rDNA genes, and the efficiency of elongation (Sanchez et al., 2016). While the underlying regulation of each of these is incredibly complex, we will use established, standard methods for assaying each.

## 6.3 TP53 reactivation revisited

Interfering with pol I in cancer cells appears to induce a DNA damage-like phenotype leading to nucleolar stress, a TP53 dependent phenomenon. In the absence of wild type TP53, the use of pol I inhibitors is still functional but appears to be more cytostatic, inducing autophagy and senescence in cancer cells while still leaving normal cells intact. Chemical reactivation of TP53 is currently in clinical trials with several small molecule reactivators under investigation as well as multiple trials using viral mediated TP53 reintroduction. By dissecting the mechanisms of pol I inhibitor efficacy in diverse TP53 mutational backgrounds we can use this information to find synergistic therapeutic targets that enhance its efficacy. Unfortunately, TP53 reactivation has never been shown conclusively to work. The research has also been marred by a series of retractions making it a difficult body of work to navigate.

# 7 MATERIALS AND METHODS

### 7.1.1 Reagents and Cell Culture

CX-5461 was purchased from ChemScene and dissolved in 50 mmol/L NaH2PO4 (pH 4.5) to make 10mmol/L stock solution. The chemosensitive and chemoresistant ovarian cancer cell line matched pairs A2780ip2, A2780cp20, HeyA8, HeyA8MDR, SKOV3ip1, and SKOV3TRip2 as well as PEO1, PEO4, ES2, OVCAR3, CaOV3, OV90, OAW42, OVSAHO and HIO-180 (Buick et al.,

1985; Halder et al., 2006; Landen et al., 2008; Louie et al., 1985; Moore et al., 1997; D. Yu et al., 1993) were maintained in RPMI-1640 medium supplemented with 10% FBS (Hyclone). A2780cp20 (platinum resistant), HeyA8MDR (paclitaxel resistant), and SKOV3TRip2 (paclitaxel resistant, a kind gift of Dr. Michael Seiden) (Duan et al., 1999) were previously generated by sequential exposure to increasing concentrations of chemotherapy. All ovarian cancer cell lines were routinely screened for Mycoplasma species (GenProbe Detection Kit; Fisher) with experiments carried out at 70% to 80% confluent cultures. Purity of stock cell lines was confirmed with short tandem repeat genomic analysis, and all cell lines were used within 20 passages from stocks.

### 7.1.2 Ovarian Cancer Patient Derived Xenograft Model

Multiple ovarian cancer PDX models were developed and previously characterized by our group (Dobbin et al., 2014). Briefly, freshly-collected untreated patient tumors collected at the time of primary debulking were implanted subcutaneously into SCID mice and allowed to propagate. For 6 models, mice with established PDX tumors (approximately 1cm in greatest diameter) were treated with weekly doses of carboplatin (90 mg/kg) and paclitaxel (20 mg/kg), intraperitoneally, for 4 weeks. All tumors had greater than 50% reduction in size, and were collected in multiple formats. RNA-Seq was performed on treated and untreated tumors, and expression profiles in matched pairs were compared using IPA software.

To evaluate changes in nucleoli after chemotherapy staff pathologists took four random images of cancer cell rich areas of H&E stained PDX tissue samples before and after combined carboplatin/paclitaxel therapy. Cells were counted both manually and automatically with CellProfiler, quantitating nucleolar area changes and determining statistical significance using GraphPad Prism (La Jolla, CA).

For examination of the efficacy of CX-5461 in PDX models, previously-collected PDX tumors either 2 or 3 generations from original collection, from 5 separate patients, were reimplanted into 5 mice per model, with 2 tumors per mouse. After tumors reached 1cm in at least 1 dimension, treatment with single-agent CX-5461 (50 mg/kg q3d) was initiated and response was followed using caliper measurements. Tumor volume was calculated using the formula (Length x $Width^2$)/2. Tumors response was averaged among all tumors and presented as change in volume compared to volume at initiation of treatment.

### 7.1.3 Assessment of cell viability and cell cycle analysis

2,000 cells/well were plated on 96-well plates and treated the next day with increasing concentrations of CX-5461, alone or in combination with carboplatin or paclitaxel, in triplicate. Viability was assessed by 2-hour incubation with 0.15% MTT (Sigma) and spectrophotometric analysis at OD570 (optical density at 570 nm). IC50 calculation was performed in GraphPad Prism® (version 6.0, La Jolla, CA) using variable slope, four-parameter logistic curve where the model is: Y=Bottom + (Top-Bottom)/(1+10^((LogIC50-X)*HillSlope)).

Drug concentration data (log transformed) and the percent inhibition data compared to vehicle control were fitted to the four-parameter model.

For cell-cycle analysis, cells were treated with vehicle alone or CX-5461 at the IC50 and IC90 dose for 48 hours, trypsinized, and fixed in 100% ethanol overnight. Cells were then centrifuged, washed in PBS, and suspended in PBS containing 0.1% Triton X-100 (v/v) 200 µg/ml DNase-free RNase A, and 20 µg/mL Propidium iodide (PI). PI fluorescence was assessed by flow cytometry and the percentage of cells in sub-$G_0$, $G_0$-$G_1$, S-, and $G_2$-M phases was calculated by the cell-cycle analysis module for Flow Cytometry Analysis Software (FlowJo v7.6.1, Ashland, OR).

### 7.1.4 Immunoblotting and Immunofluorescence

For immunoblotting: 15 µg of cell lysate was run on 10% Bis-Tris NuPage gels (ThermoFisher), transferred to 0.45 µM PVDF using a semi-dry Midi transfer system (ThermoFisher), blocked in 5% (w/v) non-fat dry milk and incubated overnight with primary antibodies at 4 degrees C. After detection with the appropriate HRP-conjugated secondary antibody, blots were developed using SuperSignal™ West Pico Chemiluminescent Substrate (ThermoFisher) and imaged using X-ray film. Antibodies used were incubated at manufacturer's recommended dilutions: pCHK1(ser317)(1:1000), pCHK2(T68)(1:1000), pCDC2(Tyr15)(1:1000), γH2AX(ser139)(1:2000), Cyclin-B(1:500), α-tubulin(1:5000), and p21(1:1000) from Cell Signaling (Danvers, MA).

For immunofluorescence, cells were plated in 8-well chamber slides (Nunc Lab-Tek II CC2, Thermo Scientific) at densities of 10,000 cells per well. Cells were fixed with 4% paraformaldehyde and blocked with 2% BSA for one hour. After primary antibody incubation, wells were detected with anti-rabbit/mouse Alexa-Flour 568 (Cell Signaling, Danvers, MA) at 1:500 dilution for one hour at room temperature and counterstained/mounted with DAPI in Prolong Gold Antifade (Invitrogen). A random selection of multiple images was captured using a Photometrix CoolSnapHQ CCD camera with a 20x objective and analyzed both manually and with the image analysis software CellProfiler (Broad Institute, Cambridge, MA).

### 7.1.5 RNA Extraction and Reverse Transcription

Total RNA was extracted from the ovarian cancer cell lines and ovarian cancer PDX tumors using the RNeasy Mini kit per the manufacturer's instructions (Qiagen, Frederick, MD). The concentrations of all RNA samples were quantified using spectrophotometric absorbance at 260/280 nm. cDNA was prepared using the High Capacity Reverse Transcription Kit (Applied Biosystems, Foster City, CA).

### 7.1.6 Quantitative PCR

Primer and probe sets for *UBTF* (Hs01115792_g1)*, 18S* (Hs99999901_s1)*, RNA28S* (Hs03654441_s1)*, β-Actin* (Hs01060665_g1, Housekeeping Gene), *POLR1B* (Hs00219263_m1)*,* and *RRN3* (Hs01592557_m1) were obtained from Applied Biosystems and used according

to manufacturer's instructions. *ITS1*, pre-rRNA, was custom ordered from Applied Biosystems (Forward Primer: CCGCGCTCTACCTTACCTACCT, 3'-Primer: GCATGGCTTAATCTTTGAGACAAG, Probe: TTGATCCTGCCAGTAGC). PCR amplification was performed on an ABI Prism 7900HT and gene expression was calculated using the comparative $C_T$ method as previously described (Steg et al., 2006).

### 7.1.7 Polysome fraction assay

For an assessment of ribosomal subunit populations and efficiency of translation, sucrose gradient fractionation was performed. Cells were grown to ~70% confluence in RPMI (10% FBS), treated with cycloheximide (100 µg/ml), washed in PBS, and cytoplasmic extracts were layered onto 10% to 50% linear sucrose gradients and centrifuged at 30,000 rpm in a Beckman SW41 ultracentrifuge rotor for 5 hours. To visualize ribosome populations, 60% sucrose was pumped into the bottom of each column and absorbance at 254 nm was monitored during elution from the top. Three different biological replicates were performed for each cell line, and representative traces are shown.

### 7.1.8 Chromatin immunoprecipitation

SKOV3ip1 and SKOV3TRip2 cells were grown to ~80% confluence and treated with formaldehyde (1% final concentration) for 10 minutes and then incubated in 0.125M glycine for an additional 5 minutes. Cells were washed in cold 1x PBS, and then processed for ChIP as described previously (Karita

Peltonen et al., 2014). Immunoprecipitation was performed with an anti-RPA194 antibody (Santa Cruz Biotechnology; SC-48385).

### 7.1.9 Isotopic labeling of cellular RNA

Cells were grown to ~80% confluence as described above in six well dishes. At time zero, $^{32}$P-orthophosphate was added to each well (20 µCi/ml) and incubated for the indicated times. Medium was removed and Triazole was added directly to the cells for harvest. RNA was purified and run on a 1% formaldehyde:agarose denaturing gel. RNA was transferred from the gel onto Zeta-Probe blotting membrane (BioRad, Hercules, CA), dried and analyzed by phosphoimaging.

### 7.1.10 RNAseq

Illumina Truseq was performed on cell lines after Rneasy RNA extraction and purification (Qiagen, Germantown, MD). Gene set association analysis was performed by importing counts from Kallisto alignment into GSAAseq (Xiong et al., 2015).

### 7.1.11 Immunoblotting and Immunofluorescence for STING results

All antibodies were purchased from Cell Signaling and procedures for immunoblotting and immunofluorescence were performed as in our previous publication (Cornelison, Dobbin, et al., 2017). For R-loop immunofluorescence the protocol was modified where cells were fixed by 4% paraformaldehyde in PBS for 5 minutes and permeabilized with 0.01% saponin in PBS. Live staining

with Sir-hoechst (Spirochrome Inc) was performed following manufacturer instructions at 1µM concentration in media.

Mice were implanted as per our previous publication (Cornelison, Dobbin, et al., 2017), treated once a week for 3 weeks by oral gavage using 50mg/kg in 50 mmol/L $NaH_2PO_4$ (pH 4.5) or by vehicle only. Tumors were collected, weighed, submerged in TPER (Thermo), and homogenized using an Omni tissue homogenizer (Omni International) for 3 bursts of 15 seconds and quantitated using BCA assay (Pierce). Luminex was performed per manufacturer instructions with normalization to protein concentration.

# 8 References

Abdullah, L. N., & Chow, E. K.-H. (2013). Mechanisms of chemoresistance in cancer stem cells. *Clinical and Translational Medicine*, *2*(1), 3. https://doi.org/10.1186/2001-1326-2-3

Abubaker, K., Latifi, A., Luwor, R., Nazaretian, S., Zhu, H., Quinn, M. A., Thompson, E. W., Findlay, J. K., & Ahmed, N. (2013). Short-term single treatment of chemotherapy results in the enrichment of ovarian cancer stem cell-like cells leading to an increased tumor burden. *Molecular Cancer*, *12*(1), 24. https://doi.org/10.1186/1476-4598-12-24

Adams. (n.d.). *Babraham Bioinformatics - FastQC A Quality Control tool for High Throughput Sequence Data*. Retrieved August 9, 2020, from http://www.bioinformatics.babraham.ac.uk/projects/fastqc/

Agarwal, R., & Kaye, S. B. (2003a). Ovarian cancer: strategies for overcoming resistance to chemotherapy. *Nature Reviews Cancer*, *3*(7), 502–516. https://doi.org/10.1038/nrc1123

Agarwal, R., & Kaye, S. B. (2003b). Ovarian cancer: strategies for overcoming resistance to chemotherapy. *Nature Reviews Cancer*, *3*(7), 502–516. https://doi.org/10.1038/nrc1123

Andrews, W. J., Panova, T., Normand, C., Gadal, O., Tikhonova, I. G., & Panov, K. I. (2013). Old drug, new target: Ellipticines selectively inhibit RNA polymerase I transcription. *Journal of Biological Chemistry*, *288*(7), 4567–

4582. https://doi.org/10.1074/jbc.M112.411611

Armstrong, D. K. (2002). Relapsed ovarian cancer: challenges and management strategies for a chronic disease. *The Oncologist*, *7 Suppl 5*, 20–28. http://www.ncbi.nlm.nih.gov/pubmed/12324630

Artero-Castro, A., Castellvi, J., García, A., Hernández, J., Ramón y Cajal, S., & Lleonart, M. E. (2011). Expression of the ribosomal proteins Rplp0, Rplp1, and Rplp2 in gynecologic tumors. *Human Pathology*, *42*(2), 194–203. https://doi.org/10.1016/j.humpath.2010.04.020

Ashworth, A. (2008a). A Synthetic Lethal Therapeutic Approach: Poly(ADP) Ribose Polymerase Inhibitors for the Treatment of Cancers Deficient in DNA Double-Strand Break Repair. *Journal of Clinical Oncology*, *26*(22), 3785–3790. https://doi.org/10.1200/JCO.2008.16.0812

Ashworth, A. (2008b). Drug resistance caused by reversion mutation. *Cancer Research*, *68*(24), 10021–10023. https://doi.org/10.1158/0008-5472.CAN-08-2287

Atherton-Fessler, S., Liu, F., Gabrielli, B., Lee, M. S., Peng, C. Y., & Piwnica-Worms, H. (1994). Cell cycle regulation of the p34cdc2 inhibitory kinases. *Molecular Biology of the Cell*, *5*(9), 989–1001.

Aust, S., Felix, S., Auer, K., Bachmayr-Heyda, A., Kenner, L., Dekan, S., Meier, S. M., Gerner, C., Grimm, C., & Pils, D. (2017). Absence of PD-L1 on tumor cells is associated with reduced MHC I expression and PD-L1 expression

increases in recurrent serous ovarian cancer. *Scientific Reports*, *7*, 42929. https://doi.org/10.1038/srep42929

Babaier, A., & Ghatage, P. (2020). Mucinous Cancer of the Ovary: Overview and Current Status. *Diagnostics (Basel, Switzerland)*, *10*(1). https://doi.org/10.3390/diagnostics10010052

Bache, M., Pigorsch, S., Dunst, J., Würl, P., Meye, A., Bartel, F., Schmidt, H., Rath, F. W., & Taubert, H. (2001). Loss of G2/M arrest correlates with radiosensitization in two human sarcoma cell lines with mutant p53. *International Journal of Cancer*, *96*(2), 110–117. https://doi.org/10.1002/ijc.1002

Bagnato, A., & Rosanò, L. (2012). Understanding and overcoming chemoresistance in ovarian cancer: emerging role of the endothelin axis. *Current Oncology (Toronto, Ont.)*, *19*(1), 36–38. https://doi.org/10.3747/co.19.895

Bagnoli, M., Granata, A., Nicoletti, R., Krishnamachary, B., Bhujwalla, Z. M., Canese, R., Podo, F., Canevari, S., Iorio, E., & Mezzanzanica, D. (2016). Choline Metabolism Alteration: A Focus on Ovarian Cancer. *Frontiers in Oncology*, *6*, 153. https://doi.org/10.3389/fonc.2016.00153

Beard, J. L., & Connor, J. R. (2003). I <scp>RON</scp> S <scp>TATUS AND</scp> N <scp>EURAL</scp> F <scp>UNCTIONING</scp>. In *Annual Review of Nutrition* (Vol. 23, Issue 1, pp. 41–58). https://doi.org/10.1146/annurev.nutr.23.020102.075739

Bell, D., Berchuck, A., Birrer, M., Chien, J., Cramer, D. W., Dao, F., Dhir, R., DiSaia, P., Gabra, H., Glenn, P., Godwin, A. K., Gross, J., Hartmann, L., Huang, M., Huntsman, D. G., Iacocca, M., Imielinski, M., Kalloger, S., Karlan, B. Y., … Thomson, E. (2011). Integrated genomic analyses of ovarian carcinoma. *Nature*, *474*(7353), 609–615. https://doi.org/10.1038/nature10166

Berek, J. S., Crum, C., & Friedlander, M. (2012). Cancer of the ovary, fallopian tube, and peritoneum. *International Journal of Gynaecology and Obstetrics: The Official Organ of the International Federation of Gynaecology and Obstetrics*, *119 Suppl*, S118-29. https://doi.org/10.1016/S0020-7292(12)60025-3

Bewersdorf, J., Bennett, B. T., & Knight, K. L. (2006). H2AX chromatin structures and their response to DNA damage revealed by 4Pi microscopy. *Proceedings of the National Academy of Sciences of the United States of America*, *103*(48), 18137–18142. https://doi.org/10.1073/pnas.0608709103

Binkhathlan, Z., & Lavasanifar, A. (2013). P-glycoprotein inhibition as a therapeutic approach for overcoming multidrug resistance in cancer: current status and future perspectives. *Current Cancer Drug Targets*, *13*(3), 326–346. http://www.ncbi.nlm.nih.gov/pubmed/23369096

Borst, P. (2012). Cancer drug pan-resistance: pumps, cancer stem cells, quiescence, epithelial to mesenchymal transition, blocked cell death pathways, persisters or what? *Open Biology*, *2*(5), 120066.

https://doi.org/10.1098/rsob.120066

Boulon, S., Westman, B. J., Hutten, S., Boisvert, F. M., & Lamond, A. I. (2010). The Nucleolus under Stress. *Molecular Cell*, *40*(2), 216–227. https://doi.org/10.1016/j.molcel.2010.09.024

Boussios, S., Mikropoulos, C., Samartzis, E., Karihtala, P., Moschetta, M., Sheriff, M., Karathanasi, A., Sadauskaite, A., Rassy, E., & Pavlidis, N. (2020). Wise Management of Ovarian Cancer: On the Cutting Edge. *Journal of Personalized Medicine*, *10*(2). https://doi.org/10.3390/jpm10020041

Bowtell, D. D., Böhm, S., Ahmed, A. A., Aspuria, P.-J., Bast, R. C., Beral, V., Berek, J. S., Birrer, M. J., Blagden, S., Bookman, M. A., Brenton, J. D., Chiappinelli, K. B., Martins, F. C., Coukos, G., Drapkin, R., Edmondson, R., Fotopoulou, C., Gabra, H., Galon, J., … Balkwill, F. R. (2015). Rethinking ovarian cancer II: reducing mortality from high-grade serous ovarian cancer. *Nature Reviews Cancer*, *15*(11), 668–679. https://doi.org/10.1038/nrc4019

Bray, N. L., Pimentel, H., Melsted, P., & Pachter, L. (2016). Near-optimal probabilistic RNA-seq quantification. *Nature Biotechnology*, *34*(5), 525–527. https://doi.org/10.1038/nbt.3519

Buchbinder, E. I., & Desai, A. (2016). CTLA-4 and PD-1 Pathways: Similarities, Differences, and Implications of Their Inhibition. *American Journal of Clinical Oncology*, *39*(1), 98–106. https://doi.org/10.1097/COC.0000000000000239

Buick, R. N., Pullano, R., & Trent, J. M. (1985). Comparative properties of five

human ovarian adenocarcinoma cell lines. *Cancer Research*, *45*(8), 3668–3676. http://www.ncbi.nlm.nih.gov/pubmed/4016745

Burger, K., Mühl, B., Harasim, T., Rohrmoser, M., Malamoussi, A., Orban, M., Kellner, M., Gruber-Eber, A., Kremmer, E., Hölzel, M., & Eick, D. (2010). Chemotherapeutic drugs inhibit ribosome biogenesis at various levels. *Journal of Biological Chemistry*, *285*(16), 12416–12425. https://doi.org/10.1074/jbc.M109.074211

Burma, S., Chen, B. P., Murphy, M., Kurimasa, A., & Chen, D. J. (2001). ATM phosphorylates histone H2AX in response to DNA double-strand breaks. *The Journal of Biological Chemistry*, *276*(45), 42462–42467. https://doi.org/10.1074/jbc.C100466200

Bywater, M. J., Pearson, R. B., McArthur, G. A., & Hannan, R. D. (2013). Dysregulation of the basal RNA polymerase transcription apparatus in cancer. *Nature Reviews. Cancer*, *13*(5), 299–314. https://doi.org/10.1038/nrc3496

Bywater, M. J., Poortinga, G., Sanij, E., Hein, N., Peck, A., Cullinane, C., Wall, M., Cluse, L., Drygin, D., Anderes, K., Huser, N., Proffitt, C., Bliesath, J., Haddach, M., Schwaebe, M. K., Ryckman, D. M., Rice, W. G., Schmitt, C., Lowe, S. W., … Hannan, R. D. (2012). Inhibition of RNA Polymerase I as a Therapeutic Strategy to Promote Cancer-Specific Activation of p53. *Cancer Cell*, *22*(1), 51–65. https://doi.org/10.1016/j.ccr.2012.05.019

Calo, E., Flynn, R. A., Martin, L., Spitale, R. C., Chang, H. Y., & Wysocka, J.

(2014). RNA helicase DDX21 coordinates transcription and ribosomal RNA processing. *Nature*, *518*(7538), 249–253. https://doi.org/10.1038/nature13923

Cancer Genome Atlas Research Network, Bell, D., Berchuck, a., Birrer, M., Chien, J., Cramer, D. W., Dao, F., Dhir, R., DiSaia, P., Gabra, H., Glenn, P., Godwin, a. K., Gross, J., Hartmann, L., Huang, M., Huntsman, D. G., Iacocca, M., Imielinski, M., Kalloger, S., … Thomson, E. (2011). Integrated genomic analyses of ovarian carcinoma. *Nature*, *474*(7353), 609–615. https://doi.org/10.1038/nature10166

Cannistra, S. A. (2004). Cancer of the Ovary. *N Engl J Med*, *35124351*(24), 2519–2529. https://doi.org/10.1136/bmj.2.6195.935-c

Cerami, E., Gao, J., Dogrusoz, U., Gross, B. E., Sumer, S. O., Aksoy, B. A., Jacobsen, A., Byrne, C. J., Heuer, M. L., Larsson, E., Antipin, Y., Reva, B., Goldberg, A. P., Sander, C., & Schultz, N. (2012). The cBio Cancer Genomics Portal: An open platform for exploring multidimensional cancer genomics data. *Cancer Discovery*, *2*(5), 401–404. https://doi.org/10.1158/2159-8290.CD-12-0095

Cheaib, B., Auguste, A., & Leary, A. (2015). The PI3K/Akt/mTOR pathway in ovarian cancer: therapeutic opportunities and challenges. *Chinese Journal of Cancer*, *34*(1), 4–16. https://doi.org/10.5732/cjc.014.10289

Chen, A. (2011). PARP inhibitors: its role in treatment of cancer. *Chinese Journal of Cancer*, *30*(7), 463–471. https://doi.org/10.5732/cjc.011.10111

Chen, C. D., Welsbie, D. S., Tran, C., Baek, S. H., Chen, R., Vessella, R., Rosenfeld, M. G., & Sawyers, C. L. (2004). Molecular determinants of resistance to antiandrogen therapy. *Nature Medicine*, *10*(1), 33–39. https://doi.org/10.1038/nm972

Chen, E. Y., Tan, C. M., Kou, Y., Duan, Q., Wang, Z., Meirelles, G. V., Clark, N. R., & Ma'ayan, A. (2013). Enrichr: interactive and collaborative HTML5 gene list enrichment analysis tool. *BMC Bioinformatics*, *14*, 128. https://doi.org/10.1186/1471-2105-14-128

Chung, F. S., Santiago, J. S., Jesus, M. F. M. De, Trinidad, C. V, & See, M. F. E. (2016). Disrupting P-glycoprotein function in clinical settings: what can we learn from the fundamental aspects of this transporter? *American Journal of Cancer Research*, *6*(8), 1583–1598. http://www.ncbi.nlm.nih.gov/pubmed/27648351

Colis, L., Peltonen, K., Sirajuddin, P., Liu, H., Sanders, S., Ernst, G., Barrow, J. C., & Laiho, M. (2014). DNA intercalator BMH-21 inhibits RNA polymerase I independent of DNA damage response. *Oncotarget*, *5*(12), 4361–4369. https://doi.org/10.18632/oncotarget.2020

Cooke, S. L., & Brenton, J. D. (2011). Evolution of platinum resistance in high-grade serous ovarian cancer. *The Lancet Oncology*, *12*(12), 1169–1174. https://doi.org/10.1016/S1470-2045(11)70123-1

Cornelison, R., Dobbin, Z. C., Katre, A. A., Jeong, D. H., Zhang, Y., Chen, D., Petrova, Y., Llaneza, D. C., Steg, A. D., Parsons, L., Schneider, D. A., &

Landen, C. N. (2017). Targeting RNA-polymerase I in both chemosensitive and chemoresistant populations in epithelial ovarian cancer. *Clinical Cancer Research*, *23*(21), 6529–6540. https://doi.org/10.1158/1078-0432.CCR-17-0282

Cornelison, R., Llaneza, D. C., & Landen, C. N. (2017). Emerging Therapeutics to Overcome Chemoresistance in Epithelial Ovarian Cancer: A Mini-Review. *International Journal of Molecular Sciences*, *18*(10). https://doi.org/10.3390/ijms18102171

Cortez, A. J., Tudrej, P., Kujawa, K. A., & Lisowska, K. M. (2018). Advances in ovarian cancer therapy. *Cancer Chemotherapy and Pharmacology*, *81*(1), 17. https://doi.org/10.1007/S00280-017-3501-8

Coward, J. I., Middleton, K., & Murphy, F. (2015). New perspectives on targeted therapy in ovarian cancer. *International Journal of Women's Health*, *7*, 189–203. https://doi.org/10.2147/IJWH.S52379

Crane, E. K., Kwan, S.-Y., Izaguirre, D. I., Tsang, Y. T. M., Mullany, L. K., Zu, Z., Richards, J. S., Gershenson, D. M., & Wong, K.-K. (2015). Nutlin-3a: A Potential Therapeutic Opportunity for TP53 Wild-Type Ovarian Carcinomas. *PloS One*, *10*(8), e0135101. https://doi.org/10.1371/journal.pone.0135101

Cree, I. A., & Charlton, P. (2017). Molecular chess? Hallmarks of anti-cancer drug resistance. *BMC Cancer*, *17*(1), 10. https://doi.org/10.1186/s12885-016-2999-1

Crum, C. P., Mehrad, M., Ning, G., Drapkin, R., McKeon, F. D., Xian, W., & Crum, C. P. (2011). STICS SCOUTs and p53 signatures a new language for pelvic serous carcinogenesis. *Frontiers in Bioscience*, *E3*(2), 275. https://doi.org/10.2741/e275

Davidson, B., Tropé, C. G., & Reich, R. (2012). Epithelial–Mesenchymal Transition in Ovarian Carcinoma. *Frontiers in Oncology*, *2*, 33. https://doi.org/10.3389/fonc.2012.00033

De Picciotto, N., Cacheux, W., Roth, A., Chappuis, P. O., & Labidi-Galy, S. I. (2016). Ovarian cancer: Status of homologous recombination pathway as a predictor of drug response. *Critical Reviews in Oncology/Hematology*, *101*, 50–59. https://doi.org/10.1016/j.critrevonc.2016.02.014

Deisenroth, C., & Zhang, Y. (2011). The Ribosomal Protein-Mdm2-p53 Pathway and Energy Metabolism: Bridging the Gap between Feast and Famine. *Genes & Cancer*, *2*(4), 392–403. https://doi.org/10.1177/1947601911409737

del Carmen, M. G., Birrer, M., & Schorge, J. O. (2012). Clear cell carcinoma of the ovary: a review of the literature. *Gynecologic Oncology*, *126*(3), 481–490. https://doi.org/10.1016/j.ygyno.2012.04.021

Deltcheva, E., & Nimmo, R. (2017). RUNX transcription factors at the interface of stem cells and cancer. *Biochemical Journal*, *474*(11). http://www.biochemj.org/content/474/11/1755

Derenzini, M., Montanaro, L., & Treré, D. (2009). What the nucleolus says to a

tumour pathologist. *Histopathology*, *54*(6), 753–762. https://doi.org/10.1111/j.1365-2559.2008.03168.x

Diaz-Padilla, I., & Oza, A. M. (2011). Epothilones in the treatment of ovarian cancer. *Future Oncology*, *7*(4), 559–568. https://doi.org/10.2217/fon.11.26

Dobbin, Z. C., Katre, A. A., Steg, A. D., Erickson, B. K., Shah, M. M., Alvarez, R. D., Conner, M. G., Schneider, D., Chen, D., & Landen, C. N. (2014). Using heterogeneity of the patient-derived xenograft model to identify the chemoresistant population in ovarian cancer. *Oncotarget*, *5*(18), 8750–8764. https://doi.org/10.18632/oncotarget.2373

Dockery, L. E., Gunderson, C. C., & Moore, K. N. (2017). Rucaparib: the past, present, and future of a newly approved PARP inhibitor for ovarian cancer. *OncoTargets and Therapy*, *10*, 3029–3037. https://doi.org/10.2147/OTT.S114714

Dou, Z., Ghosh, K., Vizioli, M. G., Zhu, J., Sen, P., Wangensteen, K. J., Simithy, J., Lan, Y., Lin, Y., Zhou, Z., Capell, B. C., Xu, C., Xu, M., Kieckhaefer, J. E., Jiang, T., Shoshkes-Carmel, M., Tanim, K. M. A. Al, Barber, G. N., Seykora, J. T., … Berger, S. L. (2017). Cytoplasmic chromatin triggers inflammation in senescence and cancer. *Nature*, *550*(7676), 402–406. https://doi.org/10.1038/nature24050

Dridi, M., Chraiet, N., Batti, R., Ayadi, M., Mokrani, A., Meddeb, K., Yahiaoui, Y., Raies, H., & Mezlini, A. (2018). Granulosa Cell Tumor of the Ovary: A Retrospective Study of 31 Cases and a Review of the Literature.

*International Journal of Surgical Oncology*, *2018*, 1–4. https://doi.org/10.1155/2018/4547892

Drygin, D., Siddiqui-Jain, A., O'Brien, S., Schwaebe, M., Lin, A., Bliesath, J., Ho, C. B., Proffitt, C., Trent, K., Whitten, J. P., Lim, J. K. C., Von Hoff, D., Anderes, K., & Rice, W. G. (2009). Anticancer Activity of CX-3543: A Direct Inhibitor of rRNA Biogenesis. *Cancer Research*, *69*(19), 7653–7661. https://doi.org/10.1158/0008-5472.CAN-09-1304

Drygin, Denis, Lin, A., Bliesath, J., Ho, C. B., O'Brien, S. E., Proffitt, C., Omori, M., Haddach, M., Schwaebe, M. K., Siddiqui-Jain, A., Streiner, N., Quin, J. E., Sanij, E., Bywater, M. J., Hannan, R. D., Ryckman, D., Anderes, K., & Rice, W. G. (2011). d. *Cancer Research*, *71*(4), 1418–1430. https://doi.org/10.1158/0008-5472.CAN-10-1728

Drygin, Denis, O'Brien, S. E., Hannan, R. D., McArthur, G. A., & Von Hoff, D. D. (2014). Targeting the nucleolus for cancer-specific activation of p53. *Drug Discovery Today*, *19*(3), 259–265. https://doi.org/10.1016/j.drudis.2013.08.012

Drygin, Denis, Rice, W. G., & Grummt, I. (2010). The RNA polymerase I transcription machinery: an emerging target for the treatment of cancer. *Annual Review of Pharmacology and Toxicology*, *50*(1), 131–156. https://doi.org/10.1146/annurev.pharmtox.010909.105844

Duan, Z., Feller, A. J., Toh, H. C., Makastorsis, T., & Seiden, M. V. (1999). TRAG-3, a novel gene, isolated from a taxol-resistant ovarian carcinoma cell

line. *Gene*, *229*(1–2), 75–81. http://www.ncbi.nlm.nih.gov/pubmed/10095106

Edenberg, E. R., Downey, M., & Toczyski, D. (2014). Polymerase stalling during replication, transcription and translation. *Current Biology*, *24*(10), R445–R452. https://doi.org/10.1016/j.cub.2014.03.060

Edwards, S. L., Brough, R., Lord, C. J., Natrajan, R., Vatcheva, R., Levine, D. A., Boyd, J., Reis-Filho, J. S., & Ashworth, A. (2008). Resistance to therapy caused by intragenic deletion in BRCA2. *Nature*, *451*(7182), 1111–1115. https://doi.org/10.1038/nature06548

El-Awady, R., Saleh, E., Hashim, A., Soliman, N., Dallah, A., Elrasheed, A., & Elakraa, G. (2016). The Role of Eukaryotic and Prokaryotic ABC Transporter Family in Failure of Chemotherapy. *Frontiers in Pharmacology*, *7*, 535. https://doi.org/10.3389/fphar.2016.00535

Erickson, B. K., Conner, M. G., & Landen, C. N. (2013). The role of the fallopian tube in the origin of ovarian cancer. *American Journal of Obstetrics and Gynecology*, *209*(5), 409–414. https://doi.org/10.1016/j.ajog.2013.04.019

Evans, T., & Matulonis, U. (2017). PARP inhibitors in ovarian cancer: evidence, experience and clinical potential. *Therapeutic Advances in Medical Oncology*, *9*(4), 253–267. https://doi.org/10.1177/1758834016687254

Fattaey, A., & Booher, R. N. (1997). Myt1: a Wee1-type kinase that phosphorylates Cdc2 on residue Thr14. *Progress in Cell Cycle Research*, *3*, 233–240.

Ferreira, R., Schneekloth, J. S., Panov, K. I., Hannan, K. M., & Hannan, R. D. (2020). Targeting the RNA Polymerase I Transcription for Cancer Therapy Comes of Age. *Cells*, *9*(2), 266. https://doi.org/10.3390/cells9020266

FF, N., I, D., & O, P. (2014). Germ Cell Tumors of the Ovary: An Update. *Archives of Pathology & Laboratory Medicine*, *138*(3). https://doi.org/10.5858/ARPA.2012-0547-RA

Frei, E., Karon, M., Levin, R. H., Freireich, E. J., Taylor, R. J., Hananian, J., Selawry, O., Holland, J. F., Hoogstraten, B., Wolman, I. J., Abir, E., Sawitsky, A., Lee, S., Mills, S. D., Burgert, E. O., Spurr, C. L., Patterson, R. B., Ebaugh, F. G., James, G. W., & Moon, J. H. (1965). The effectiveness of combinations of antileukemic agents in inducing and maintaining remission in children with acute leukemia. *Blood*, *26*(5), 642–656. http://www.ncbi.nlm.nih.gov/pubmed/5321112

Friedlander, M. L., Russell, K., Millis, S., Gatalica, Z., Bender, R., & Voss, A. (2016). Molecular Profiling of Clear Cell Ovarian Cancers: Identifying Potential Treatment Targets for Clinical Trials. *International Journal of Gynecological Cancer : Official Journal of the International Gynecological Cancer Society*, *26*(4), 648–654. https://doi.org/10.1097/IGC.0000000000000677

Fu, Y., Hu, D., Qiu, J., Xie, X., Ye, F., & Lu, W.-G. (2011). Overexpression of glycogen synthase kinase-3 in ovarian carcinoma cells with acquired paclitaxel resistance. *International Journal of Gynecological Cancer : Official*

*Journal of the International Gynecological Cancer Society*, *21*(3), 439–444. https://doi.org/10.1097/IGC.0b013e31820d7366

Gabut, M., Bourdelais, F., & Durand, S. (2020). Ribosome and Translational Control in Stem Cells. *Cells*, *9*(2), 497. https://doi.org/10.3390/cells9020497

Gadducci, A., & Cosio, S. (2020). Therapeutic Approach to Low-Grade Serous Ovarian Carcinoma: State of Art and Perspectives of Clinical Research. *Cancers*, *12*(5). https://doi.org/10.3390/cancers12051336

Gao, J., Aksoy, B. A., Dogrusoz, U., Dresdner, G., Gross, B., Sumer, S. O., Sun, Y., Jacobsen, A., Sinha, R., Larsson, E., Cerami, E., Sander, C., & Schultz, N. (2013). Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. *Science Signaling*, *6*(269). https://doi.org/10.1126/scisignal.2004088

Gentilella, A., Kozma, S. C., & Thomas, G. (2015). A liaison between mTOR signaling, ribosome biogenesis and cancer. *Biochimica et Biophysica Acta - Gene Regulatory Mechanisms*, *1849*(7), 812–820. https://doi.org/10.1016/j.bbagrm.2015.02.005

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

Gibson, J.-M., Alzghari, S., Ahn, C., Trantham, H., & La-Beck, N. M. (2013). The role of pegylated liposomal doxorubicin in ovarian cancer: a meta-analysis of

randomized clinical trials. *The Oncologist*, *18*(9), 1022–1031. https://doi.org/10.1634/theoncologist.2013-0126

Golomb, L., Volarevic, S., & Oren, M. (2014). P53 and ribosome biogenesis stress: The essentials. *FEBS Letters*, *588*(16), 2571–2579. https://doi.org/10.1016/j.febslet.2014.04.014

Goodfellow, S. J., & Zomerdijk, J. C. B. M. (2013). Basic mechanisms in RNA polymerase I transcription of the ribosomal RNA genes. *Subcellular Biochemistry*, *61*. https://doi.org/10.1007/978-94-007-4525-4_10

Goodspeed, A., Heiser, L. M., Gray, J. W., & Costello, J. C. (2016). Tumor-Derived Cell Lines as Molecular Models of Cancer Pharmacogenomics. *Molecular Cancer Research : MCR*, *14*(1), 3. https://doi.org/10.1158/1541-7786.MCR-15-0189

Gorringe, K. L., Cheasley, D., Wakefield, M. J., Ryland, G. L., Allan, P. E., Alsop, K., Amarasinghe, K. C., Ananda, S., Bowtell, D. D. L., Christie, M., Chiew, Y.-E., Churchman, M., DeFazio, A., Fereday, S., Gilks, C. B., Gourley, C., Hadley, A. M., Hendley, J., Hunter, S. M., … Scott, C. L. (2020). Therapeutic options for mucinous ovarian carcinoma. *Gynecologic Oncology*, *156*(3), 552–560. https://doi.org/10.1016/j.ygyno.2019.12.015

Gottesman, M. M., Fojo, T., & Bates, S. E. (2002). MULTIDRUG RESISTANCE IN CANCER: ROLE OF ATP-DEPENDENT TRANSPORTERS. *Nature Reviews Cancer*, *2*(1), 48–58. https://doi.org/10.1038/nrc706

Gottesman, M. M., & Pastan, I. H. (2015). The Role of Multidrug Resistance Efflux Pumps in Cancer: Revisiting a JNCI Publication Exploring Expression of the MDR1 (P-glycoprotein) Gene. *Journal of the National Cancer Institute*, *107*(9), djv222. https://doi.org/10.1093/jnci/djv222

Goudarzi, K. M., Nistér, M., & Lindström, M. S. (2014). mTOR inhibitors blunt the p53 response to nucleolar stress by regulating RPL11 and MDM2 levels. *Cancer Biology and Therapy*, *15*(11), 1499–1514. https://doi.org/10.4161/15384047.2014.955743

Groen, R. S., Gershenson, D. M., & Fader, A. N. (2015). Updates and emerging therapies for rare epithelial ovarian cancers: One size no longer fits all. *Gynecologic Oncology*, *136*(2), 373–383. https://doi.org/10.1016/j.ygyno.2014.11.078

Groenendijk, F. H., & Bernards, R. (2014). Drug resistance to targeted therapies: Déjà vu all over again. *Molecular Oncology*, *8*(6), 1067–1083. https://doi.org/10.1016/j.molonc.2014.05.004

Grummt, I. (2003). Life on a planet of its own: Regulation of RNA polymerase I transcription in the nucleolus. *Genes and Development*, *17*(14), 1691–1702. https://doi.org/10.1101/gad.1098503R

Grummt, I. (2007). Different epigenetic layers engage in complex crosstalk to define the epigenetic state of mammalian rRNA genes. *Human Molecular Genetics*, *16*(R1), R21–R27. https://doi.org/10.1093/hmg/ddm020

Grummt, I., & Längst, G. (2013). Epigenetic control of RNA polymerase I transcription in mammalian cells. *Biochimica et Biophysica Acta - Gene Regulatory Mechanisms*, *1829*(3–4), 393–404. https://doi.org/10.1016/j.bbagrm.2012.10.004

Gupta, N., Xu, Z., El-Sehemy, A., Steed, H., & Fu, Y. (2013). Notch3 induces epithelial–mesenchymal transition and attenuates carboplatin-induced apoptosis in ovarian cancer cells. *Gynecologic Oncology*, *130*(1), 200–206. https://doi.org/10.1016/j.ygyno.2013.03.019

Haddach, M., Schwaebe, M. K., Michaux, J., Nagasawa, J., O'Brien, S. E., Whitten, J. P., Pierre, F., Kerdoncuff, P., Darjania, L., Stansfield, R., Drygin, D., Anderes, K., Proffitt, C., Bliesath, J., Siddiqui-Jain, A., Omori, M., Huser, N., Rice, W. G., & Ryckman, D. M. (2012a). Discovery of CX-5461, the first direct and selective inhibitor of RNA polymerase I, for cancer therapeutics. *ACS Medicinal Chemistry Letters*, *3*(7), 602–606. https://doi.org/10.1021/ml300110s

Haddach, M., Schwaebe, M. K., Michaux, J., Nagasawa, J., O'Brien, S. E., Whitten, J. P., Pierre, F., Kerdoncuff, P., Darjania, L., Stansfield, R., Drygin, D., Anderes, K., Proffitt, C., Bliesath, J., Siddiqui-Jain, A., Omori, M., Huser, N., Rice, W. G., & Ryckman, D. M. (2012b). Discovery of CX-5461, the first direct and selective inhibitor of RNA polymerase I, for cancer therapeutics. *ACS Medicinal Chemistry Letters*, *3*(7), 602–606. https://doi.org/10.1021/ml300110s

Halder, J., Kamat, A. A., Landen, C. N., Han, L. Y., Lutgendorf, S. K., Lin, Y. G., Merritt, W. M., Jennings, N. B., Chavez-Reyes, A., Coleman, R. L., Gershenson, D. M., Schmandt, R., Cole, S. W., Lopez-Berestein, G., & Sood, A. K. (2006). Focal adhesion kinase targeting using in vivo short interfering RNA delivery in neutral liposomes for ovarian carcinoma therapy. *Clinical Cancer Research : An Official Journal of the American Association for Cancer Research*, *12*(16), 4916–4924. https://doi.org/10.1158/1078-0432.CCR-06-0021

Hamanishi, J., Mandai, M., Ikeda, T., Minami, M., Kawaguchi, A., Murayama, T., Kanai, M., Mori, Y., Matsumoto, S., Chikuma, S., Matsumura, N., Abiko, K., Baba, T., Yamaguchi, K., Ueda, A., Hosoe, Y., Morita, S., Yokode, M., Shimizu, A., … Konishi, I. (2015). Safety and Antitumor Activity of Anti–PD-1 Antibody, Nivolumab, in Patients With Platinum-Resistant Ovarian Cancer. *Journal of Clinical Oncology*, *33*(34), 4015–4022. https://doi.org/10.1200/JCO.2015.62.3397

Hanahan, D., & Weinberg, R. A. (2011). Hallmarks of Cancer: The Next Generation. *Cell*, *144*(5), 646–674. https://doi.org/10.1016/j.cell.2011.02.013

Hannan, K. M., Sanij, E., Rothblum, L. I., Hannan, R. D., & Pearson, R. B. (2013). Dysregulation of RNA polymerase I transcription during disease. *Biochimica et Biophysica Acta - Gene Regulatory Mechanisms*, *1829*(3–4), 342–360. https://doi.org/10.1016/j.bbagrm.2012.10.014

Hannan, R. D., Drygin, D., & Pearson, R. B. (2013). Targeting RNA polymerase I

transcription and the nucleolus for cancer therapy. *Expert Opinion on Therapeutic Targets*, *17*(8), 873–878. https://doi.org/10.1517/14728222.2013.818658

Harries, M., & Kaye, S. B. (2001). Recent advances in the treatment of epithelial ovarian cancer. *Expert Opinion on Investigational Drugs*, *10*(9), 1715–1724. https://doi.org/10.1517/13543784.10.9.1715

Hashimoto, O., Shinkawa, M., Torimura, T., Nakamura, T., Selvendiran, K., Sakamoto, M., Koga, H., Ueno, T., & Sata, M. (2006). Cell cycle regulation by the Wee1 Inhibitor PD0166285, Pyrido [2,3-d] pyimidine, in the B16 mouse melanoma cell line. *BMC Cancer*, *6*(1), 292. https://doi.org/10.1186/1471-2407-6-292

Haslehurst, A. M., Koti, M., Dharsee, M., Nuin, P., Evans, K., Geraci, J., Childs, T., Chen, J., Li, J., Weberpals, J., Davey, S., Squire, J., Park, P. C., & Feilotter, H. (2012). EMT transcription factors snail and slug directly contribute to cisplatin resistance in ovarian cancer. *BMC Cancer*, *12*(1), 91. https://doi.org/10.1186/1471-2407-12-91

He, J., Yu, J. J., Xu, Q., Wang, L., Zheng, J. Z., Liu, L. Z., & Jiang, B. H. (2015). Downregulation of ATG14 by EGR1-MIR152 sensitizes ovarian cancer cells to cisplatin-induced apoptosis by inhibiting cyto-protective autophagy. *Autophagy*, *11*(2), 373–384. https://doi.org/10.1080/15548627.2015.1009781

Heckl, M., Schmoeckel, E., Hertlein, L., Rottmann, M., Jeschke, U., & Mayr, D.

(2018). The ARID1A, p53 and ß-Catenin statuses are strong prognosticators in clear cell and endometrioid carcinoma of the ovary and the endometrium. *PLOS ONE*, *13*(2), e0192881. https://doi.org/10.1371/journal.pone.0192881

Hedditch, E. L., Gao, B., Russell, A. J., Lu, Y., Emmanuel, C., Beesley, J., Johnatty, S. E., Chen, X., Harnett, P., George, J., Williams, R. T., Flemming, C., Lambrechts, D., Despierre, E., Lambrechts, S., Vergote, I., Karlan, B., Lester, J., Orsulic, S., … Henderson, M. J. (2014). ABCA transporter gene expression and poor outcome in epithelial ovarian cancer. *Journal of the National Cancer Institute*, *106*(7). https://doi.org/10.1093/jnci/dju149

Heijink, A. M., Blomen, V. A., Bisteau, X., Degener, F., Matsushita, F. Y., Kaldis, P., Foijer, F., & van Vugt, M. A. T. M. (2015). A haploid genetic screen identifies the $G_1$/S regulatory machinery as a determinant of Wee1 inhibitor sensitivity. *Proceedings of the National Academy of Sciences*, *112*(49), 15160–15165. https://doi.org/10.1073/pnas.1505283112

Hein, N., Hannan, K. M., George, A. J., Sanij, E., & Hannan, R. D. (2013). The nucleolus: An emerging target for cancer therapy. *Trends in Molecular Medicine*, *19*(11), 643–654. https://doi.org/10.1016/j.molmed.2013.07.005

Herzog, T. J., & Monk, B. J. (2017). Bringing new medicines to women with epithelial ovarian cancer: what is the unmet medical need? *Gynecologic Oncology Research and Practice*, *4*, 13. https://doi.org/10.1186/s40661-017-0050-0

Ho, G. Y., Woodward, N., & Coward, J. I. G. (2016). Cisplatin versus carboplatin:

comparative review of therapeutic management in solid malignancies. *Critical Reviews in Oncology/Hematology*, *102*, 37–46. https://doi.org/10.1016/j.critrevonc.2016.03.014

Hong, C., Tijhuis, A. E., & Foijer, F. (2019). The cGAS Paradox: Contrasting Roles for cGAS-STING Pathway in Chromosomal Instability. *Cells*, *8*(10). https://doi.org/10.3390/cells8101228

Horta, M., & Cunha, T. M. (2015). Sex cord-stromal tumors of the ovary: a comprehensive review and update for radiologists. *Diagnostic and Interventional Radiology (Ankara, Turkey)*, *21*(4), 277–286. https://doi.org/10.5152/dir.2015.34414

Hsieh, A. C., Truitt, M. L., & Ruggero, D. (2011). Oncogenic AKTivation of translation as a therapeutic target. *British Journal of Cancer*, *105*(3), 329–336. https://doi.org/10.1038/bjc.2011.241

Huang, R.-Y., Francois, A., McGray, A. R., Miliotto, A., & Odunsi, K. (2017). Compensatory upregulation of PD-1, LAG-3, and CTLA-4 limits the efficacy of single-agent checkpoint blockade in metastatic ovarian cancer. *Oncoimmunology*, *6*(1), e1249561. https://doi.org/10.1080/2162402X.2016.1249561

Huen, M. S. Y., & Chen, J. (2008). The DNA damage response pathways: at the crossroad of protein modifications. *Cell Research*, *18*(1), 8–16. https://doi.org/10.1038/cr.2007.109

Hunter, T. (1995). Protein kinases and phosphatases: the yin and yang of protein phosphorylation and signaling. *Cell*, *80*(2), 225–236.

Issaeva, N., Thomas, H. D., Djurenovic, T., Jaspers, J. E., Stoimenov, I., Kyle, S., Pedley, N., Gottipati, P., Zur, R., Sleeth, K., Chatzakos, V., Mulligan, E. A., Lundin, C., Gubanova, E., Kersbergen, A., Harris, A. L., Sharma, R. A., Rottenberg, S., Curtin, N. J., … Helleday, T. (2010). 6-Thioguanine Selectively Kills BRCA2-Defective Tumors and Overcomes PARP Inhibitor Resistance. *Cancer Research*, *70*(15), 6268–6276. https://doi.org/10.1158/0008-5472.CAN-09-3416

James, A., Wang, Y., Raje, H., Rosby, R., & DiMario, P. (2014). Nucleolar stress with and without p53. *Nucleus (Austin, Tex.)*, *5*(5), 1–25. https://doi.org/10.4161/nucl.32235

Javle, M., & Curtin, N. J. (2011). The role of PARP in DNA repair and its therapeutic exploitation. *British Journal of Cancer*, *105*(8), 1114–1122. https://doi.org/10.1038/bjc.2011.382

Jayson, G. C., Kohn, E. C., Kitchener, H. C., & Ledermann, J. A. (2014). Ovarian cancer. *The Lancet*, *384*(9951), 1376–1388. https://doi.org/10.1016/S0140-6736(13)62146-7

Jelovac, D., & Armstrong, D. K. (2011). Recent Progress in the Diagnosis and Treatment of Ovarian Cancer. *CA Cancer J Clin*, *61*(3), 183–203. https://doi.org/10.3322/caac.20113

Jin, Y., Li, Y., Pan, L., Napoli, M., Cecere, S. C., Tambaro, R., Facchini, G., & Pignata, S. (2014). The target therapy of ovarian clear cell carcinoma. *OncoTargets and Therapy*, *7*(8), 1647. https://doi.org/10.2147/OTT.S49993

Johansen, B., Seberg, O., Petersen, G., & Arctander, P. (1995). DOES DAPI DETECT CYTOPLASMIC DNA? *American Journal of Botany*, *82*(10), 1215–1219. https://doi.org/10.1002/j.1537-2197.1995.tb12654.x

Johnatty, S. E., Beesley, J., Paul, J., Fereday, S., Spurdle, A. B., Webb, P. M., Byth, K., Marsh, S., McLeod, H., Harnett, P. R., Brown, R., deFazio, A., Chenevix-Trench, G., & Chenevix-Trench, G. (2008). ABCB1 (MDR 1) Polymorphisms and Progression-Free Survival among Women with Ovarian Cancer following Paclitaxel/Carboplatin Chemotherapy. *Clinical Cancer Research*, *14*(17), 5594–5601. https://doi.org/10.1158/1078-0432.CCR-08-0606

Johnatty, Sharon E., Beesley, J., Gao, B., Chen, X., Lu, Y., Law, M. H., Henderson, M. J., Russell, A. J., Hedditch, E. L., Emmanuel, C., Fereday, S., Webb, P. M., Goode, E. L., Vierkant, R. A., Fridley, B. L., Cunningham, J. M., Fasching, P. A., Beckmann, M. W., Ekici, A. B., … Chenevix-Trench, G. (2013). ABCB1 (MDR1) polymorphisms and ovarian cancer progression and survival: A comprehensive analysis from the Ovarian Cancer Association Consortium and The Cancer Genome Atlas. *Gynecologic Oncology*, *131*(1), 8–14. https://doi.org/10.1016/j.ygyno.2013.07.107

Kajiyama, H., Shibata, K., Terauchi, M., Yamashita, M., Ino, K., Nawa, A., &

Kikkawa, F. (2007). Chemoresistance to paclitaxel induces epithelial-mesenchymal transition and enhances metastatic potential for epithelial ovarian carcinoma cells. *International Journal of Oncology*, *31*(2), 277–283. http://www.ncbi.nlm.nih.gov/pubmed/17611683

Kapse-Mistry, S., Govender, T., Srivastava, R., & Yergeri, M. (2014). Nanodrug delivery in reversing multidrug resistance in cancer cells. *Frontiers in Pharmacology*, *5*, 159. https://doi.org/10.3389/fphar.2014.00159

Kavallaris, M., Kuo, D. Y., Burkhart, C. A., Regl, D. L., Norris, M. D., Haber, M., & Horwitz, S. B. (1997). Taxol-resistant epithelial ovarian tumors are associated with altered expression of specific beta-tubulin isotypes. *Journal of Clinical Investigation*, *100*(5), 1282–1293. https://doi.org/10.1172/JCI119642

Kelemen, L. E., & Köbel, M. (2011). Mucinous carcinomas of the ovary and colorectum: Different organ, same dilemma. In *The Lancet Oncology* (Vol. 12, Issue 11, pp. 1071–1080). Lancet Oncol. https://doi.org/10.1016/S1470-2045(11)70058-4

Kerbel, R. S., Kobayashi, H., & Graham, C. H. (1994). Intrinsic or acquired drug resistance and metastasis: Are they linked phenotypes? *Journal of Cellular Biochemistry*, *56*(1), 37–47. https://doi.org/10.1002/jcb.240560108

Kim, D.-W., Wu, N., Kim, Y.-C., Cheng, P. F., Basom, R., Kim, D., Dunn, C. T., Lee, A. Y., Kim, K., Lee, C. S., Singh, A., Gazdar, A. F., Harris, C. R., Eisenman, R. N., Park, K.-S., & MacPherson, D. (2016). Genetic

requirement for Mycl and efficacy of RNA Pol I inhibition in mouse models of small cell lung cancer. *Genes & Development*, *30*(11), 1289–1299. https://doi.org/10.1101/gad.279307.116

Kinner, A., Wu, W., Staudt, C., & Iliakis, G. (2008). Gamma-H2AX in recognition and signaling of DNA double-strand breaks in the context of chromatin. *Nucleic Acids Research*, *36*(17), 5678–5694. https://doi.org/10.1093/nar/gkn550

Klein, J., & Grummt, I. (1999). Cell cycle-dependent regulation of RNA polymerase I transcription: the nucleolar transcription factor UBF is inactive in mitosis and early G1. *Proceedings of the National Academy of Sciences of the United States of America*, *96*(11), 6096–6101. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=26841&tool=pmcentrez&rendertype=abstract

Kondo, T., Kobayashi, J., Saitoh, T., Maruyama, K., Ishii, K. J., Barber, G. N., Komatsu, K., Akira, S., & Kawai, T. (2013). DNA damage sensor MRE11 recognizes cytosolic double-stranded DNA and induces type I interferon by regulating STING trafficking. *Proceedings of the National Academy of Sciences*, *110*(8), 2969–2974. https://doi.org/10.1073/pnas.1222694110

Konno, H., Konno, K., & Barber, G. N. (2013). Cyclic Dinucleotides Trigger ULK1 (ATG1) Phosphorylation of STING to Prevent Sustained Innate Immune Signaling. *Cell*, *155*(3), 688–698. https://doi.org/10.1016/j.cell.2013.09.049

Korsholm, L. M., Gál, Z., Lin, L., Quevedo, O., Ahmad, D. A., Dulina, E., Luo, Y.,

Bartek, J., & Larsen, D. H. (2019). Double-strand breaks in ribosomal RNA genes activate a distinct signaling and chromatin response to facilitate nucleolar restructuring and repair. *Nucleic Acids Research*, *47*(15), 8019–8035. https://doi.org/10.1093/nar/gkz518

Krzystyniak, J., Ceppi, L., Dizon, D. S., & Birrer, M. J. (2016). Epithelial ovarian cancer: The molecular genetics of epithelial ovarian cancer. *Annals of Oncology : Official Journal of the European Society for Medical Oncology / ESMO*, *27*(suppl 1), i4–i10. https://doi.org/10.1093/annonc/mdw083

Kuleshov, M. V, Jones, M. R., Rouillard, A. D., Fernandez, N. F., Duan, Q., Wang, Z., Koplev, S., Jenkins, S. L., Jagodnik, K. M., Lachmann, A., McDermott, M. G., Monteiro, C. D., Gundersen, G. W., & Ma'ayan, A. (2016). Enrichr: a comprehensive gene set enrichment analysis web server 2016 update. *Nucleic Acids Research*, *44*(W1), W90-7. https://doi.org/10.1093/nar/gkw377

Kumar, R., Ichihashi, Y., Kimura, S., Chitwood, D. H., Headland, L. R., Peng, J., Maloof, J. N., & Sinha, N. R. (2012). A High-Throughput Method for Illumina RNA-Seq Library Preparation. *Frontiers in Plant Science*, *3*, 202. https://doi.org/10.3389/fpls.2012.00202

Kuo, C.-Y., & Ann, D. K. (2018). When fats commit crimes: fatty acid metabolism, cancer stemness and therapeutic resistance. *Cancer Communications*, *38*. https://doi.org/10.1186/S40880-018-0317-9

Kurman, R. J., & Shih, I.-M. (2008). Pathogenesis of Ovarian Cancer.

*International Journal of Gynecological Pathology*, *PAP*(2), 151–160. https://doi.org/10.1097/PGP.0b013e318161e4f5

Kusnadi, E. P., Hannan, K. M., Hicks, R. J., Hannan, R. D., Pearson, R. B., & Kang, J. (2015). Regulation of rDNA transcription in response to growth factors, nutrients and energy. *Gene*, *556*(1), 27–34. https://doi.org/10.1016/j.gene.2014.11.010

L Hollis, R., Gourley, C., L Hollis, R., & Gourley, C. (2016). Genetic and molecular changes in ovarian cancer. *Cancer Biology & Medicine*, *13*(2), 236–247. https://doi.org/10.20892/j.issn.2095-3941.2016.0024

Labidi-Galy, S. I., Papp, E., Hallberg, D., Niknafs, N., Adleff, V., Noe, M., Bhattacharya, R., Novak, M., Jones, S., Phallen, J., Hruban, C. A., Hirsch, M. S., Lin, D. I., Schwartz, L., Maire, C. L., Tille, J.-C., Bowden, M., Ayhan, A., Wood, L. D., … Velculescu, V. E. (2017). High grade serous ovarian carcinomas originate in the fallopian tube. *Nature Communications*, *8*(1), 1093. https://doi.org/10.1038/s41467-017-00962-1

Landen, C. N., Kim, T.-J., Lin, Y. G., Merritt, W. M., Kamat, A. A., Han, L. Y., Spannuth, W. A., Nick, A. M., Jennnings, N. B., Kinch, M. S., Tice, D., & Sood, A. K. (2008). Tumor-selective response to antibody-mediated targeting of alphavbeta3 integrin in ovarian cancer. *Neoplasia (New York, N.Y.)*, *10*(11), 1259–1267. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2570602&tool=pmcentrez&rendertype=abstract

Lang, S. M., Mills, A. M., & Cantrell, L. A. (2017a). Malignant Brenner tumor of the ovary: Review and case report. *Gynecologic Oncology Reports*, *22*, 26–31. https://doi.org/10.1016/j.gore.2017.07.001

Lang, S. M., Mills, A. M., & Cantrell, L. A. (2017b). Malignant Brenner tumor of the ovary: Review and case report. *Gynecologic Oncology Reports*, *22*, 26–31. https://doi.org/10.1016/j.gore.2017.07.001

Larsen, D. H., & Stucki, M. (2016). Nucleolar responses to DNA double-strand breaks. *Nucleic Acids Research*, *44*(2), 538–544. https://doi.org/10.1093/nar/gkv1312

Latifi, A., Abubaker, K., Castrechini, N., Ward, A. C., Liongue, C., Dobill, F., Kumar, J., Thompson, E. W., Quinn, M. A., Findlay, J. K., & Ahmed, N. (2011). Cisplatin treatment of primary and metastatic epithelial ovarian carcinomas generates residual cells with mesenchymal stem cell-like profile. *Journal of Cellular Biochemistry*, *112*(10), 2850–2864. https://doi.org/10.1002/jcb.23199

Leary, D. J., & Huang, S. (2001). Regulation of ribosome biogenesis within the nucleolus. *FEBS Letters*, *509*(2), 145–150. https://doi.org/10.1016/S0014-5793(01)03143-X

Lee, J.-H., & Paull, T. T. (2005). ATM Activation by DNA Double-Strand Breaks Through the Mre11-Rad50-Nbs1 Complex. *Science*, *308*(5721), 551–554. https://doi.org/10.1126/science.1108297

Lee, Y.-C., Lee, C.-H., Tsai, H.-P., An, H.-W., Lee, C.-M., Wu, J.-C., Chen, C.-S., Huang, S.-H., Hwang, J., Cheng, K.-T., Leiw, P.-L., Chen, C.-L., & Lin, C.-M. (2015). Targeting of Topoisomerase I for Prognoses and Therapeutics of Camptothecin-Resistant Ovarian Cancer. *PloS One*, *10*(7), e0132579. https://doi.org/10.1371/journal.pone.0132579

Lee, Y., Miron, A., Drapkin, R., Nucci, M., Medeiros, F., Saleemuddin, A., Garber, J., Birch, C., Mou, H., Gordon, R., Cramer, D., McKeon, F., & Crum, C. (2007). A candidate precursor to serous carcinoma that originates in the distal fallopian tube. *The Journal of Pathology*, *211*(1), 26–35. https://doi.org/10.1002/path.2091

Leggett, R. M., Ramirez-Gonzalez, R. H., Clavijo, B. J., Waite, D., & Davey, R. P. (2013). Sequencing quality assessment tools to enable data-driven informatics for high throughput genomics. *Frontiers in Genetics*, *4*. https://doi.org/10.3389/FGENE.2013.00288

Leijen, S., Beijnen, J. H., & Schellens, J. H. M. (2010). Abrogation of the G2 checkpoint by inhibition of Wee-1 kinase results in sensitization of p53-deficient tumor cells to DNA-damaging agents. *Current Clinical Pharmacology*, *5*(3), 186–191.

Lengyel, E. (2010). Ovarian cancer development and metastasis. *The American Journal of Pathology*, *177*(3), 1053–1064. https://doi.org/10.2353/ajpath.2010.100105

Leslie, M. (2014). Central command. *Science (New York, N.Y.)*, *345*(6196), 506–

507. https://doi.org/10.1126/science.345.6196.506

Li, G., Shen, J., Cao, J., Zhou, G., Lei, T., Sun, Y., Gao, H., Ding, Y., Xu, W., Zhan, Z., Chen, Y., & Huang, H. (2018). Alternative splicing of human telomerase reverse transcriptase in gliomas and its modulation mediated by CX-5461. *Journal of Experimental & Clinical Cancer Research*, *37*(1), 78. https://doi.org/10.1186/s13046-018-0749-8

Li, L., Li, Y., Zhao, J., Fan, S., Wang, L., Li, X., Li, L., Li, Y., Zhao, J., Fan, S., & Wang, L. (2016). CX-5461 induces autophagy and inhibits tumor growth via mammalian target of rapamycin-related signaling pathways in osteosarcoma. *OncoTargets and Therapy*, *9*, 5985–5997. https://doi.org/10.2147/OTT.S104513

Liang, Y., Lin, S.-Y., Brunicardi, F. C., Goss, J., & Li, K. (2009). DNA damage response pathways in tumor suppression and cancer treatment. *World Journal of Surgery*, *33*(4), 661–666. https://doi.org/10.1007/s00268-008-9840-1

Lin, J. H., & Lu, A. Y. (2001). Interindividual variability in inhibition and induction of cytochrome P450 enzymes. *Annu.Rev.Pharmacol Toxicol*.

Lindström, M. S., Jurada, D., Bursac, S., Orsolic, I., Bartek, J., & Volarevic, S. (2018). Nucleolus as an emerging hub in maintenance of genome stability and cancer pathogenesis. *Oncogene*, *37*(18), 2351–2366. https://doi.org/10.1038/s41388-017-0121-z

Ling, K.-S., Chen, G.-D., Tsai, H.-J., Lee, M.-S., Wang, P.-H., & Liu, F.-S. (2005). Mechanisms Involved in Chemoresistance in Ovarian Cancer. *Taiwanese Journal of Obstetrics and Gynecology*, *44*(3), 209–217. https://doi.org/10.1016/S1028-4559(09)60143-1

Liu, J., Jiao, X., & Gao, Q. (2020). Neoadjuvant chemotherapy-related platinum resistance in ovarian cancer. *Drug Discovery Today*, *25*(7), 1232–1238. https://doi.org/10.1016/J.DRUDIS.2020.04.015

Liu, X., Chan, D., & Ngan, H. (2012). Mechanisms of Chemoresistance in Human Ovarian Cancer at a Glance. *Gynecology & Obstetrics*, *2*(3), 1000e104. https://doi.org/10.4172/2161-0932.1000e104

Louie, K. G., Behrens, B. C., Kinsella, T. J., Hamilton, T. C., Grotzinger, K. R., McKoy, W. M., Winker, M. A., & Ozols, R. F. (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), 2110–2115. http://www.ncbi.nlm.nih.gov/pubmed/3986765

Love, M. I., Huber, W., & Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology*, *15*(12), 550. https://doi.org/10.1186/s13059-014-0550-8

Low, J. J. H., Ilancheran, A., & Ng, J. S. (2012). Malignant ovarian germ-cell tumours. *Best Practice & Research Clinical Obstetrics & Gynaecology*, *26*(3), 347–355. https://doi.org/10.1016/J.BPOBGYN.2012.01.002

Lukinavičius, G., Blaukopf, C., Pershagen, E., Schena, A., Reymond, L., Derivery, E., Gonzalez-Gaitan, M., D'Este, E., Hell, S. W., Gerlich, D. W., & Johnsson, K. (2015). SiR-Hoechst is a far-red DNA stain for live-cell nanoscopy. *Nature Communications*, *6*(1), 1–7. https://doi.org/10.1038/ncomms9497

Lyu, L., Wang, H., Li, B., Qin, Q., Qi, L., Nagarkatti, M., Nagarkatti, P., Janicki, J. S., Wang, X. L., & Cui, T. (2015). A critical role of cardiac fibroblast-derived exosomes in activating renin angiotensin system in cardiomyocytes. *Journal of Molecular and Cellular Cardiology*, *89*(Pt B), 268–279. https://doi.org/10.1016/j.yjmcc.2015.10.022

Mabuchi, S., Sugiyama, T., & Kimura, T. (2016). Clear cell carcinoma of the ovary: molecular insights and future therapeutic perspectives. *Journal of Gynecologic Oncology*, *27*(3), e31. https://doi.org/10.3802/jgo.2016.27.e31

Maoz, A., Matsuo, K., Ciccone, M. A., Matsuzaki, S., Klar, M., Roman, L. D., Sood, A. K., & Gershenson, D. M. (2020). Molecular pathways and targeted therapies for malignant ovarian germ cell tumors and sex cord– stromal tumors: A contemporary review. In *Cancers* (Vol. 12, Issue 6). MDPI AG. https://doi.org/10.3390/cancers12061398

Marchetti, C., Muzii, L., Romito, A., & Panici, P. B. (2019). First-line treatment of women with advanced ovarian cancer: focus on bevacizumab. *OncoTargets and Therapy*, *12*, 1095. https://doi.org/10.2147/OTT.S155425

Marchini, S., Fruscio, R., Clivio, L., Beltrame, L., Porcu, L., Nerini, I. F., Cavalieri,

D., Chiorino, G., Cattoretti, G., Mangioni, C., Milani, R., Torri, V., Romualdi, C., Zambelli, A., Romano, M., Signorelli, M., Giandomenico, S. di, & D'Incalci, M. (2013). Resistance to platinum-based chemotherapy is associated with epithelial to mesenchymal transition in epithelial ovarian cancer. *European Journal of Cancer*, *49*(2), 520–530. https://doi.org/10.1016/j.ejca.2012.06.026

Matheson, C. J., Backos, D. S., & Reigan, P. (2016). Targeting WEE1 Kinase in Cancer. *Trends in Pharmacological Sciences*, *37*(10), 872–881. https://doi.org/10.1016/j.tips.2016.06.006

Matos-Perdomo, E., & Machín, F. (2019). Nucleolar and Ribosomal DNA Structure under Stress: Yeast Lessons for Aging and Cancer. *Cells*, *8*(8). https://doi.org/10.3390/cells8080779

McCluggage, W. G., Lyness, R. W., Atkinson, R. J., Dobbs, S. P., Harley, I., McClelland, H. R., & Price, J. H. (2002). Morphological effects of chemotherapy on ovarian carcinoma. *Journal of Clinical Pathology*, *55*(1), 27–31. http://www.ncbi.nlm.nih.gov/pubmed/11825920

McGowan, C. H., & Russell, P. (1993). Human Wee1 kinase inhibits cell division by phosphorylating p34cdc2 exclusively on Tyr15. *The EMBO Journal*, *12*(1), 75–85.

McLachlan, J., George, A., & Banerjee, S. (2016). The current status of PARP inhibitors in ovarian cancer. *Tumori Journal*, *102*(5), 433–440. https://doi.org/10.5301/tj.5000558

Mi, Y.-J., Liang, Y.-J., Huang, H.-B., Zhao, H.-Y., Wu, C.-P., Wang, F., Tao, L.-Y., Zhang, C.-Z., Dai, C.-L., Tiwari, A. K., Ma, X.-X., To, K. K. W., Ambudkar, S. V, Chen, Z.-S., & Fu, L.-W. (2010). Apatinib (YN968D1) reverses multidrug resistance by inhibiting the efflux function of multiple ATP-binding cassette transporters. *Cancer Research*, *70*(20), 7981–7991. https://doi.org/10.1158/0008-5472.CAN-10-0111

Miow, Q. H., Tan, T. Z., Ye, J., Lau, J. a, Yokomizo, T., Thiery, J., & Mori, S. (2015). Epithelial-mesenchymal status renders differential responses to cisplatin in ovarian cancer. *Oncogene*, *34*(March), 1899–1907. https://doi.org/10.1038/onc.2014.136

Montanaro, L., Treré, D., & Derenzini, M. (2008). Nucleolus, Ribosomes, and Cancer. *The American Journal of Pathology*, *173*(2), 301–310. https://doi.org/10.2353/ajpath.2008.070752

Moore, D. H., Allison, B., Look, K. Y., Sutton, G. P., & Bigsby, R. M. (1997). Collagenase expression in ovarian cancer cell lines. *Gynecologic Oncology*, *65*(1), 78–82. https://doi.org/10.1006/gyno.1997.4628

Mukhtar, E., Adhami, V. M., & Mukhtar, H. (2014). Targeting Microtubules by Natural Agents for Cancer Therapy. *Molecular Cancer Therapeutics*, *13*(2), 275. https://doi.org/10.1158/1535-7163.MCT-13-0791

Musella, A., Vertechy, L., Romito, A., Marchetti, C., Giannini, A., Sciuga, V., Bracchi, C., Tomao, F., Di Donato, V., De Felice, F., Monti, M., Muzii, L., & Benedetti Panici, P. (2017). Bevacizumab in Ovarian Cancer: State of the

Art and Unanswered Questions. *Chemotherapy*, *62*(2), 111–120. https://doi.org/10.1159/000448942

Narla, A., & Ebert, B. L. (2011). Translational medicine: ribosomopathies. *Blood*, *118*(16), 4300–4301. https://doi.org/10.1182/blood-2011-08-372250

Neijenhuis, S., Bajrami, I., Miller, R., Lord, C. J., & Ashworth, A. (2013). Identification of miRNA modulators to PARP inhibitor response. *DNA Repair*, *12*(6), 394–402. https://doi.org/10.1016/j.dnarep.2013.02.003

Nguyen, L. X. T., Raval, A., Garcia, J. S., & Mitchell, B. S. (2015). Regulation of Ribosomal Gene Expression in Cancer. *Journal of Cellular Physiology*, *230*(6), 1181–1188. https://doi.org/10.1002/jcp.24854

Nojima, K., Hochegger, H., Saberi, A., Fukushima, T., Kikuchi, K., Yoshimura, M., Orelli, B. J., Bishop, D. K., Hirano, S., Ohzeki, M., Ishiai, M., Yamamoto, K., Takata, M., Arakawa, H., Buerstedde, J.-M., Yamazoe, M., Kawamoto, T., Araki, K., Takahashi, J. A., … Sonoda, E. (2005). Multiple repair pathways mediate tolerance to chemotherapeutic cross-linking agents in vertebrate cells. *Cancer Research*, *65*(24), 11704–11711. https://doi.org/10.1158/0008-5472.CAN-05-1214

Olive, K. P., Jacobetz, M. A., Davidson, C. J., Gopinathan, A., McIntyre, D., Honess, D., Madhu, B., Goldgraben, M. A., Caldwell, M. E., Allard, D., Frese, K. K., DeNicola, G., Feig, C., Combs, C., Winter, S. P., Ireland-Zecchini, H., Reichelt, S., Howat, W. J., Chang, A., … Tuveson, D. A. (2009). Inhibition of Hedgehog Signaling Enhances Delivery of

Chemotherapy in a Mouse Model of Pancreatic Cancer. *Science*, *324*(5933), 1457–1461. https://doi.org/10.1126/science.1171362

Orr, G. A., Verdier-Pinard, P., McDaid, H., & Horwitz, S. B. (2003). Mechanisms of Taxol resistance related to microtubules. *Oncogene*, *22*(47), 7280–7295. https://doi.org/10.1038/sj.onc.1206934

Osman, A. A., Monroe, M. M., Ortega Alves, M. V., Patel, A. A., Katsonis, P., Fitzgerald, A. L., Neskey, D. M., Frederick, M. J., Woo, S. H., Caulin, C., Hsu, T.-K., McDonald, T. O., Kimmel, M., Meyn, R. E., Lichtarge, O., & Myers, J. N. (2015). Wee-1 Kinase Inhibition Overcomes Cisplatin Resistance Associated with High-Risk TP53 Mutations in Head and Neck Cancer through Mitotic Arrest Followed by Senescence. *Molecular Cancer Therapeutics*, *14*(2), 608–619. https://doi.org/10.1158/1535-7163.MCT-14-0735-T

Paik, E. S., Kim, T. J., Choi, C. H., Kim, B. G., Bae, D. S., & Lee, J. W. (2018). Clinical outcomes of patients with clear cell and endometrioid ovarian cancer arising from endometriosis. *Journal of Gynecologic Oncology*, *29*(2), e18. https://doi.org/10.3802/jgo.2018.29.e18

Panov, K. I., Friedrich, J. K., Russell, J., & Zomerdijk, J. C. B. M. (2006). UBF activates RNA polymerase I transcription by stimulating promoter escape. *The EMBO Journal*, *25*(14), 3310–3322. https://doi.org/10.1038/sj.emboj.7601221

Patch, A.-M., Christie, E. L., Etemadmoghadam, D., Garsed, D. W., George, J.,

Fereday, S., Nones, K., Cowin, P., Alsop, K., Bailey, P. J., Kassahn, K. S., Newell, F., Quinn, M. C. J., Kazakoff, S., Quek, K., Wilhelm-Benartzi, C., Curry, E., Leong, H. S., Hamilton, A., … Bowtell, D. D. L. (2015). Whole–genome characterization of chemoresistant ovarian cancer. *Nature*, *521*(7553), 489–494. https://doi.org/10.1038/nature14410

Patel, A. G., Sarkaria, J. N., & Kaufmann, S. H. (2011). Nonhomologous end joining drives poly(ADP-ribose) polymerase (PARP) inhibitor lethality in homologous recombination-deficient cells. *Proceedings of the National Academy of Sciences of the United States of America*, *108*(8), 3406–3411. https://doi.org/10.1073/pnas.1013715108

Pathan, M., Keerthikumar, S., Ang, C.-S., Gangoda, L., Quek, C. Y. J., Williamson, N. A., Mouradov, D., Sieber, O. M., Simpson, R. J., Salim, A., Bacic, A., Hill, A. F., Stroud, D. A., Ryan, M. T., Agbinya, J. I., Mariadason, J. M., Burgess, A. W., & Mathivanan, S. (2015). FunRich: An open access standalone functional enrichment and interaction network analysis tool. *Proteomics*, *15*(15), 2597–2601. https://doi.org/10.1002/pmic.201400515

Pathan, M., Keerthikumar, S., Chisanga, D., Alessandro, R., Ang, C.-S., Askenase, P., Batagov, A. O., Benito-Martin, A., Camussi, G., Clayton, A., Collino, F., Di Vizio, D., Falcon-Perez, J. M., Fonseca, P., Fonseka, P., Fontana, S., Gho, Y. S., Hendrix, A., Hoen, E. N.-’t, … Mathivanan, S. (2017). A novel community driven software for functional enrichment analysis of extracellular vesicles data. *Journal of Extracellular Vesicles*, *6*(1),

1321455. https://doi.org/10.1080/20013078.2017.1321455

Pectasides, D., Pectasides, E., & Kassanos, D. (2008). Germ cell tumors of the ovary. *Cancer Treatment Reviews*, *34*(5), 427–441. https://doi.org/10.1016/j.ctrv.2008.02.002

Pelletier, J., Thomas, G., & Volarević, S. (2018). Ribosome biogenesis in cancer: new players and therapeutic avenues. *Nature Reviews Cancer*, *18*(1), 51–63. https://doi.org/10.1038/nrc.2017.104

Peltonen, K., Colis, L., Liu, H., Jaamaa, S., Zhang, Z., af Hallstrom, T., Moore, H. M., Sirajuddin, P., & Laiho, M. (2014). Small Molecule BMH-Compounds That Inhibit RNA Polymerase I and Cause Nucleolar Stress. *Molecular Cancer Therapeutics*, *13*(11), 2537–2546. https://doi.org/10.1158/1535-7163.MCT-14-0256

Peltonen, Karita, Colis, L., Liu, H., Trivedi, R., Moubarek, M. S., Moore, H. M., Bai, B., Rudek, M. A., Bieberich, C. J., & Laiho, M. (2014). A targeting modality for destruction of RNA polymerase I that possesses anticancer activity. *Cancer Cell*, *25*(1), 77–90. https://doi.org/10.1016/j.ccr.2013.12.009

Pépin, G., Nejad, C., Ferrand, J., Thomas, B. J., Stunden, H. J., Sanij, E., Foo, C.-H., Stewart, C. R., Cain, J. E., Bardin, P. G., Williams, B. R. G., & Gantier, M. P. (2017). Topoisomerase 1 Inhibition Promotes Cyclic GMP-AMP Synthase-Dependent Antiviral Responses. *MBio*, *8*(5), e01611-17. https://doi.org/10.1128/mBio.01611-17

Perez, E. A. (2009). Microtubule inhibitors: Differentiating tubulin-inhibiting agents based on mechanisms of action, clinical activity, and resistance. *Molecular Cancer Therapeutics*, *8*(8), 2086–2095. https://doi.org/10.1158/1535-7163.MCT-09-0366

Pevzner, P. A., Tang, H., & Waterman, M. S. (2001). An Eulerian path approach to DNA fragment assembly. *Proceedings of the National Academy of Sciences of the United States of America*, *98*(17), 9748–9753. https://doi.org/10.1073/pnas.171285098

Pickard, A. J., & Bierbach, U. (2013). The cell's nucleolus: An emerging target for chemotherapeutic intervention. *ChemMedChem*, *8*(9), 1441–1449. https://doi.org/10.1002/cmdc.201300262

Pierson, W. E., Peters, P. N., Chang, M. T., Chen, L.-M., Quigley, D. A., Ashworth, A., & Chapman, J. S. (2020). An integrated molecular profile of endometrioid ovarian cancer. *Gynecologic Oncology*, *157*(1), 55–61. https://doi.org/10.1016/j.ygyno.2020.02.011

Pines, J., & Hunter, T. (1994). The differential localization of human cyclins A and B is due to a cytoplasmic retention signal in cyclin B. *The EMBO Journal*, *13*(16), 3772–3781.

Pinter, M., & Jain, R. K. (2017). Targeting the renin-angiotensin system to improve cancer treatment: Implications for immunotherapy. *Science Translational Medicine*, *9*(410). https://doi.org/10.1126/scitranslmed.aan5616

Planting, A. S. T., Sonneveld, P., van der Gaast, A., Sparreboom, A., van der Burg, M. E. L., Luyten, G. P. M., de Leeuw, K., de Boer-Dennert, M., Wissel, P. S., Jewell, R. C., Paul, E. M., Purvis, N. B., & Verweij, J. (2005). A phase I and pharmacologic study of the MDR converter GF120918 in combination with doxorubicin in patients with advanced solid tumors. *Cancer Chemotherapy and Pharmacology*, *55*(1), 91–99. https://doi.org/10.1007/s00280-004-0854-6

Poortinga, G., Quinn, L., & Hannan, R. (2014). Targeting RNA polymerase I to treat MYC-driven cancer. *Oncogene*, *34*(4), 403–412. https://doi.org/10.1038/onc.2014.13

PosthumaDeBoer, J., Würdinger, T., Graat, H. C., van Beusechem, V. W., Helder, M. N., van Royen, B. J., & Kaspers, G. J. (2011). WEE1 inhibition sensitizes osteosarcoma to radiotherapy. *BMC Cancer*, *11*(1), 156. https://doi.org/10.1186/1471-2407-11-156

Potapova, T. A., Unruh, J. R., Yu, Z., Rancati, G., Li, H., Stampfer, M. R., & Gerton, J. L. (2019). Superresolution microscopy reveals linkages between ribosomal DNA on heterologous chromosomes. *The Journal of Cell Biology*, *218*(8), 2492–2513. https://doi.org/10.1083/jcb.201810166

Pradeep, S., Kim, S., Wu, S., Nishimura, M., Chaluvally-Raghavan, P., Miyake, T., Pecot, C., Kim, S. J., Choi, H., Bischoff, F., Mayer, J., Huang, L., Nick, A., Hall, C., Rodriguez-Aguayo, C., Zand, B., Dalton, H., Arumugam, T., Lee, H., … Sood, A. (2014). Hematogenous Metastasis of Ovarian Cancer:

Rethinking Mode of Spread. *Cancer Cell*, *26*(1), 77–91. https://doi.org/10.1016/j.ccr.2014.05.002

Prat, J. (2012). New insights into ovarian cancer pathology. *Annals of Oncology : Official Journal of the European Society for Medical Oncology*, *23 Suppl 10*, x111-7. https://doi.org/10.1093/annonc/mds300

Prieto, J.-L., & McStay, B. (2005). Nucleolar biogenesis: the first small steps. *Biochemical Society Transactions*, *33*(Pt 6), 1441–1443. https://doi.org/10.1042/BST20051441

Quin, J., Chan, K. T., Devlin, J. R., Cameron, D. P., Diesch, J., Cullinane, C., Ahern, J., Khot, A., Hein, N., George, A. J., Hannan, K. M., Poortinga, G., Sheppard, K. E., Khanna, K. K., Johnstone, R. W., Drygin, D., McArthur, G. A., Pearson, R. B., Sanij, E., & Hannan, R. D. (2016). Inhibition of RNA polymerase I transcription initiation by CX-5461 activates non-canonical ATM/ATR signaling. *Oncotarget*. https://doi.org/10.18632/oncotarget.10452

Quin, J. E., Devlin, J. R., Cameron, D., Hannan, K. M., Pearson, R. B., & Hannan, R. D. (2014). Targeting the nucleolus for cancer intervention. *Biochimica et Biophysica Acta - Molecular Basis of Disease*, *1842*(6), 802–816. https://doi.org/10.1016/j.bbadis.2013.12.009

Ramalingam, P. (2016). Morphologic, Immunophenotypic, and Molecular Features of Epithelial Ovarian Cancer. *Oncology (Williston Park, N.Y.)*, *30*(2), 166–176. http://www.ncbi.nlm.nih.gov/pubmed/26892153

Ramos, R. N., Piaggio, E., & Romano, E. (2017). Mechanisms of Resistance to Immune Checkpoint Antibodies. In *Handbook of experimental pharmacology*. https://doi.org/10.1007/164_2017_11

Ray, S., Panova, T., Miller, G., Volkov, A., Porter, A. C. G., Russell, J., Panov, K. I., & Zomerdijk, J. C. B. M. (2013). Topoisomerase IIα promotes activation of RNA polymerase I transcription by facilitating pre-initiation complex formation. *Nature Communications*, *4*, 1598. https://doi.org/10.1038/ncomms2599

Reade, C. J., McVey, R. M., Tone, A. A., Finlayson, S. J., McAlpine, J. N., Fung-Kee-Fung, M., & Ferguson, S. E. (2014). The Fallopian Tube as the Origin of High Grade Serous Ovarian Cancer: Review of a Paradigm Shift. *Journal of Obstetrics and Gynaecology Canada*, *36*(2), 133–140. https://doi.org/10.1016/S1701-2163(15)30659-9

Reinbolt, R. E., & Hays, J. L. (2013). The Role of PARP Inhibitors in the Treatment of Gynecologic Malignancies. *Frontiers in Oncology*, *3*, 237. https://doi.org/10.3389/fonc.2013.00237

Reinthaller, A. (2016). Antiangiogenic therapies in ovarian cancer. *Memo*, *9*(3), 139–143. https://doi.org/10.1007/s12254-016-0282-4

Ricci, F., Affatato, R., Carrassa, L., & Damia, G. (2018). Recent Insights into Mucinous Ovarian Carcinoma. *International Journal of Molecular Sciences*, *19*(6). https://doi.org/10.3390/ijms19061569

Ricciardi, E., Baert, T., Ataseven, B., Heitz, F., Prader, S., Bommert, M., Schneider, S., du Bois, A., & Harter, P. (2018). Low-grade Serous Ovarian Carcinoma. *Geburtshilfe Und Frauenheilkunde*, *78*(10), 972–976. https://doi.org/10.1055/a-0717-5411

Rivera, E., & Gomez, H. (2010). Chemotherapy resistance in metastatic breast cancer: the evolving role of ixabepilone. *Breast Cancer Research : BCR*, *12 Suppl 2*(Suppl 2), S2. https://doi.org/10.1186/bcr2573

Rodionov, V. I., & Borisy, G. G. (1997). Microtubule Treadmilling in Vivo. *Science*, *275*(5297), 215–218. https://doi.org/10.1126/SCIENCE.275.5297.215

Rodriguez-Antona, C., & Ingelman-Sundberg, M. (2006). Cytochrome P450 pharmacogenetics and cancer. *Oncogene*, *25*(11), 1679–1691. https://doi.org/10.1038/sj.onc.1209377

Rogakou, E. P., Pilch, D. R., Orr, A. H., Ivanova, V. S., & Bonner, W. M. (1998). DNA double-stranded breaks induce histone H2AX phosphorylation on serine 139. *The Journal of Biological Chemistry*, *273*(10), 5858–5868. http://www.ncbi.nlm.nih.gov/pubmed/9488723

Roque, D. M., Ratner, E. S., Silasi, D.-A., Azodi, M., Rutherford, T. J., Schwartz, P. E., Nelson, W. K., & Santin, A. D. (2015). Weekly ixabepilone with or without biweekly bevacizumab in the treatment of recurrent or persistent uterine and ovarian/primary peritoneal/fallopian tube cancers: A retrospective review. *Gynecologic Oncology*, *137*(3), 392–400.

https://doi.org/10.1016/j.ygyno.2015.03.008

Rosano, L., Cianfrocca, R., Spinella, F., Di Castro, V., Nicotra, M. R., Lucidi, A., Ferrandina, G., Natali, P. G., & Bagnato, A. (2011). Acquisition of Chemoresistance and EMT Phenotype Is Linked with Activation of the Endothelin A Receptor Pathway in Ovarian Carcinoma Cells. *Clinical Cancer Research*, *17*(8), 2350–2360. https://doi.org/10.1158/1078-0432.CCR-10-2325

Rosano, L., Cianfrocca, R., Tocci, P., Spinella, F., Di Castro, V., Caprara, V., Semprucci, E., Ferrandina, G., Natali, P. G., & Bagnato, A. (2014). Endothelin A Receptor/ -Arrestin Signaling to the Wnt Pathway Renders Ovarian Cancer Cells Resistant to Chemotherapy. *Cancer Research*, *74*(24), 7453–7464. https://doi.org/10.1158/0008-5472.CAN-13-3133

Rossi, L., Verrico, M., Zaccarelli, E., Papa, A., Colonna, M., Strudel, M., Vici, P., Bianco, V., & Tomao, F. (2017). Bevacizumab in ovarian cancer: A critical review of phase III studies. *Oncotarget*, *8*(7), 12389. https://doi.org/10.18632/ONCOTARGET.13310

Rottenberg, S., Jaspers, J. E., Kersbergen, A., van der Burg, E., Nygren, A. O. H., Zander, S. A. L., Derksen, P. W. B., de Bruin, M., Zevenhoven, J., Lau, A., Boulter, R., Cranston, A., O'Connor, M. J., Martin, N. M. B., Borst, P., & Jonkers, J. (2008). High sensitivity of BRCA1-deficient mammary tumors to the PARP inhibitor AZD2281 alone and in combination with platinum drugs. *Proceedings of the National Academy of Sciences*, *105*(44), 17079–17084.

https://doi.org/10.1073/pnas.0806092105

Rubin, S. C., Randall, T. C., Armstrong, K. A., Chi, D. S., & Hoskins, W. J. (1999). Ten-year follow-up of ovarian cancer patients after second-look laparotomy with negative findings. *Obstetrics and Gynecology*, *93*(1), 21–24. http://www.ncbi.nlm.nih.gov/pubmed/9916949

Ruggero, D. (2012). Revisiting the nucleolus: from marker to dynamic integrator of cancer signaling. *Science Signaling*, *5*(241), pe38. https://doi.org/10.1126/scisignal.2003477

Russell, J., & Zomerdijk, J. C. B. M. (2005). RNA-polymerase-I-directed rDNA transcription, life and works. *Trends in Biochemical Sciences*, *30*(2), 87–96. https://doi.org/10.1016/j.tibs.2004.12.008

Russell, J., & Zomerdijk, J. C. B. M. (2006). The RNA polymerase I transcription machinery. *Biochemical Society Symposium*, *73*, 203–216. https://doi.org/10.1007/978-1-4614-0514-6

Sakamoto, T., Kobayashi, S., Yamada, D., Nagano, H., Tomokuni, A., Tomimaru, Y., Noda, T., Gotoh, K., Asaoka, T., Wada, H., Kawamoto, K., Marubashi, S., Eguchi, H., Doki, Y., & Mori, M. (2016). A Histone Deacetylase Inhibitor Suppresses Epithelial-Mesenchymal Transition and Attenuates Chemoresistance in Biliary Tract Cancer. *PLOS ONE*, *11*(1), e0145985. https://doi.org/10.1371/journal.pone.0145985

Salomon-Perzyński, A., Salomon-Perzyńska, M., Michalski, B., & Skrzypulec-

Plinta, V. (2017). High-grade serous ovarian cancer: the clone wars. *Archives of Gynecology and Obstetrics*, *295*(3), 569–576. https://doi.org/10.1007/s00404-017-4292-1

Sanchez, C. G., Teixeira, F. K., Czech, B., Preall, J. B., Zamparini, A. L., Seifert, J. R. K., Malone, C. D., Hannon, G. J., & Lehmann, R. (2016). Regulation of Ribosome Biogenesis and Protein Synthesis Controls Germline Stem Cell Differentiation. *Cell Stem Cell*, *18*(2). https://doi.org/10.1016/j.stem.2015.11.004

Sanij, E., Diesch, J., Lesmana, A., Poortinga, G., Hein, N., Lidgerwood, G., Cameron, D. P., Ellul, J., Goodall, G. J., Wong, L. H., Dhillon, A. S., Hamdane, N., Rothblum, L. I., Pearson, R. B., Haviv, I., & Hannan, T. M. R. D. (2015). A novel role for the pol I transcription factor ubtf in maintaining genome stability through the regulation of highly transcribed pol II genes. *Genome Research*, *25*(2), 201–212. https://doi.org/10.1101/gr.176115.114

Sanij, E., Hannan, K. M., Xuan, J., Yan, S., Ahern, J. E., Trigos, A. S., Brajanovski, N., Son, J., Chan, K. T., Kondrashova, O., Lieschke, E., Wakefield, M. J., Frank, D., Ellis, S., Cullinane, C., Kang, J., Poortinga, G., Nag, P., Deans, A. J., … Pearson, R. B. (2020). CX-5461 activates the DNA damage response and demonstrates therapeutic efficacy in high-grade serous ovarian cancer. *Nature Communications*, *11*(1), 2641. https://doi.org/10.1038/s41467-020-16393-4

Santoro, R., Li, J., & Grummt, I. (2002). The nucleolar remodeling complex NoRC

mediates heterochromatin formation and silencing of ribosomal gene transcription. *Nature Genetics*, *32*(3), 393–396. https://doi.org/10.1038/ng1010

Sanz, G., Leray, I., Dewaele, A., Sobilo, J., Lerondel, S., Bouet, S., Grébert, D., Monnerie, R., Pajot-Augy, E., & Mir, L. M. (2014). Promotion of cancer cell invasiveness and metastasis emergence caused by olfactory receptor stimulation. *PLoS ONE*, *9*(1). https://doi.org/10.1371/journal.pone.0085110

Sarge, K. D., & Park-Sarge, O.-K. (2009). Mitotic bookmarking of formerly active genes: keeping epigenetic memories from fading. *Cell Cycle (Georgetown, Tex.)*, *8*(6), 818. https://doi.org/10.4161/CC.8.6.7849

Scala, F., Brighenti, E., Govoni, M., Imbrogno, E., Fornari, F., Treré, D., Montanaro, L., & Derenzini, M. (2016). Direct relationship between the level of p53 stabilization induced by rRNA synthesis-inhibiting drugs and the cell ribosome biogenesis rate. *Oncogene*, *35*(April), 1–13. https://doi.org/10.1038/onc.2015.147

Schinkel. (1999). P-Glycoprotein, a gatekeeper in the blood-brain barrier. *Advanced Drug Delivery Reviews*, *36*(2–3), 179–194. http://www.ncbi.nlm.nih.gov/pubmed/10837715

Schneider, D. A. (2012). RNA polymerase I activity is regulated at multiple steps in the transcription cycle: Recent insights into factors that influence transcription elongation. *Gene*, *493*(2), 176–184. https://doi.org/10.1016/j.gene.2011.08.006

Schultz, K. A. P., Harris, A. K., Schneider, D. T., Young, R. H., Brown, J., Gershenson, D. M., Dehner, L. P., Hill, D. A., Messinger, Y. H., & Frazier, A. L. (2016). Ovarian Sex Cord-Stromal Tumors. *Journal of Oncology Practice*, *12*(10), 940. https://doi.org/10.1200/JOP.2016.016261

Seiden, M. V, Swenerton, K. D., Matulonis, U., Campos, S., Rose, P., Batist, G., Ette, E., Garg, V., Fuller, A., Harding, M. W., & Charpentier, D. (2002). A phase II study of the MDR inhibitor biricodar (INCEL, VX-710) and paclitaxel in women with advanced ovarian cancer refractory to paclitaxel therapy. *Gynecologic Oncology*, *86*(3), 302–310. http://www.ncbi.nlm.nih.gov/pubmed/12217752

Selvakumaran, M., Pisarcik, D. A., Bao, R., Yeung, A. T., & Hamilton, T. C. (2003). Enhanced cisplatin cytotoxicity by disturbing the nucleotide excision repair pathway in ovarian cancer cell lines. *Cancer Research*, *63*(6), 1311–1316.

Shah, P. P., Dupre, T. V., Siskind, L. J., & Beverly, L. J. (2017). Common cytotoxic chemotherapeutics induce epithelial-mesenchymal transition (EMT) downstream of ER stress. *Oncotarget*, *8*(14), 22625–22639. https://doi.org/10.18632/oncotarget.15150

Shav-Tal, Y. (2005). Dynamic Sorting of Nuclear Components into Distinct Nucleolar Caps during Transcriptional Inhibition. *Molecular Biology of the Cell*, *16*(5), 2395–2413. https://doi.org/10.1091/mbc.E04-11-0992

Shaw, P. J., & McKeown, P. C. (2011). The structure of rDNA chromatin. *Protein*

*Reviews*, *15*, 43–55. https://doi.org/10.1007/978-1-4614-0514-6-3

Shiue, C.-N., Arabi, A., & Wright, A. P. H. (2010). Nucleolar organization, growth control and cancer. *Epigenetics*, *5*(3), 200–205. http://www.ncbi.nlm.nih.gov/pubmed/20305389

Shukla, S., Ohnuma, S., & Ambudkar, S. V. (2011). Improving cancer chemotherapy with modulators of ABC drug transporters. *Current Drug Targets*, *12*(5), 621–630. http://www.ncbi.nlm.nih.gov/pubmed/21039338

Siegel, R. L., Miller, K. D., & Jemal, A. (2020). Cancer statistics, 2020. *CA: A Cancer Journal for Clinicians*, *70*(1), 7–30. https://doi.org/10.3322/caac.21590

Sirri, V., Urcuqui-Inchima, S., Roussel, P., & Hernandez-Verdun, D. (2008). Nucleolus: the fascinating nuclear body. *Histochemistry and Cell Biology*, *129*(1), 13–31. https://doi.org/10.1007/s00418-007-0359-6

Skilling, J. S., Squatrito, R. C., Connor, J. P., Niemann, T., & Buller, R. E. (1996). p53 Gene Mutation Analysis and Antisense-Mediated Growth Inhibition of Human Ovarian Carcinoma Cell Lines. *Gynecologic Oncology*, *60*(1), 72–80. https://doi.org/10.1006/gyno.1996.0014

Smith, J., Tho, L. M., Xu, N., & Gillespie, D. A. (2010). The ATM-Chk2 and ATR-Chk1 pathways in DNA damage signaling and cancer. *Advances in Cancer Research*, *108*, 73–112. https://doi.org/10.1016/B978-0-12-380888-2.00003-0

Son, J., Hannan, K. M., Poortinga, G., Hein, N., Cameron, D. P., Ganley, A. R. D., Sheppard, K. E., Pearson, R. B., Hannan, R. D., & Sanij, E. (2020). rDNA Chromatin Activity Status as a Biomarker of Sensitivity to the RNA Polymerase I Transcription Inhibitor CX-5461. *Frontiers in Cell and Developmental Biology*, *8*, 568. https://doi.org/10.3389/fcell.2020.00568

Song, X., Ma, F., & Herrup, K. (2019). Accumulation of Cytoplasmic DNA Due to ATM Deficiency Activates the Microglial Viral Response System with Neurotoxic Consequences. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *39*(32), 6378–6394. https://doi.org/10.1523/JNEUROSCI.0774-19.2019

Srivastava, R., Srivastava, R., & Ahn, S. H. (2016). The Epigenetic Pathways to Ribosomal DNA Silencing. *Microbiology and Molecular Biology Reviews*, *80*(3), 545–563. https://doi.org/10.1128/MMBR.00005-16

Stark, R., Grzelak, M., & Hadfield, J. (2019). RNA sequencing: the teenage years. *Nature Reviews Genetics*, *20*(11), 631–656. https://doi.org/10.1038/s41576-019-0150-2

Steg, A., Wang, W., Blanquicett, C., Grunda, J. M., Eltoum, I. A., Wang, K., Buchsbaum, D. J., Vickers, S. M., Russo, S., Diasio, R. B., Frost, A. R., LoBuglio, A. F., Grizzle, W. E., & Johnson, M. R. (2006). Multiple gene expression analyses in paraffin-embedded tissues by TaqMan low-density array: Application to hedgehog and Wnt pathway analysis in ovarian endometrioid adenocarcinoma. *The Journal of Molecular Diagnostics : JMD*,

*8*(1), 76–83. https://doi.org/10.2353/jmoldx.2006.040402

Szakács, G., Paterson, J. K., Ludwig, J. A., Booth-Genthe, C., & Gottesman, M. M. (2006). Targeting multidrug resistance in cancer. *Nature Reviews Drug Discovery*, *5*(3), 219–234. https://doi.org/10.1038/nrd1984

Takekuma, M., Wong, K. K., & Coleman, R. L. (2016). A long-term surviving patient with recurrent low-grade serous ovarian carcinoma treated with the MEK1/2 inhibitor, selumetinib. *Gynecologic Oncology Research and Practice*, *3*(1), 5. https://doi.org/10.1186/s40661-016-0026-5

Tang, H., Liu, Y., Wang, X., Guan, L., Chen, W., Jiang, H., & Lu, Y. (2018). Clear cell carcinoma of the ovary. *Medicine*, *97*(21), e10881. https://doi.org/10.1097/MD.0000000000010881

Tanner, M. M., Grenman, S., Koul, A., Johannsson, O., Meltzer, P., Pejovic, T., Borg, Å., & Isola, J. J. (2000). Frequent Amplification of Chromosomal Region 20q12-q13 in Ovarian Cancer. *Clin. Cancer Res.*, *4*(12), 2925–2929. https://clincancerres.aacrjournals.org/content/6/5/1833

Tapia, G., & Diaz-padilla, I. (2013). Molecular Mechanisms of Platinum Resistance in Ovarian Cancer. *Ovarian Cancer: A Clinical and Translational Update*, 371. https://doi.org/DOI: 10.5772/55562

Thiery, J. P., Acloque, H., Huang, R. Y. J., & Nieto, M. A. (2009). Epithelial-Mesenchymal Transitions in Development and Disease. *Cell*, *139*(5), 871–890. https://doi.org/10.1016/j.cell.2009.11.007

Thomson, E., Ferreira-Cerca, S., & Hurt, E. (2013). Eukaryotic ribosome biogenesis at a glance. *Journal of Cell Science*, *126*(Pt 21), 4815–4821. https://doi.org/10.1242/jcs.111948

Tomao, F., Papa, A., Rossi, L., Caruso, D., Panici, P. B., Venezia, M., & Tomao, S. (2013). Current status of bevacizumab in advanced ovarian cancer. *OncoTargets and Therapy*, *6*, 889–899. https://doi.org/10.2147/OTT.S46301

Tomkinson, H., Kemp, J., Oliver, S., Swaisland, H., Taboada, M., & Morris, T. (2011). Pharmacokinetics and tolerability of zibotentan (ZD4054) in subjects with hepatic or renal impairment: two open-label comparative studies. *BMC Clinical Pharmacology*, *11*(1), 3. https://doi.org/10.1186/1472-6904-11-3

Turinetto, V., & Giachino, C. (2015). Multiple facets of histone variant H2AX: a DNA double-strand-break marker with several biological functions. *Nucleic Acids Research*, *43*(5), 2489–2498. https://doi.org/10.1093/nar/gkv061

Vaidyanathan, A., Sawers, L., Gannon, A.-L., Chakravarty, P., Scott, A. L., Bray, S. E., Ferguson, M. J., & Smith, G. (2016). ABCB1 (MDR1) induction defines a common resistance mechanism in paclitaxel- and olaparib-resistant ovarian cancer cells. *British Journal of Cancer*, *115*(4), 431–441. https://doi.org/10.1038/bjc.2016.203

van Sluis, M., & McStay, B. (2015). A localized nucleolar DNA damage response facilitates recruitment of the homology-directed repair machinery independent of cell cycle stage. *Genes and Development*, *29*(11), 1151–1163. https://doi.org/10.1101/gad.260703.115

van Sluis, M., & McStay, B. (2017). Nucleolar reorganization in response to rDNA damage. *Current Opinion in Cell Biology*, *46*, 81–86. https://doi.org/10.1016/j.ceb.2017.03.004

Vanpouille-Box, C., Demaria, S., Formenti, S. C., & Galluzzi, L. (2018). Cytosolic DNA Sensing in Organismal Tumor Control. *Cancer Cell*, *34*(3), 361–378. https://doi.org/10.1016/J.CCELL.2018.05.013

Venezia, T. A., Merchant, A. A., Ramos, C. A., Whitehouse, N. L., Young, A. S., Shaw, C. A., & Goodell, M. A. (2004). Molecular signatures of proliferation and quiescence in hematopoietic stem cells. *PLoS Biology*, *2*(10), e301. https://doi.org/10.1371/journal.pbio.0020301

Vishnu, P., Colon-Otero, G., Kennedy, G. T., Marlow, L. A., Kennedy, W. P., Wu, K. J., Santoso, J. T., & Copland, J. A. (2012). RhoB mediates antitumor synergy of combined ixabepilone and sunitinib in human ovarian serous cancer. *Gynecologic Oncology*, *124*(3), 589–597. https://doi.org/10.1016/j.ygyno.2011.11.019

Vlatković, N., Boyd, M. T., & Rubbi, C. P. (2014). Nucleolar control of p53: A cellular achilles' heel and a target for cancer therapy. *Cellular and Molecular Life Sciences*, *71*(5), 771–791. https://doi.org/10.1007/s00018-013-1361-x

Wandel, C., Kim, R. B., Kajiji, S., Guengerich, P., Wilkinson, G. R., & Wood, A. J. (1999). P-glycoprotein and cytochrome P-450 3A inhibition: dissociation of inhibitory potencies. *Cancer Research*, *59*(16), 3944–3948. http://www.ncbi.nlm.nih.gov/pubmed/10463589

Wang, B., Li, S., Meng, X., Shang, H., & Guan, Y. (2015). Inhibition of mdr1 by G-quadruplex oligonucleotides and reversal of paclitaxel resistance in human ovarian cancer cells. *Tumor Biology*, *36*(8), 6433–6443. https://doi.org/10.1007/s13277-015-3333-2

Wang, D., & Lippard, S. J. (2005). Cellular processing of platinum anticancer drugs. *Nature Reviews Drug Discovery*, *4*(4), 307–320. https://doi.org/10.1038/nrd1691

Wang, Y., Wang, Y., & Zheng, W. (2013). Cytologic changes of ovarian epithelial cancer induced by neoadjuvant chemotherapy. *International Journal of Clinical and Experimental Pathology*, *6*(10), 2121–2128. http://www.ncbi.nlm.nih.gov/pubmed/24133590

Wang, Z., Gerstein, M., & Snyder, M. (2009). RNA-Seq: a revolutionary tool for transcriptomics. *Nature Reviews Genetics*, *10*(1), 57–63. https://doi.org/10.1038/nrg2484

Weidner, L. D., Fung, K. L., Kannan, P., Moen, J. K., Kumar, J. S., Mulder, J., Innis, R. B., Gottesman, M. M., & Hall, M. D. (2016). Tariquidar Is an Inhibitor and Not a Substrate of Human and Mouse P-glycoprotein. *Drug Metabolism and Disposition*, *44*(2), 275–282. https://doi.org/10.1124/dmd.115.067785

Weiss, L., Huemer, F., Mlineritsch, B., & Greil, R. (2016). Immune checkpoint blockade in ovarian cancer. *Memo*, *9*, 82–84. https://doi.org/10.1007/s12254-016-0267-3

Wells, N. J., Watanabe, N., Tokusumi, T., Jiang, W., Verdecia, M. A., & Hunter, T. (1999). The C-terminal domain of the Cdc2 inhibitory kinase Myt1 interacts with Cdc2 complexes and is required for inhibition of G(2)/M progression. *Journal of Cell Science*, 3361–3371. http://www.ncbi.nlm.nih.gov/pubmed/10504341

White, E. A., Kenny, H. A., & Lengyel, E. (2014). Three-dimensional modeling of ovarian cancer. *Advanced Drug Delivery Reviews*, *79*, 184–192. https://doi.org/10.1016/j.addr.2014.07.003

White, R. J. (2008). RNA polymerases I and III, non-coding RNAs and cancer. *Trends in Genetics : TIG*, *24*(12), 622–629. https://doi.org/10.1016/j.tig.2008.10.003

Williamson, D., Lu, Y.-J., Fang, C., Pritchard-Jones, K., & Shipley, J. (2006). Nascent pre-rRNA overexpression correlates with an adverse prognosis in alveolar rhabdomyosarcoma. *Genes, Chromosomes & Cancer*, *45*(9), 839–845. https://doi.org/10.1002/gcc.20347

Wong, C.-N., Wong, C.-N., & Liu, F.-S. (2017). Continuous oral cyclophosphamide as salvage or maintenance therapy in ovarian, primary peritoneal, and fallopian tube cancers: A retrospective, single institute study. *Taiwanese Journal of Obstetrics and Gynecology*, *56*(3), 302–305. https://doi.org/10.1016/J.TJOG.2017.04.006

Woods, S. J., Hannan, K. M., Pearson, R. B., & Hannan, R. D. (2015). The nucleolus as a fundamental regulator of the p53 response and a new target

for cancer therapy. *Biochimica et Biophysica Acta*, *1849*(7), 821–829. https://doi.org/10.1016/j.bbagrm.2014.10.007

Woolnough, J. L., Atwood, B. L., Liu, Z., Zhao, R., & Giles, K. E. (2016). The regulation of rRNA gene transcription during directed differentiation of human embryonic stem cells. *PLoS ONE*, *11*(6), e0157276. https://doi.org/10.1371/journal.pone.0157276

Wu, D., Li, X., Meng, X.-N., Yan, J., & Zong, Z. (2016). MicroRNA-873 mediates multidrug resistance in ovarian cancer cells by targeting ABCB1. *Tumor Biology*, *37*(8), 10499–10506. https://doi.org/10.1007/s13277-016-4944-y

Xia, Y., & Lee, K. (2010). Targeting multidrug resistance with small molecules for cancer therapy. In *Biomolecules and Therapeutics*. https://doi.org/10.4062/biomolther.2010.18.4.375

Xiong, Q., Mukherjee, S., & Furey, T. S. (2015). GSAASeqSP: A Toolset for Gene Set Association Analysis of RNA-Seq Data. *Scientific Reports*, *4*(1), 6347. https://doi.org/10.1038/srep06347

Xu, H., Antonio, M. Di, Mckinney, S., Mathew, V., Neil, N. J. O., Santos, N. Dos, Wei, V., Garcia, J., Yap, D., Le, D., Banáth, J., Ye, F. B., Zhang, A., Thu, K., Soong, J., Lin, S., Osako, T., Algara, T., Saunders, D., … Hieter, P. (n.d.). *CX-5461 and CX-3543 are G-quadruplex stabilizers with selective synthetic lethality in BRCA1/2 deficient tumours.*

Xu, H., Di Antonio, M., McKinney, S., Mathew, V., Ho, B., O'Neil, N. J., Santos,

N. Dos, Silvester, J., Wei, V., Garcia, J., Kabeer, F., Lai, D., Soriano, P., Banáth, J., Chiu, D. S., Yap, D., Le, D. D., Ye, F. B., Zhang, A., … Aparicio, S. (2017). CX-5461 is a DNA G-quadruplex stabilizer with selective lethality in BRCA1/2 deficient tumours. *Nature Communications*, *8*(1), 14432. https://doi.org/10.1038/ncomms14432

Xu, Q., Zou, Y., & Zhang, X. F. (2018). Sertoli–Leydig cell tumors of ovary. *Medicine*, *97*(42), e12865. https://doi.org/10.1097/MD.0000000000012865

Xue, S., & Barna, M. (2012). Specialized ribosomes: a new frontier in gene regulation and organismal biology. *Nature Reviews Molecular Cell Biology*, *13*(6), 355–369. https://doi.org/10.1038/nrm3359

Yamada, H. Y., & Gorbsky, G. J. (2006). Spindle checkpoint function and cellular sensitivity to antimitotic drugs. *Molecular Cancer Therapeutics*, *5*(12), 2963–2969. https://doi.org/10.1158/1535-7163.MCT-06-0319

Yamamoto, R., Fujita, M., Kuwabara, M., Sogame, M., Ebina, Y., Sakuragi, N., Kato, H., & Fujimoto, S. (1999). Malignant Brenner Tumors of the Ovary and Tumor Markers: Case Reports. *Japanese Journal of Clinical Oncology*, *29*(6), 308–313. https://doi.org/10.1093/jjco/29.6.308

Yan, S., Frank, D., Son, J., Hannan, K., Hannan, R., Chan, K., Pearson, R., & Sanij, E. (2017). The Potential of Targeting Ribosome Biogenesis in High-Grade Serous Ovarian Cancer. *International Journal of Molecular Sciences*, *18*(1), 210. https://doi.org/10.3390/ijms18010210

Yang, X., Iyer, A. K., Singh, A., Choy, E., Hornicek, F. J., Amiji, M. M., & Duan, Z. (2015). MDR1 siRNA loaded hyaluronic acid-based CD44 targeted nanoparticle systems circumvent paclitaxel resistance in ovarian cancer. *Scientific Reports*, *5*, 8509. https://doi.org/10.1038/srep08509

Yang, X., Iyer, A. K., Singh, A., Milane, L., Choy, E., Hornicek, F. J., Amiji, M. M., & Duan, Z. (2015). Cluster of Differentiation 44 Targeted Hyaluronic Acid Based Nanoparticles for MDR1 siRNA Delivery to Overcome Drug Resistance in Ovarian Cancer. *Pharmaceutical Research*, *32*(6), 2097–2109. https://doi.org/10.1007/s11095-014-1602-1

Yap, T. A., Carden, C. P., & Kaye, S. B. (2009). Beyond chemotherapy: targeted therapies in ovarian cancer. *Nature Reviews Cancer*, *9*(3), 167–181. https://doi.org/10.1038/nrc2583

Ye, Q., Pang, S., Zhang, W., Guo, X., Wang, J., Zhang, Y., Liu, Y., Wu, X., & Jiang, F. (2017). Therapeutic Targeting of RNA Polymerase I With the Small-Molecule CX-5461 for Prevention of Arterial Injury–Induced Neointimal Hyperplasia. *Arteriosclerosis, Thrombosis, and Vascular Biology*, ATVBAHA.116.308401. https://doi.org/10.1161/ATVBAHA.116.308401

Young, M.-J., Wu, Y.-H., Chiu, W.-T., Weng, T.-Y., Huang, Y.-F., & Chou, C.-Y. (2015). All-trans retinoic acid downregulates ALDH1-mediated stemness and inhibits tumour formation in ovarian cancer cells. *Carcinogenesis*, *36*(4), 498–507. https://doi.org/10.1093/carcin/bgv018

Young, R. H. (2005). Sex cord-stromal tumors of the ovary and testis: their

similarities and differences with consideration of selected problems. *Modern Pathology*, *18*(S2), S81–S98. https://doi.org/10.1038/modpathol.3800311

Young, R. H. (2018). Ovarian Sex Cord–Stromal Tumors: Reflections on a 40-Year Experience With a Fascinating Group of Tumors, Including Comments on the Seminal Observations of Robert E. Scully, MD. *Archives of Pathology & Laboratory Medicine*, *142*(12), 1459–1484. https://doi.org/10.5858/arpa.2018-0291-RA

Yu, D., Wolf, J. K., Scanlon, M., Price, J. E., & Hung, M. C. (1993). Enhanced c-erbB-2/neu expression in human ovarian cancer cells correlates with more severe malignancy that can be suppressed by E1A. *Cancer Research*, *53*(4), 891–898. http://www.ncbi.nlm.nih.gov/pubmed/8094034

Yu, F., Shen, X., Fan, L., & Yu, Z. (2015). Analysis of histone modifications at human ribosomal DNA in liver cancer cell. *Scientific Reports*, *5*. https://doi.org/10.1038/SREP18100

Yuan, J., Adamski, R., & Chen, J. (2010). Focus on histone variant H2AX: to be or not to be. *FEBS Letters*, *584*(17), 3717–3724. https://doi.org/10.1016/j.febslet.2010.05.021

Yvon, A.-M. C., Wadsworth, P., & Jordan, M. A. (1999). Taxol Suppresses Dynamics of Individual Microtubules in Living Human Tumor Cells. *Molecular Biology of the Cell*, *10*(4), 947. https://doi.org/10.1091/MBC.10.4.947

Zagouri, F., Sergentanis, T., Chrysikos, D., Bamias, A., & Dimopoulos, A.-M.

(2015). Epothilones in epithelial ovarian, fallopian tube, or primary peritoneal cancer: a systematic review. *OncoTargets and Therapy*, *8*, 2187. https://doi.org/10.2147/OTT.S77342

Zahreddine, H., & Borden, K. L. B. (2013). Mechanisms and insights into drug resistance in cancer. *Frontiers in Pharmacology*, *4*, 28. https://doi.org/10.3389/fphar.2013.00028

Zaidi, S. K., Young, D. W., Montecino, M. A., Lian, J. B., van Wijnen, A. J., Stein, J. L., & Stein, G. S. (2010). Mitotic bookmarking of genes: a novel dimension to epigenetic control. *Nature Reviews. Genetics*, *11*(8), 583–589. https://doi.org/10.1038/nrg2827

Zhang, X.-Y., & Zhang, P.-Y. (2016). Recent perspectives of epithelial ovarian carcinoma (Review). *Oncology Letters*, *12*(5), 3055–3058. https://doi.org/10.3892/ol.2016.5107

Zhang, Y., Sriraman, S. K., Kenny, H. A., Luther, E., Torchilin, V., & Lengyel, E. (2016). Reversal of Chemoresistance in Ovarian Cancer by Co-Delivery of a P-Glycoprotein Inhibitor and Paclitaxel in a Liposomal Platform. *Molecular Cancer Therapeutics*, *15*(10), 2282–2293. https://doi.org/10.1158/1535-7163.MCT-15-0986

Zhang, Yingao, Staley, S. A., Tucker, K., & Clark, L. H. (2019). Malignant Brenner tumor of the ovary: Case series and review of treatment strategies. *Gynecologic Oncology Reports*, *28*, 29–32. https://doi.org/10.1016/j.gore.2019.02.003

Zhu, X., & Lang, J. (2017). Programmed death-1 pathway blockade produces a synergistic antitumor effect: combined application in ovarian cancer. *Journal of Gynecologic Oncology*, *28*(5), e64. https://doi.org/10.3802/jgo.2017.28.e64