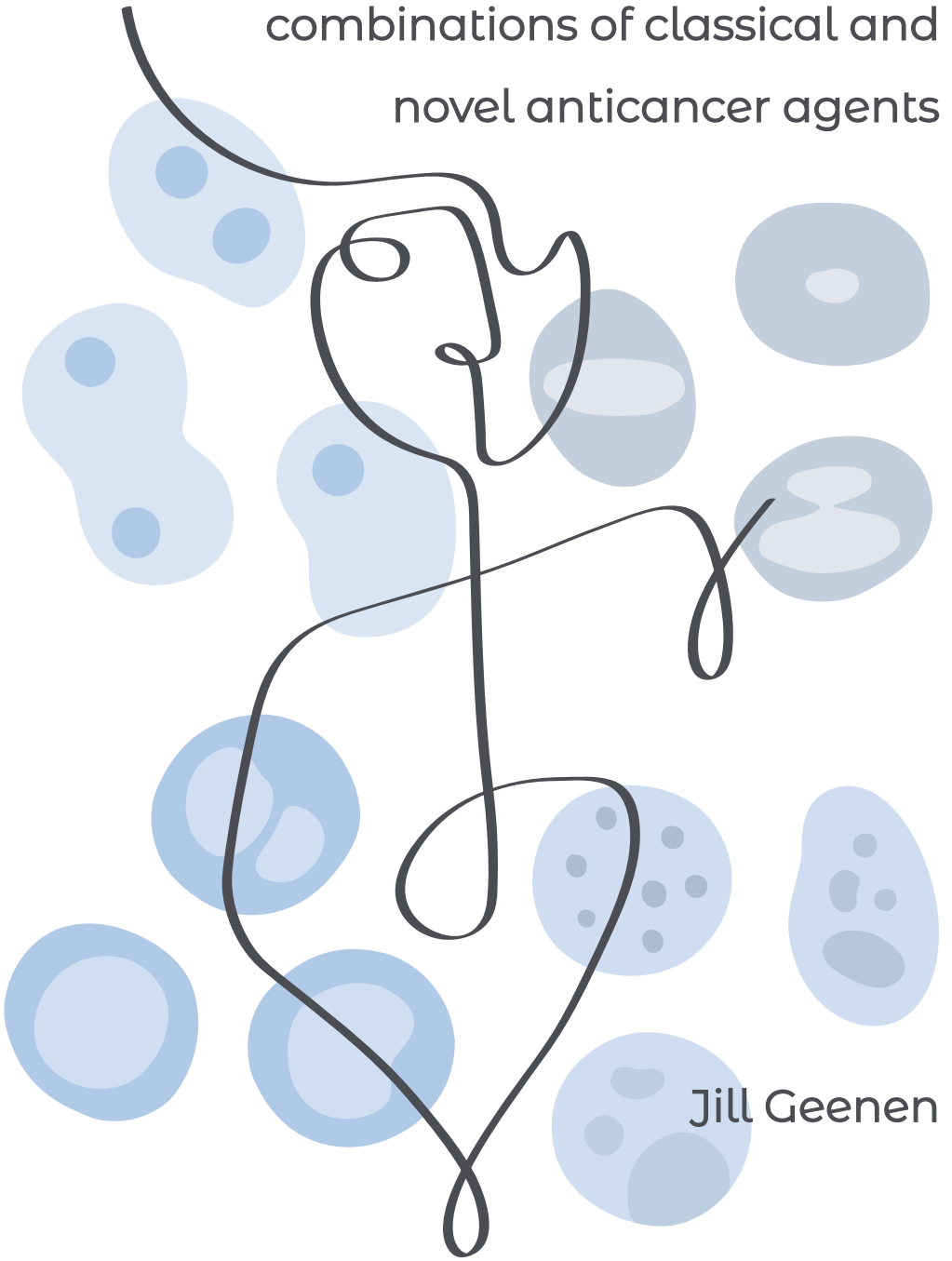
Exploiting drug targets for development of rational combinations of classical and novel anticancer agents



Jill Geenen

Muziek heeft bij het schrijven van dit proefschrift
een grote rol gespeeld. Mijn favoriete afspeellijst
is grijs gedraaid de afgelopen jaren.
Graag maak ik jullie ook hiervandeelgenoot.
Bij elk hoofdstuk vind je een van de nummers van deze
afspeellijst, met de Spotify app zijn ze te beluisteren.

Exploiting drug targets for development of rational combinations of classical and novel anticancer agents

Jill J.J. Geenen

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Exploiting drug targets for development of rational combinations of classical and novel anticancer agents

Gebruik van geneesmiddel targets voor ontwikkeling van rationele combinaties van klassieke en nieuwe antikanker middelen.
(met een samenvatting in het Nederlands)

PROEFSCHRIFT

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Jill Jacqueline Johanna Geenen

Geboren op 06 maart 1988
te Geleen

Promotoren: Prof. dr. J.H. Beijnen
Prof. dr. S.C. Linn

You treat a disease, you win, you lose.
You treat a person, I guarantee you, you'll win,
no matter what the outcome.

Robin Williams in Patch Adams

Voor Siem, Tess en jullie kleine broertje

Table of contents

Preface

Preface

Cancer is described as the rapid creation of abnormal cells that grow beyond their normal boundaries. It is the leading cause of death worldwide with an estimated 9.8 million deaths in 2018, what makes that about 1 in 6 deaths is due to cancer[1]. Breast cancer is the second most common cancer in women, it affects over 2 million people a year worldwide. In the Netherlands approximately 14000 women per year are diagnosed with invasive breast cancer each year[2]. Ovarian cancer accounts for an estimated 239000 new cases and 152000 deaths worldwide annually. The majority of the patients are diagnosed with advanced stage disease[3]. Treatment for cancer has been extensively changed the past decades. In the past, treatment was mostly based on the organ of tumor origin. The past years, treatment has been more and more based on molecular profiles and is therefore more specified and individualized. In addition to chemotherapy, radiotherapy, surgery and hormonal therapy, new treatment options for different types of cancer have found their way to clinical practice. New targets have been identified as possible focus for treatment. Also, more and more combination therapies are being explored, like new combinations of immunotherapy with chemotherapy and targeted therapy[4,5]. This thesis will describe some examples of different treatment strategies to improve the treatment of cancer patients.

In **Chapter 1** we focus on the new treatment strategy with PARP-inhibitors. **Chapter 1.1** gives an overview of the literature about the molecular features of TNBC and the feasibility of treatment with PARP-inhibitors. In this chapter we focus on the pharmacotherapeutic options for this patient group. **In Chapter 1.2** results of a phase I study of the combination of the PARP-inhibitor olaparib with carboplatin in advanced cancer are shown. In this study the maximum tolerable dose (MTD) and recommended phase II dose (RP2D) of the combination of both was established by a dose-escalation study in a 3+3 design. In **Chapter 1.3,** the evidence found in literature regarding administration of several PARP-inhibitors to patients with brain metastases is set out.
Chapter 2 describes treatment of cancer patients with a Wee1 inhibitor, a small molecule inhibitor. **Chapter 2.1** gives an overview of the preclinical and clinical features of Wee1 inhibition in cancer.

In **Chapter 2.2** the results of an interim analysis of an additional safety cohort of a large phase III trial with the combination of AZD1775 and carboplatin are shown. In this trial, patients with advanced epithelial ovarian cancer were treated with this combination in a three-weekly schedule. In **Chapter 2.3** results of a phase II study with the combination of the Wee1 inhibitor adavosertib in combination with chemotherapy in patients with ovarian, fallopian tube or peritoneal cancer are presented.
Overexpression of PD-L1 is found in many tumors and could therefore be a target for therapy. Several conventional chemotherapies like paclitaxel, carboplatin and cyclophosphamide may have immunogenic effects. This has led to the design of the phase Ib study described in **Chapter 3.** In this dose-finding study we combined cyclophosphamide, carboplatin and atezolizumab, a humanized monoclonal antibody that targets human PD-L1 and inhibits its interactions with its receptors. This combination was administered to patients with TNBC, ovarian, cervical or endometrial cancer.
Targeted therapy are drugs or other substances that block the growth and spread of cancer by interfering with specific molecules that are involved in spread, growth and progression of cancer. Antibody-drug conjugates (ADCs) are a novel class of anticancer agents that combines the cytotoxic potential of chemotherapeutic drugs with the selectivity of targeted therapy. **Chapter 4** shows the results of a phase I expansion cohort study of the ADC SYD985 in heavily pretreated patients with HER2-positive metastatic breast cancer. In this study patients with both high and low HER2 expression were treated with trastuzumab-duocarbamazine.
Finally, a summary of the conclusions of the combined results of this research will be described in **Chapter 5** and future perspectives and challenges will be discussed.

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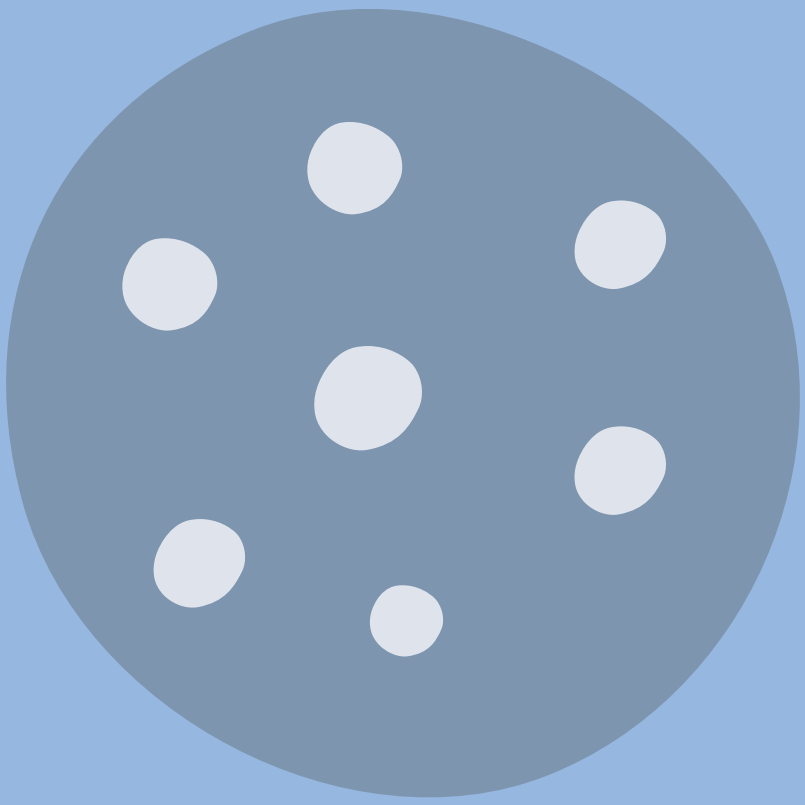
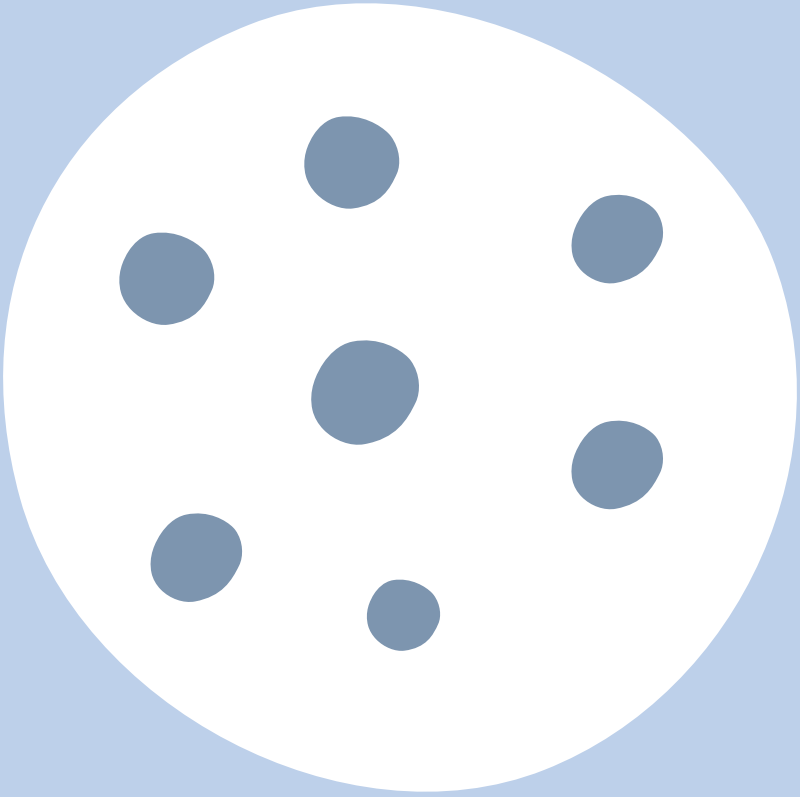


Chapter 1

PARP-inhibition



Chapter 1.1

PARP-inhibitors in the treatment of triple negative breast cancer

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Summary

Breast cancer is a heterogeneous disease, manifesting in a broad differentiation in phenotypes and morphologic profiles. This results in variable clinical behavior. Between 10-20% of all breast cancers are triple negative. Triple negative breast cancer (TNBC) lacks the expression of Human Epidermal growth factor Receptor 2 (HER2) and hormone receptors, therefore up to now chemotherapy remains the backbone of the treatment. TNBC tends to be aggressive and has a high histological grade, resulting in a poor 5-year prognosis. Triple negative breast cancer has a high prevalence of *BRCA1* mutations and an increased Ki-67 expression. This subtype usually responds well to taxanes, and/or platinum compounds and Poly (ADP-ribose) polymerase (PARP)-inhibitors. Studies with PARP-inhibitors have demonstrated promising results in the treatment of *BRCA* mutated breast- and ovarian cancer. PARP-inhibitors have been studied as monotherapy and in combination with cytotoxic therapy or radiotherapy. PARP-inhibitor efficacy on PAR formation *in vivo* can be quantified by pharmacodynamic assays that measure PAR activity in peripheral blood mononuclear cells (PBMC). Biomarkers such as *TP53*, *ATM*, *PALB2* and *RAD51C* might be prognostic or predictive indicators for treatment response. These markers could also provide targets for novel treatment strategies. In summary, this review provides an overview of the treatment options for basal-like TNBC including PARP-inhibitors, and focuses on the pharmacotherapeutic options in these patients.

Introduction

Breast cancer is the most common cancer in women worldwide[1]. Unfortunately worldwide the incidence of breast cancer is still rising. The past few decades enormous progress has been made in the understanding of the molecular pathways involving breast cancer leading to development of more personalized therapies. Despite this, the 5-years survival of metastatic breast cancer remains low[2]. Breast cancer is a very heterogeneous disease, manifesting in a broad differentiation in phenotypes and morphologic profiles, resulting in different clinical behaviors[3]. Based on their immunohistochemical features, breast cancers can be divided into three main types: hormone receptor (HR) positive, Human Epidermal Growth Factor Receptor 2 (HER2) positive and triple negative (TN) tumors[4]. Between 10 and 20% of breast cancers are triple negative. Triple negative breast cancers (TNBC) are characterized by the absence of estrogen (ER) and progresterone (PR) receptor and HER2 expression[5]. TNBC tends to be aggressive, occurs at younger age and has a higher grade. TNBC has a high recurrence rate and a poor 5-year prognosis compared to other types of breast cancer[6]. TNBC cannot be treated with targeted therapy like endocrine therapy or trastuzumab because they lack their cellular targets[7]. Treatment of TNBC remains therefore challenging. In this review we describe the molecular features of TNBC, the relationship with *BRCA* mutation status and the treatment with a novel class of anticancer agents, the Poly (ADP-ribose) polymerase (PARP)-inhibitors. We will also discuss patient selection, biomarkers and individualization of dosing schedules employing new pharmacodynamics assays.

Homologous recombination deficiency

If the process of homologous recombination is unavailable or impaired, this is referred to as 'homologous recombination deficiency' (HRD). In this situation, DNA repair is more error-prone which leads to genomic instability[8,9]. Bunting et al. showed that loss of *53BP1* restored HR activity in *BRCA1* mutant cells with HR deficiency[10]. In addition, Bouwman et al. showed, using a cell-based screen, that *53BP1* is essential for continuing the growth arrest induced by *BRCA1* mutation[11]. Other mechanisms of resistance to *BRCA*-targeted therapies could be through secondary mutations, which

could restore *BRCA1* and *BRCA2* function. These secondary somatic mutations predict resistance to platinum chemotherapy and PARP-inhibitors in women with *BRCA1/2* mutations[12,13]. Tumors with HRD can be sensitive to DNA cross-linking agents, such as alkylators and platinum drugs[14-16]. *BRCA1* and *BRCA2* play a pivotal role in the repair of double strand breaks (DSBs) in the DNA by the process of homologous recombination. Loss of either *BRCA1* or *BRCA2* function leads to HRD[17]. However, sensitivity to alkylators and other agents can be lost if *53BP1* is lost in addition to impaired *BRCA* function. At present it is unclear whether the presence of HRD is a requirement for tumor cells to be sensitive to alkylating agents, platinum compounds and other drugs. In addition, it is unclear which diagnostic test best enriches for tumors with HRD. Finally, it is unclear what is most needed in the clinic: a test that reliably measures HRD or a test that reliably measures sensitivity to a certain agent, or combination of agents.

BRCA mutations and other potentially HRD inducing mutations

Of the newly diagnosed TNBC patients, about 10% harbors a mutation in genes encoding for breast cancer susceptibility protein 1/2 (*BRCA 1/2*)[18-20]. A large part of the triple negative breast neoplasms has similar characteristics as tumors that harbor a germline *BRCA1* or *BRCA2* mutation. Of the breast cancer patients with a germline *BRCA1* mutation, more than 80% has a triple negative breast tumor[21]. Tumors that harbor a *BRCA* mutation are often highly sensitive to drugs that induce DNA double strand breaks like alkylating agents and less sensitive to spindle poisons[22]. Treating TNBC patients with platinum compounds is based on the fact that TNBC has molecular similarities to *BRCA* mutated breast cancers, which are sensitive to platinum compounds[23]. Besides sensitivity to platinum compounds, TN tumors and *BRCA1* mutant breast cancers have various concordances like a basal-like profile, frequent *TP53* mutations and a high load of genomic aberrations like loss of heterozygosity[24]. Byrski et al. showed in a phase II trial that cisplatin chemotherapy is highly active in women with a *BRCA1* germline mutation[25]. They showed that platinum-based chemotherapy is effective in a high proportion of patients with *BRCA1* associated cancer[26]. Tumors without germline *BRCA1* mutation that have absence or reduced *BRCA1* expression may be linked to hypermethylation of the *BRCA1* promotor

region[27]. About 9.1-37% of sporadic breast cancers have hypermethylation of *BRCA1*, a condition that is associated with high tumor grade, ER negativity, basal marker expression, younger age at diagnosis and reduction or loss in *BRCA1* mRNA expression[8,27-29]. In the absence of a *BRCA1* mutation, *BRCA1* promotor hypermethylation could be an indicator of an impaired *BRCA* function[30]. Two neoadjuvant clinical trials have shown that part of the sporadic, non-*BRCA1* mutated TNBC is sensitive to platinum compounds[24]. Heterogeneity of TNBC makes treatment challenging. Gathering more insight in the heterogeneity could lead to better and more focused therapy[31]. Rare inactivating mutations in several genes in the DSB repair pathway are associated with the development of cancer. These genes, like *RAD51c*, *ATM*, *PALB2* are involved in a small fraction of the disease. *RAD51c* works with *BRCA1* and *BRCA2* to repair DNA double strand breaks. The overall mutation frequency of *RAD51c* in familial breast cancer is low[32]. *PALB2* is a breast cancer susceptibility gene and interacts with *BRCA 2*. *ATM* interacts with *BRCA1*[33]. Depletion of ATM in breast cancer cells could sensitize these cells to PARP-inhibition. This suggests a treatment potential for breast cancers with low *ATM* protein expression[34].

Molecular features of TNBC

Recently six subtypes of the triple negative breast cancer have been identified: basal-like 1 (BL-1), basal-like 2 (BL-2), immunomodulatory (IM), mesenchymal (M), mesenchymal stem-like (MSL) and luminal androgen receptor (LAR positive)[6]. Understanding and insight in these molecular subtypes could contribute to better treatment strategies due to more individualized treatments. The Basal-like 1 subtype is characterized by an enriched cell cycle and DNA damage response gene expression[35]. The enrichment in proliferation genes and increased Ki-67 expression in basal-like TNBC could explain why this subtype responded well to antimitotic agents like taxanes[36,37], although the notion that high proliferative tumors respond better to spindle poisons contradicts with findings in the Oxford Overview where patients with well differentiated ER positive breast cancers appeared to benefit more from taxane-containing regimens than patients with moderately and poorly differentiated ER positive breast cancers in the adjuvant setting[38]. Either response rate does not match with long term survival, or spindle poisons do not only frustrate mitosis, but have another,

yet unknown mechanism of action as well. Two clinical trials show that patients who received taxanes as neoadjuvant therapy have a higher pCR rate (63%, p=0.042) when they compared the basal-like subtype to the mesenchymal-like type (31 %) or the LAR (14%) type[6]. Masuda et al. showed that BL1 tumors have the highest rate of pCR (52%) with taxane based neoadjuvant regimens, compared to other subtypes[39]. Basal-like breast cancers show high prevalence of *BRCA1* mutations[40]. Turner et al. found in their study that 63% of metaplastic breast cancers, a rare type of basal-like cancers, had *BRCA1* promoter methylation, compared to 12% in the control group (p<0.0001). This high incidence of *BRCA1* methylation might point to a new treatment strategy for patients with basal-like breast cancer[41]. Basal-like TNBC has similarities to *BRCA1* mutated tumors, such as the morphological features and the immunohistochemical profile like a similar pattern of cell cycle protein expression[42,43]. Therefore, another promising target for the treatment of basal-like 1 breast tumors are PARP-inhibitors. Sensitivity for particular agents is not restricted to *BRCA* mutated tumors. It is thought that about 30% of the sporadic breast cancers also have defects in homologous recombination repair, a phenotype that is referred to as 'BRCAness'. Several studies have shown that breast cancer that harbors a *BRCA1* or *BRCA2* mutation has a characteristic pattern of DNA gains and losses in an array comparative genomic hybridization (aCGH) assay[44-47]. Vollebergh et al. showed that a subgroup of HER2 negative tumors characterized by BRCA1-like aCGH (*BRCA* 1-like ^{CGH}) pattern had benefit from high dose platinum therapy. Patients with *BRCA1* loss (not *BRCA* mutated) can be found by aCGH and this could thereby identify patients who could have benefit from DNA DSB inducing chemotherapy[48]. Lips et al. found a *BRCA2*-like CGH (*BRCA* 2-like^{CGH}) pattern and found this to be present in some sporadic breast cancers[8]. The BRCA 2-like^{CGH} pattern was in contrast to the *BRCA1*-like^{CGH} pattern frequently observed in ER-positive tumors. In a follow-up study Vollebergh et al. explored besides ER-negative, also ER-positive breast cancer patients could be identified that could have benefit from DNA crosslinking agents. Fifty-one percent (41/81) of the *BRCA*-like^{CGH} tumors were ER-positive. They showed that patients with *BRCA2*-like^{CGH} tumors have more benefit from intensified DNA double strand break inducing agents when compared to standard chemotherapy, like patients with the *BRCA1*-like^{CGH} pattern[49]. The *BRCA*-like^{CGH} could be helpful in selecting patients that may have benefit from intensified DNA double strand break inducing agents in combination with autologous stem cell rescue.

PARP-inhibitors

PARP is a damage recognition repair protein of a single strand break and plays an important role in the initiation of repair of single strand breaks in the DNA by base excision repair. Inhibition of PARP results in accumulation of single strand breaks, which can lead to the formation of double strand breaks. Cells that are *BRCA* mutant are not able to repair double strand breaks error free, which ultimately can lead to cell death. When a deficiency in one gene does not lead to cell death, but a combination of two or more deficiencies do, like the combination of a PARP-inhibitor and a *BRCA* mutation, this is called synthetic lethality[50].

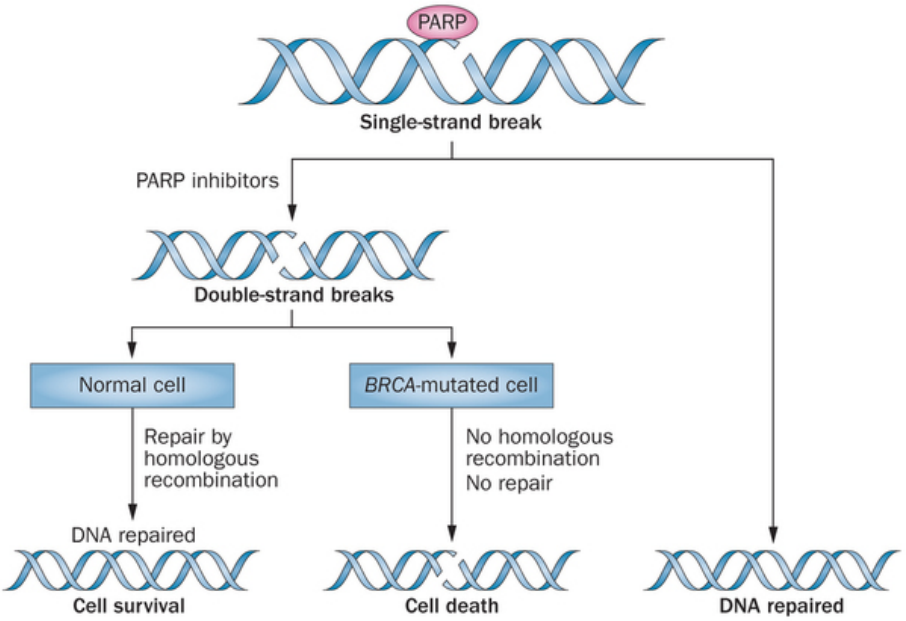

Figure from: Sonnenblick et al. An update on PARP-inhibitors- moving to the adjuvant setting. Nature reviews clinical oncology 12, 27-41 (2015).

Olaparib

Olaparib is a potent oral PARP-inhibitor that can be lethal to cells harboring a *BRCA1* or *BRCA2* mutation[51]. Fong et al. demonstrated in a phase I trial the pharmacokinetic and pharmacodynamic characteristics of the olaparib

capsule formulation. Pharmacokinetic parameters of twice daily dosing showed a fast absorption and elimination. The peak plasma concentration is reached at 1-3 hours after oral intake of olaparib. This is followed by a biphasic decline in plasma concentrations, with a terminal elimination half-life of 5-7 hours[50]. The main metabolism of olaparib occurs via dehydrogenation and oxidation, with a number of components that were further metabolized by the glucuronide or sulphate conjugation[52]. Ang et al. performed a mass balance study of olaparib and found that the excretion of the drug occurs mostly via faeces (42%) and urine (44%)[53]. CYP3A4 is the main metabolizing enzyme of olaparib. Coadministration of olaparib with strong or moderate CYP3A4 inducers or inhibitors is therefore not recommended. Fong et al. showed in this dose-escalation study that an increase in the olaparib dose led to a linear increase in exposure until dose levels of 100 mg olaparib. The exposure did not increase proportionally with the increase of olaparib at dose levels higher than 100 mg. The mean apparent volume of distribution is 40 L with a mean plasma clearance of 4.6 L/h. There were no severe toxicities reported, besides mild gastro-intestinal toxicities. Dirix et al. investigated the effect of the CYP3A4 inhibitor itraconazole and the CYP3A4 inducer rifampin on the pharmacokinetics of olaparib. They conducted two phase I studies in patients with advanced solid tumors. Patients received olaparib alone and in coadministration with itraconazol or rifampin. Co-administration of olaparib with itraconazole resulted in a statistically significant increase in the relative bioavailability of olaparib with a C_{max} treatment ratio of 1.42 (90% CI, 1.33-1.52) and a mean AUC treatment ratio, 2.70 (90% CI, 2.44-2.97). They found a reduction of the mean CL/F and V_z /F. Coadministration with rifampin resulted in a statistically significant reduction of the relative bioavailability of olaparib with a C_{max} treatment ratio, 0.29 (90% CI, 0.24-0.33) and a mean AUC treatment ratio, 0.13 (90% CI, 0.11-0.16). These results show that potent CYP3A4 induces and inhibitors should be avoided during treatment with olaparib[54]. Recently a pharmacokinetic and pharmacodynamic phase I/Ib study of olaparib tablets and carboplatin was published by Lee et al. They treated 77 patients with olaparib and carboplatin. Patients received either olaparib on days 1-7, carboplatin on day 8 or carboplatin on day 1 followed by olaparib on days 2-8. The clearance of olaparib was increased by approximately 50% when carboplatin was given 24 hours before olaparib. This increased clearance resulted in a 25% lower AUC_{last} ($p=0.046$) and a 28% shorter $T_{½}$. These results suggest administration

of carboplatin prior to olaparib[55]. Fong et al. performed a follow up study to explore the anti-tumor activity of olaparib in patients with ovarian, primary peritoneal, and fallopian tube cancer. In total, 50 patients were treated with olaparib in doses ranging from 40 mg daily to 600 mg twice daily, of whom 48 had a germline *BRCA1/2* mutation, in a dose-escalation scheme with olaparib monotherapy. Of the 50 patients, 24 had platinum-resistant disease and 13 had platinum-refractory disease. The overall benefit rate was 46%, with a median response duration of 28 weeks. Seventeen patients were treated for more than six months[56]. A proof of concept study by Audeh et al.[57] confirmed the clinical benefit of olaparib. They treated 55 patients with *BRCA1/2* recurrent ovarian cancer, primary peritoneal or fallopian tube carcinoma with 400 mg olaparib twice daily (n=33) or 100 mg olaparib twice daily (n=24). All patients had recurrence after a previous chemotherapy regimen. The objective response rate (ORR) was significantly better in the 400 mg twice daily cohort compared to the 100 mg twice daily cohort (33% vs. 12.5%). All patients except one, had at least one adverse event. Most common toxicities were comparable to those observed in previous studies, namely nausea and fatigue. Grade 3 or 4 adverse events were, not as expected, reported slightly more often in the 100 mg twice daily cohort compared to the 400 mg twice daily cohort (58% vs. 45%). However, fewer patients discontinued in the 100 mg twice daily cohort (4%) compared to the 400 mg twice daily cohort (12%). In terms of anti-tumor activity, the 100 mg twice daily dose seemed less effective compared to the 400 mg twice daily (BID) dose, however the patients in the lower dosing cohort appeared to have less favorable prognostic factors at start[57]. The clinical benefit of olaparib was also confirmed in a study of Tutt et al, who showed also a higher ORR in the *BRCA* mutated advanced breast cancer group that received 400 mg BID compared to the 100 mg cohort BID (41% vs. 22%). Both groups were comparable. All patients were pretreated with at least one chemotherapy regimen. Olaparib showed also activity in patients who were heavily pretreated[58]. As a result of these studies, the European Medicine Agency (EMA) approved olaparib monotherapy as treatment for advanced *BRCA* mutated ovarian cancer in 2014. After this, the Food and Drug Administration (FDA) also approved olaparib for this indication. Plummer et al. studied the effect of food on the pharmacokinetics of olaparib. They showed that the absorption of olaparib (once 300 mg) was lower in the presence of (high fat) food (Tmax delayed by 2.5h), resulting in a decreased plasma peak concentration (Cmax) of olaparib of 21%[59]. There was only a

slight increase in the olaparib exposure ($AUC_{0-\infty}$), from 43.0 µg h/mL in the fasted state to 45.4 µg h/mL in the fed state. Besides Olaparib there are several other PARP-inhibitors that have been studied. Recently the results of the phase III OlympiAD trial were presented. Robson et al. conducted a randomized, open label, phase III trial in which they compared olaparib monotherapy to standard chemotherapy in patients with BRCA mutated, HER2 negative metastatic breast cancer. Patients were not allowed to have received more than two previous lines of chemotherapy for metastatic disease. They received olaparib (300 mg twice daily) or standard 'physician's choice' chemotherapy (capecitabine, eribulin or vinorelbin). In total 302 patients received treatment, of whom 205 patients were assigned to the olaparib cohort. The median progression free survival was significantly longer in the olaparib group than in the standard therapy group (7.0 months vs. 4.2 months; $p<0.001$). The response rate was 59.9% in the olaparib group and 28.8% in the standard-therapy group[60].

Veliparib

Veliparib is also an oral poly (ADP-ribose) polymerase inhibitor. Rugo et al. investigated the combination of veliparib and carboplatin in early breast cancer in the neoadjuvant setting. Patients were randomized to receive either paclitaxel monotherapy or paclitaxel and the combination of veliparib and carboplatin. In both treatment arms this was followed by four cycles of doxorubicin and cyclophosphamide. The estimated rates of pathological complete response (pCR) in the triple negative breast tumors were 51% in the paclitaxel-veliparib-carboplatin group and 26% in the control group. Adding the combination of veliparib-carboplatin to standard chemotherapy in patients with triple negative breast cancer, leads to an increase in pCR rate compared to standard therapy[61]. Mizugaki et al. conducted a phase I trial to investigate the pharmacokinetics of veliparib in combination with carboplatin and paclitaxel in patients with non-small cell lung carcinoma. They showed that the addition of carboplatin and paclitaxel had no significant effect on the veliparib Tmax, dose-normalized Cmax or dose-normalized area under the plasma concentration-time curve (AUC). There was also no evidence of an effect of veliparib on the pharmacokinetics of paclitaxel and carboplatin[62]. Nuthalpati et al. conducted a mass balance study of veliparib in subjects with nonhematologic malignancies. They

showed pharmacokinetic parameters of veliparib and its metabolite M8 for different doses. Veliparib was rapidly absorbed after oral dosing with a median Tmax of 1 h hour, for its metabolite M8 this was two hours. The systemic exposure of veliparib increased proportional to dose in the dose ranges of 10-80 mg twice daily. This was also seen for the M8 metabolite. The renal elimination of veliparib seemed to be independent of the veliparib dose. Renal elimination seems to be the major route of elimination of veliparib[63]. Veliparib single agent therapy has not been investigated much. Coleman et al. performed a multicenter, open label, phase II trial with veliparib monotherapy and showed activity of the single agent in patients with BRCA-mutated epithelial ovarian, fallopian tube or primary peritoneal cancer. They met an ORR of 26%. This is the first phase II study with veliparib monotherapy[64].

Niraparib

Niraparib is a potent oral PARP-1 and PARP-2 inhibitor with a half maximum inhibitory concentration (IC_{50}) of 3.8 nmol/L for PARP-1 and 2.1 nmol/L for PARP-2[65]. Sandhu et al. conducted a phase I dose-escalation study in which 100 patients were enrolled. The AUC was proportional to increase of the dose. Absorption is rapid with a mean plasma concentration peak 3-4 h after a dose followed by biphasic decrease and a mean terminal half-life of 36.4 h (range 32.8-46.0). Two of four *BRCA* mutated breast cancer cases reached partial responses confirmed by RECIST[66]. Van Andel et al. showed in a human mass balance study of ^{14}C-niraparib that both renal and hepatic pathways are involved in excretion of niraparib and its metabolites. The mean total radioactivity recovered in faeces and urine was 86.3% (71.1-91.0%) of the total administered dose of which 38.8% (28.3%-47.0%) was recovered in faeces and 47.5% (33.4-60.2%) in urine. The elimination of ^{14}C-niraparib was biphasically and slow with $T_{1/2}$ in plasma of on average 92.5 h. They were able to detect two major metabolites: M1 (amide hydrolysed niraparib) and the glucuronide of M1[67]. Very recently, the U.S. Food and Drug Administration approved niraparib for the maintenance treatment of adult patients with recurrent high-grade serous epithelial ovarian, fallopian tube, or primary peritoneal cancer who are in complete or partial response to platinum-based chemotherapy.

Table 1.1.1 A selection of PARP-inhibitors often tested in breast cancer and their pharmacological characteristics.

	Tmax (h)	T1/2 (h)	AUC (ug h/mL)	Cmax (ug/mL)	CL/F (L/h)	V_z/F	Reference
Olaparib capsule formulation 300 mg	1.49 (0.57-3.05)	13.02(8.23)	55.20 (67.4)	8.05 (24.3)	6.36 (3.47)	112.1 (59.84)	Dirix et al. Clin. Ther. 2016;38:2286-2299
Olaparib tablet formulation 300 mg single dose (fasted)[59]	1.50 (0.50-5.85)[1]	12.2h (5.31)[1]	43.6 (54.3) (AUC_{0-t}) 43.0 (55.2) (AUC_{inf})	7.00 (35.0)[1]	7.95 (4.23)	146 (142)	Plummer et al. Cancer Chemoth Pharmacol. 2015;76:723-729
Olaparib tablet formulation 300 mg single dose (fed)[59]	4.00 (1.00-12.0)	12.2h (5.31)	46.0 (56.6) (AUC_{0-t}) 45.4 (57.1) (AUC_{inf})	5.48 (40.5)	7.55 (3.99)	127 (107)	Plummer et al. Cancer Chemoth Pharmacol. 2015;76:723-729
Veliparib monotherapy 40 mg (4x10 mg fasting)[81]	1.2 ± 0.8	5.9 ± 1.3	2.23 ± 0.82 (AUC_t) 2.43 ± 1.07 (AUC_{inf})	0.36 ± 0.13	19.0 ± 7.36	NA	Mostafa et al. Cancer Chemother Pharmacol. 2014;74:583-591
Veliparib monotherapy 40 mg (4x10 mg fed)[81]	1.2 ± 0.7	5.8 ± 1.2	2.45 ± 0.93 (AUC_t) 2.65 ± 1.17 (AUC_{inf})	0.37 ± 0.12	17.3 ± 6.41	NA	Mostafa et al. Cancer Chemother Pharmacol. 2014;74:583-591
Veliparib monotherapy 40 mg (1x40 mg fasting)[81]	1.3 ± 0.9	5.8 ± 1.3	2.24 ± 0.98 (AUC_t) 2.45 ± 1.24 (AUC_{inf})	0.34 ± 0.12	19.5 ± 7.66	NA	Mostafa et al. Cancer Chemother Pharmacol. 2014;74:583-591
Veliparib monotherapy 40 mg (1x40 mg fed)[81]	2.5 ± 1.1	5.8 ± 1.4	2.14 ± 0.80 (AUC_t) 2.35 ± 1.06 (AUC_{inf})	0.28 ± 0.09	19.7 ± 7.51	NA	Mostafa et al. Cancer Chemother Pharmacol. 2014;74:583-591
Veliparib metabolite M8	2.4 (3.5-9.8)	-	0.3-1.9 (AUC_{int})	0.011 (0.007-0.014)	NA	NA	Wiegand et al. J Chromatogr B 2010;878(0):333-9
Niraparib 300 mg/day[66]	3.1 (2.0-6.1)	§	14.117 (AUC_{0-24})*	1.921*	NA	NA	Sandhu et al. Lancet Oncol. 2013;14:882-92
Niraparib metabolite: unlabeled M1 plasma	9.02	78.4	41.2 (AUC_{inf})	476	NA	NA	Van Andel et al. Invest New Drugs. 2017. doi:10.1007/s10637-017-0451-2

* Area under the curve 0–12 h and concentration after 12 h reported because sampling ended at 12 h on day 1 of the second course. § Intensive pharmacokinetic sampling was not done (no drug holiday between fi rst and second courses before the third protocol amendment, and thus half-life could not be calculated for the first two doses).

Differentiation

Olaparib is a potent PARP-1, PARP-2 and PARP-3 inhibitor whereas niraparib and veliparib are inhibitors of PARP-1 and PARP-2. Phase I trials showed rapid absorption of both niraparib and olaparib. The peak plasma concentration of olaparib seems to be reached earlier for olaparib (1-3 hours) than for niraparib (3-4 hours). The decrease in plasma concentration of niraparib is 5-7 times slower compared to olaparib. Mizugaki et al. showed that veliparib in the recommended phase II dose (120 mg twice daily) has a peak plasma concentration comparable to niraparib (3.3 hours). Both niraparib and olaparib are eliminated via urine en faeces, whereas elimination of veliparib occurs mainly by urine.

Combination therapy

PARP-inhibitors have been studied as monotherapy and in combination with radiotherapy or cytotoxic chemotherapy. The putative benefit of combining PARP-inhibitors with cytotoxic chemotherapy or radiotherapy is reaching a better efficacy. However more severe toxicity has been seen in combination trials with chemotherapy and radiotherapy. PARP-inhibitors have been studied in several combination studies with cytotoxic agents like platinum compounds. Oza et al. assessed the tolerability and efficacy of olaparib in combination with chemotherapy followed by olaparib monotherapy, compared to chemotherapy alone in patients with high-grade serous ovarian cancer. Patients were randomized between the combination of olaparib (200 mg BID), plus paclitaxel (175 mg/m^2, administered intravenously on day 1) and carboplatin (AUC 4 min*mg/ml, administered intravenously on day 1), followed by olaparib monotherapy (400 mg BID) until progression or to paclitaxel (175 mg/m^2 on day 1) and carboplatin (AUC 6 min*mg/ml, on day 1), not followed by other chemotherapy. Progression free survival was significantly longer in the olaparib plus chemotherapy group (median 12.2 months [95% CI 9.7–15.0]) than in the chemotherapy alone group (median 9.6 months [95% CI 9.1–9.7) (HR 0.51 [95% CI 0.34-0.77]; p=0.0012), especially in patients with *BRCA* mutations (HR 0.21 [0.08-0.55]; p=0.0015). Adverse events that were more common in the combination group compared to the chemotherapy group alone were alopecia (60 [74%] of 81 vs. 44 [59%] of 75), nausea (56 [69%] vs. 43 [57%]), neutropenia (40

[49%] vs. 29 [39%]), diarrhea (34 [42%] vs. 20 [27%]), headache (27 [33%] vs. seven [9 %]), peripheral neuropathy (25 [31%] vs. 14 [19%]), and dyspepsia (21 [26%] vs. 9 [12%]); most were of mild-to-moderate intensity[68]. Del Conte et al. studied the combination of olaparib and liposomal doxorubicin in patients with advanced solid tumors. Patients received either continuously (day 1-28) or intermittently (days 1-7) olaparib plus liposomal doxorubicin (40 mg m^2, day 1). The recommended dose was found after dose-escalation of olaparib in seven cohorts (50-400 mg BID). They showed that the Cmax and $AUC_{0\text{-}10h}$ of olaparib increased with dose and that the olaparib concentration tended to be higher in the presence of liposomal doxorubicin. During the 28 days of treatment, the minimum plasma concentrations (Cmin) were maintained. The most common related toxicities (any grade) were nausea and stomatitis[69]. Van der Noll et al. demonstrated that continuous long-term daily olaparib was safe and tolerable with manageable side effects and promising anti-tumor effects. These patients (10 with breast cancer, 9 with ovarian and 2 with fallopian tube cancer) received olaparib monotherapy after treatment with olaparib combined with carboplatin or paclitaxel. The median treatment duration with single agent olaparib was 52 (7-113) weeks[70]. Another potential combination therapy of PARP-inhibitors could be combination with Wee1 inhibitors. Wee1 is a protein kinase that regulates the G2 checkpoint and prevents entry to mitosis in response to DNA damage[71]. Cells with a defective p53 expression are not able to arrest the cell cycle in the G1 phase in order to repair damaged DNA. These cells rely on the G2 checkpoint of the cell cycle for DNA repair[72]. AZD1775 (formerly MK-1775) is a specific inhibitor of the Wee1 kinase. Previous studies have shown a promising safety profile and anti-tumor activity of AZD1775 administered with cytotoxics like gemcitabine, cisplatin or carboplatin[73,74]. Karnak et al. performed a study to evaluate the radiosensitization of the combination of the Wee1 inhibitor AZD1775 in combination with olaparib. Their hypothesis was that Wee1 and PARP-inhibitors together would give more radiosensitization than either of them alone. They treated pancreatic cells with AZD1775 and olaparib and found that the combination of these agents significantly increased radiosensitivity in these cell lines compared to Wee1 or PARP-inhibition alone[75]. Wee1 inhibition could sensitize cells to PARP-inhibition through abrogation of the G2 checkpoint and inhibition of homologous recombination repair. The combination of these agents resulted in more unrepaired DNA and therefore to cell death. About >80% of the TNBC have a TP53 mutation, therefore they

may be highly sensitive to this combination regimen. Further studies are warranted to investigate this combination in clinical trials.

Pharmacodynamic assays

Pharmacodynamic (PD) assay methodologies are designed to determine the effect of the drug on its target[76]. The optimal dose and duration of therapy could be determined and thereby supporting clinical decision making. There are several PD assays that measure PARP activity in tumor cells and in peripheral blood mononuclear cells (PBMCs) or lymphocytes[77,78]. PARP plays a role in the repair of single strand breaks in the DNA. PARP produces poly (ADP-ribose) polymers (PAR). Inhibition of PARP is thought to decrease PAR levels. Ji et al. validated a PD assay that quantifies PAR levels both in tumor cells and PBMCs, using an ELISA method[78] They applied the PD assay to a clinical trial of ABT-888. Kummar et al. performed a phase 0 clinical trial of the Poly (ADP-ribose) Polymerase Inhibitor ABT-888 (veliparib) in patients with advanced malignancies. They determined PAR levels in PBMCs and in tumor samples after administration of a single dose of ABT-888. An immunoassay with purified monocloncal antibody to PAR as the capture reagent and rabbit anti-PAR antiserum as the detecting agent were used. There was a statistically significant inhibition of PAR levels in both tumors and PBMCs after a single dose of ABT-888[79]. However, PAR levels in PBMCs can be very low, which makes quantification of PAR levels difficult[78,79]. De Haan et al. aimed to develop a clinically applicable pharmacodynamic assay for quantification of PAR levels and PAR reduction upon PARP-inhibition in PBMCs[80]. PBMCs were isolated from the blood of healthy volunteers and from non-small cell lung cancer (NSCLC) patients before, during and after treatment with chemoradiation and olaparib. Low levels of PAR are based on low levels of endogenous DNA damage. Therefore in this study DNA damage was induced by ex-vivo irradiation of the PBMCs. Radiation resulted in increase of PAR levels in a dose dependent and linear manner. Another important step in the assay was the incubation on ice after irradiation. This resulted in improved PAR signal strength. Clinical studies may benefit from this new assay, due to the increased sensitivity and the opportunity to correlate the individual patient PD values with individual PARP-inhibitor drug response. As a result, more individualization of treatment could be applied.

Conclusion

This review summarizes the PARP-inhibitor treatment of TNBC, and focuses also on patient selection, biomarkers, combination therapy and pharmaco-dynamics assays.
TNBC has several characteristics that make treatment challenging; it tends to be aggressive, has a high recurrence rate and a poor 5-year prognosis compared to other types of breast cancer. Mutations in the *BRCA1/2* protein lead to a more error-prone repair pathway due to the function of these genes in the repair of DNA double strand breaks. Often TNBC has similarities with tumors that harbor a *BRCA1* mutation. Breast cancer that harbors a *BRCA1* or 2 mutation is characterized by a specific pattern of DNA gains and losses in an aCGH assay. This *BRCA1/2* –like^{CGH} pattern could help selecting patients that may have benefit from high dose DNA double strand break inducing agents. Six unique subtypes of TNBC have been identified. Basal-like 1 is the most well-known subtype, it has shown high incidence of *BRCA1* methylation and demonstrated similarities to *BRCA1* mutated tumors. *TP53* is a potential biomarker for patients with TNBC. Since more than 80% of the TNBC have a *TP53* mutation, the combination of the Wee1 inhibitor AZD1775 with a PARP-inhibitor could be promising. Given the fact that basal-like TNBC shows similarities with *BRCA1* mutated cells, PARP-inhibition could be a treatment option for these patients. The combination of PARP-inhibition and *BRCA 1 or 2* mutated tumors shows synthetic lethality, leading to cell death. Olaparib is the most well-known PARP-inhibitor and has shown manageable side effects in both short and long term, and promising anti-tumor activity has been demonstrated. Combining olaparib with cytotoxic agents or radiotherapy reaches more efficacy than olaparib monotherapy. Individualization of olaparib treatment is possible due to the use of PD assays. These assays are able to measure PARP activity in tumor cells and PBMCs. Individual patient PD values could be correlated with the clinical parameters to determine whether the dose has to be adjusted. Currently, PARP-inhibitors are studied in several combination schedules with cytotoxic agents and radiotherapy. Besides in patients with BRCA mutation, PARP-inhibition is being studied in patients with the BRCAness phenotype. This could lead to a broader application of PARP-inhibitors, since 30% of the sporadic tumors have the BRCAness phenotype, clinical trials must show whether there is an increased anti-tumor effect combining these agents, with manageable side effects. Individualization of treatment plays more and

more a role in daily practice. When PD assays will be generally applied in treatment with PARP-inhibitors, under- and overdosing could be prevented. However, this concept needs prospective clinical validation.

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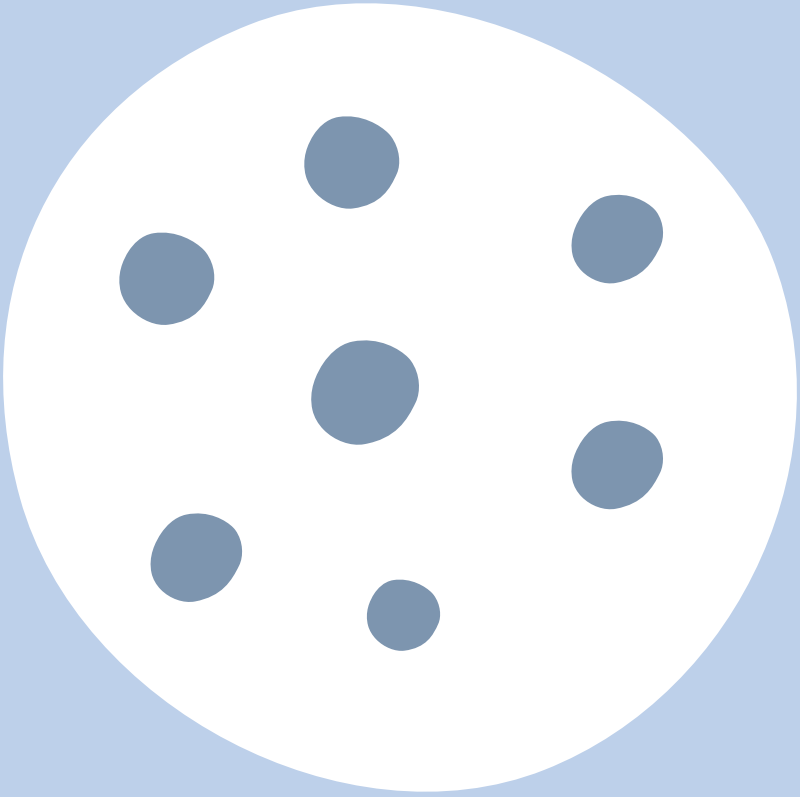

Chapter 1.2

A Phase I dose-escalation study of two cycles carboplatin-olaparib followed by olaparib monotherapy in patients with advanced cancer

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Summary

Preclinical studies have shown synergistic effects when combining PARP1/2 inhibitors and platinum drugs in BRCA1/2 mutated cancer cell models. After a formulation change of olaparib from capsules to tablets, we initiated a dose finding study of olaparib tablets bidaily (BID) continuously with carboplatin to prepare comparative studies in this patient group. Patients were included in a 3+3 dose-escalation schedule: olaparib 25 mg BID and carboplatin area under the curve (AUC) 3 mg*min/mL d1/d22, olaparib 25 mg BID and carboplatin AUC 4 mg*min/mL d1/d22, followed by increasing dose-levels of olaparib from 50 mg BID, 75 mg BID, to 100 mg BID with carboplatin at AUC 4 mg*min/mL d1/d22. After two cycles, patients continued olaparib 300 mg BID as monotherapy. Primary objective was to assess the maximum tolerable dose (MTD). Twenty-four patients with a confirmed diagnosis of advanced cancer were included. Most common adverse events were nausea (46%), fatigue (33%) and platelet count decrease (33%) Dose-level 3 (olaparib 75 mg BID and carboplatin AUC 4 mg*min/mL; n=6) was defined as MTD. Fourteen out of 24 patients (56%) had a partial response as best response (RECIST 1.1). Systemic exposure of the olaparib tablet formulation appeared comparable to the previous capsule formulation with olaparib tablet AUC0-12 of 16.3 µg/mL*h at MTD. Polymers of ADP-ribose levels in peripheral blood mononuclear cells were reduced by 98.7% ± 0.14% at Day 8 compared to Day 1 for dose-level 3. Olaparib tablets 75 mg BID and carboplatin AUC 4 mg*min/mL for two cycles preceding olaparib monotherapy 300 mg is a feasible and tolerable treatment schedule for patients with advanced cancer.

Introduction

BReast CAncer 1(*BRCA1*) and BReast CAncer 2 (*BRCA2*) are the most important breast cancer susceptibility genes. The lifetime risk of breast cancer in *BRCA1*- and *BRCA2*-mutation carriers is 45-80%[1,2]. *BRCA1* and *BRCA2* play important roles in the process of homologous recombination and the repair of DNA double strand breaks (DSB)[3]. *BRCA*-mutated tumors are often highly sensitive to drugs that induce DNA double strand breaks, such as alkylating agents[4]. Poly(ADP-ribose) Polymerase (PARP) plays an important role in the repair of DNA single strand breaks (SSB). Trapping of PARP on the DNA results in persistence of SSB leading to DSB[5]. The genetic interaction between PARP and *BRCA* can be described as synthetic lethality, which occurs where individual loss of either gene is compatible with cell survival, but simultaneous loss of both genes results in cell death[6]. Several preclinical studies have demonstrated that *BRCA* deficient cells are sensitive to PARP1/2 inhibition[7-9]. Furthermore, in clinical studies several selective PARP1/2-inhibitors (talazoparib, niraparib, veliparib) and more broad PARP1,2,3,4,12,15,16-inhibitors (rucaparib) have proven to be effective in patients with *BRCA* mutations. These studies have demonstrated efficacy of PARP1/2-inhibitors in breast- and ovarian cancer, and showed a tolerable safety profile[10-14]. Most clinical studies have been performed with the PARP1/2-inhibitor olaparib. Proof of concept regarding olaparib treatment for advanced *BRCA*-mutated breast cancer was shown by Tutt et al.[15]. In a phase I trial with olaparib in an oral capsule formulation, pharmacokinetic measurements showed a rapid absorption followed by a biphasic decline in plasma concentration. However, the area under the concentration-time curve (AUC) relationship showed nonlinear absorption pharmacokinetics[16]. Olaparib has also been investigated in combination with cytotoxic agents like paclitaxel, carboplatin and doxorubicin for solid tumors like ovarian- and breast cancer. The benefit in efficacy of combining PARP1/2 inhibitors with cytotoxic chemotherapy has been shown, but more severe toxicity could be the result of combining these agents[17-19]. Olaparib combined with carboplatin showed more bone marrow toxicity compared to carboplatin alone[17,19]. Olaparib has been approved as maintenance treatment of patients with platinum sensitive high-grade ovarian cancer[20]. Recently, there was a change in olaparib formulation from capsules to tablets. The approved capsule formulation of 400 milligram (mg) bidaily (BID) required intake of eight 50 mg capsules twice daily. A tablet formulation has been designed and

registered to overcome these disadvantages. The oral bioavailability of the tablet formulation is higher compared to the previous capsule formulation[21]. The AUC of the tablet formulation (300 mg) is 13% higher than the capsule formulation (400 mg)[22]. As a result of the OlympiAD trial, olaparib tablets recently have been approved by the Food and Drug Administration (FDA) as monotherapy for advanced BRCA-mutated breast cancer[23]. Previous studies have determined MTD of the olaparib capsule formulation when administered in combination with carboplatin. The maximum tolerable dose was found to be olaparib capsules 400 mg BID day 1-7 and carboplatin target AUC 5 mg*min/ml once per 21-day cycle[24]. Another phase I study in which olaparib tablets were combined with carboplatin and paclitaxel, showed increased myelosuppression requiring frequent dose modifications, including interruptions, delays and reductions. This toxicity appeared to be more frequent and severe with increasing doses of olaparib (ranging from 50 to 400 mg BID)[25,26]. This supports lower dose olaparib in combination with carboplatin. The aim of this study was to investigate the MTD of the combination of olaparib in tablet formulation administered in combination with carboplatin for two cycles, followed by olaparib monotherapy.

Patients and methods

Patient selection

Patients were eligible if they were at least 18 years old and had a confirmed histological or cytological diagnosis of advanced cancer. A maximum of one prior line systemic chemotherapy and any number of prior lines of endocrine therapy for advanced disease was allowed. Patients were only included if benefit from the combination of olaparib and carboplatin could be expected. All patients had an Eastern Cooperative Oncology Group performance status (ECOG-PS) of ≤2, adequate organ function and evaluable disease according to RECIST version 1.1[27].

Study design and drug treatment

The study design has been previously described elsewhere[28]. In brief, this was an investigator initiated 3+3 traditional phase I dose-escalation trial with predefined dose-levels, conducted at the Netherlands Cancer Institute in Amsterdam, the Netherlands. Patients received two cycles of carboplatin

intravenously with olaparib tablets, followed by olaparib monotherapy. Patients received carboplatin in 30 minute infusions on day 1 of the first two cycles at a dose resulting in a target platinum AUC of 3 mg*min/ml (dose-level -1) or AUC 4 mg*min/ml (all other dose-levels). Olaparib was administered from day 0 onwards at a dose ranging from 25 mg BID (dose-level -1 and dose-level 1) to 100 mg BID (dose-level 4) continuously for a 21-days cycle. After the first two cycles, patients continued with olaparib monotherapy at a dose of 300 mg BID (Supplemental Figure S1.2.1 shows an overview of the study design). Study treatment was continued until disease progression (≥20% increase in the sum of diameters of target lesions), unacceptable toxicity despite dose modifications or patient withdrawal. The Calvert formula, in which glomerular filtration rate was estimated using the Cockcroft-Gault equation was used to determine the carboplatin dose[29].

Objectives

The primary objective was to determine the MTD of two cycles carboplatin with olaparib tablets followed by olaparib monotherapy. Secondary objectives were to investigate the systemic exposure of the olaparib tablet formulation, the pharmacodynamics and the preliminary response rate of this combination.

Dose-escalation and dose-limiting toxicities

A traditional 3+3 dose-escalation scheme was used. The starting dose was olaparib 25 mg BID with carboplatin AUC 3 mg*min/ml followed by olaparib monotherapy 300 mg BID. Patients were enrolled per protocol in sequential cohorts of 3 patients based on the occurrence of dose-limiting toxicity (DLT) within the first 21- days (one cycle) and only after study committee approval. If 1 of 3 patients experienced a DLT in the first cycle, the cohort was expanded to 6 patients. If a DLT was found in at least 2 out of 6 patients, the dose-level was considered to be unsafe. The MTD was the highest dose-level in which not more than one patient experienced a DLT. A DLT was defined as any of the following drug-related adverse events (AEs) occurring in the first cycle of treatment (day 1-21): development of > grade 2 toxicity during the DLT assessment period, toxicity that resulted in missing more than 5 doses of olaparib or that delayed the administration of carboplatin more than 7 days, a dose delay of 7 days or more of the second cycle of olaparib-carboplatin (Supplemental Table S1.2.1). For some toxicities > grade

2 toxicity was accepted as supportive treatment was available. For those the DLT was defined when patients experienced: hematological toxicity: grade 4 anemia, grade 4 neutropenia ≥ 7 consecutive days, grade 3 or 4 febrile neutropenia, grade 4 thrombocytopenia or grade 3 thrombocytopenia with bleeding events. Non-hematological toxicities: ≥ grade 3 diarrhea, vomiting and nausea despite adequate supportive treatment, increased liver biochemistry (AST, ALT,ALP, γGT, LD) ≥ grade 3 lasting >3 days. In case of a DLT, dosing was interrupted until the toxicity was recovered to less than grade 2. Dose modifications according to protocol were allowed in the best interest of the patient.

Olaparib and platinum measurements

An HPLC-MS/MS method was used to determine olaparib in human plasma using Olaparib-d8 (deuterated) as internal standard. The compounds were extracted from the plasma by liquid-liquid extraction with tert-Butyl methyl ether (TBME). Chromatographic separation was performed on a Phenomenex HPLC Gemini C18 column using gradient elution. For detection an AB Sciex API4000 tandem mass spectrometer equipped with an electrospray ionization interface (ESI) was used operating in the positive ion mode. Further details have been described before[30]. An Inductively Coupled Plasma Mass Spectometry (ICPMS) method was used to determine platinum from carboplatin in human plasma and plasma ultrafiltrate using Iridium as internal standard. For detection a Varian 810-MS ICPMS is used[31].

Safety and assessments

During screening information was gathered about medical history and demographics. At baseline and throughout treatment physical examination, vital signs, ECOG-PS, concomitant medication and laboratory (hematology, chemistry, urine analysis) assessments were performed. Tumor response was evaluated using Computer tomography (CT) scans at baseline and every two cycles according to RECIST version 1.1. Adverse events were graded according to the National Cancer Institute Common Terminology Criteria (CTC) version 4.03[32].

Statistical analysis

No formal sample size calculation was performed prior to this study because of the traditional 3+3 design. Upfront we expected to enroll 15-20 patients in this study. Data were summarized with descriptive statistics and graphs. Disease progression was summarized with Kaplan-Meier method. All analyses were performed using R software version 3.3.3.

Pharmacokinetic assessments

To determine the pharmacokinetic parameters of olaparib from tablets in combination with carboplatin, an intensive blood sampling scheme was used. For olaparib 21 blood samples (21x4 ml) were collected: nine on day 0: pre-dose and 0.5, 1, 2,4,6,8,10,and 12 hours after administration. Twelve on day 1 pre-dose and 0.5, 1, 2, 2.25, 2.5, 3,4,6,8,10, 12 hours after olaparib administration. For carboplatin 10 blood samples (10x4 ml) were collected: 0.25, 0.5, 1, 2, 4, 6, 8,10, 24 and 48 hours after infusion of carboplatin. For carboplatin pharmacokinetics, concentrations of platinum were measured in both plasma and plasma ultrafiltrates (31). Calculation of pharmacokinetic (PK) parameters included maximum concentration (C_{max}), time to reach maximum concentration (t_{max}), AUC from 0 to time t (AUC_{0-t}; 12 hours for olaparib and 48 hours for carboplatin), AUC from time 0 to t_{last} (AUC_{last}), and half-life ($t_{1/2}$).

Pharmacodynamic assessments

Poly(ADP) ribose levels were determined in peripheral blood mononuclear cells (PBMCs) using the Radiation-Enhanced-Polymers of ADP-ribose (PAR) pharmacodynamic assay (REP assay)[33]. In brief,16 mL venous blood was collected in mononuclear cell preparation citrate tubes (CPT) pre-dose at day 0, and at day 8. PAR levels were assessed in three independent samples, containing $2x10^6$ PBMCs, which were irradiated ex vivo with 8 gray (Gy) on ice and incubated for 1 hour on ice. Cellular PAR levels were measured by using the HT-PARP in vivo pharmacodynamics Assay II, following the National Cancer Institute (NCI) protocol[34] using a Tecan-Infinite-200-Pro. PAR levels on day 8 of cycle 1 treatment were compared with the PAR levels before start of treatment to determine the balance of PARP and poly (ADP-ribose) glycohydrolase (PARG) activity and the resulting relative reduction in PAR levels after 8 days of treatment.

The relative reduction in PAR levels was defined as ((PAR levels day 0 - PAR levels day 8)/PAR levels day 0) * 100%.

Results

Patients

Between July 2015 and October 2017 we enrolled 24 eligible patients with advanced malignancies: 18 patients had breast cancer, three had ovarian cancer, one had eye melanoma, one colorectal cancer, and one esophageal cancer. One patient had received more than one line of treatment in the advanced setting and was therefore not considered to be evaluable for safety and efficacy. Baseline characteristics of the 24 patients are presented in Table 1.2.1. The median patient age was 49 years (range, 27-70). Most patients had a WHO performance status of zero (19/24;79%). Nineteen patients (19/24; 79%) had a germline *BRCA* mutation (*BRCA1* or *BRCA2*).

Treatment

Patients were enrolled in predefined dose cohorts (Supplemental Table S1.2.2); the lowest dose-level started with 25 mg olaparib BID and carboplatin AUC 3 mg*min/ml, the highest dose-level explored olaparib 100 mg BID and carboplatin AUC 4 mg*min/ml. Three patients were treated in the lowest dose-level, six patients were treated at each dose-levels with olaparib 50 mg BID, olaparib 75 mg BID and olaparib 100 mg BID, respectively. Since there were two dose-limiting toxicities at the highest dose-level of 100 mg BID, the MTD was determined to be olaparib 75 mg BID and carboplatin AUC 4 mg*min/ml. Dose-reductions were applied in five patients. Two patients received olaparib maintenance at 250 mg BID instead of 300 mg BID because of hematological toxicity. Two patients received olaparib 200 mg BID instead of 300 mg because of malaise. One patient was allocated to the olaparib 100mg BID and carboplatin AUC 4 mg*min/ml cohort and was switched to olaparib 75 mg BID in the second cycle because of hematologic toxicity in the first cycle. Supplemental Figure S1.2.2 shows an overview of the dose-escalation scheme.

Table 1.2.1 Table showing the baseline characteristics for all 24 evaluable patients included in this study.

	N	%
Gender		
Female	22	92
Male	2	8
Age (median)(range) years	49 (27-70)	-
Tumor type primary disease		
Breast	18	75
Ovarian	3	13
Colorectal	1	4
Esophageal	1	4
Eye melanoma	1	4
Ethnicity		
Caucasian	24	100
WHO performance status		
WHO 0	19	79
WHO 1	4	17
WHO 2	1	4
BRCA-status		
BRCA-1 mutated	8	33
BRCA-2 mutated	11	47
BRCA-2 like	2	8
Non-carrier	1	4
Unknown	2	8
Previous platinum treatment		
Yes	6	25
No	18	75
Lines of chemotherapy in adjuvant setting		
0	7	29
1	17	71
Lines of chemotherapy for M1 disease		
0	18	25
1	6	75
Previous hormonal therapy		
Yes	12	50
No	12	50

Safety

Three DLT events were observed in this trial. In dose-level 2 (olaparib 50 mg BID and carboplatin AUC 4 mg*min/ml), one patient developed a grade 3 liver biochemistry increase lasting for more than 3 days. In dose-level 4 (olaparib 100 mg BID and carboplatin AUC 4 mg*min/ml), two patients experienced a DLT consisting of ≥7 days dose delay of cycle 2 or missing ≥5 doses of olaparib due to hematologic toxicity. Therefore, the preceding dose-level (olaparib 75 mg BID and carboplatin AUC 4 mg*min/ml) was expanded to 6 patients. No DLTs were observed at this dose-level

(Supplemental Table S1.2.2). Hence, the MTD was determined to be olaparib 75 mg BID combined with carboplatin AUC 4 mg*min/ml. The most common grade 1/2 AEs observed in this study were nausea (11/24; 46%), fatigue (8/24; 33%) and platelet count decrease (8/24; 33%). The majority of AEs (20/24; 83%) were grade 1/2 in severity (supplemental table 3). Most common grade 3/4 AE were hematological events: anemia (4/24;17%), neutrophil count decrease (2/24; 8%) and platelet count decrease (2/24; 8%). Table 1.2.2 provides an overview of adverse events that were possibly related to the treatment administration.

Pharmacokinetics

Pharmacokinetic parameters for all patients included in this phase I study are presented in Supplemental Table S1.2.4 The mean maximum concentration (Cmax) of olaparib in the different dose-levels show an increase with increasing olaparib dose. At MTD the median AUC_{0-12} on day 0 was 15.5 µg/ml*h and on day 1 16.3 µg/ml*h indicating minimal accumulation. Figure 1.2.1 shows summarized PK profiles of olaparib tablet formulation by dose-level after receipt of a single olaparib dose. Mean AUC_{0-48} for carboplatin target AUC 4 mg*min/ml, determined in plasma ultrafiltrates was 5.10 mg/ml*min. Supplemental Figure S1.2.3 shows the pharmacokinetic profile of carboplatin measured in plasma-ultrafiltrate after a single dose.

Pharmacodynamics

Supplemental Figure S1.2.4 shows the relative remaining PAR levels as a net result of the balance of PARP and PARG activity in PBMCs at day 8 of cycle 1. The mean relative reduction in PAR levels at day 8 was 97.5% ± 0.1%. The relative reduction in PAR levels increased with an increase of the olaparib dose and reached 99.1% ±0.1% at dose-level 4.

Table 1.2.2 Adverse events at least possibly related to the treatment administration and occurring in >10% of patients in case of grade 1/2 events and all events in case of grade 3/4 events. All events were graded according to the Common Terminology for Adverse Events (CTCAE) version 4.03.

	Grade 1/2						Grade 3/4					
Dose-level	-1	1	2	3	4	All (%)	-1	1	2	3	4	All (%)
Adverse event												
ALT increased	1	1	1			3 (13)			1			1 (4)
Anemia				1		1 (4)			1	2	1	4 (17)
Anorexia			1	2	1	4 (17)						
AST increased	1		1			2 (8)			1			1 (4)
Dysgeusia			2		1	3 (13)						
Fatigue		2	2	1	3	8 (33)						
Nausea			6	3	2	11 (46)						
Neutrophil count decreased		1	1	2	1	4 (17)				2		2 (8)
Platelet count decreased			4	2	2	8 (33)				1	1	2 (8)
Vomiting			1	1	2	4 (17)						

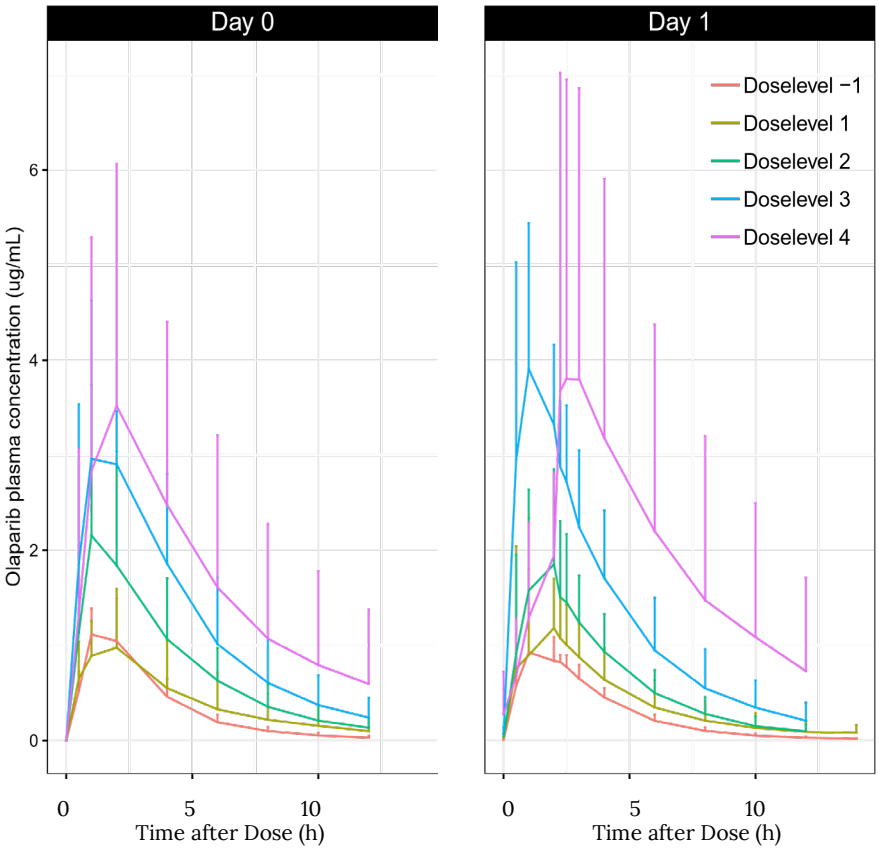

Figure 1.2.1 Olaparib plasma concentration

Efficacy

A total of 24 patients were evaluable for response. In one patient physical examination was used for tumor evaluation, because of extensive skin metastases. Fourteen patients (14/24;58%) had a partial response as best response. According to RECIST 1.1, this was confirmed after at least 4 weeks. The group with partial responses was represented by patients treated at all dose-levels explored. Almost all patients achieved partial response within the first 12 weeks of treatment (13/14; 93%). In seven patients (7/24;29%) stable disease was the best response observed (Supplemental Table 1.2.5). The waterfall plot in Figure 1.2.2 shows the maximum change in target lesion diameter compared to baseline. Six patients (6/24;25%) had a prolonged response of >12 months (Figure 1.2.3). The median progression free survival was 7 months (Supplemental Figure 1.2.5).

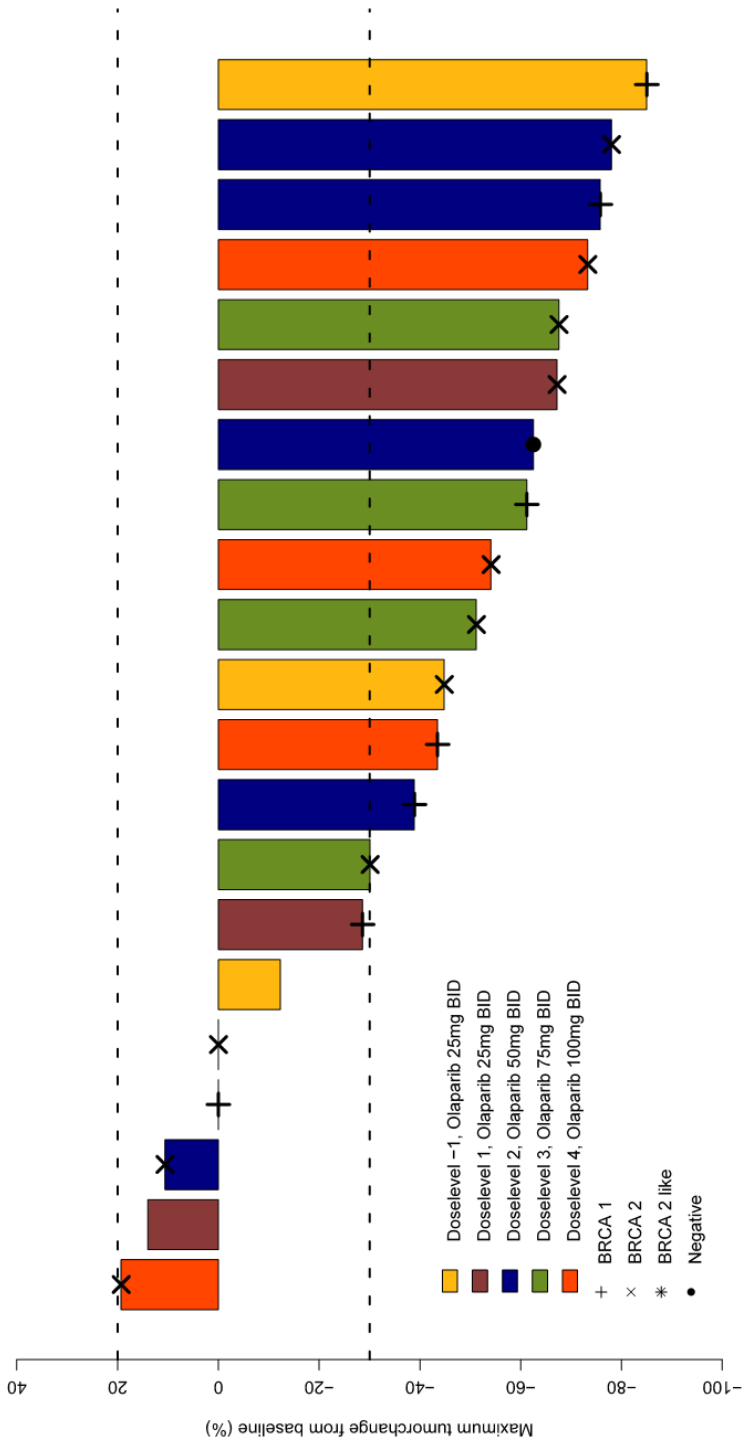

Figure 1.2.2 Waterfall plot

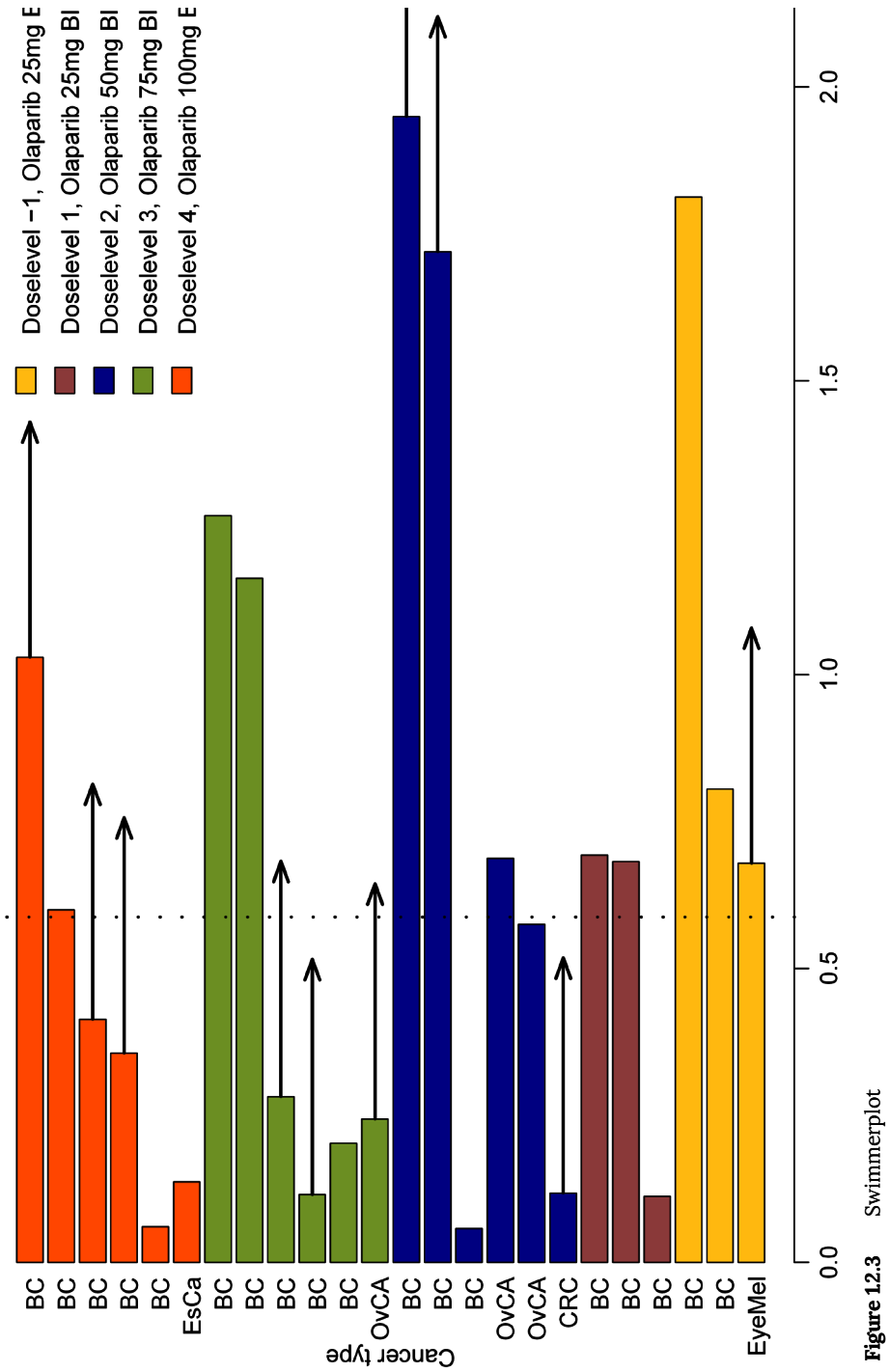

Figure 12.3 Swimmerplot

Discussion

This phase I trial of olaparib tablets, an oral PARP1/2 inhibitor, combined with carboplatin, showed that the combination is safe and has an acceptable side-effect profile. The MTD was found to be olaparib tablets 75 mg BID and carboplatin AUC 4 mg*min/ml. Although the target AUC of carboplatin was AUC 4 mg*min/ml in 21 patients (dose-level 1-4), the mean AUC_{0-48} measured in carboplatin ultrafiltrates of these 21 patients was 5.1 mg*min/ml. Therefore, the exposure of carboplatin was higher than the targeted AUC 4 mg*min/ml. This study shows that the tablet formulation of olaparib can be administered safely in combination with carboplatin, compared to the previous capsule formulation[16]. Comparing the tablet pharmacokinetics with the previous capsule formulation shows comparable exposure at the maximum tolerable dose, although there are slight differences in timeframe[16]. In our study the AUC_{0-12} is 16.3 µg/ml*h at 75 mg olaparib tablet BID, which is comparable to the AUC_{0-12} of 13.2 µg/ml*h at 80 mg olaparib capsule BID[16]. However, in previous pharmacokinetic studies of olaparib tablet and capsule formulations it was found that the exposure of the olaparib tablets was higher than the capsule formulation[22]. In our study this was not confirmed. This could be explained by the low dosing of olaparib in this study compared with the bioequivalence testing dose of 400 mg before. At lower dose, non-linear pharmacokinetics may be less prominent which might explain this discrepancy. Pharmacodynamic analyses showed a high relative reduction in PAR levels, as read-out for the balance of PARP and PARG activity in PBMCs, of 97.5% ± 0.1% at the lowest dose-level indicating that already at the level of 25 mg olaparib BID there is almost complete PAR downregulation. Pharmacodynamic analyses showed a slight further relative reduction in PAR levels with an increase of the olaparib dose. Since olaparib treatment response may be dose dependent, we choose to abide to the registered monotherapy tablet dosage of 300 mg BID for maintenance therapy[15]. Future studies are necessary in order to explore whether pharmacodynamically-guided reduction of maintenance dosing could lead to fewer adverse events without compromising treatment response.

Before, only one validated enzyme-linked immunosorbent assay (ELISA) for quantifying basal PAR levels was available for pharmacodynamic assessment of the effect of PARP1/2 inhibitors on the balance of PARP and PARG activity. Notably, the overall level of polymers of ADP-ribose measured is a reflection

of both synthesis and degradation[35]. In that study PAR levels were measured in PBMCs of patients who were administered veliparib in combination with topotecan, which resulted in a greater than 50% reduction in PAR levels in 19 out of 23 patients with measurable PAR levels[36]. A limitation of this previous PD assay was that it is only applicable to patients with sufficient high levels of PAR. In our study, the REP-assay was used. The REP-assay uses 8 Gy of ex vivo radiation to strongly enhance the basal PARP1/2 activity in PBMCs, which allowed the sensitive determination of the lowest PAR levels present in PBMCs even from patients treated at the highest dose-level[33]. This higher sensitivity of the REP-assay probably explains the impressive relative reduction in PAR levels observed at all dose-levels compared to previous studies and provides a better representation of the true biological inhibitory effect of PARP1/2 inhibitors on the balance of PARP and PARG activity.

Furthermore, since carboplatin induces DNA double strand breaks it could therefore lead to higher PARP1/2 activity and higher PAR levels. So far, this has only been confirmed in nucleotide excision repair (NER) deficient tumor cell lines, but not in NER proficient tumor cell lines nor PBMCs[37]. Furthermore, the REP assay used in this study increased baseline PARP activity in PBMCs on average 121-fold, which probably far outweighs any possible additional effect of carboplatin on PARP1/2 activity.

The most common AEs were mild and self-limiting. However, in three patients dose reductions were applied because of hematological toxicities. Anemia was the most common grade 3/4 AE (4/24;17%). The level of hematological toxicities observed was comparable to the one reported in trials with olaparib monotherapy[38]. Serious toxicities that previously have been described for olaparib, such as pneumonitis or the development of secondary malignancies were not observed[39], this could be related to the relatively short follow up. Regarding the tumor response, the patient with the most pronounced decrease in tumor volume was treated with the lowest dose of the olaparib-carboplatin combination explored in this study. However, there was only slight difference in olaparib dose between the different dose-levels. Furthermore, an almost complete relative reduction in PAR levels was observed at the lowest dose-levels. This raises the question whether treatment at a higher dose-level would have an additional therapeutic effect. The increase in dose could lead to a more durable response but this study was not aimed at nor powered for in-depth analyses

of progression free survival (PFS) or overall survival (OS), so conclusions on differences in response duration are not possible.
Recently the results of the OlympIAD trial were published in which olaparib monotherapy was administered to patients with advanced *BRCA*-mutated breast cancer[40]. The progression free survival in the olaparib group was significantly longer compared to the reference group (non- platinum containing therapy) (7 vs. 4.2 months). The main question is what the addition of carboplatin to olaparib would do on the end points of progression free survival and overall survival. Looking at the mechanism of action, the addition of carboplatin is a rational choice. However, the addition of carboplatin to olaparib could result in more and more severe (hematological) toxicities[17], although not observed in this study even with the higher exposure to carboplatin of 5 mg*min/ml instead of the targeted AUC 4 mg*min/ml. Carboplatin monotherapy is also a promising therapy in patients with advanced *BRCA*-mutated triple negative breast cancer[41]. Comparing in a 3-arm study olaparib monotherapy with the combination olaparib-carboplatin and with carboplatin monotherapy might give useful information. Although our study provides valuable information on safety and anti-tumor effect, some questions remain. Firstly, it would have been interesting to have tumor tissue available from the time of progression in order to study the resistance mechanisms involved. Secondly, we did not measure PAR levels at the end of treatment. It would be interesting to see whether there is still sufficient reduction in PAR levels at time of progressive disease. Thirdly, we did not perform any pretreatment genotyping of the tumor. Although most patients harbored a *BRCA1* or *BRCA2* mutation, treatment responses varied considerably. This may have been due to multiple factors[42-44], including the molecular make-up of the tumor, tumor heterogeneity or differences in the tumor microenvironment. Future studies addressing all these factors are highly desirable in order to select the most appropriate treatment for a certain patient. Finally, the small number of patients (n=24) in this trial makes it difficult to draw firm conclusions on anti-tumor activity and a prospective trial comparing olaparib with or without carboplatin would be needed.
Overall, this study provided the maximum tolerable dose of olaparib tablets in combination with two cycles of carboplatin. Furthermore, this study showed that this combination can be applied safely and is reasonably well tolerated. The observed preliminary anti-tumor activity is encouraging with 58% of the patients having a decrease in tumor volume of more than 30%.

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Supplemental files

Table S1.2.1 Criteria for defining dose limiting toxicities (DLTs) occurring during the DLT assessment period (3 weeks).

Toxicity	DLT definition*
Hematological toxicity	• Grade 4 anemia • Grade 4 neutropenia > 7 consecutive days • Grade 3 or 4 febrile neutropenia • Grade 4 thrombocytopenia or Grade 3 thrombocytopenia associated with bleeding events
Non-hematological toxicity	• Diarrhea ≥ Grade 3 despite adequate supportive treatment and interruption of study drugs • Vomiting ≥ Grade 3 despite adequate supportive treatment use and interruption of study drugs • Nausea ≥ Grade 3 despite adequate supportive treatment • Increases in liver biochemistry (AST, ALT, ALP, yGT, LDH) ≥ Grade 3 lasting > 3 days
Other	• >grade 2 toxicity during the DLT assessment period toxicity that delays the administration of carboplatin • > 7 days or results in missing more than 5 doses of olaparib during the DLT assessment period • A dose delay of ≥7 days of the second cycle of carboplatin-olaparib

*The toxicity or delay should be possibly, probably or definitely related to study drugs.
AST=aspartate aminotransferase, ALT=alanine aminotransferase, ALP=alkaline phosphatase, yGT=gamma glutamyltransferase, LDH=lactate dehydrogenase, DLT=dose limiting toxicity

Table S1.2.2 Dose-escalation cohorts for the phase-Ib part of this study including a total of n=24 patients .

Dose-level*	Patients (n)	Carboplatin (AUC)	Olaparib (mg)	DLT (CTCAE version 4.03)
-1	3	3	25	0
1	3	4	25	0
2	6	4	50	1
3	6	4	75	0
4	6	4	100	2

*In dose-level 2 one patient experienced a grade 3 increase in liver biochemistry lasting for more than 3 days. In dose-level 4, two patients experienced a DLT consisting of ≥7 days dose delay of cycle 2 or missing >5 olaparib doses
AUC = area under the curve, mg = milligram, DLT = dose limiting toxicity, CTCAE

Table S1.2.3 Adverse events at least possibly related to the treatment administration. AEs were graded according to the Common Terminology for Adverse Events (CTCAE) version 4.03.

	Grade 1/2						Grade 3/4					
Dose-level	**-1**	**1**	**2**	**3**	**4**	**All (%)**	**-1**	**1**	**2**	**3**	**4**	**All (%)**
Adverse event												
Agitation			1			1 (4)						
ALT increased	1	1	1			3 (13)			1			1 (4)
Alopecia				1		1 (4)						
Anemia				1		1 (4)			1	2	1	4 (17)
Anorexia			1	2	1	4 (17)						
AST increased	1		1			2 (8)			1			1 (4)
Concentration impairment				1		1 (4)						
Creatinine increased		1				1 (4)						
Depressive disorder			1			1 (4)						
Diarrhea			1			1 (4)						
Dizziness			1			1 (4)						
Dysgeusia			2		1	3 (13)						
Dyspepsia		1				1 (4)						
Dyspnea			1			1 (4)						
Erythema multiforme	1					1 (4)						
Fatigue		2	2	1	3	8 (33)						
Flatulence					1	1 (4)						
Flu like symptoms				1		1 (4)						
Headache			1		1	2 (8)						
Hematoma					1	1 (4)						
Localised edema			1			1 (4)						
Malaise			1		1	2 (8)						
Muscle spasm			1			1 (4)						
Nausea			6	3	2	11 (46)						
Neutrophil count decreased		1	1	2	1	4 (17)				2		2 (8)
Pain in extremity		1				1 (4)						
Peripheral sensory neuropathy	1					1 (4)						

Table S1.2.4 Pharmacokinetic parameters for all patients included in the phase-I part of the revival study by dose-level and cycle day.

Dose-level	**-1**	**1**	**2**	**3**	**4**
	N=3	N=3 25	N=6 50	N=6 75	N=6 100
Olaparib dose (mg)	25				
Olaparib	**Cycle 1 day 0**				
C_{max} (µg/mL)(mean)	1.35	1.07	2.38	3.75	4.06
C_{max} (µg/mL)(SD)	0.21	0.54	1.37	0.71	2.16
T_{max} (h)(median)	1	2	1	1	1.5
T_{max} (h)(range)	1-2	1-2	1-6	0.5-4	0.5-4
AUC_{0-12}(µg/ml*h)(mean)	4.31	5.06	9.60	15.46	20.56
AUC_{0-12}(µg/ml*h)(SD)	1.10	4.21	5.28	3.79	17.66
Olaparib	**Cycle 1 day 1**				
C_{max} (µg/mL)(mean)	1.27	1.48	2.08	4.53	4.60 3.50
C_{max} (µg/mL)(SD)	0.51	0.96	1.03	0.81	2.13
T_{max} (h)(median)	2	2	2	1	1-3
T_{max} (h)(range)	1-2	1-2.25	0.5-3	0.5-2	22.81
AUC_{0-12}(µg/ml*h)(mean)	3.97	5.67	8.02	16.32	19.55
AUC_{0-12}(µg/ml*h)(SD)	1.24	4.63	3.74	5.63	
AUC		**3**		**4**	
		N=3		**N=21**	
Carboplatin	**Cycle 1 day 1 (plasma)**				
C_{max} (mg/mL)(mean)		0.02		0.03 0.01 7.41	
C_{max} (mg/mL)(SD)		0.004		1.36	
AUC_{0-48}(mg*min/ml)(mean)		5.71			
AUC_{0-48}(mg*min/ml)(SD)		0.97			
Carboplatin	**Cycle 1 day 1 (plasma ultrafiltrate)**				
C_{max} (mg/mL)(mean)		0.03		0.04 0.01 5.10	
C_{max} (mg/mL)(SD)		0.006		0.92	
AUC_{0-48}(mg*min/ml)(mean)		3.87			
AUC_{0-48}(mg*min/ml)(SD)		0.72			

Table S1.2.5 Best overall response according to the RECIST 1.1 criteria.

	Responders	
Best response according to RECIST 1.1	N	%
Progressive Disease (PD)	2	8
Stable Disease (SD)	7	29
Partial Response (PR)	14	59
Unknown	1	4

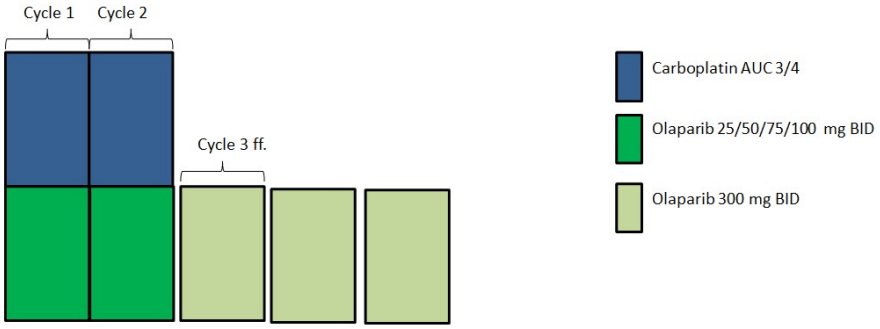


Figure S1.2.1 Study schedule for the phase-Ib part of this study.
AUC=area under the curve, BID= bi-daily ff= and following.

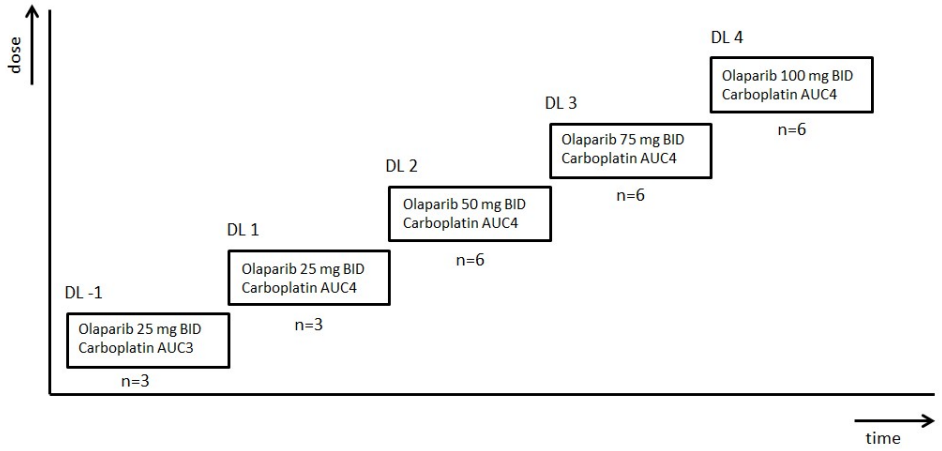


Figure S1.2.2 Dose-escalation scheme for this phase-Ib study.
AUC in mg*min/ml, AUC=area under the curve, BID=bi-daily, DL=dose level, mg=milligram, min=minute, ml=milliliter.

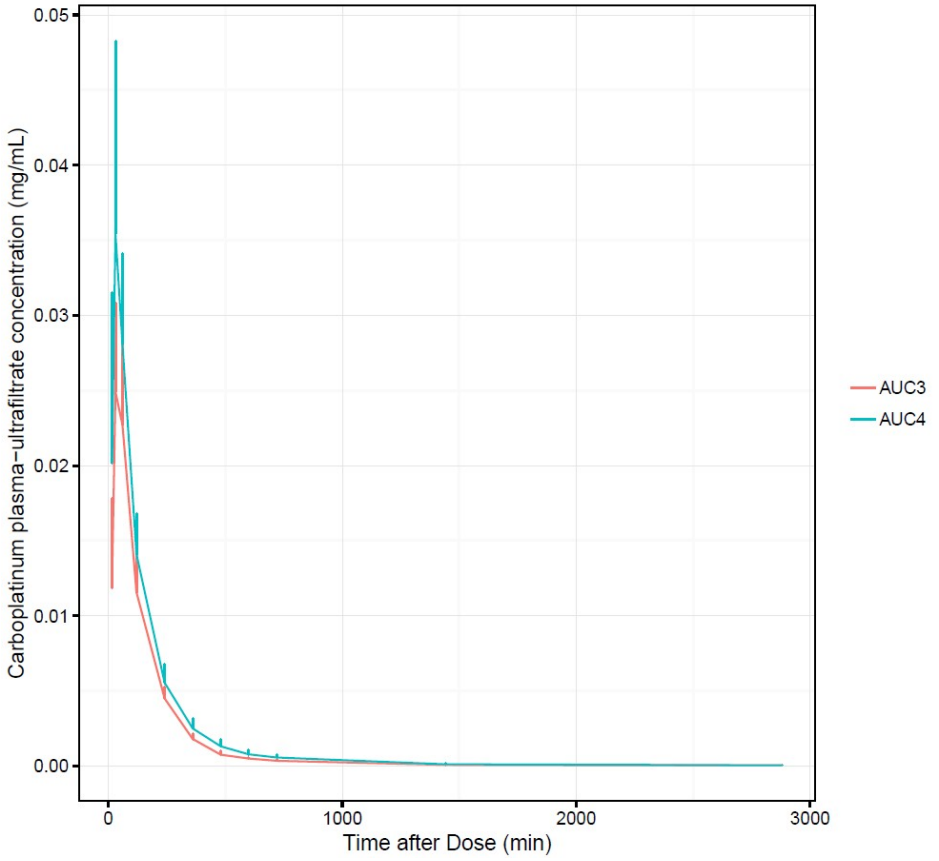


Figure 1.2.3 Summarized pharmacokinetic profile of carboplatin measured in plasmaultrafiltrate after a single intravenous dose at t=2 of day 1.
Carboplatin was administered at AUC 3 during Dose-level -1, patients in all other dose-levels (Dose-level 1- Dose-level 4) received carboplatin AUC 4. Vertical bars represent the inter-patient variablity. AUC=area under the curve, mg=milligram, min=minute, mL=milliliter.

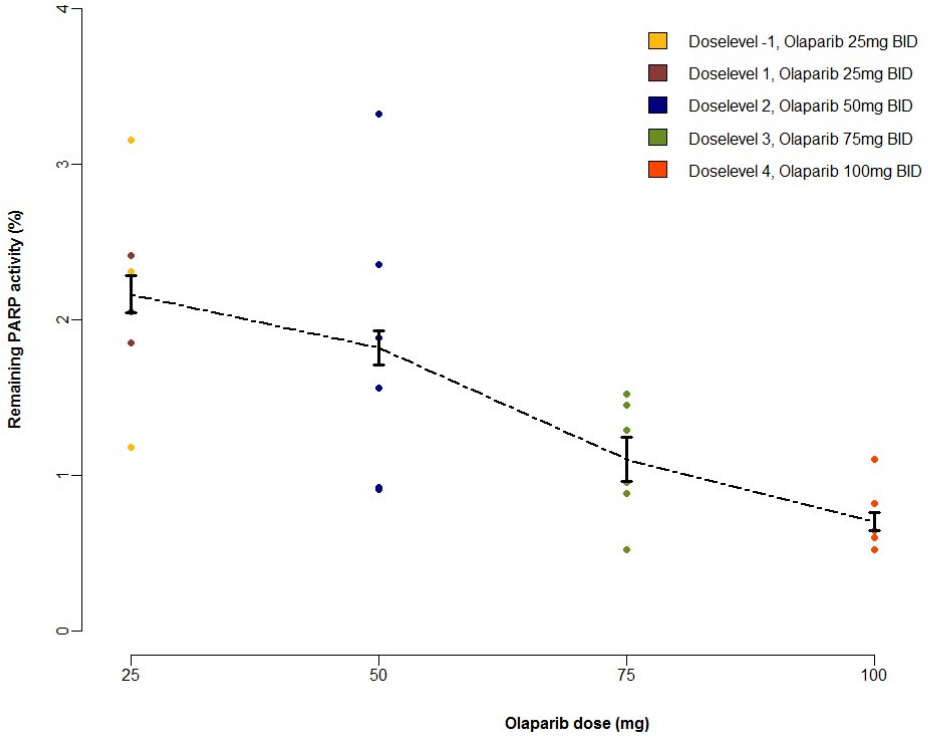


Figure S1.2.4 Relative remaining PAR levels as a net result of the balance of poly (ADP-ribose) polymerase (PARP) and poly (ADP-ribose) glycohydrolase (PARG) activity, denoted as 'Remaining.
BID=bi-daily, mg=milligram, PARP=poly(ADP) ribose Polymerase, PBMC=peripheral mononuclear cell

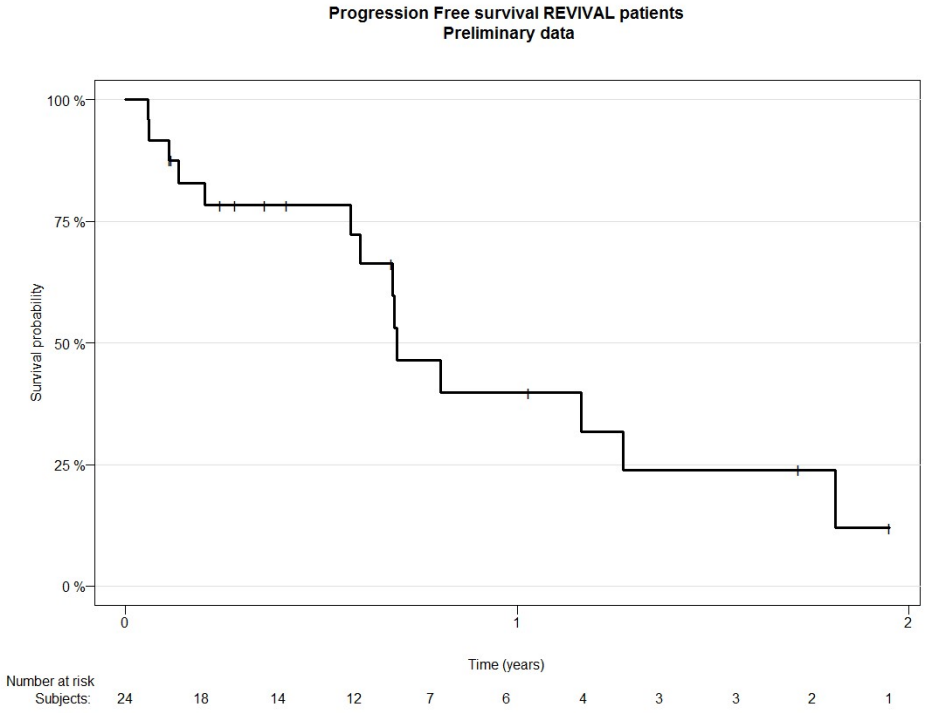
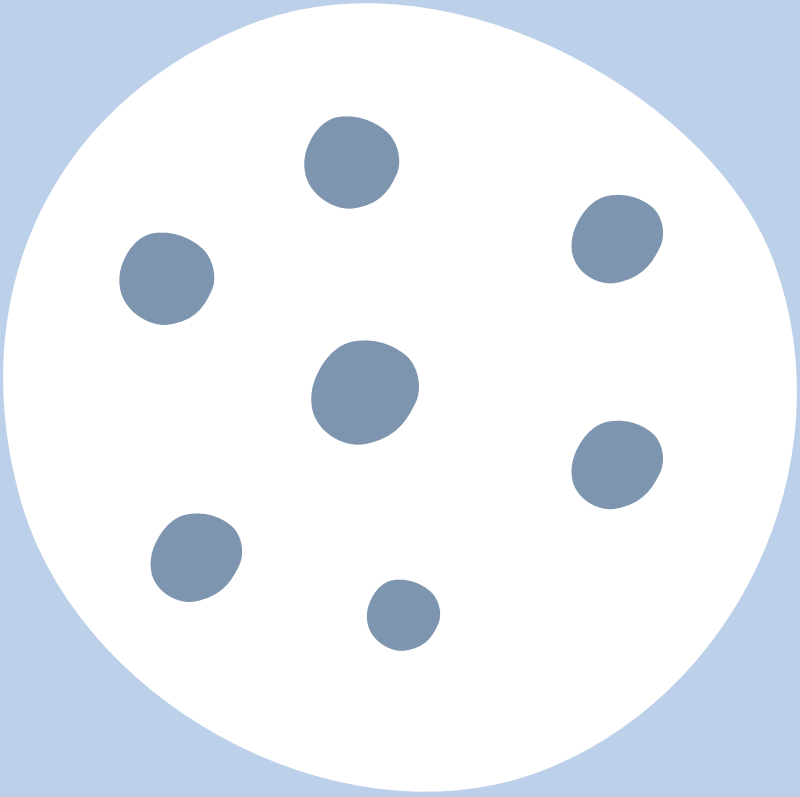


Figure S1.2.5 Kaplan Meier curve showing Progression Free Survival (PFS) for all 24 patients included in this phase-Ib study.



Chapter 1.3

PARP-inhibitors in the treatment of brain metastases

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Manuscript in preparation

Summary

The past decade poly(adenosine diphosphate [ADP]-ribose) polymerase (PARP) inhibitors have conquered place in the treatment of several cancer types like breast and ovarian cancer, either as monotherapy or in combination with other anticancer drugs. PARP inhibitors like olaparib, veliparib, niraparib, rucaparib and talazoparib have been studied extensively over the past years. This has resulted in the registration of olaparib in 2014 as the first representative of this class of compounds. We present a 70-year-old female patient with advanced breast cancer, treated successfully for many years with olaparib in combination with carboplatin. Despite the ongoing systemic response, she developed several brain metastases while on study treatment. This has raised the question whether patients with brain metastases could anyway successfully be treated with PARP inhibition. Patients with brain metastases however, are generally excluded from clinical trials and are therefore not able to receive PARP inhibitors. The patient presented was treated several times with radiation therapy for the brain metastases. It was decided to continue olaparib and carboplatin therapy because of the ongoing systemic response observed. We have reviewed pharmacological literature and conclude that of all PARP inhibitors, veliparib seems to have the best odds to treat brain metastases due to the lack of being a P-glycoprotein (P-gp) substrate. But evidence is thin and data are contradictory. Including patients with brain metastases in clinical trials in order to evaluate the efficacy of PARP inhibitors on these metastases should be worth considering.

Case description

A 70-year-old female was referred to our hospital because of a second opinion regarding the treatment of her advanced breast cancer. Four years before referral, she was diagnosed with a pT2N0 triple negative breast tumor of the left breast for which she underwent breast conserving surgery. She declined additional treatment post-surgery. One year later she presented with pT2a grade 2 urothelial cell carcinoma, low risk, for which she underwent transurethral resection (TUR) of the bladder. Because of a germline (g) BReast CAncer 1 (BRCA1) mutation, a prophylactic ablation of the right breast and a bilateral salpingo-oophorectomy was performed. Pathology showed no signs of malignancy. In 2016 she presented with multiple sub pleural lung lesions and pathological enlarged lymph nodes of the right hilus on Computer Tomography (CT) scan. Pathology report confirmed metastases of the breast tumor. In 2016 she started study treatment with the combination of the PARP inhibitor olaparib combined with carboplatin in a three-weekly schedule. After two cycles of treatment she had a partial response (PR). After more than one year of treatment with olaparib and carboplatin, she was diagnosed with transient global amnesia caused by a brain metastasis on the left parietal side. She received radiotherapy (2400 centigray (cGy)) and it was decided that she could continue treatment with olaparib and carboplatin because of the ongoing systemic response. In 2018, almost two years after start of the olaparib/carboplatin treatment and one year after the initial radiotherapy on the brain, she received Gamma Knife treatment because of a new asymptomatic brain metastasis. The olaparib and carboplatin treatment was interrupted shortly during the Gamma Knife treatment. Gamma knife treatment is a radio surgical treatment that has been used to treat metastatic brain tumors and other intracranial diseases. The Gamma Knife is a radiation machine with 192 radioactive cobalt sources, which are focused at a target point in the brain using stereotactic guidance. This results in bloodless, closed-cranial destruction or inactivation of intracranial tumors[1]. After this, the olaparib and carboplatin were continued because of the ongoing systemic response. In 2019 she developed a new brain metastasis for which she received again 1 fraction (2500 cGy) of Gamma Knife treatment. Olaparib and carboplatin were shortly interrupted because of the Gamma Knife treatment but were resumed thereafter (Figure 1.3.1). The patient has currently still ongoing systemic response on three-weekly

olaparib and carboplatin regimen but she developed several times new brain metastases under this treatment regimen. When discussing this patient, the question raised if brain metastases from breast-and ovarian cancer are exposed and could be sensitive to PARP inhibition and if there is difference in response between the current available PARP inhibitors. In this viewpoint we will discuss the literature regarding PARP inhibitors and brain metastases both in preclinical and in clinical setting in order to answer our research question.

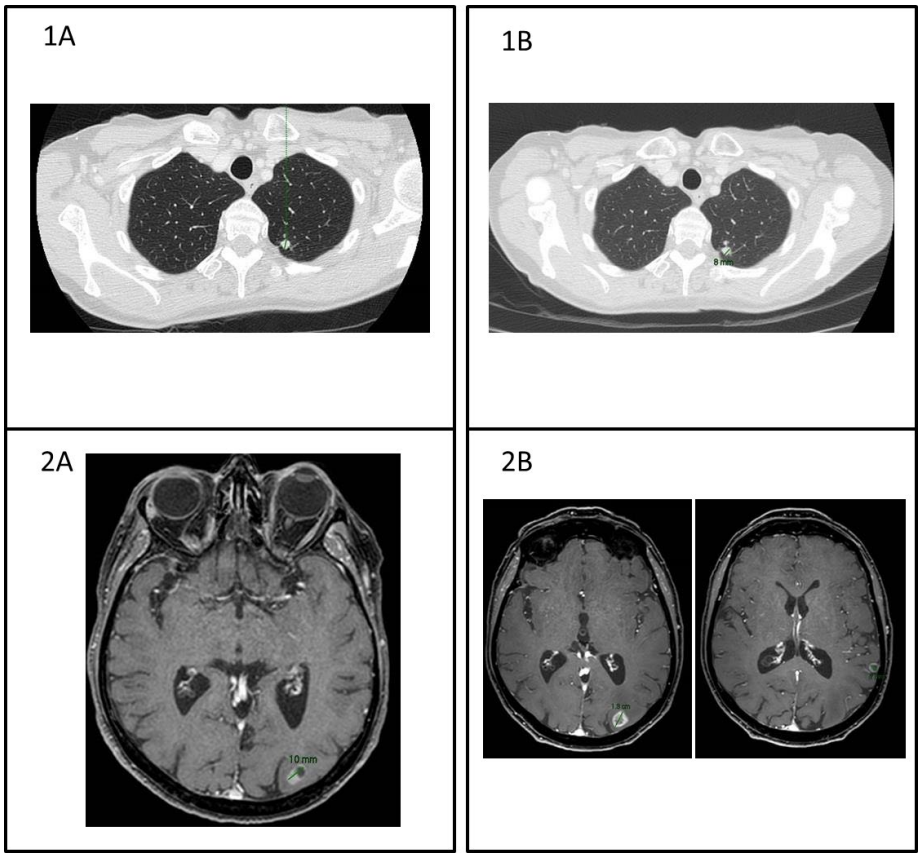

Figure 1.3.1 CT scan.
1A/2A: The patient was treated for more than one year with olaparib and carboplatin followed by olaparib monotherapy. CT chest showed an ongoing partial response, however, she was newly diagnosed with a brain metastasis.
1B/2B: after almost 3 years of treatment there was still an ongoing systemic response. Unfortunately, she developed a new brain metastasis.

Background PARP inhibitors

The past decade PARP inhibitors have been developed for the treatment of breast and ovarian cancer either as monotherapy or in combination with other anticancer drugs. PARP is a family of proteins involved in the repair of DNA single strand breaks (SSB). As a result of PARP inhibition, accumulation of the SSBs occurs, which can lead to DNA double strand breaks (DSB)[2]. Cells that have a homologous repair deficiency (HRD), like cells that are *BRCA* mutant, are not able to repair DSBs error free. This can ultimately lead to cell death. When a combination of deficiencies in two genes leads to cell death, but the deficiency in one gene does not, it is called synthetic lethality[3]. There are 17 PARP family members identified, of which PARP-1, PARP-2 and PARP-3 play a role in DNA damage repair. PARP inhibitors like olaparib, veliparib, niraparib, talazoparib and rucaparib have been studied extensively the past years[4-8]. This has resulted in the registration of several agents in this class for different indications. Olaparib has been licensed for the treatment of patients with deleterious or suspected deleterious (*g*)*BRCA*-mutated advanced ovarian cancer, for maintenance treatment of adult patients with recurrent epithelial ovarian, fallopian tube, or primary peritoneal cancer, who are in complete or partial response to platinum-based chemotherapy. Last year, it was also approved for the treatment of metastatic *BRCA* mutated breast cancer, based on the results of the OlympIAD trial, where olaparib monotherapy showed a significant benefit over standard therapy. The median progression-free survival (PFS) was significantly longer in the olaparib group compared to the standard-therapy group (7.0 months vs. 4.2 months; hazard ratio for disease progression or death, 0.58; 95% confidence interval, 0.43 to 0.80; p<0.001)[9]. Niraparib has been approved for maintenance treatment of patients with platinum-sensitive, recurrent epithelial ovarian cancer, fallopian tube or primary peritoneal cancer. Approval was based on a randomized trial that showed a statistically significant improvement in PFS in the niraparib group compared with placebo regardless of the presence or absence of *gBRCA* mutations or HRD status (21.0 vs. 5.5 months in de *gBRCA* cohort, 12.9 vs. 3.8 months in the non-*gBRCA* cohort with tumors with HRD and 9.3 months vs. 3.9 months in the overall non-*gBRCA* cohort; P<0.001 for all three comparisons)[10]. More recently, talazoparib, a relatively unknown PARP inhibitor, was approved for gBRCA mutated advanced or metastatic breast cancer, based on the EMBRACA trial, which showed a significantly longer PFS in favor of the

talazoparib group[11]. The Food and Drug Administration (FDA) granted Orphan Drug Designation to veliparib, being investigated in combination with chemotherapies, such as carboplatin and paclitaxel, or radiation for the treatment of advanced squamous non-small cell lung cancer (NSCLC)[12]. Despite these different registered indications, patients with brain metastases from breast-and ovarian cancer are usually excluded from treatment with PARP inhibitors or if allowed with non-symptomatic brain metastases, no additional information is available regarding this subgroup. In this viewpoint we will provide a concise overview of the incidence of brain metastases in breast-and ovarian cancer, the role of the blood-brain barrier and discuss the different PARP inhibitors and their efficacy in treating brain metastases.

Brain metastases from breast and ovarian cancer

Between 10-30% of all breast cancer patients develop brain metastases[13]. Young age, high grade, tumor size ≥ 5cm, human epidermal growth factor receptor 2 (HER2)-positive and estrogen receptor (ER)-negative disease are factors associated with the development of brain metastases[14]. Patients with *BRCA*-mutated breast cancer are more likely to develop brain metastases in a short time interval compared with *BRCA* negative controls[15]. Due to more advanced imaging techniques to detect brain metastases earlier and the introduction of novel systemic therapies which results in longer survival from the primary breast cancer, the incidence of brain metastases is increasing[16]. Brain metastases in ovarian cancer on the other hand are rare, with a reported average incidence of 2.5%[17]. The majority of the ovarian cancer patients developing brain metastases have high stage disease (according to the International Federation of Gynecology and Obstetrics (FIGO) staging). Treatment options for brain metastases in ovarian cancer are similar to those of breast cancer: whole brain radiotherapy, stereotactic radiotherapy, surgery, systemic chemotherapy or a combination of those. The median survival after diagnosis of ovarian cancer brain metastases is 8.2 months, based on a systemic review which included 57 studies[17]. With the registration of several PARP inhibitors, and the promising clinical responses observed, the obvious question raises, if these compounds could also be used to treat patients with brain metastases from breast and ovarian cancer, which usually have a poor prognosis[17,18].

Blood brain barrier

When systemically treating brain metastases with chemotherapy, the blood brain barrier (BBB) plays an important role. The BBB consists of a tight layer of endothelial cells. It forms a selective barrier for systemic treatments to enter brain tissue. In the BBB drug-uptake and drug-efflux transporters are present, like P-gp and Breast Cancer Resistance Protein (BCRP)[19]. Transport takes place by passive diffusion or by active transport. Drugs that are small molecular lipophilic compounds can pass more easily the barrier, compared to large hydrophilic drugs[20]. The efflux transporters, like P-gp can transport anticancer agents such as vinblastine and paclitaxel out of the endothelium and thereby prevent uptake into brain tissue[21,22]. Several new anticancer drugs are also substrate for the efflux transporters and do not penetrate brain tissue and therefore seem not eligible for treating brain metastases[23]. The function of the BBB in metastatic disease is however controversial and may have significant implications for new treatment strategies[24]. Based on animal data and limited clinical data, the suggestion arises that the BBB can be compromised in the presence of overt metastatic disease. Animal studies showed the role of tumor size in the BBB permeability. Tumors larger than 0.5 mm were shown to be permeable to fluorescein, a marker for BBB permeability. They also found in more than 70% of the tumors larger than 0.5 mm signs of central necrosis indicating an ischemic environment[25]. This is in line with the finding that metastatic brain tumors have a lower density of blood vessels compared to the surrounding tissue, most prominently in the central region of the tumor[26,27]. Blood vessels at the outer margin of the tumor might be more dense, but these vessels are still abnormal[26]. As a result of the compromised BBB, drugs could more easily reach the target in the brain. On the contrary, another possibility is that the BBB remains intact or mostly intact at the infiltrating edge of the tumor so in case of disrupted BBB, the drug is only delivered at the necrotic center part of the tumor[24]. Nevertheless, the BBB is considered a hurdle and challenge in the development of effective treatment options for brain metastases. Regarding the question whether PARP inhibitors could be effective in the treatment of brain metastases of breast and ovarian cancer, it is important to know if and if yes, to what extent the PARP inhibitor crosses the BBB and reaches the metastatic site. Table 1.3.1 gives an overview of the PARP-inhibitors mentioned in this viewpoint and their characteristics.

Veliparib

Veliparib is a potent, oral PARP-1 and PARP-2 inhibitor and has a good penetration into the brain[28]. In a study about PARP inhibitors as P-gp substrates, it was found that veliparib is no substrate of P-gp[29]. Veliparib was evaluated in rats in which drug concentrations were measured in plasma and in brain and brain tumor tissue. After multiple dosing of veliparib (50 mg/kg/day) the concentration of veliparib 2 hours after dosing (reaching Cmax) was 1.36 ± 0.16 μg/mL, 0.72 ± 0.12 μg/g, and 3.00 ± 0.16 μg/g in plasma, brain, and brain tumor tissues, respectively[28]. Drug concentration in brain tissue could be higher at later time point, but data is not available for later time points. In addition, veliparib was found to be a temozolomide sensitizer in a subset of Patient Derived Xenograft (PDX) models with glioblastoma (GBM)[30]. Preclinical models showed that veliparib improves cell death in combination with radiation therapy[28,31]. Veliparib inhibits PARP significantly as was shown in a phase 0 trial in patients with advanced malignancies[32]. A phase I trial of the combination of whole brain radiotherapy (WBRT) and veliparib showed a median survival time of 7.7 months in the breast cancer group compared to a nomogram-model-predicted median survival time of 4.9 months in the group treated with WBRT alone. There were no additional toxicities identified when combining veliparib and WBRT[33]. A phase 2 randomized trial evaluating the efficacy and safety of veliparib in combination with WBRT (versus placebo plus WBRT) in patients with metastases from NSCLC did not find a statistically significant difference in overall survival (OS), intracranial response rate, time to clinical or radiographic progression and adverse events between the groups[34]. This is in contradiction to the previous preclinical and early clinical data that suggested that veliparib might potentiate the efficacy of radiotherapy. Therefore, the benefit of administration of veliparib in patients with brain metastases remains uncertain. On clinicaltrials.gov one study (ClinicalTrials.gov Identifier: NCT02595905) is recruiting patients with recurrent or metastatic triple negative and/or BRCA mutated associated breast cancer with or without brain metastases, who will be treated with cisplatin with or without veliparib. This randomized phase II trial has a brain metastases cohort where they compare the efficacy of cisplatin with or without veliparib on PFS in these patients. This study will provide valuable information on the addition of veliparib for treating brain metastases from

patients with triple negative and/or *g*BRCA mutation-associated breast cancer.

Olaparib

The PARP inhibitor olaparib is an oral PARP-1 and PARP-2 inhibitor[35]. It has been studied in preclinical and clinical trials (9, 36-39). Olaparib was studied in two P-gp overexpressing drug-resistant cell lines. Both cell models were resistant to olaparib. The resistance appeared to be reversible with addition of a P-gp inhibitor like elacradir, zosuquidar and valspodar. Olaparib thus appears to be a P-gp substrate[29]. Results of a phase I trial of olaparib in combination with temozolomide in patients with relapsed GBM also showed that olaparib was a substrate for P-gp. The efflux of olaparib was blocked by ketoconazole (P-gp inhibitor). Olaparib was detected in 24/24 resected GBM tissues from eight patients treated with olaparib and ketoconazole. The concentrations found in tumor tissue in this trial were similar to those found in previous studies[40]. Despite the relatively high olaparib concentrations in these resected GBM tissues, other data suggest limited penetration of olaparib into the brain with minor to moderate tumor activity[41]. This is probably due to a high efflux rate for olaparib by the P-gp transporter. In literature one case report about a patient with a sustained clinical and radiological response to olaparib for leptomeningeal metastases with BRCA2 mutated ovarian cancer was found[42]. Another case report describes a Japanese patient who had a persistent clinical and radiological response on olaparib treatment for brain metastases from primary peritoneal cancer. This patient received both WBRT and olaparib, so it is difficult to conclude that the response was due to olaparib treatment, but the brain metastases were still decreasing 22 months after starting the olaparib therapy[43]. Since there are only a few reports on the treatment of brain metastases with olaparib, the evidence is thin. There are currently multiple trials investigating olaparib as monotherapy or in combination with several different anti-cancer agents for different tumor types, according to clinicaltrials.gov. In some studies, the presence of brain metastases is not mentioned in the in-or exclusion criteria of the trial. In others, symptomatic uncontrolled brain metastases are an exclusion criterion. In all of these latter studies there is no additional information available on how these brain metastases will be evaluated and if there are any planned analyses on this

patient group. This is unfortunate because knowing the value of olaparib in treating brain metastases would be important information.

Talazoparib

Talazoparib is a novel inhibitor of PARP-1 and PARP-2 and in addition to the PARP inhibitory effect, talazoparib is the most potent PARP inhibitor in trapping PARP-DNA complexes, contributing to cell death[44]. Talazoparib combined with temozolomide was investigated in GBM models. The average brain and plasma talazoparib concentrations at 2 hours after a single dose (0.15 mg/kg) were 0.49±0.07 ng/g and 25.5±4.1 ng/g, respectively, indicating that a small amount of talazoparib enters the brain tissue. The brain/plasma ratio in P-gp knock out mice was higher than in wildtype (WT) mice (0.23 vs. 0.02, $p<0.001$). This result indicates that talazoparib is a substrate for P-gp which restricts delivery across the blood-brain barrier[45]. A first in human phase I trial showed single agent antitumor activity and a tolerable safety profile. The maximum tolerable dose (MTD) was found to be 1 mg/day. At this dose, responses were observed in 50% of the breast cancer and in 42% of the ovarian cancer patients[46]. However, in this study patients with brain metastases were excluded. In the phase 3 EMBRACA trial, the efficacy and safety of talazoparib were compared with standard chemotherapy of physician's choice for the treatment of locally advanced or metastatic *g*BRCA1/2 breast cancer. Patients with CNS metastases were eligible if they had stable CNS lesions on repeat brain imaging and were receiving low-dose or no glucocorticoids. The objective response rate (ORR) of patients treated with talazoparib with a history of CNS metastases (n=38) was similar to the patients without a history of CNS metastases (n=181) (ORR 63.2% versus 62.4%). However, no data is available on the response of the CNS metastases to talazoparib[11]. Clinicaltrials.gov shows several clinical trials recruiting patients for treatment with talazoparib, in which patients with active or symptomatic known brain metastases are excluded. This means patients with adequately treated, non-symptomatic brain metastases are allowed to be included in these trials. None of the trials is primarily focusing on patients with brain metastases and it is unclear if response of brain metastases on talazoparib will be monitored.

Table 1.3.1 Overview of PARP-inhibitors, brain to plasma ratio and available clinical data.

PARP-inhibitor	P-gp and/or BCRP substrate	Brain/plasma ratio (reference)	Clinical data	References
Veliparib	no	0.53 (28)	Preclinical: Improves cell death in combination with radiotherapy Phase I: Improved median survival of WBRT + veliparib compared to prediction of efficacy of WBRT alone. Phase II: no difference in OS, response rate, time to clinical or radiological progression between veliparib and WBRT and WBRT alone in NSCLC.	[28,31] [33] [34]
Olaparib	yes	0.03 (52)	Case report: sustained clinical and radiological response to olaparib for leptomeningeal metastases with BRCA2 mutated ovarian cancer. Case report: persistent clinical and radiological response on olaparib + WBRT for brain metastases from peritoneal cancer.	[42] [43]
Talazoparib	yes	0.02 (45)	Phase III: EMBRACA trial shows comparable ORR of pts treated with talazoparib with or without history of CNS metastases. No data on response of CNS metastases itself.	[11]
Rucaparib	yes	unknown	Patients with brain metastases were excluded from clinical trials.	[51]
Niraparib	no	0.3 (52)	No clinical studies have been performed to evaluate the effect of niraparib on brain tumors. Ongoing studies that allow pts with known brain metastases, unknown if subgroup analyses will take place.	-

Rucaparib

Rucaparib is an orally available, potent small molecule inhibitor of the PARP-1 and PARP-2 enzyme. In a preclinical study it was investigated whether the efflux transporters BCRP and P-gp have influence on the bioavailability and brain penetration of rucaparib in mice. It was shown that both P-gp and BCRP restrict the brain accumulation of rucaparib[47]. Parrish and colleagues performed a pre-clinical evaluation of rucaparib in combination with temozolomide in GBM xenograft models. Their results demonstrate that rucaparib is excluded from the CNS by the BBB and has limited efficacy in an orthotopic GBM xenograft model. Brain accumulation of rucaparib was significantly increased in mice knockout for P-gp and BCRP, showing that rucaparib accumulation within the CNS is limited due to P-gp and BCRP activity at the BBB[48]. Rucaparib has been studied in multiple clinical studies, as monotherapy and in combination with cytotoxic chemotherapy agents like carboplatin or temozolomide[49,50]. Clinical trials, like the phase II multicenter study by Drew et al excluded patients with brain metastases, probably because of the lack of efficacy shown in preclinical data due to the influence of efflux transporters on the brain rucaparib accumulation[51]. Rucaparib is currently investigated in combination therapy regimens with for example immunotherapy. In these studies, found at clinicaltrials.gov, patients with known (active) CNS disease are excluded for participation.

Niraparib

Niraparib is an oral bioavailable PARP-1 and PARP-2 inhibitor. Previous data have indicated that niraparib is able to penetrate the brain in rodents and has sufficient exposure to have therapeutic effect in an intracranial *BRCA* mutated human xenograft model[41]. In a preclinical study, the exposure of niraparib and olaparib was investigated in MDA-MB-436 triple negative breast cancer (TNBC) xenograft models. The brain to plasma exposure (AUC) ratio of niraparib and olaparib was significantly different (niraparib ~0.3, olaparib ~0.03). Furthermore, the niraparib exposure was highly sustainable during time. Niraparib appears to be a substrate for P-gp, although the efflux rate of niraparib is much lower compared to the efflux rate of olaparib. As a result, niraparib could lead to higher brain tissue exposure and retention compared to olaparib[52]. Furthermore, niraparib showed 53%

tumor growth inhibition (TGI), tested in mice bearing subcutaneously inoculated Capan-1 tumor cells, which is a higher TGI compared to olaparib. The safety and recommended phase II dose (RP2D) were demonstrated in a phase I trial in solid tumors. The RP2D of 300 mg/day was well tolerated[53]. The FDA approval of niraparib was based on a double-blind, randomized, placebo-controlled trial (NOVA) in which 553 patients with platinum-sensitive recurrent epithelial ovarian, fallopian tube or primary peritoneal cancer were randomized to receive niraparib or placebo. It is not clear whether the increased CNS penetration (as showed above, compared to olaparib) is associated with clinical activity in patients with brain metastases[54]. To our knowledge no clinical studies have been performed to evaluate the effect of niraparib on brain tumors. There are ongoing studies with niraparib as monotherapy or in combination regimens with for example everolimus, that allow inclusion of patients with known, not symptomatic brain metastases. For these studies it is unknown if the patients with stable brain metastases at start of treatment will be studied in a subgroup analysis regarding the response on niraparib of their brain metastases.

Discussion

PARP inhibitors are moving forward towards registration for several indications, including advanced breast- and ovarian cancer. The incidence of brain metastases in ovarian cancer is low but the incidence in breast cancer is higher, especially in *BRCA* mutated breast cancer. Unfortunately, treatment options for patients with brain metastases from breast-or ovarian cancer are limited[17,18]. Since the life expectancy of cancer patients increases, due to better diagnostics and more and better treatment options, the cumulative risk for the development of brain metastases increases as well. Therefore, there is an unmet need for alternative treatments to treat these CNS metastases.

The patient described in this viewpoint has advanced breast cancer with brain metastases. She has an ongoing systemic response on treatment with olaparib and carboplatin for many years now. However, she developed multiple brain metastases during treatment with these compounds. On the one hand she responds very well on the systemic level, on the other hand she develops new brain metastases during olaparib with carboplatin therapy. This suggests that either the treatment does not reach the target in the

brain, or the brain metastasis are not sensitive to this treatment. Since olaparib is a substrate for P-gp, low drug exposure in brain tissue might explain this. However, this patient has advanced disease of which we assume that patients with metastatic disease have a compromised BBB, indicating that there would be less obstacles for the drug to reach target. In this patient, also the decreased density of blood vessels in the center of the tumor could have a negative effect on the drug delivery in the tumor. Whatever the reason, the net effect here has not resulted in reduction of the patient's brain metastases. An additional question is, if carboplatin could have contributed to a reduction of the brain metastases. Carboplatin is a water-soluble chemotherapy agent with a molecular weight of 371 g/mol. This results in impaired delivery of carboplatin across the BBB. Several strategies have been developed to increase drug delivery of carboplatin across the BBB. A randomized controlled trial of carboplatin and the bradykinin analog RMP-7 versus carboplatin and placebo has been performed. RMP-7 temporarily increases the permeability of the BBB to chemotherapeutic drugs. In this phase II study the use of RMP-7 did not improve the efficacy of carboplatin. This was in contrast to a preclinical study where the infusion of RMP-7 before carboplatin infusion led to significant uptake effects of carboplatin[55]. It is unlikely that carboplatin would have had any additional value in the treatment of brain metastases of our patient.

During WBRT the olaparib therapy was interrupted because of protocol requirements. Since there is evidence that PARP inhibitors could sensitize brain tumors to radiotherapy, it would be interesting to know to what extent olaparib could have contributed to tumor inhibition if it would have been continued during radiation therapy. For this patient, it was decided to resume olaparib and carboplatin after WBRT, but stopping treatment because of new brain metastases was also considered. Because of the ongoing clinical benefit and the good tolerability, it was decided to continue treatment with olaparib and carboplatin.

Based on our literature review, most PARP inhibitors (olaparib, talazoparib, rucaparib and to a lesser extent niraparib), appear substrates for efflux transporters like P-gp and thereby exhibit poor penetration into brain tissue. As a result, these PARP inhibitors seem less attractive options for treating brain metastases. Of note, it was shown in preclinical data that niraparib is a more potent tumor growth inhibitor in brain tissue compared to olaparib (Table 1.3.1)[52]. Veliparib is no substrate for P-gp, what makes this

potentially a more attractive compound for treating brain metastases. Data regarding the efficacy of veliparib for treating brain metastases however remain contradictory (Table 1.3.1), making treatment choice difficult. Of all PARP inhibitors, veliparib and niraparib probably show the most advantages for treatment of brain metastases, however evidence is thin.

To learn more about the potential of PARP inhibition treatment for brain metastases there are, in our opinion, three possible options. First, collection of data on patients with asymptomatic brain metastases that have been enrolled in clinical trials with PARP-inhibitors. Looking into these patients as a subgroup would give important information on the response of their brain metastases on PARP inhibitor treatment. Secondly, specific clinical trials for treating patients with brain metastases with PARP-inhibitors could be initiated. Regarding the evidence found in literature, veliparib and niraparib would be the most suitable PARP-inhibitors to investigate in these trials. Thirdly, because the efflux ratio seems to play an important role in the tissue accumulation, an option could be to investigate the addition of a P-gp inhibitor to the treatment with PARP inhibitors to counteract efflux of the PARP inhibitor by P-g, but risk of relatively high concentrations of the PARP inhibitor in the CNS then exists, which requires very careful application. Clearly, more research regarding this topic is warranted to improve the outlook of breast and gynecological patients with brain metastases.

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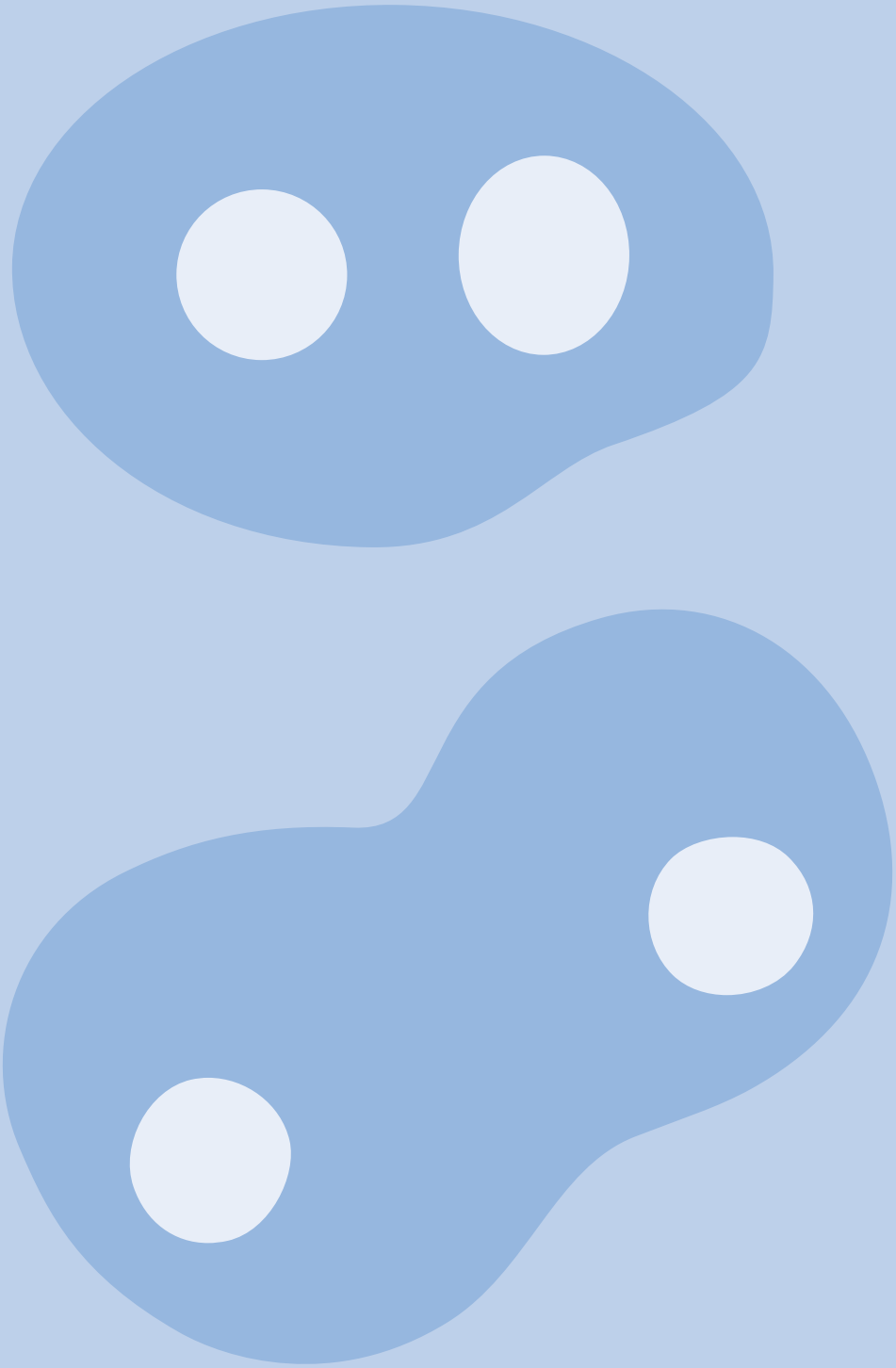
Chapter 2

Wee-1 inhibition



Chapter 2.1

Molecular Pathways: Targeting the Protein Kinase Wee1 in Cancer

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Summary

Wee1 is a protein kinase that regulates the G2 checkpoint and prevents entry into mitosis in response to DNA damage. Cyclin-dependent kinases (CDKs) are a family of 14 serine/threonine protein kinases, which coordinate the progression through the cell cycle. The Cdc2/cyclin B complex controls the progression from G2 into mitosis. There are two mechanisms by which the G2 checkpoint is initiated in response to DNA damage: phosphorylation of Cdc25c by CHK1 and of Wee1 kinase, which phosphorylates Cdc2. Blockade at the G2 checkpoint is especially important for p53 mutant cells because these tumors mainly rely on DNA repair at the G2 checkpoint. AZD1775 (formerly MK-1775) is a small molecule pyrazol-pyrimidine derivative and potent and ATP-competitive specific inhibitor of the Wee1 kinase. Several preclinical and clinical studies demonstrated encouraging anti-tumor effects with manageable side effects of the combination of Wee1 inhibition and DNA-damaging agents. Promising combination schedules are being investigated at the moment, e.g. combining PARP-inhibition and Wee1 inhibition. Also a weekly schedule with carboplatin and AZD1775 warrants investigation aimed at further improving the anti-tumor effect.

Background

Wee1 is a protein kinase that regulates the G2 checkpoint and prevents entry into mitosis in response to DNA damage (Figure 2.1.1)[1]. The cell cycle is a highly controlled process. There are several mechanisms by which cells can modulate progression through the cell cycle in case of DNA damage or other factors that affect DNA replication. There are cell-cycle checkpoints that provide cells time to repair damaged DNA before transmission into mitosis[2]. Cyclin-dependent kinases (CDKs) are a family of 14 serine/ threonine protein kinases, which coordinate the progression through the cell cycle[3]. The progression from G2 into mitosis is controlled by the Cdc2/cyclin B complex, also known as CDK1/cyclin B. This complex is activated by dephosphorylation of tyrosine 15 (Tyr15) on Cdc2 by the phosphatase of Cdc25c[4]. There are two mechanisms by which the G2 checkpoint is initiated in response to DNA damage. First there is the phosphorylation of Cdc25c by CHK1, which leads to its degradation[5]. As a result, the activation of the Cdc2/cyclin B complex is prevented. Second, inactivation of the Cdc2/cyclin B complex takes place by phosphorylation of Wee1 kinase[6]. This blockade at the G2 checkpoint is especially important for p53 mutant cells. P53 wild-type cells have the opportunity to arrest the cell cycle at the G1 checkpoint in order to repair damaged DNA. Cells with a defective p53 pathway rely mainly on DNA repair at the G2 checkpoint[7]. Since p53 mutant cells rely on the G2 checkpoint for DNA damage control, several small molecule inhibitors of the G2 checkpoint have been developed, which sensitize mostly p53 mutant tumor cells to DNA-damaging agents[8,9]. Ataxia-telangiectasia mutated (ATM) protein kinase or ataxia-telangiectasia-related (ATR) protein kinase pathways are activated in the presence of DNA damage[1]. ATM is activated by stress factors that result in double strand breaks. ATM activates CHK2, resulting in phosphorylation of Cdc25c. Suppression of Cd25c leads to inhibition of phosphorylation of the CDK1/cyclin B complex[10]. ATR is activated by stress factors that result in single strand breaks[11,12]. ATR plays a role in the activation and phosphorylation of CDK1. CDK1 phosphorylates Wee1 and Cdc25c, which results in activation of the Wee1 kinase and inactivation of the Cdc25c phosphatase activity. Next, Wee1 phosphorylates and inactivates the CDK1/cyclin B complex on tyrosine 15, resulting in cell-cycle arrest in G2 and time for DNA damage repair. Overexpression of Wee1 has been reported in several cancer types, like breast (luminal, HER2 positive)[13,14], ovarian[15],

colorectal[16], gastric[17], malignant melanoma[18] and sarcoma[19]. In ovarian cancer, melanoma and glioma tumors, high expression of Wee1 is associated with poor outcome.

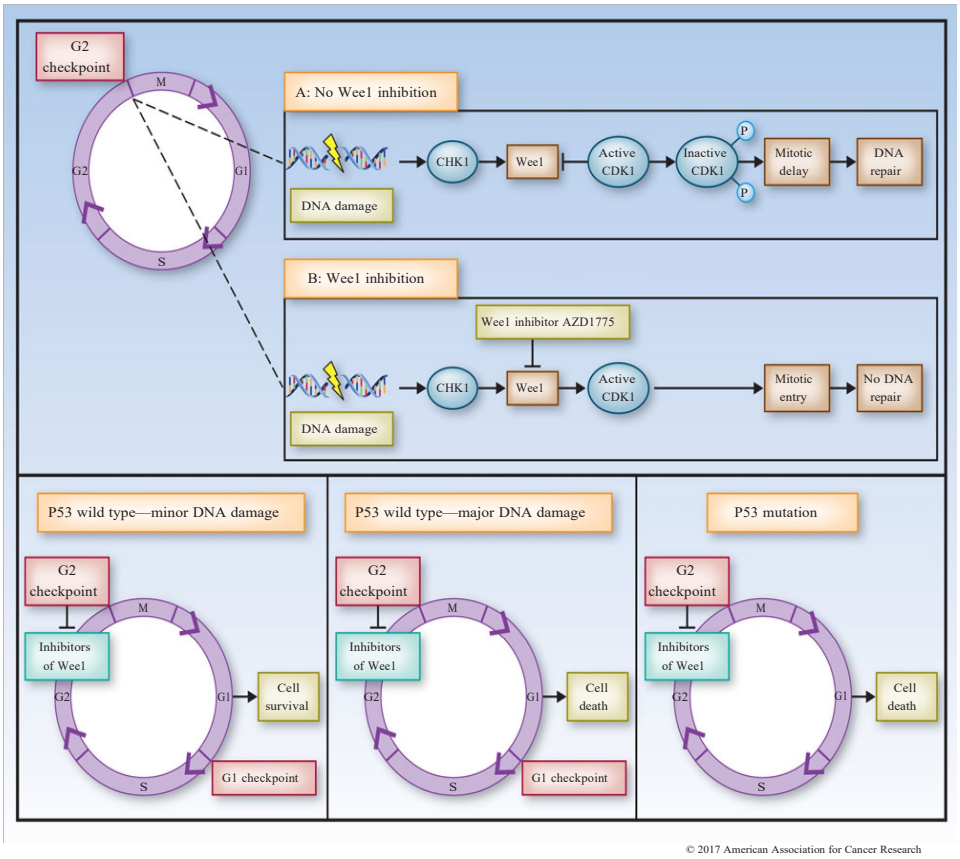

Figure 2.1.1 Involvement of Wee1 in G_2–M regulation and possible consequences of Wee1 inhibition in p53-mutant cells upon exposure to DNA damage.

Clinical-Translational advances

AZD1775 (formerly MK-1775), a pyrazol-pyrimidine derivate, is a potent and ATP-competitive specific small molecule inhibitor[20,21] of the Wee1 kinase. An IC_{50} of 5.2 nM has been reported[20]. *In vivo*, AZD1775 has a relatively short terminal half-life (t1/2) ranging from 9 to 12 hours. Results of a study of Cuneo et al demonstrated that sensitization to radiation by Wee1 inhibition occurred in both p53 mutant and wild-type cells. They showed that in two

cell lines with TP53 null and TP53 mutant cells there was an increase in histone H3 phosphorylation, indicative of G2 checkpoint abrogation, leading to early mitosis. In addition, in TP53 wild-type cells that were treated with AZD1775 there was a minimal effect on histone H3 phosphorylation. This was probably related to the fact that these cells are able to arrest at the G1 checkpoint[22]. However, this study shows no proof for the functionality of the entire p53 pathway. Also other studies that have demonstrated activity of Wee1 inhibition in p53 wildtype tumors did not show the functionality of the whole p53 pathway. Another possible explanation why p53 wildtype tumors could benefit from Wee1 inhibition is the existence of an alternative mechanism for the synthetic lethality caused by p53 mutation and Wee1 inhibition. Kato et al described that aberrations in the CDKN2A locus, frequently present in diverse tumor types, can contribute to dysregulation of the G1-M checkpoint that could lead to synthetic lethality when combined with Wee1 inhibition[23]. *In vitro* studies showed that simultaneous treatment of a Wee1 inhibitor and for example gemcitabine or Wee1 inhibition followed by gemcitabine resulted in increased cell death. In comparison, sequential treatment with first gemcitabine followed by Wee1 inhibition increased cell death to a greater extent. These data suggest that optimal treatment is sequential administration of first the DNA damaging agent, followed by the Wee1 inhibitor. This makes sense because the mechanism of action of the DNA damaging agents is induction of DNA damage. When followed by Wee1 inhibition this will lead to not (fully) repaired DNA due to lack of cell-cycle arrest. The anti-tumor effect of AZD1775 in combination with carboplatin was investigated in a rat xenograft model with human cervical adenocarcinoma cells. AZD1775 (doses of 10, 20 and 30 mg/kg) was administered 24 hours after carboplatin (50 mg/kg). AZD1775 dose-dependently enhanced the anti-tumor effect of carboplatin in these tumor models[20]. These anti-tumor effects were also found in clinical studies investigating combination therapies with DNA damaging agents and Wee1 inhibition. However, dosing schedules as used in preclinical research cannot be extrapolated directly to clinical trials. The dose used in *in vitro* studies is often high in comparison to the safe dose used in clinical trials; 50 mg/kg carboplatin in preclinical data would suggest an ultrahigh dose of approximately 3500 mg carboplatin for an average person. Hirai et al first reported increased sensitivity to various anti-tumor agents by co-administration of AZD1775 in an ovarian cancer cell line with TP53 mutation[20]. This study and following studies demonstrated that AZD1775

induces cell death and sensitizes p53 defective tumor cells in response to radiotherapy and to gemcitabine, carboplatin and cisplatin[20,21,24,25]. Kim et al investigated the role of Wee1 in gastric cancer. They conducted a combination treatment with AZD1775 and 5-fluorouracil (5-FU) and paclitaxel in gastric cancer cells and established a mouse model. The cells were treated with AZD1775 alone, 5-FU or paclitaxel alone or a combination of AZD1775 and 5-FU or paclitaxel. This study demonstrated that AZD1775 treatment alone is effective in reducing gastric tumor size, but combination therapy further reduced growth of the gastric tumors[17]. Leijen et al performed a phase II study of AZD1775 combined with carboplatin in patients with p53-mutated ovarian cancer patients. They included 24 patients and achieved an overall response rate of 43% (95% CI, 22% to 66%), including one patient with a prolonged partial response. Patients were platinum refractory, or resistant with progression within three months after the end of first line standard carboplatin-paclitaxel therapy. Leijen et al showed evidence that AZD1775 enhances carboplatin efficacy in *TP53*-mutated tumors[22]. Other studies demonstrated that sensitization of tumor cells also occurred in p53 wild-type tumor cells[26]. AZD1775 demonstrated anti-tumor activity as a single agent in both p53 wild-type as well as in p53 mutant tumors. However, in the wild-type cells integrity of the entire p53 pathway was not demonstrated. Further studies are warranted to demonstrate if and to what extent Wee1 inhibition is dependent on p53 mutation status and p53 pathway integrity. Hirai and colleagues investigated several dosing schedules of AZD1775 in combination with 5-FU or capecitabine. They tested once weekly, twice weekly and five times weekly schedules. However all schedules resulted in enhancement of the anti-tumor effect of 5-FU, whereby both the twice weekly and the five weekly schedules seemed to be more effective[27]. The relatively short half-life, ranging from 9 to 11 hours, as well as the results of preclinical data suggest that dosing multiple times per week would lead to enhanced anti-tumor effect. As a result, the 2.5 days treatment was introduced.

Combination therapy of DNA damaging agents with Wee1 inhibition shows promising results. Question is, whether a Wee1 inhibitor could be administered as single dose to achieve (comparable to combination therapy) anti-tumor activity as well. Krealing et al showed that AZD1775 is effective as monotherapy in sarcoma cells independent of the p53 status. Although there was no p53 mutation, it was not clear whether the functionality of the entire p53 pathway was undisturbed. They found a similar level of cell death in

cells with a defective p53 system, p53 null and p53 wild-type cells. Guertin, who conducted a preclinical evaluation of AZD1775 as single-agent anticancer therapy, found similar results. In the applied non-small cell lung cancer (NSCLC) xenograft model, treatment with AZD1775 resulted in a decrease of approximately 50% compared to the initial tumor volume. These anti-tumor effects were also observed in additional xenograft models[28]. Do et al conducted a phase I study of single agent AZD1775 in patients with refractory solid tumors. The dosing schedule was based on previous combination trials with AZD1775 and chemotherapeutic agents. The MTD was found to be 225 mg BID over 2.5 days per week for 2 weeks per 21-day cycle. Toxicities at this dose-level were manageable and mainly grade 1-2 according to the Common Terminology Criteria for Adverse Events (CTCAE). There were two partial responses shown in patients with a BRCA mutation. Min and colleagues found that Poly (ADP) ribose polymerase (PARP) binding to Chk1 at stalled replication forks is needed for S-phase checkpoint activation[29]. PARP-inhibitors like olaparib play a pivotal role in the repair of DNA single strand breaks. BRCA 1/2 mutant or other homologous recombination deficient cancers lack the ability to properly repair double strand breaks. The combination of a PARP inhibitor and an impaired homologous recombination system, e.g. by BRCA 1/2 mutation, leads to synthetic lethality. Radiosensitization with PARP inhibition is more effective in cells with double strand break repair defective tumors. Wee1 indirectly inhibits homologous recombination repair (HRR). Karnak et al performed a study to test the combination of AZD1775 and olaparib in pancreatic cancer as a radiosensitizing strategy. They found indeed that the combination produced significantly more radiation damage in pancreatic cells than inhibition of Wee1 or PARP alone. They showed that Wee1 inhibition leads to inhibition of HRR and abrogation of the G2 checkpoint combined with olaparib. Further investigation in clinical trials of this combination is warranted[30]. Another strategy for combination therapy could be weekly carboplatin and AZD1775. Increasing evidence shows that administration of a platinum compound in a 'dose-dense' schedule, which includes more frequent scheduling with increased dose intensity, could lead to improvement of anti-tumor effect and decreased resistance[31]. By more frequent carboplatin administration, AZD1775 should be administered also more frequently to achieve a sequential administration of both. There is no evidence yet, if lower and more frequent dosing of Wee1 would lead to effective exposure in the tumor. A pharmacokinetic and -dynamic study of

weekly carboplatin and Wee1 should be performed to investigate the systemic exposure to both drugs. Secondary the safety and preliminary anti-tumor activity could be studied. Currently there are several clinical trials ongoing with AZD1775 in different tumor types. AZD1775 is being combined with irinotecan, radiation therapy, docetaxel, cytarabine and other anti-cancer drugs and is being studied in colorectal cancer, lung cancer, acute myeloid leukemia and pancreatic cancer (clinicaltrials.gov, last visit 27 JAN). An optimal dose-schedule biomarker has not been found yet. Another promising biomarker would be a marker to test the p53 pathway in its entirety. Not only p53 mutant cells but also cells with phenotypical loss of p53 function could be found. As a result, Wee1 inhibition could be wider applied and more patients could have benefit from this treatment.

Toxicity of Wee1 inhibitors

The toxicity profile of AZD1775 was first studied in rats and showed that the majority of organs that were affected were proliferation-dependent organs such as lymphoid and hematopoietic organs and the gastrointestinal tract. By the end of a 2-week or longer recovery period there was evidence of reversibility. Based on these studies and the histomorphological examination of the bone marrow, it is expected that hematological changes will fully recover. Leijen et al found that monotherapy given as single dose was well tolerated and the maximum tolerated dose (MTD) was not reached. The most common adverse events in the combination part (with gemcitabine, cisplatin or carboplatin) where fatigue, nausea, vomiting, diarrhea and hematologic toxicity[25]. The subsequent phase II study with AZD1775 combined with carboplatin in TP53 mutant platinum refractory or resistant ovarian cancer patients, demonstrated also manageable toxicity. The most common adverse events were overall manageable (CTCAE grade 1-2) and were mostly fatigue (87%), nausea (78%), thrombocytopenia (70%), diarrhea (70%), and vomiting (48%)[32]. Based on the safety data from six completed clinical trials and preliminary data from ongoing studies, mainly hematological disorders should be observed closely. Next, this promising combination will be applied to other TP53 mutant tumor types. It is of interest to test the activity and safety of a schedule intensive weekly carboplatin-AZD1775 combination with the aim to further increase the efficacy of the combination.

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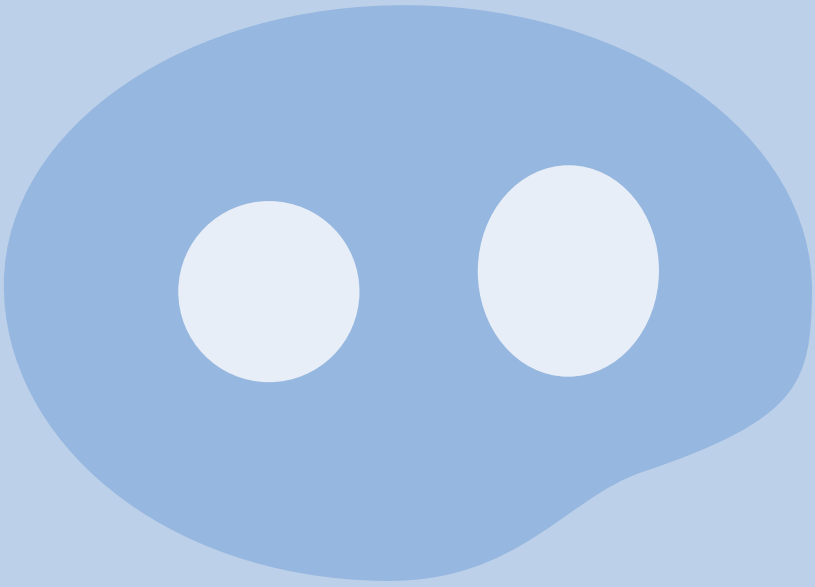

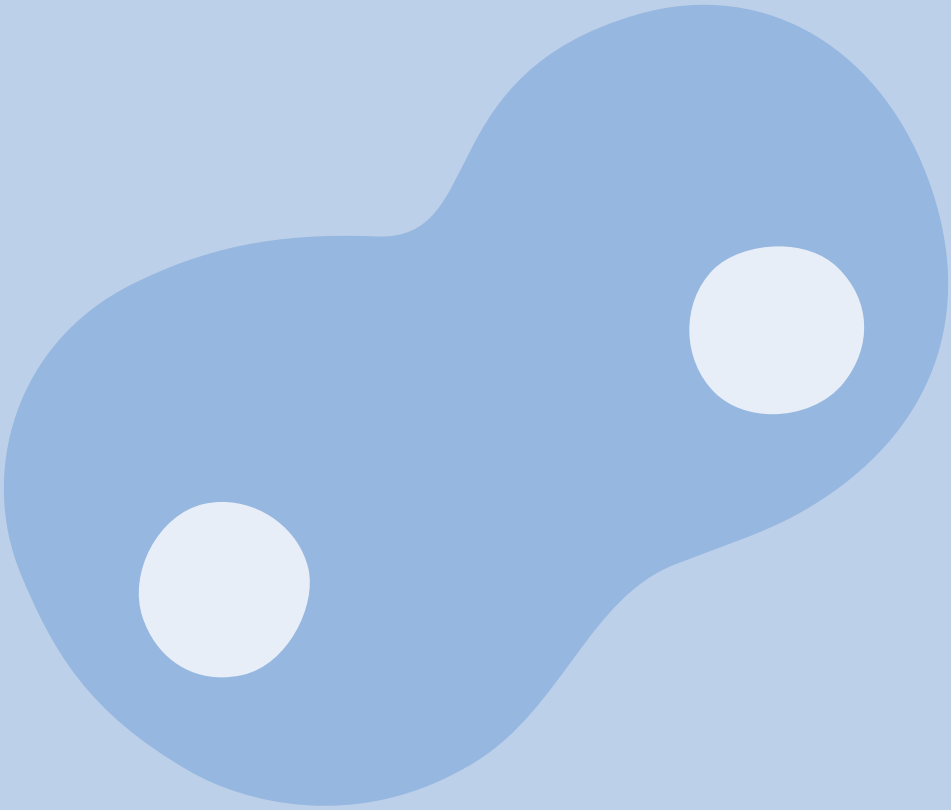

Chapter 2.2

Wee-1 inhibitor AZD1775 in advanced p53 mutated ovarian cancer–interim analysis

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Manuscript in preparation

Summary

Background
AZD1775 is a potent and selective inhibitor of Wee-1 kinase. Wee1 is a tyrosine kinase that regulates the G2 checkpoint and prevents entry into mitosis in response to DNA damage. In the first part of the current study, it was shown that the combination of AZD1775 and carboplatin was safe and effective in patients with p53 mutated ovarian cancer. The aim of this report this paper was to present an update of the safety and efficacy in of the combination of AZD1775 and carboplatin in p53 mutated platinum resistant or refractory ovarian cancer.

Methods
Phase II, open-label, non-randomized study. Patients received carboplatin intravenously with a target area under the curve (AUC) of 5 mg/ml·min in a 30-minute infusion, combined with AZD1775 225 mg orally twice day for 2.5 days (the third day only 1 administration) in a 21-day cycle. The primary end point of this additional cohort was to assess safety and preliminary anti-tumor activity (according to RECIST 1.1) of AZD1775 in combination with carboplatin in *p*53 mutated epithelial ovarian cancer in a 21-day schedule.

Results
To date, 10 patients were enrolled into the additional safety and activity cohort and started treatment (Table 2.2.1). Two patients were not evaluable for safety and efficacy. The median age of the patients was 59 years (range, 42 to 70 years). The majority of the patients (75%) had an Eastern Cooperative Oncology Group performance status of 0. Bone marrow toxicity (thrombocytopenia, anemia and neutrophil count decrease), nausea, vomiting and fatigue were the most common adverse events. Grade 3 and 4 thrombocytopenia and/or grade 4 neutropenia resulted in dose reductions. Five patients (68%) had a partial response as best response.

Conclusion
AZD1775 225 mg 2.5 days and carboplatin target AUC 5 could be safely administered to patients with refractory or resistant ovarian cancer. This combination shows a promising anti-tumor effect. However, bone marrow toxicity has led to dose-reductions and dose delays in 5 patients. This remains a point of attention combining AZD1775 and carboplatin.

Introduction

Ovarian cancer is the most common cause of death among women with gynecologic malignancies[1]. The majority of ovarian cancers are of epithelial origin (90%). These patients are often diagnosed in late-stage disease. Despite high initial responses (>80%) in first line treatment with platinum compounds in combination with taxanes after debulking surgery, the overall prognosis is poor because most patients relapse due to the development of tumors that are resistant to the initially used agents[2]. Approximately 25% of the epithelial ovarian cancers are platinum resistant, with disease recurrence within 6 months after finishing first-line therapy. Both patients with refractory ovarian cancer and with resistant ovarian cancer have a poor prognosis[3]. Platinum based agents exert a cytotoxic effect by damaging DNA, which eventually results in DNA strand breaks and apoptosis. Apoptosis induced by DNA damage is dependent on tumor suppressor protein p53. P53 wild type cells have the opportunity to arrest cells at the G1 checkpoint in order to repair damaged DNA. Cells with a p53 mutation mainly rely on DNA repair at the G2 checkpoint[4]. The p53 gene is the most commonly mutated gene in human cancer and p53 inactivation occurs by a mutation of one allele followed by loss of the remaining wild type gene[5]. In absence of a functional p53 gene and therefore a functional G1 checkpoint, damaged DNA relies on the G2 checkpoint for repair. Annulment of the G2 checkpoint may therefore make p53 deficient tumor cells more susceptible to anti-cancer agents. Wee1 is a tyrosine kinase that regulates the G2 checkpoint and prevents entry into mitosis in response to DNA damage[6]. Mitosis can be triggered by binding of cyclin B to cyclin-dependent kinase (CDK1), whereas inhibition of this complex of Cyclin B and CDK1 by Wee1 induced phosphorylation of CDK1 at tyrosine 15 will result in cell cycle arrest and allows time for DNA repair. Overexpression of Wee1 has been reported in several cancer types, like ovarian cancer[7]. Inhibition of Wee1 could therefore be a strategy to abrogate G2 cell cycle arrest and in absence of G1 checkpoint arrest (in case of p53 mutation) this could lead to apoptosis. AZD1775 is a potent and selective inhibitor of Wee-1 kinase, a kinase that regulates the G2/M checkpoint. Proof of principle was demonstrated in several preclinical studies[8,9] and clinical studies have been shown ananti-tumor effect of AZD1775 in combination with several anti-cancer drugs[6,10].
In the first part of the current study, it was shown that the combination of AZD1775 and carboplatin was safe and effective in patients with p53 mutated

ovarian cancer. Patients were platinum refractory, or resistant with progression within three months after the end of first line standard carboplatin-paclitaxel therapy. AZD was dosed 225 mg for 2.5 days (the third day only one administration) in 21-day schedule. Carboplatin was dosed target AUC 5 mg/ml·min. This study report has been published before[11]. Following the published part of this study, we wrote an amendment to the protocol for an additional safety and activity cohort. The aim of the current cohort reported now is to gain more safety and efficacy information of the combination of AZD1775 and carboplatin in p53 mutated platinum resistant or refractory ovarian cancer. Secondary objectives include time to progression and to observe pharmacodynamics changes induced by AZD1775 in combination with carboplatin in circulating tumor cells (CTC).

Patients and methods

Patient selection

All patients had a histological diagnosis of epithelial ovarian cancer with a *TP53* mutation determined by sequencing of exon 2-10 by polymerase chain reaction. All patients received first-line platinum therapy previously and showed evidence of disease recurrence during or within 6 months after the end of this treatment according to Response Evaluation Criteria in Solid Tumors (RECIST version 1.1)[12]. All patients were ≥18 years old and had a Eastern Cooperative Oncology Group Performance status of ≤1, adequate organ function, and evaluable disease according to RECIST version 1.1.

Study design and treatment

This phase II, open-label, non-randomized study was conducted at the Netherlands Cancer Institute in Amsterdam, The Netherlands and in Utrecht University Medical Centre. The study (ClinicalTrials.gov identifier: NCT01164995) received approval of the institutional medical ethical review board and was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice (GCP). All patients gave written informed consent before start. Results of a previous ovarian cancer cohort of this study was published before[11]. After this publication an amendment was submitted to include another 29 patients in an additional safety and activity ovarian cohort. Here we will discuss the first 10 patients treated in this additional

safety cohort. Patients received carboplatin intravenously with a target area under the curve (AUC) of 5 mg/ml·min in a 30 minute infusion, combined with AZD1775 225 mg orally twice day for 2.5 days (the third day only 1 administration) in a 21-day cycle. The modified Calvert formula was used to calculate the carboplatin dose[13]. Glomerular filtration rate was estimated using the Cockcroft-Gault estimation. The first dose of AZD1775 was administered concomitantly with the infusion of carboplatin.

Safety and assessments

Medical history and demographic data and were collected during screening. Physical examination, vital signs, and other safety assessments (Eastern Cooperative Oncology Group performance status, registration of concomitant medication, hematology, biochemistry, and urine analysis) were performed at baseline, and hematology, biochemistry and toxicity assessments were performed throughout treatment. At baseline and every two cycles radiologic evaluations were performed using computed tomography scans. Tumor response was graded according to RECIST version 1.1[12]. Adverse events were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (version 4.03)[14].

Statistical analysis

The primary end point of this additional cohort was to assess safety and preliminary anti-tumor activity (according to RECIST 1.1) of AZD1775 in combination with carboplatin in *p*53 mutated epithelial ovarian cancer in a 21 day schedule. For the ovarian cancer cohort, proof-of-concept has been shown in the phase II proof-of-concept trial. A response of 5% or less would definitely indicate no efficacy of interest. Of the 29 included patients, at least 4 patients should have a response (PR or CR) to declare an efficacy of at least a 20% (α=0.05). Of note, a response rate of 20% will be considered a strong signal, as the patients did not show a response to first line platinum containing chemotherapy.

Pharmacodynamic assay

To determine circulating tumor cells in peripheral blood, blood samples were drawn before start, in case of response (partial response (PR), or

complete response (CR) and at progressive disease. Per time point, 3 CPT tubes of 8 mL each were drawn.

P53 status

TP53 mutation status was analyzed in archival tumor tissue, if not already known. Standard IHC and mutation analysis by Sanger sequencing as routinely performed in our institute were performed before inclusion, with proven *TP53* mutation as inclusion criterion.

Results

Patient population

To date, 10 patients were enrolled into the additional safety and activity cohort and started treatment (Table 2.2.1). Two patients were not evaluable for safety and efficacy. One patient withdrew informed consent after the first administration and the second patient did not complete two cycles of treatment due to clinical deterioration. This patient developed a neutropenic fever for which she was treated with intravenous antibiotics, erythrocyte transfusion and mineral suppletion. There were no positive cultures. Despite these interventions she detoriated clinically very rapidly.
The median age of the patients was 59 years (range, 42 to 70 years). The majority of the patients (75%) had an Eastern Cooperative Oncology Group performance status of 0 at baseline. These findings are in line what can be expected from this particular patient group. Most patients (75%) were treated with one line of systemic therapy before. All patients showed radiologic evaluable disease before study start. Eight patients were evaluable for toxicity.

Safety

The main treatment-related and clinically significant adverse events per patient are listed in Table 2.2.2. Bone marrow toxicity (thrombocytopenia, anemia and neutrophil count decrease), nausea, vomiting and fatigue were the most common adverse events. Grade 3 and 4 thrombocytopenia and/or grade 4 neutropenia resulted in carboplatin dose reductions 6 times and in AZD1775 dose reductions in 3 times. In one patient the carboplatin dose was

reduced because of ongoing fatigue grade 2. One patient had prolonged hospitalization because of grade 3 diarrhea. The initial hospitalization was due to an infusion related reaction. The patient received loperamide and oral rehydration solution and recovered within one day. In one patient, the AZD1775 dose was reduced twice because of ongoing malaise. The median exposure to the combination of AZD was 200 mg and AUC 4 5 mg/ml·min carboplatin.

Table 2.2.1 **P**atient characteristics from 8 evaluable patients.

Characteristics	**N**
Median age (years)	59 (42-70)
Previous lines of chemotherapy	
1	6 (75%)
2	2 (25%)
WHO performance status	
0	6 (75%)
1	2 (25%)
Best response*	
PR	5 (63%)
SD	3 (38%)
Platinum resistance/refractory	
Yes	8 (100%)
No	0
BRCA mutation	
Yes	1 (13%)
No	5 (63%)
Unknown	2 (25%)
P53 mutation	
Missense mutation	6 (75%)
Nonsense mutation	0
Deletion	1 (13%)
Other	1 (13%)
Uknown	0

Table 2.2.2 Adverse events at least possibly related to study drug in 8 evaluable patients. Graded according to CTCAE version 4.03.

Adverse event	**Grade 1/2**		**Grade 3/4**	
	n	**%**	**n**	**%**
Nausea	7	88	0	
Diarrhea	5	63	1	13
Vomiting	5	63	0	
Neutrophil count decrease	1	13	4	50
Platelet count decrease	2	25	3	38
Anemia	3	38	1	13
Fatigue	4	50	0	
Malaise	3	38	0	
Infusion related reaction	2	25	0	
Anorexia	1	13	0	

Antitumor activity

Of the 10 patients who started study treatment, two patients did not receive at least two cycles of study treatment and did not reach the first response evaluation after 6 weeks of treatment. One patient withdrew informed consent shortly after the first admission of AZD1775 and carboplatin. The second patient did not reach the first response evaluation as a result of clinical deterioration. Of the 8 evaluable patients, five patients (63%) showed partial response as best response. Three patients (38%) showed stable disease as best response. At time of interim analysis, 3 patients were still ongoing.

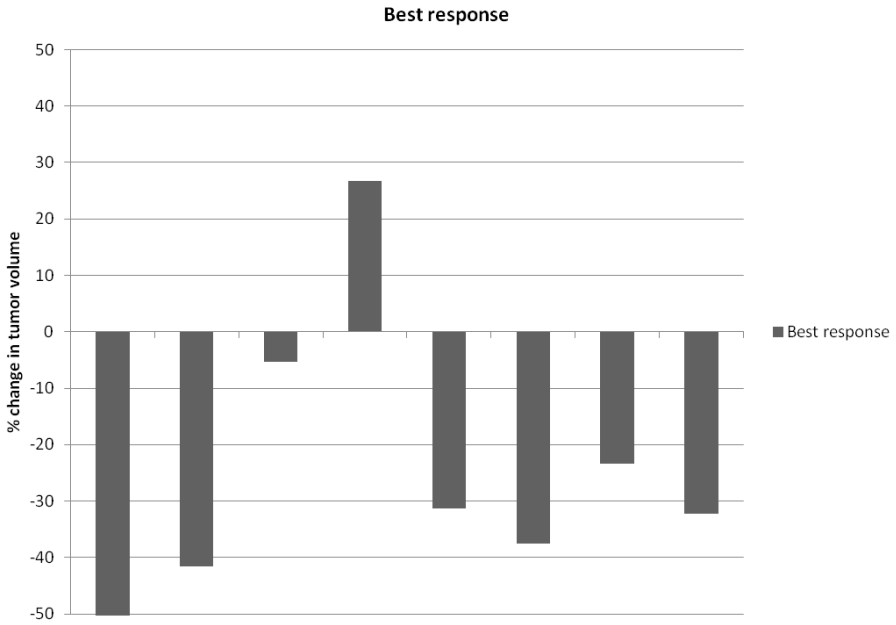

Figure 2.2.1 Waterfall plot showing the maximum change in tumor volume according to RECIST 1.1 criteria.

Pharmacodynamics

Blood samples for pharmacodynamics measurements in circulating tumor cells were obtained of 7 patients before start of treatment. Of the five patients with a partial response, for 3 patients the circulating tumor cells were measured at time of response. Of one patient, these samples were obtained after data lock so these were not included. Of 4 patients samples

were collected at the end of treatment. One patient showed decrease of circulating tumor cells at response. One patient showed significant increase in circulating tumor cells at time of progressive disease. Results of the pharmacodynamics are shown in Figure 2.2.3.

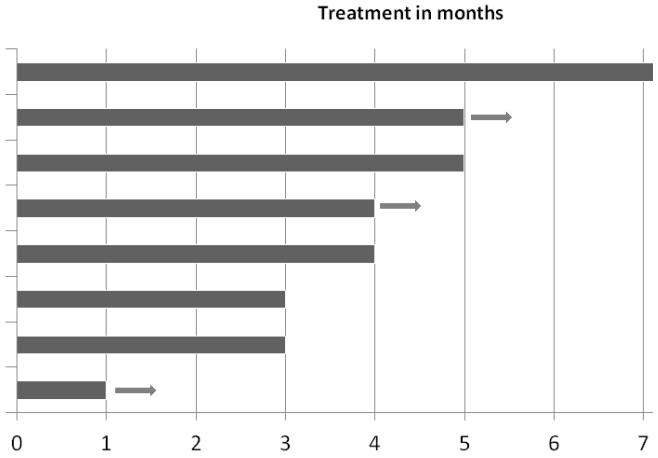

Figure 2.2.2 Swimmerplot showing the time on treatment in months.
Arrows indicate patients that were ongoing at time of the interim analysis.

Table 2.2.3 Dose reductions. Dose reductions were executed as per protocol recorded.

Carboplatin	**Number of reductions (n)**	**Reason**
AUC 5 → AUC 4	5	1 (bad tolerance) 2 (thrombocytopenia) 1 (neutropenia) 1 (fatigue)
AUC 4 → AUC 3	1	1 (neutropenia)[1] 2 (thrombocytopenia)[1]
AUC 3 → AUC 2	1	1 (thrombocytopenia)

AZD1775	**Number of reductions (n)**	**Reason**
225 mg BID → 175 mg BID	4	1 (malaise) 2 (thrombocytopenia) 1 (nausea)[2] 1 (vomiting)[2]
175 mg BID → 125 mg BID	2	1 (malaise) 1 (thrombocytopenia)

[1] in one patient, both thrombocytopenia and neutropenia were reason for dose-reduction. Counted as 1 dose-reduction.
[2] in one patient, both nausea and vomiting were reason for dose-reduction. Counted as 1 dose-reduction.
[3] AUC in mg/ml·min.

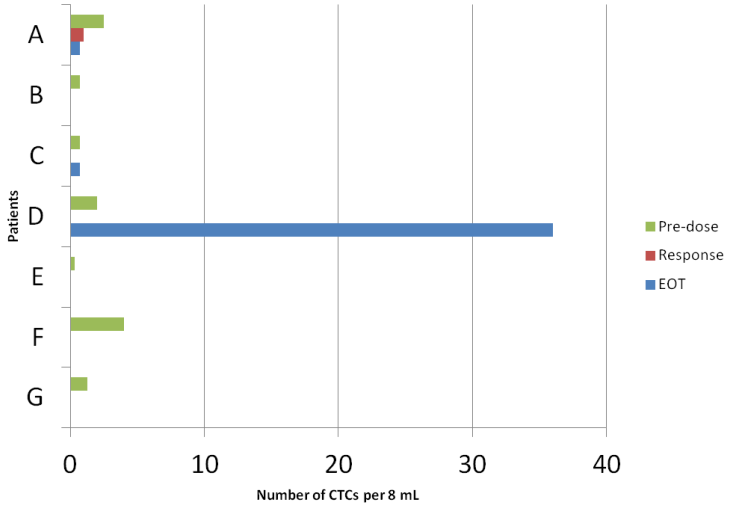

Figure 2.2.3 Pharmacodynamic assay.
Patient G: response sample was collected after data cutt off.
Patient E: response samples were obtained, however samples contained little amount of blood and were therefore not useful.

Discussion

We report the interim analysis of an additional safety and activity cohort of an investigator-initiated, proof-of-principle, phase II study with the first-in-class WEE1 inhibitor AZD1775. The previous ovarian cancer cohort of this trial was published before[11]. The inclusion and exclusion criteria of the previous reported cohort and this additional cohort were almost similar except the timeframe for resistant disease, which was expanded from 3 to 6 months in the additional cohort. The Gynecologic Oncology Group (GOG) defined platinum resistance as relapse under six months of last platinum therapy as platinum resistant. Therefore, we expanded the timeframe for resistant disease from 3 to 6 months[15]. In addition, for the additional safety cohort it was allowed to have received second line (non-platinum containing) chemotherapy instead of only first line therapy. This was supported by the findings in the previous phase I trial with AZD1775 administered as monotherapy and in combination with gemcitabine,

cisplatin or carboplatin to patients with solid tumors[10]. In this trial 202 patients were included, from whom 176 patients were evaluable for efficacy. Despite the extensive pretreatment of this patient group, both partial responses and prolonged stable diseases were observed. This has led to the widening of this inclusion criterion regarding lines of pretreatment, of course with acceptable bone marrow reserves and good clinical condition at baseline. The dosing regimen of AZD1775 and carboplatin were similar in this cohort compared to the previous cohort. AZD1775 in combination with carboplatin in the additional safety cohort showed a similar toxicity profile as in the previous study, with nausea, vomiting, fatigue and bone marrow suppression as major adverse events. This is also in line with a previous phase I study with AZD1775 and carboplatin (or cisplatin or gemcitabine) in patients with advanced solid tumors[10]. Grade 3 and 4 thrombocytopenia and grade 4 neutropenia did not lead to complications, however dose-reductions were often mandatory in order to continue treatment. In the previous cohort, most common bone marrow toxicity was thrombocytopenia, occurring in 70% of the patients. Most patients had grade 4 thrombocytopenia (11,48%). Bone marrow toxicity (grade 4 thrombocytopenia and/or grade 2 or 4 neutropenia) resulted in dose reductions 11 times (in 11 patients). In the current cohort grade 3 and 4 thrombocytopenia and grade 4 neutropenia resulted in carboplatin dose reductions in 6 times and AZD1775 dose-reductions 3 times to achieve a tolerable safety profile. One patient was treated on the lowest dose-level of carboplatin target AUC 2 mg/ml·min and AZD1775 125 mg for 5 doses. This patient had stable disease as best response. Gastro-intestinal toxicity was also commonly observed. An extensive pre-medication scheme of dexamethasone, granisetron, magnesiumhydroxide and metoclopramide was applied to reduce nausea and vomiting toxicities. Of the eight evaluable patients, five patients achieved a partial response as best response, three patients had stable disease as best response. Despite these high response numbers, the duration of response was not very long. In the previous cohort, patients had sustainable responses up to more than 2 years of treatment. In our cohort, the patient with the longest treatment duration was treated for 8 months. This difference could be due to the extra line of pretreatment allowed in this additional cohort and therefore a lower probability of response rate also due to lower bone marrow reserves. In the current cohort, but also in the previous cohort, it is noticeable that some patients respond very well for a longer period of time, while others have a very short time of response.

Probably, development of resistance to AZD1775 plays a role in this phenomenon. So far, it is unknown whether this plays a role in the response and duration of response in these patients. Upfront selection of patients with best chances of durable response would be wishful. Therefore, one is busy investigating tumor tissue and blood samples obtained from patients treated with AZD1775 in order to find biomarkers that could be used to develop this upfront screening. In our study, we combined the Wee1 inhibitor AZD775 with carboplatin. The combination of AZD1775 and a cytotoxic agent like gemcitabine, carboplatin or cisplatin was studied before. It was found that AZD1775 sensitizes p53 defective tumor cells to chemotherapeutics[8,10,16,17]. Whether AZD1775 is also effective as monotherapy is questionable. Do and colleagues conducted a phase I study of single agent AZD1775 in patients with refractory solid tumors. Toxicities at the maximum tolerable dose (MTD) level were acceptable according to the CTCAE criteria. Two partial responses were observed[6]. Another possible combination regimen could be AZD1775 with a poly (ADP-ribose) polymerase (PARP)-inhibitor. PARP-inhibitors such as olaparib play an important role in the repair of single strand DNA breaks. BRCA 1/2 mutant or other homologous recombination deficient cancers have no ability to repair double strand DNA breaks. Combining PARP-inhibition and an impaired homologous recombination system, like BRCA 1/2 mutation, leads to synthetic lethality. Wee1 indirectly inhibits homologous recombination repair (HRR). Karnak and colleagues combined AZD1775 and olaparib as radio sensitizing strategy in pancreatic cancer. They found that the combination of AZD1775 and olaparib led to more radiation damage compared to single agent sensitization alone. Their results show that Wee1 inhibition leads to inhibition of HRR and abrogation of the G2 checkpoint when combined with olaparib[18]. Combining AZD1775 with other agents could lead to more side effects, so feasibility should be examined. Wee1 inhibition leads to blockade of the G2 checkpoint in the cell cycle. This is especially important for p53 mutant cells. P53 wild type cells have the opportunity to arrest the cell cycle at the G1 checkpoint in order to repair damaged DNA. Administration of a Wee1 inhibitor to p53 mutant tumors does not result in cell cycle arrest and therefore no DNA repair will take place. There are studies that have showed activity of Wee1 inhibition independent of p53 status[19,20]. Although there wasn't a p53 mutation found, it is unknown whether the entire p53 pathway is undisturbed. In this cohort patients with p53 mutation were included, because of the rationale as described above. The aim of measuring

circulating tumor cells is to investigate whether is possible to detect response or progression in the blood based on the amount of circulating tumor cells in the blood at that time point. Circulating tumor cells are cells that have migrated into the vasculature and are derived from the primary tumor. A response to treatment would, in theory, lead to less circulating tumor cells in the peripheral blood, whereas progressive disease would show an increase in circulating tumor cells in the peripheral blood. In our patients, the amount of circulating tumor cells at baseline was low. One patient showed a significant increase in circulating tumor cells at time of response (from 2 to 36 CTCs per 8 mL). Other patients show no or little difference between pre-dose and at progressive disease. Since we have only two patient samples at response, nothing can be concluded from this. The number of obtained samples at time of this interim analyses is low, so no conclusions can be drawn at this moment. Since twenty-nine patients will be included in this cohort, more conclusions can be drawn after finishing inclusion.

Regarding the side effects shown in our study, it would be interesting to investigate if lower dose AZD1775 and carboplatin could also lead to enough inhibition and damage to achieve tumor response. With lower dose, the probability of developing side effects is lower. Especially bone marrow toxicity like thrombocytopenia often has led to dose-reductions and treatment interruption. Because of the delay in dosing, the time between to cycles increased. This has led to less dose intensity in these patients as has been foreseen. It would be worth investigating, whether a dose-dense schedule of lower dose and more frequent dosing, would lead to less side effects and therefore better tolerability. This might also lead to higher dose intensity, and possibly to better and more durable responses. Hirai and colleagues investigated several dosing schedules of AZD1775 combined with 5-FU or capecitabine. All schedules resulted in enhancement of the anti-tumor effect, whereby the twice weekly and five weekly dosing schedules were favorably. AZD1775 has a relatively short half-life of 9-11 hours, which supports the two times daily administration. One of our patients was treated on the lowest dose-level of carboplatin AUC 2 mg/ml·min and AZD1775 125 mg BID had stable disease for five cycles on this regimen, which implicates that there is enough Wee1 inhibition at the lowest dose-level. Since the occurrence of bone-marrow toxicity in many patients, it would be worth finding a balance between dose, administration, schedule and combination on the one hand and toxicity on the other hand, with the aim to

maximize the antitumor activity with manageable toxicity. Currently, multiple AZD1775 containing studies are ongoing, with AZD1775 as monotherapy and in combination with chemotherapy or radiotherapy, with different strategies for AZD1775 development[21]. Inclusion for this study will continue with the inclusion of an additional 21 patients in order to gain more safety data on the combination of carboplatin and AZD1775 in pre-treated patients with advanced p53 mutated ovarian cancer. In conclusion, AZD1775 225 mg 2.5 days and carboplatin target AUC 5 mg/ml·min could be safely administered to patients with refractory or resistant ovarian cancer. This combination shows a promising anti-tumor effect. However, bone marrow toxicity has led to dose-reductions and dose delays in 5 patients. This remains a point of attention combining AZD1775 and carboplatin.

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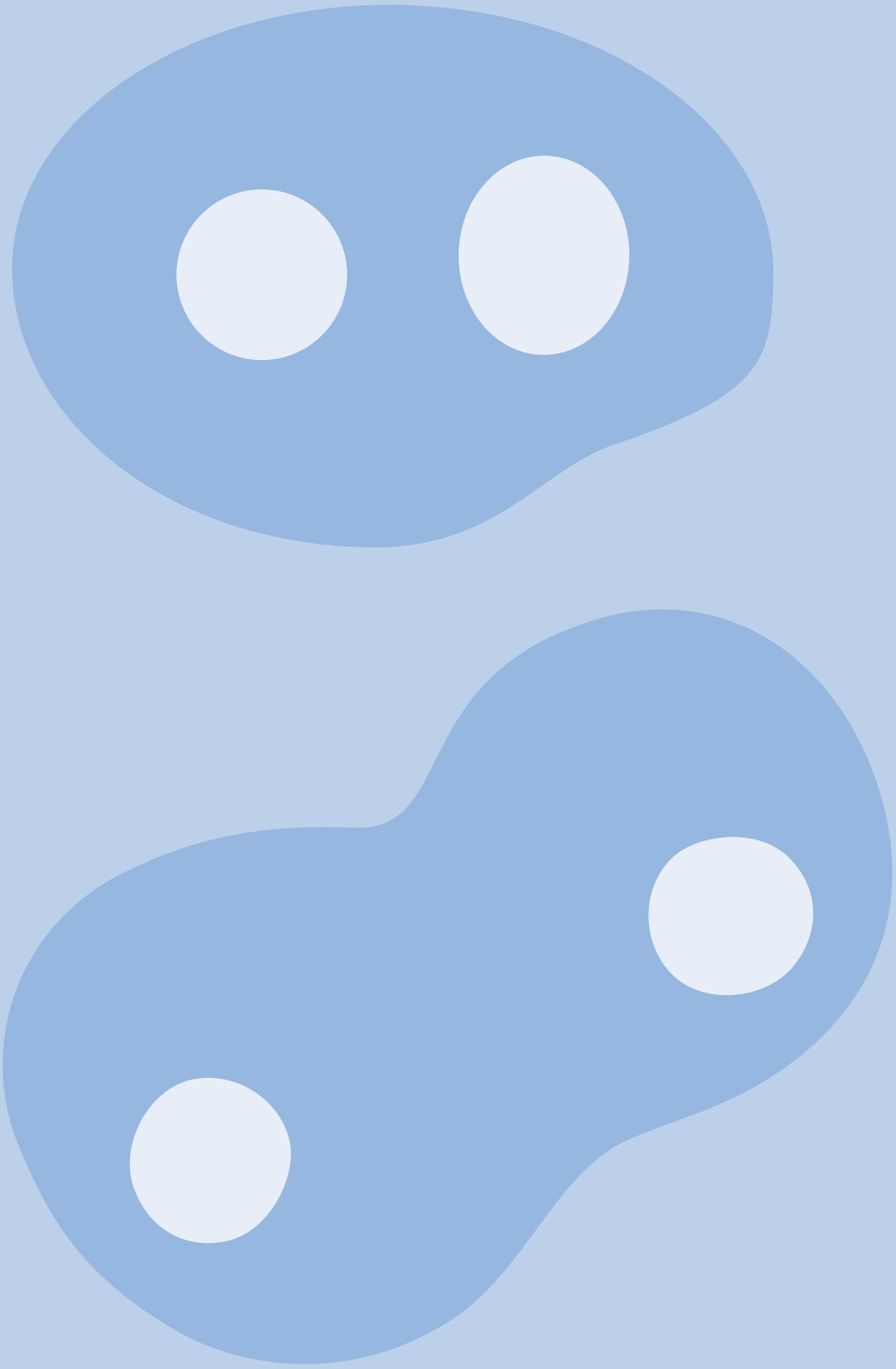

Chapter 2.3

Adavosertib with Chemotherapy in Patients with Primary Platinum-Resistant Ovarian, Fallopian Tube, or Peritoneal Cancer: an Open-Label, Four-Arm, Phase II Study

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Clinical Cancer Research.2022;28:36-44

Summary

Purpose

This study assessed the efficacy, safety, and pharmacokinetics of adavosertib in combination with four chemotherapy agents commonly used in patients with primary platinum-resistant ovarian cancer.

Patients and methods

Women with histologically or cytologically confirmed epithelial ovarian, fallopian tube, or peritoneal cancer with measurable disease were enrolled between January 2015 and January 2018 in this open-label, four-arm, multicenter, Phase II study. Patients received adavosertib (oral capsules, 2 days on/5 days off or 3 days on/4 days off) in six cohorts from 175 mg once daily to 225 mg twice daily combined with gemcitabine, paclitaxel, carboplatin, or pegylated liposomal doxorubicin. The primary outcome measurement was overall response rate.

Results

Three percent of patients (3/94) had confirmed complete response and 29% (27/94) had confirmed partial response. The response rate was highest with carboplatin plus weekly adavosertib, at 66.7%, with 100% disease control rate, and median progression-free survival of 12.0 months. The longest median duration of response was in the paclitaxel cohort (12.0 months). The most common grade {greater than or equal to}3 adverse events across all cohorts were neutropenia (45/94 [47.9%] patients), anemia (31/94 [33.0%]), thrombocytopenia (30/94 [31.9%]), and diarrhea and vomiting (10/94 [10.6%] each).

Conclusions

Adavosertib showed preliminary efficacy when combined with chemotherapy. The most promising treatment combination was adavosertib 225 mg twice daily on days 1-3, 8-10, and 15-17 plus carboplatin every 21 days. However, hematologic toxicity was more frequent than would be expected for carboplatin monotherapy, and the combination requires further study to optimize the dose, schedule, and supportive medications.

Introduction

Standard-of-care treatment for newly diagnosed cases of epithelial ovarian, fallopian tube, or peritoneal cancer (EOC) involves a combination of cytoreductive surgery and adjuvant platinum- and taxane-based chemotherapy[1,2]. While recurrent disease is treatable and most patients initially achieve remission with front-line therapy, tumors become resistant to currently available chemotherapies over time, and patients succumb to their disease[3]. Outcomes for patients with primary platinum-resistant (recurrence <6 months following frontline platinum chemotherapy), recurrent EOC remain particularly poor, with low response rates to further chemotherapy (10-20%), median progression-free survival (mPFS) of 3-4 months, and a median overall survival (mOS) of less than 14 months (3-5). Even these estimates may be optimistic given the results from JAVELIN 200 (NCT02580058)[6]. In this randomized Phase III trial of avelumab + pegylated liposomal doxorubicin (PLD) versus avelumab or PLD monotherapy in platinum-resistant disease,the overall response rate (ORR) for PLD was 4.2%. This study was heavily populated with patients who had primary platinum-resistant disease[7]. Development of novel drugs for use in the recurrent resistant setting is critical. Progress has been made in the clinical application of molecularly targeted agents designed to shift EOC treatment away from broad-based cytotoxic use towards more tailored therapeutic interventions[8-10]. Although the ORR is quite low, for patients who have platinum resistance[11,12], targeting the DNA repair process is still an attractive possibility for improving response rates and survival. The ubiquitous loss of TP53[13] and dependence on DNA cell cycle checkpoint 2 (G2/M) makes checkpoint 2 inhibition of interest. Cell cycle and DNA replication control involves cyclin-dependent kinases (CDKs), specifically CDK1 and CDK2, which are regulated by the tyrosine kinase Wee1. CDK1 regulates the G2/M checkpoint; inhibition of Wee1, combined with DNA-damaging agents, causes mitotic entry without completion of DNA repair and replication, leading to mitotic catastrophe[14]. CDK2 deregulation through Wee1 inhibition also causes DNA replication stress, due to increased replication-origin firing and nucleotide depletion[15]. Adavosertib (AZD1775) is a potent, selective, small-molecule Wee1 inhibitor. In preclinical studies, adavosertib enhanced antitumor effects of chemotherapy and radiation[15-20],especially for *TP53*-mutated cells[15,19,20]. Evidence from Phase I and II clinical trials indicates that adavosertib plus chemotherapy appears to be an active combination for

consideration in the treatment of platinum-resistant ovarian cancer (PROC)[16,21-23]. In a Phase I dose-escalation study in patients with solid tumors, the maximum tolerated dose (MTD) of adavosertib was 175 mg when given 2 days per week for 3 consecutive weeks, in combination with gemcitabine (1000 mg/m^2 weekly for 3 consecutive weeks) in a 4-week cycle[16]. In the same study, adavosertib 225 mg twice daily (bid) orally for 2.5 days per 21-day cycle (five doses across days 1, 2, and morning of day 3) was the MTD, in combination with intravenous infusion of carboplatin (area under the concentration–time curve, concentration of 5 mg/mL·min [AUC5]) on day 1[16]. This dose achieved the target exposure of 240 nmol/L for 8 hours, which was associated with maximum efficacy in preclinical xenograft studies[16]. The schedule of 2.5 days per 21-day cycle was designed to provide continued inhibition of Wee1 by adavosertib at the G2/M checkpoint for up to 60 hours (approximate doubling time of a tumor cell), thus maximizing the number of tumor cells that experience premature checkpoint escape. In a Phase II trial in women with platinum-sensitive *TP53*-mutant ovarian cancer, adavosertib (225 mg bid for 2.5 days per 21-day cycle) in combination with paclitaxel (175 mg/m^2) and carboplatin (AUC5) was considered tolerable and showed signs of efficacy[21]. Additionally, paclitaxel at 80 mg/m^2 every week for 4 weeks for the first three cycles (12 weekly doses) followed by three consecutive weekly doses during each 4-week cycle appeared to be efficacious in chemotherapy-resistant ovarian cancer[24]. Pegylated liposomal doxorubicin (PLD) is one of the standard treatments in platinum-resistant ovarian cancer, with an approved dose ranging from 20 to 50 mg/m^2, depending on the cancer type. A stealth liposomal (pegylated) construct increases the circulation half-life of doxorubicin while minimizing the off-target toxicity[25]. Potentiation of doxorubicin activity was observed when co-administered with other DNA damage response agents[26]. Hence, combination of adavosertib with PLD may have increased efficacy compared with monotherapy. Adavosertib is primarily metabolized by CYP3A4 and FMO3 and is a weak inhibitor of CYP3A, CYP1A2 and CYP2C19[27]; therefore, the likelihood of drug interactions between adavosertib and chemotherapies such as carboplatin, paclitaxel, gemcitabine, and PLD is low. Gemcitabine is metabolized by cytidine deaminase, carboplatin is cleared mostly unchanged, and paclitaxel is metabolized by CYP2C8 and CYP3A4. In a Phase I study,the pharmacokinetics of adavosertib were approximately linear, increased in a dose168 proportional manner, and were not significantly changed in combination with chemotherapy[16].We therefore conducted a

multisite trial exploring the efficacy, safety, and pharmacokinetics of several adavosertib and chemotherapy combinations in patients with primary PROC: adavosertib 175 mg 2 days per week for 3 consecutive weeks + gemcitabine (1000 mg/m^2 weekly for 3 consecutive weeks, reduced to 800 mg/m^2 weekly following a protocol amendment) in a 4-week cycle; adavosertib 225 mg bid for 2.5 days on weeks 1, 2, and 3 of a 28-day cycle + paclitaxel 80 mg/m^2 every week for 4 weeks; adavosertib 225 mg bid (five doses on days 1-3 or on days 1-3, 8–10, and 15–17 per 21-day cycle) + carboplatin (AUC5) on day 1; and adavosertib (175 mg or 225 mg bid for 2.5 days) + 40 mg/m^2 PLD.

Methods

This study was conducted by Sarah Cannon Research Institute (SCRI) at 20 global investigational sites in the USA, Canada, and the Netherlands according to ethical principles that have their origin in the Declaration of Helsinki, the International Council for Harmonisation (ICH)/Good Clinical Practice (GCP) guidance, and the AstraZeneca policy of bioethics. The institutional review boards of all participating sites approved the study, and patients were enrolled following written informed consent. This trial was registered with
ClinicalTrials.gov (NCT02272790) and the European Clinical Trials Database (EudraCT2015-000886-30).

Study design

This open-label, four-arm, Phase II study with safety lead-in was designed to evaluate the ORR, safety, pharmacokinetics (PK), and tolerability of adavosertib combined with chemotherapy agents in women with primary PROC. Treatment arms are described in Table 2.3.1.

Eligibility criteria

Women with histologically or cytologically confirmed EOC with measurable disease according to Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1[28] were eligible. All patients had disease progression within 6 months of completing (but without progression during) ≥4 cycles of first-line platinum-based chemotherapy for stage III/IV disease and had ≤4 prior

treatment regimens. For treatment arms D and D2, only patients without any prior anthracycline exposure were eligible. Additional entry criteria included age >18 years, Eastern Cooperative Oncology Group (ECOG) performance status 0-1, and adequate hematologic, liver, and renal function. *TP53* mutation status was not required for study entry.

Table 2.3.1 Treatment arms (N=94).

Treatment arm	Adavosertib dosing	Chemotherapy agent	Chemotherapy dosing	Cycle length
Arm A (N=9)	175 mg PO daily days 1-2, 8-9, 15-16	Gemcitabine	1000 or 800 mg/m^2 IV days 1, 8, 15[a]	28 days
Arm B (N=38)	225 mg PO bid x 5 doses days 1-3, 8-10,15-17	Paclitaxel	80 mg/m^2 IV days 1, 8, 15	28 days
Arm C (N=23)	225 mg PO bid x 5 doses days 1-3	Carboplatin	AUC5 IV day 1	21 days
Arm C2 (N=12)	225 mg PO bid x 5 doses days 1-3, 8-10,15-17	Carboplatin	AUC5 IV day 1	21 days
Arm D 175 mg (N=6)	175 mg PO bid x 5 doses days 1-3	PLD	40 mg/m^2 IV day 1	28 days
Arm D2 225 mg (N=6)	225 mg PO bid x 5 doses days 1-3	PLD	40 mg/m^2 IV day 1	28 days

[a]A protocol amendment was implemented to reduce the gemcitabine dose to 800 mg/m^2 after the first four patients experienced toxicity (four patients were dosed at 1000 mg/m^2 and five patients were dosed at 627 800 mg/m^2). AUC5, area under the concentration–time curve concentration of 5 mg/min·mL; bid, twice 628 daily; IV, intravenous; PLD, pegylated liposomal doxorubicin; PO, oral.

Safety lead-in and dose-limiting toxicity

A six-patient safety lead-in for each drug combination was conducted during cycle 1 of treatment. Dose-limiting toxicities (DLTs) were defined as any of the following toxicities not attributable to the disease that occurred during cycle 1: grade 4 hematologic toxicity lasting >7 days; grade 3 thrombocytopenia associated with hemorrhage; grade ≥3 non-hematologic toxicity; and other toxicity that was clinically significant and/or unacceptable, was unresponsive to supportive care, resulted in a disruption of dosing schedule of >7 days, or was judged to be a DLT by the investigators.

Dose modifications

Dose modifications for each drug were specified in the protocol and management was detailed for anticipated adavosertib- and chemotherapy-related toxicities. Patients received a serotonin 5-HT_3 antagonist and dexamethasone prior to each dose of adavosertib to prevent nausea and vomiting. If one drug was held as a result of toxicity, treatment with the other drug was allowed to continue as appropriate. If treatment was delayed for >4 weeks because of toxicity, the patient was discontinued from the study. Patients who benefited from treatment were allowed to continue the non-offending medication. Grade 3 or 4 toxicity required stopping treatment with the offending agent until the toxicity improved to grade ≤1. All patients were followed up for toxicity in accordance with National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) version 4.03[29] from informed consent until 30 days after the end of the last investigational product administration.
Any patient who developed a grade 3 or 4 non-hematologic toxicity that did not resolve to grade ≤1 within 21 days was removed from the study treatment unless approved by the medical monitor. Patients requiring >2 dose reductions of adavosertib and the chemotherapy were discontinued from study treatment. Dose re-escalation was not permitted.

Determination of response

Patients in arms A, B, D, and D2 were evaluated for response every 8 weeks, and patients in arm C were evaluated every 6 weeks. All patients were assessed according to RECIST version 1.1[23]. Patients with elevated cancer antigen 125 (CA-125) serum levels that could be monitored for response were also assessed according to the Gynecological Cancer Intergroup (GCIG) CA-125 response criteria[30].

Pharmacokinetics and exploratory analysis

PK sample collection was based on treatment schedules of adavosertib and the four chemotherapeutic agents. PK analysis was designed to characterize the exposure of analytes in the safety lead-in group, help determine the cause of any adverse events (AEs), and assess the drug interaction between adavosertib and each chemotherapeutic agent. Exploratory, unblinded analysis of efficacy was also conducted according to the presence of

potential genomic biomarkers determined from archival formalin-fixed and paraffinembedded tissue samples (collected prior to adavosertib treatment) using the FoundationOne® assay and analyzed using Foundation Medicine, Inc's F1 classification rules[31]. Targeted genomic profiling was presented using an in-house bioinformatics platform and correlated with clinical outcomes. All tissue samples were shipped at ambient temperature to a central laboratory for processing. Patients provided additional informed consent for the optional collection of genetic material from archival tumor tissue. Germline and somatic variants were reported if they were known pathogenic, likely pathogenic, or variants of unknown significance (VUS; defined as a variant that cannot be determined to be either pathogenic or benign); only pathogenic or likely pathogenic aberrations were correlated with clinical response, regardless of whether they were somatic or germline.

Statistical analysis

Statistical analyses were performed using SAS® statistical analysis software (SAS Institute, Cary, NC) by Sarah Cannon Development Innovations under the direction of the Biometrics Group, AstraZeneca. All patients who received ≥1 dose of study treatment were included in the safety analyses, and all patients who received ≥1 dose of investigational drug and had measurable disease at baseline were included in the efficacy analysis. The primary efficacy endpoint was ORR, defined as the proportion of patients with measurable disease with ≥1 confirmed complete response (CR; disappearance of all target lesions since baseline) or partial response (PR; ≥30% decrease in the sum of the diameters of target lesions). An exact two-sided 80%/95% confidence interval (CI) for the ORR was computed using the Clopper and Pearson method. Secondary endpoints included duration of response (DoR), disease control rate (DCR; defined as CR + PR + stable disease [neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for progressive disease for ≥7 weeks for arms A, B, D, and D2, and for ≥5 weeks for arms C and C2]), PFS, overall survival (OS), PK parameters, and toxicity. Arm B was designed to enroll 30 patients based on a 20-30% ORR historical reference for paclitaxel alone. Arm C enrollment was based on a primary endpoint of ORR (null hypothesis of 10% vs. an alternative hypothesis of 30% ORR). Arm C2 enrolled an additional 12 patients to assess weekly adavosertib in combination with carboplatin on a 21-day cycle. As

arms A, D, and D2 were exploratory, no formal sample-size calculations were conducted.

Results

Disposition and patient characteristics

Ninety-four patients were enrolled between January 28, 2015 and January 29, 2018. The majority of patients were Caucasian (77.7%), with a median (range) age of 60 (34-85) years. Demographics and tumor characteristics are listed in Table 2.3.2.

The median (range) number of initiated cycles for the overall population was 4 (1-23). Reasons for treatment discontinuation were progressive disease (57.4%), AEs (12.8%), patient decision (3.2%), physician decision (2.1%), death, clinical progression, and study closure at site (1.1% each).

Efficacy and safety

Efficacy for the overall study population, as well as each cohort of the study, is presented in Table 2.3.3, and a waterfall response plot is shown in Figure 2.3.1. A Kaplan–Meier plot of PFS by cohort is provided in Supplementary Figure S2.3.1.

Arm A: Adavosertib 175 mg once daily (qd) on days 1-2, 8-9, and 15-16 + gemcitabine 1000 mg/m^2 intravenous (IV) on days 1, 8, and 15 (every 28 days; N=9). Two of the six safety lead-in patients experienced a DLT of grade 4 neutropenia. Gemcitabine was reduced from 1000 to 800 mg/m^2 after the first four patients experienced hematologic toxicity (5/9 patients were dosed at 800 mg/m^2). The most common non-hematologic AEs were nausea (55.6%), vomiting (44.4%), diarrhea, and fatigue (33.3% each). The most common hematologic AEs were neutropenia (88.9%), thrombocytopenia, and anemia (33.3% each; Table 2.3.4). Two patients (22.2%) experienced an AE leading to dose reduction of adavosertib, and six patients (66.7%) experienced an AE leading to dose reduction of gemcitabine.

Arm B: Adavosertib 225 mg bid x 5 doses on days 1-3, 8-10, and 15-17 + paclitaxel 80 mg/m^2 IV on days 1, 8, and 15 (every 28 days; N=38). One of the six safety lead-in patients experienced a DLT of grade 4 neutropenia. The most common non-hematologic AEs included nausea (60.5%), fatigue (60.5%), diarrhea (81.6%), and vomiting (50.0%). The most common

hematologic AEs included neutropenia (65.8%), anemia (63.2%), and thrombocytopenia (39.5%; Table 2.3.4). Eighteen patients (47.4%) experienced an AE leading to dose reduction of adavosertib, and 19 patients (50.0%) experienced an AE leading to dose reduction of paclitaxel. One patient (1.1%) of three (7.9%) died of neutropenic sepsis causally related to chemotherapy (paclitaxel) and adavosertib.

Arm C: Adavosertib 225 mg bid x 5 doses on days 1-3 + carboplatin AUC5 IV on day 1 (every 21 days; N=23). Two of the six safety lead-in patients experienced a DLT of grade 2 diarrhea, and one of these patients experienced additional DLTs of grade 3 nausea and vomiting. The most common non-hematologic AEs were nausea (82.6%), fatigue (73.9%), diarrhea (69.6%), and vomiting (56.5%). Abdominal pain (34.8%) and headache (30.4%) were also reported (Table 2.3.4). Five patients (21.7%) experienced an AE leading to dose reduction of adavosertib, and eight patients (34.8%) experienced an AE leading to dose reduction of carboplatin.

Arm C2: Adavosertib 225 mg bid x 5 doses on days 1-3, 8-10, and 15-17 (weeks 1-3) + carboplatin AUC5 IV on day 1 (every 21 days; N=12). No DLTs were reported for any of the six safety lead-in patients. The most common non-hematologic AEs were nausea (83.3%), fatigue (66.7%), diarrhea (50.0%), and vomiting (33.3%). Hematologic AEs were notable and included neutropenia (91.7%), anemia (75.0%), and thrombocytopenia (91.7%; Table 2.3.4). Eleven patients (91.7%) experienced an AE leading to dose reduction of adavosertib, and 11 patients (91.7%) experienced an AE leading to dose reduction of carboplatin.

Patients in arm C2 experienced the highest rate of grade ≥3 AEs (100%), grade ≥3 AEs that were considered by the investigator to be causally related to adavosertib (100%), and grade ≥3 AEs that were considered by the investigator to be causally related to chemotherapy (100%).

Arms D and D2: Adavosertib 175 or 225 mg bid x 5 doses on days 1-3 + PLD 40 mg/m^2 IV on day 1 (every 28 days; N=6 for each dose). No DLTs were reported for any of the six safety lead-in patients at each dose. With the increase in dose of adavosertib, there was increased toxicity, including diarrhea (16.7% to 83.3%), fatigue (50.0% to 83.3%), neutropenia (16.7% to 33.3%), and thrombocytopenia (0% to 16.7%). Notably, the proportion of patients reporting anemia and vomiting decreased with increased dose (Table 2.3.4). No patients experienced an AE leading to dose reduction of adavosertib or PLD.

Table 2.3.2 Demographics and prior systemic therapy (N=94).

Demographic characteristics	Arm A (N=9)	Arm B (N=38)	Arm C (N=23)	Arm C2 (N=12)	Arm D (N=6)	Arm D2 (N=6)	Overall (N=94)
Median age, years (range)	63 (46–72)	60 (45–76)	62 (34–85)	58.5 (52–76)	58.5 (40–72)	60.5 (54–70)	60 (34–85)
Age <65 years, *n* (%)	5 (55.6)	26 (68.4)	14 (60.9)	8 (66.7)	3 (50.0)	3 (50.0)	59 (62.8)
Age ≥65 years, *n* (%)	4 (44.4)	12 (31.6)	9 (39.1)	4 (33.3)	3 (50.0)	3 (50.0)	35 (37.2)
ECOG performance status, *n* (%)							
0	5 (55.6)	19 (50.0)	13 (56.5)	4 (33.3)	1 (16.7)	3 (50.0)	45 (47.9)
1	4 (44.4)	19 (50.0)	10 (43.5)	8 (66.7)	5 (83.3)	3 (50.0)	49 (52.1)
Histology, *n* (%)							
Serous	9 (100.0)	33 (86.8)	21 (91.3)	12 (100.0)	4 (66.7)	6 (100.0)	85 (90.4)
Endometrioid	0	1 (2.6)	0	0	0	0	1 (1.1)
Clear cell	0	2 (5.3)	1 (4.3)	0	1 (16.7)	0	4 (4.3)
Mucinous	0	0	0	0	1 (16.7)	0	1 (1.1)
Mixed	0	1 (2.6)	0	0	0	0	1 (1.1)
Missing	0	1 (2.6)	1 (4.3)	0	0	0	2 (2.1)
Histological grade, n (%)							
G1 – well differentiated	1 (11.1)	1 (2.6)	0	2 (16.7)	0	0	4 (4.3)
G2 – moderately differentiated	0	1 (2.6)	1 (4.3)	0	0	0	2 (2.1)
G3 – poorly differentiated	5 (55.6)	28 (73.7)	15 (65.2)	9 (75.0)	5 (83.3)	3 (50.0)	65 (69.1)
G4 – undifferentiated	0	3 (7.9)	2 (8.7)	0	1 (16.7)	1 (16.7)	7 (7.4)
GX – could not be assessed/not applicable	2 (22.2)	3 (7.9)	3 (13.0)	1 (8.3)	0	2 (33.3)	11 (11.7)
Missing	1 (11.1)	2 (5.3)	2 (8.7)	0	0	0	5 (5.3)
Number of prior regimens, n (%)							
1	3 (33.3)	12 (31.6)	8 (34.8)	4 (33.3)	3 (50.0)	2 (33.3)	33 (35.1)
2	6 (66.7)	16 (42.1)	9 (39.1)	5 (41.7)	3 (50.0)	4 (66.7)	42 (44.7)
3	0	10 (26.3)	6 (26.1)	2 (16.7)	0	0	18 (19.1)
4	0	0	0	1 (8.3)	0	0	1 (1.1)
Prior bevacizumab, n (%)							
Yes	2 (22.2)	12 (31.6)	7 (30.4)	5 (41.7)	3 (50.0)	3 (50.0)	32 (34.0)
No	7 (77.8)	26 (68.4)	16 (69.6)	7 (58.3)	3 (50.0)	3 (50.0)	62 (66.0)
Prior surgery, n (%)							
Yes	8 (88.9)	35 (92.1)	22 (95.7)	11 (91.7)	6 (100.0)	6 (100.0)	88 (93.6)
No	1 (11.1)	3 (7.9)	1 (4.3)	1 (8.3)	0	0	6 (6.4)

Table 2.3.2 (continued)

Demographic characteristics	Arm A (N=9)	Arm B (N=38)	Arm C (N=23)	Arm C2 (N=12)	Arm D (N=6)	Arm D2 (N=6)	Overall (N=94)
tBRCA1, n/N (%)a							
Yes	1/9 (11.1)	3/31 (9.7)	1/16 (6.3)	0/11 (0)	0/5 (0)	0/4 (0)	5/76 (6.6)
No	8/9 (88.9)	28/31 (90.3)	15/16 (93.8)	11/11 (100)	5/5 (100)	4/4 (100)	71/76 (93.4)
tBRCA2, n/N (%)a							
Yes	1/9 (11.1)	0/31 (0)	1/16 (6.3)	1/11 (9.1)	0/5 (0)	0/4 (0)	3/76 (3.9)
No	8/9 (88.9)	31/31 (100)	15/16 (93.8)	10/11 (90.9)	5/5 (100)	4/4 (100)	73/76 (96.1)

Arm A: adavosertib 175 mg qd on days 1-2, 8-9, and 15-16 + gemcitabine 1000 mg/m^2 IV on days 1, 8, and 15 (every 28 days); arm B: adavosertib 225 mg bid x 5 doses on days 1-3, 8-10, and 15-17 + paclitaxel 80 mg/m^2 IV on days 1, 8, and 15 (every 28 days); arm C: adavosertib 225 mg bid x 5 doses on days 1-3 + carboplatin AUC5 IV on day 1 (every 21 days); arm C2: adavosertib 225 mg bid x 5 doses on days 1-3, 8-10, and 15-17 (weeks 1-3) + carboplatin AUC5 IV on day 1 (every 21 days); arms D and D2: adavosertib 175 or 225 mg bid x 5 doses on days 1-3 + PLD 40 mg/m^2 IV on day 1 (every 28 days). [a]Determined from optional tumor biopsy samples, which were not provided by all patients. AUC5, area under the concentration–time curve concentration of 5 mg/min·mL; bid, twice daily; ECOG, Eastern Cooperative Oncology Group; IV, intravenous; PLD, pegylated liposomal doxorubicin; qd, once daily; tBRCA1/2, tumor breast cancer gene 1/2.

Table 2.3.3 Response and survival rates (N=94).

Arm	CR *n* (%)	PR *n* (%)	SD *n* (%)	ORR *n*/N (%)	DCR *n*/N (%)	CA-125 response rate *n*/N (%)	Median PFS months (95% CI)	Median OS months (90% CI)
Arm A	0	1 (11.1)	2 (22.2)	1/9 (11.1)	3/9 (33.3)	2/8 (25.0)	1.7 (0.3–5.5)	16.0 (2.2–NC)
Arm B	1 (2.6)	10 (26.3)	16 (42.1)	11/38 (28.9)	27/38 (71.1)	15/28 (53.6)	5.5 (3.7–7.4)	NC (11.6–NC)
Arm C	1 (4.3)	6 (26.1)	12 (52.2)	7/23 (30.4)	19/23 (82.6)	4/15 (26.7)	4.2 (2.8–8.9)	8.9 (6.5–NC)
Arm C2	1 (8.3)	7 (58.3)	4 (33.3)	8/12 (66.7)	12/12 (100.0)	7/11 (63.6)	12.0 (2.7–NC)	19.2 (12.4–19.2)
Arm D	0	2 (33.3)	1 (16.7)	2/6 (33.3)	3/6 (50.0)	1/4 (25.0)	2.7 (0.5–NC)	6.2 (2.0–NC)
Arm D2	0	1 (16.7)	4 (66.7)	1/6 (16.7)	5/6 (83.3)	1/4 (25.0)	NC (NC–NC)	NC (NC–NC)
Overall[a]	3/94 (3.2)	27/94 (28.7)	39/94 (41.5)	30/94 (31.9)	69/94 (73.4)	30/70 (42.9)	5.5 (3.9–7.2)	19.2 (12.4–19.2)

Arm A: adavosertib 175 mg qd on days 1–2, 8–9, and 15–16 + gemcitabine 1000 mg/m^2 IV on days 1, 8, and 15 (every 28 days); arm B: adavosertib 225 mg bid x 5 doses on days 1–3, 8–10, and 15–17 + paclitaxel 80 mg/m^2 IV on days 1, 8, and 15 (every 28 days); arm C: adavosertib 225 mg bid x 5 doses on days 1–3 + carboplatin AUC5 IV on day 1 (every 21 days); arm C2: adavosertib 225 mg bid x 5 doses on days 1–3, 8–10, and 15–17 (weeks 1–3) + carboplatin AUC5 IV on day 1 (every 21 days); arms D and D2: adavosertib 175 or 225 mg bid x 5 doses on days 1–3 + PLD 40 mg/m^2 IV on day 1 (every 28 days). [a]Overall values are presented as *n*/N (%) for CR, PR, SD, ORR, DCR, and CA125 response. AUC5, area under the concentration–time curve concentration of 5 mg/min·mL; bid, twice daily; CA-125, cancer antigen 125; CR, complete response; DCR, disease control rate; IV, intravenous; NC, not calculable; ORR, objective response rate; OS, overall survival; PFS, progression-free survival; PLD, pegylated liposomal doxorubicin; PR, partial response; qd, once daily; SD, stable disease.

Table 2.3.4 Most frequent adverse events (N=94).

MedDRA preferred term, n (%)	Arm A (N=9)	Arm B (N=38)	Arm C (N=23)	Arm C2 (N=12)	Arm D (N=6)	Arm D2 (N=6)	Overall (N=94)
Patients with at least one adverse event	9 (100.0)	38 (100.0)	23 (100.0)	12 (100.0)	6 (100.0)	6 (100.0)	94 (100.0)
Nausea	5 (55.6)	23 (60.5)	19 (82.6)	10 (83.3)	4 (66.7)	4 (66.7)	65 (69.1)
Diarrhea	3 (33.3)	31 (81.6)	16 (69.6)	6 (50.0)	1 (16.7)	5 (83.3)	62 (66.0)
Fatigue	3 (33.3)	23 (60.5)	17 (73.9)	8 (66.7)	3 (50.0)	5 (83.3)	59 (62.8)
Anemia/hemoglobin decreased	3 (33.3)	24 (63.2)	14 (60.9)	9 (75.0)	3 (50.0)	2 (33.3)	55 (58.5)
Neutropenia/neutrophil count decreased	8 (88.9)	25 (65.8)	8 (34.8)	11 (91.7)	1 (16.7)	2 (33.3)	55 (58.5)
Thrombocytopenia/platelet count decreased	3 (33.3)	15 (39.5)	16 (69.6)	11 (91.7)	0	1 (16.7)	46 (48.9)
Vomiting	4 (44.4)	19 (50.0)	13 (56.5)	4 (33.3)	3 (50.0)	2 (33.3)	45 (47.9)
Abdominal pain	2 (22.2)	8 (21.1)	8 (34.8)	1 (8.3)	1 (16.7)	2 (33.3)	22 (23.4)
Leukopenia/white blood cell count decreased	2 (22.2)	13 (34.2)	5 (21.7)	0	1 (16.7)	1 (16.7)	22 (23.4)
Dyspnea	1 (11.1)	10 (26.3)	4 (17.4)	4 (33.3)	0	1 (16.7)	20 (21.3)
Hypomagnesemia/blood magnesium decreased	1 (11.1)	8 (21.1)	7 (30.4)	2 (16.7)	0	0	18 (19.1)
Headache	1 (11.1)	8 (21.1)	7 (30.4)	1 (8.3)	0	0	17 (18.1)
Decreased appetite	2 (22.2)	7 (18.4)	5 (21.7)	1 (8.3)	1 (16.7)	0	16 (17.0)
Back pain	1 (11.1)	6 (15.8)	4 (17.4)	3 (25.0)	1 (16.7)	0	15 (16.0)
Constipation	1 (11.1)	4 (10.5)	5 (21.7)	3 (25.0)	2 (33.3)	0	15 (16.0)
Hypokalemia/blood potassium decreased	1 (11.1)	4 (10.5)	3 (13.0)	2 (16.7)	1 (16.7)	0	11 (11.7)
Edema peripheral	0	10 (26.3)	0	4 (33.3)	0	0	14 (14.9)
Pyrexia	4 (44.4)	8 (21.1)	1 (4.3)	0	1 (16.7)	0	14 (14.9)
Dysgeusia	1 (11.1)	4 (10.5)	3 (13.0)	4 (33.3)	1 (16.7)	0	13 (13.8)
Hyperglycemia	1 (11.1)	6 (15.8)	3 (13.0)	0	1 (16.7)	0	11 (11.7)
Insomnia	1 (11.1)	6 (15.8)	2 (8.7)	1 (8.3)	1 (16.7)	0	11 (11.7)
Urinary tract infection	0	6 (15.8)	2 (8.7)	1 (8.3)	1 (16.7)	1 (16.7)	11 (11.7)

Arm A: adavosertib 175 mg qd on days 1-2, 8-9, and 15-16 + gemcitabine 1000 mg/m^2 IV on days 1, 8, and 15 (every 28 days); arm B: adavosertib 225 mg bid x 5 doses on days 1-3, 8-10, and 15-17 + paclitaxel 80 mg/m^2 IV on days 1, 8, and 15 (every 28 days); arm C: adavosertib 225 mg bid x 5 doses on days 1-3 + carboplatin AUC5 IV on day 1 (every 21 days); arm C2: adavosertib 225 mg bid x 5 doses on days 1-3, 8-10, and 15-17 (weeks 1-3) + carboplatin AUC5 IV on day 1 (every 21 days); arms D and D2: adavosertib 175 or 225 mg bid x 5 doses on days 1-3 + PLD 40 mg/m^2 IV on day 1 (every 28 days). MedDRA, Medical Dictionary for Regulatory Activities.

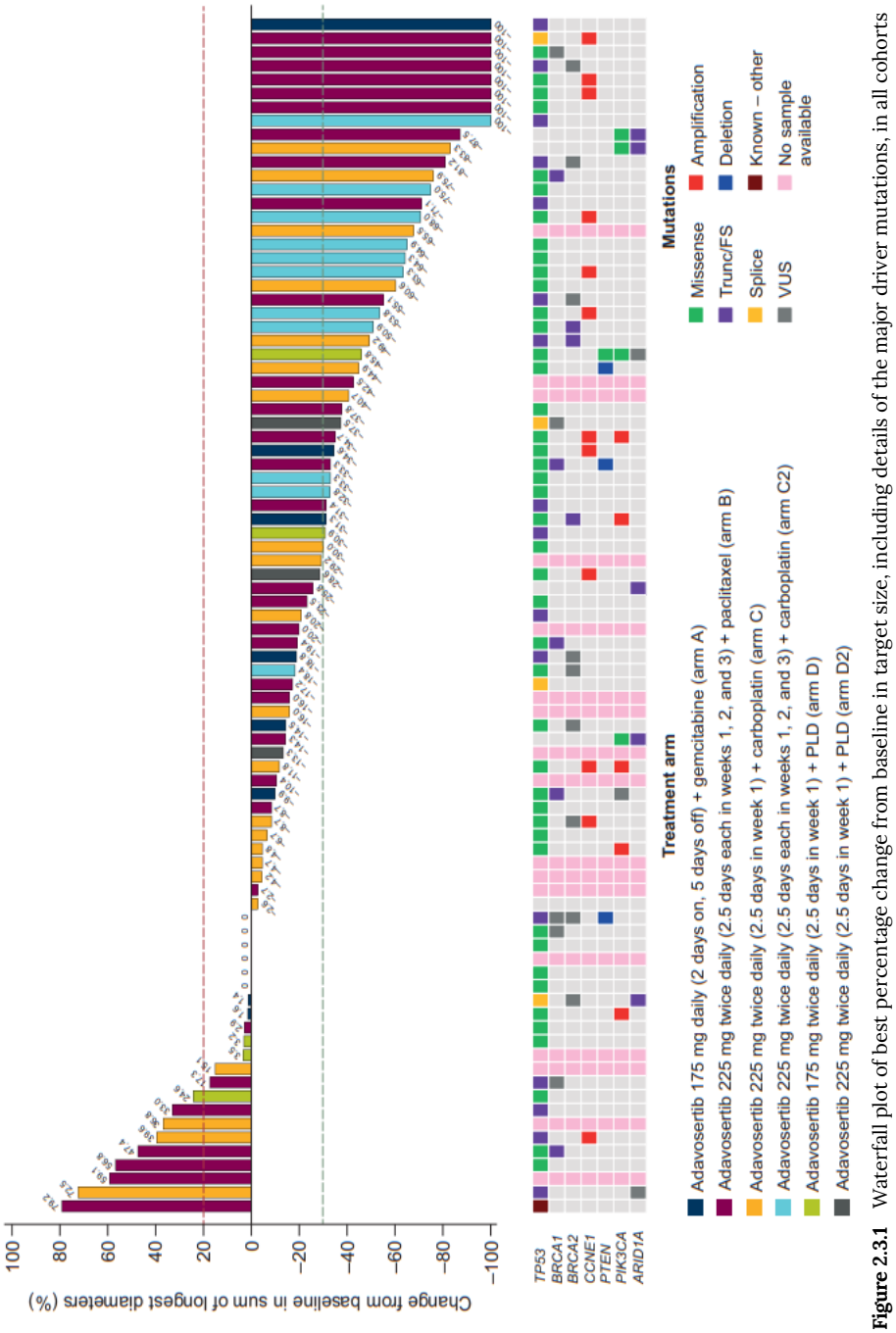

Figure 2.3.1 Waterfall plot of best percentage change from baseline in target size, including details of the major driver mutations, in all cohorts

The most common (≥10%) AEs are listed in Table 2.3.4. The most common (≥10%) grade ≥3 treatment-related AEs are listed in Supplementary Table S2.3.1. A total of 46.8% of patients overall experienced serious AEs (SAEs), including 27.7% who experienced adavosertib related SAEs (Supplementary Table S2.3.2).

Pharmacokinetics

Adavosertib was steadily absorbed following oral administration of the drug in combination with infusion of chemotherapy agents. Median time to maximum plasma concentration (t_{max}) values was 2.00-4.08 hours after a single dose on cycle 1 day 1 and 2.88-3.92 hours after multiple bid doses on cycle 1 day 3. After reaching maximum plasma concentration (C_{max}), adavosertib was slowly eliminated, with concentrations remaining relatively constant through 8 hours post-dose; geometric mean plasma concentrations at 8 hours post-dose were approximately 42-92% and 56% of the corresponding geometric mean C_{max} after single and multiple dosing, respectively.

Following a single dose of adavosertib 175 mg plus gemcitabine 1000 mg/m^2, adavosertib C_{max} and AUC from time zero to time t (AUC_{0-t}) values were slightly higher than with gemcitabine 800 mg/m^2. Mean systemic exposure (C_{max} and AUC_{0-t}) to adavosertib following a single dose of adavosertib 225 mg plus paclitaxel 80 mg/m^2 or carboplatin AUC5 was similar.

After multiple bid doses of adavosertib plus PLD, mean C_{max} was 42- to 44-fold higher and mean AUC_{0-t} was 36- to 46-fold higher than after single-dose adavosertib plus other chemotherapy agents. As the adavosertib dose increased from 175 to 225 mg (1.29-fold increase), adavosertib mean C_{max} increased 5.7-fold. This higher adavosertib plasma exposure associated with PLD had not been observed in any previous adavosertib studies, and PLD was not expected to result in a drug interaction with adavosertib. Additional investigations (bioanalytical interference, *in vitro* metabolism, and binding to liposomes) did not reveal a possible mechanism for higher exposure. The PLD-associated increased adavosertib concentration did not result in additional toxicity.

Genetic biomarkers

Exploratory analyses of response and next-generation sequencing (NGS) of pretreatment samples showed that the *TP53* mutation was the most

common genetic aberration found across all cohorts (range, 87.1-100%; Supplementary Figure S2.3.2). All functional *TP53* mutations were somatic. Only one KRAS hotspot mutation (G12V) was identified; all others were amplifications (Supplementary Table S2.3.3). No statistically significant correlation was observed between genomic markers and clinical response.

Discussion

In this multisite, multi-arm, Phase II trial of adavosertib in combination with chemotherapy in the treatment of primary PROC, a notable efficacy signal was observed with the combination of adavosertib and carboplatin, particularly for patients in arm C2. The ORR in this arm was 66.7% and the efficacy signals were durable, with mPFS of 12.0 months and mOS of 19.2 months. These findings are significant when one considers historical controls for ORR and time-toevent endpoints for primary platinum-resistant disease. In clinical trials of single-agent gemcitabine, paclitaxel, carboplatin, or PLD, overall tumor response rates ranged from 5% to 30% in platinum-resistant and platinum-refractory patients[32-37]. At a median of 12.0 months, PFS was longer than usually observed in patients with PROC (3-4 months). The JAVELIN 200 ovarian cancer trial observed an ORR of 4.2%, mPFS of 3.5 months, and mOS of 13.1 months for patients treated with PLD[6]. The results presented here are consistent with a Phase II study in which patients with *TP53*-mutated, recurrent EOC with relapse within 3 months following primary platinum-based chemotherapy were given adavosertib plus carboplatin[16]. The ORR was 43% among all evaluable patients and 47% for patients with serous tumors, median PFS was 5.3 months, and mOS was 12.6 months[22]. The time to relapse of ≤3 months following primary platinum treatment differed from the time to relapse of ≤6 months in this study. Furthermore, here, the efficacy signal in the carboplatin arms was not limited to the *TP53*-mutant cases. Two CRs were observed with the combination of adavosertib and carboplatin, both in patients without a *TP53* mutation: in arm C, a patient with clear-cell histology, a loss-of-function mutation in *ARID1A*, a hotspot mutation in *PIK3CA*, and amplification of *MET*, *ERBB2*, and *ZNF217*; in arm C2, a patient with serous histology, a loss-of-function mutation in *ARID1A*, and a hotspot mutation in *PIK3CA*. Owing to the known risk of gastrointestinal toxicity with adavosertib, premedication with a 5-HT3 antagonist and dexamethasone was mandatory prior to each

adavosertib dose, regardless of study arm (aprepitant and fosaprepitant were not permitted because of the risk of drug–drug interactions). Vigorous antidiarrheal treatment with loperamide was also mandated at the first onset of diarrhea according to American Society of Clinical Oncology guidelines[38]. Toxicity was considered generally manageable with dose delays, dose reductions, intermittent dosing, and/or the use of supportive care. Hematologic toxicity was more frequent in arm C2 than in the other arms and was also more frequent than would be expected for single-agent chemotherapy. This is an expected challenge, and additional studies with larger cohorts are required to further optimize the dose schedule and supportive medications for the combination of adavosertib and chemotherapy. The results here are in accordance with previous trials investigating the combination of adavosertib and chemotherapy. In patients with primary platinum-refractory or early platinum-resistant disease, hematologic toxicity was severe with adavosertib in combination with carboplatin, with 44% having grade 4 thrombocytopenia and 39% grade ≥3 neutropenia[22]. Hematologic toxicity was also observed in a randomized Phase II trial of gemcitabine with or without adavosertib in patients with platinum-resistant, measurable disease, with grade ≥3 anemia in 31% versus 18%, thrombocytopenia in 31% versus 6%, and neutropenia in 62% versus 30% of patients[23]. Platinum-based chemotherapy remains an important treatment option for ovarian cancer. As recently outlined in ovarian cancer treatment recommendations, patients who are defined as 'inappropriate for platinum', based on true progression during receipt of platinum or an allergy, may benefit from the addition of novel drugs such as adavosertib that disrupt the DNA damage response and potentiate the benefit of platinum treatment[40]. It is noteworthy that the vast majority of patients in this study had grade 3 or 4 histology; therefore, further studies are required to explore adavosertib plus chemotherapy in other histologies. In this study, the combination with gemcitabine did not appear to have preliminary activity, with an ORR of 11.1%. This differs from a recent study of gemcitabine with and without adavosertib in PROC presented by Lheureux and colleagues, which found that the addition of adavosertib improved mPFS from 3 to 4.6 months, mOS from 7.2 to 11.5 months, and ORR from 1% to 21%[23]. However, the Lheureux et al. study allowed many prior lines of therapy, so it is likely that patients had acquired platinum resistance. Patients in this current study all had primary platinum resistance, which carries a poorer prognosis[41]. There were no apparent PK drug interactions

between adavosertib and gemcitabine, paclitaxel, or carboplatin when co-administered. As previously reported by Leijen et al., plasma exposure in this work increased dose proportionally in the combination therapy arms, and the PK parameters were not different between the chemotherapy groups, with the exception of the PLD combination[16]. Several studies are investigating adavosertib combined with chemotherapy in ovarian cancer (NCT02272790, NCT02101775) and other tumor types. Different adavosertib monotherapy schedules are also being examined (NCT02482311, NCT02610075). Studies are selecting genetic aberrations that may affect response, including breast cancer gene 1/2 (BRCA1/2) mutations and *CCNE1* amplifications, which are usually mutually exclusive (NCT02482311, NCT02511795)[42]. *CCNE1*-amplified tumors have a poor prognosis and are generally refractory to therapies[43]. In the present study, no clear correlation was observed between genomic markers and clinical response. However, the number of patients included in each arm was too small to reach meaningful conclusions.

In conclusion, adavosertib showed preliminary efficacy when combined with chemotherapy in primary platinum-resistant EOC. The most promising treatment combination was adavosertib 225 mg bid on days 1-3, 8-10, and 15-17 plus carboplatin every 21 days. The mPFS of 12 months was longer than usually observed in patients with PROC (3-4 months). However, hematologic toxicity was more frequent in this cohort than in the other cohorts, as well as higher than would be expected for carboplatin monotherapy. This clinical trial adds to the mounting data regarding efficacy of adavosertib in combination with chemotherapy. However, its long-term tolerability profile and generalized use may not be feasible at the explored doses and regimens. As previously stated, future studies are planned to evaluate the efficacy of alternative dosing strategies, combination partners, and biomarker enrichment in an effort to individualize this therapy to those most likely to benefit, while also establishing the optimal safety and tolerability profile[21].

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Table S2.3.1 Treatment-related adverse events of CTCAE grade ≥3 by preferred term (full analysis set).

MedDRA preferred term, *n* (%)	Arm A (N=9)	Arm B (N=38)	Arm C (N=23)	Arm C2 (N=12)	Arm D (N=6)	Arm D2 (N=6)	Overall (N=94)
Neutropenia/neutrophil count decreased	7 (77.8)	20 (52.6)	5 (21.7)	9 (75.0)	0	2 (33.3)	43 (47.7)
Thrombocytopenia/platelet count decreased	2 (22.2)	4 (10.5)	12 (52.2)	10 (83.3)	0	1 (16.7)	29 (30.9)
Anemia/hemoglobin decreased	1 (11.1)	9 (23.7)	11 (47.8)	7 (58.3)	0	0	28 (29.8)
Leukopenia/white blood cell count decreased	1 (11.1)	10 (26.3)	3 (13.0)	0	0	1 (16.7)	15 (16.0)
Diarrhea	0	4 (10.5)	4 (17.4)	1 (8.3)	0	0	9 (9.6)
Febrile neutropenia	0	5 (13.2)	1 (4.3)	2 (16.7)	0	0	8 (8.5)
Vomiting	0	3 (7.9)	3 (13.0)	1 (8.3)	1 (16.7)	0	8 (8.5)
Fatigue	0	7 (18.4)	0	0	0	0	7 (7.4)
Nausea	0	0	3 (13.0)	1 (8.3)	1 (16.7)	0	5 (5.3)
Pulmonary embolism	0	1 (2.6)	0	2 (16.7)	0	0	3 (3.2)
Hypokalemia/blood potassium decreased	0	1 (2.6)	2 (4.3)	0	0	0	3 (3.2)
Bacteremia	0	1 (2.6)	0	1 (8.3)	0	0	2 (2.1)
Hypophosphatemia	0	1 (2.6)	0	1 (8.3)	0	0	2 (2.1)
Infusion-related reaction	0	0	2 (4.3)	0	0	0	2 (2.1)
Sepsis	0	1 (2.6)	0	1 (8.3)	0	0	2 (2.1)
Hyponatremia	0	1 (2.6)	0	0	0	0	1 (1.1)
Urinary tract infection	0	1 (2.6)	0	0	0	0	1 (1.1)
Syncope	0	0	1 (4.3)	0	0	0	1 (1.1)
Alanine aminotransferase increased	0	1 (2.6)	0	0	0	0	1 (1.1)
Anaphylactic reaction	0	0	0	1 (8.3)	0	0	1 (1.1)
Blood magnesium decreased	0	0	1 (4.3)	0	0	0	1 (1.1)
Decreased appetite	1 (11.1)	0	0	0	0	0	1 (1.1)
Fungal sepsis	0	1 (2.6)	0	0	0	0	1 (1.1)
Hepatic infection	0	1 (2.6)	0	0	0	0	1 (1.1)
Hypermagnesemia	0	0	1 (4.3)	0	0	0	1 (1.1)
Liver abscess	0	1 (2.6)	0	0	0	0	1 (1.1)
Lymphocyte count decreased	0	1 (2.6)	0	0	0	0	1 (1.1)
Neutropenic sepsis	0	1 (2.6)	0	0	0	0	1 (1.1)
Pancytopenia	0	1 (2.6)	0	0	0	0	1 (1.1)

Table S2.3.1 (continued)

MedDRA preferred term, *n* (%)	Arm A (N=9)	Arm B (N=38)	Arm C (N=23)	Arm C2 (N=12)	Arm D (N=6)	Arm D2 (N=6)	Overall (N=94)
Peripheral sensory neuropathy	0	1 (2.6)	0	0	0	0	1 (1.1)
Septic shock	0	1 (2.6)	0	0	0	0	1 (1.1)
Stomatitis	0	0	0	1 (8.3)	0	0	1 (1.1)

Arm A: adavosertib 175 mg qd on days 1-2, 8-9, and 15-16 + gemcitabine 1000 mg/m^2 (subsequently reduced to 800 mg/m^2 following a protocol amendment) IV on days 1, 8, and 15 (every 28 days); arm B: adavosertib 225 mg bid x 5 doses on days 1-3, 8-10, and 15-17 + paclitaxel 80 mg/m^2 IV on days 1, 8, and 15 (every 28 days); arm C: adavosertib 225 mg bid x 5 doses on days 1-3 + carboplatin AUC5 IV on day 1 (every 21 days); arm C2: adavosertib 225 mg bid x 5 doses on days 1-3, 8-10, and 15-17 (weeks 1-3) + carboplatin AUC5 IV on day 1 (every 21 days); arms D and D2: adavosertib 175 or 225 mg bid x 5 doses on days 1-3 + PLD 40 mg/m^2 IV on day 1 (every 28 days). AUC5, area under the concentration–time curve, concentration of 5 mg/mL·min; bid, twice daily; CTCAE, Common Terminology Criteria for Adverse Events; IV, intravenous; MedDRA, Medical Dictionary for Regulatory Activities; PLD, pegylated liposomal doxorubicin; qd, once daily.

Table S2.3.2 Adavosertib-related serious adverse events by preferred term (full analysis set).

MedDRA preferred term, *n* (%)	Arm A (N=9)	Arm B (N=38)	Arm C (N=23)	Arm C2 (N=12)	Arm D (N=6)	Arm D2 (N=6)	Overall (N=94)
Thrombocytopenia	0	0	3 (13.0)	5 (41.7)	0	0	8 (8.5)
Febrile neutropenia	0	5 (13.2)	1 (4.3)	1 (8.3)	0	0	7 (7.4)
Vomiting	0	1 (2.6)	2 (8.7)	1 (8.3)	1 (16.7)	0	5 (5.3)
Anemia	0	0	4 (17.4)	0	0	0	4 (4.3)
Nausea	0	1 (2.6)	2 (8.7)	0	1 (16.7)	0	4 (4.3)
Neutropenia	0	1 (2.6)	0	2 (16.7)	0	1 (16.7)	4 (4.3)
Pulmonary embolism	0	0	0	2 (16.7)	0	0	2 (2.1)
Bacteremia	0	1 (2.6)	0	1 (8.3)	0	0	2 (2.1)
Diarrhea	0	0	2 (8.7)	0	0	0	2 (2.1)
Platelet count decreased	0	0	2 (8.7)	0	0	0	2 (2.1)
Leukopenia	0	0	1 (4.3)	0	0	0	1 (1.1)
Liver abscess	0	1 (2.6)	0	0	0	0	1 (1.1)
Neutropenic sepsis	0	1 (2.6)	0	0	0	0	1 (1.1)
Neutrophil count decreased	0	0	1 (4.3)	0	0	0	1 (1.1)
Pancytopenia	0	1 (2.6)	0	0	0	0	1 (1.1)
Sepsis	0	0	0	1 (8.3)	0	0	1 (1.1)
Septic shock	0	1 (2.6)	0	0	0	0	1 (1.1)
Vascular device infection	0	1 (2.6)	0	0	0	0	1 (1.1)

Arm A: adavosertib 175 mg qd on days 1-2, 8-9, and 15-16 + gemcitabine 1000 mg/m^2 (subsequently reduced to 800 mg/m^2 following a protocol amendment) IV on days 1, 8, and 15 (every 28 days); arm B: adavosertib 225 mg bid x 5 doses on days 1-3, 8-10, and 15-17 + paclitaxel 80 mg/m^2 IV on days 1, 8, and 15 (every 28 days); arm C: adavosertib 225 mg bid x 5 doses on days 1-3 + carboplatin AUC5 IV on day 1 (every 21 days); arm C2: adavosertib 225 mg bid x 5 doses on days 1-3, 8-10, and 15-17 (weeks 1-3) + carboplatin AUC5 IV on day 1 (every 21 days); arm D and D2: adavosertib 175 or 225 mg bid x 5 doses on days 1-3 + PLD 40 mg/m^2 IV on day 1 (every 28 days). AUC5, area under the concentration–time curve, concentration of 5 mg/mL·min; bid, twice daily; IV, intravenous; MedDRA, Medical Dictionary for Regulatory Activities; PLD, pegylated liposomal doxorubicin; qd, once daily.

Table S2.3.3 Details of genetic mutations in all patients with available archival tumor tissue (FoundationOne®) by treatment arm.

#	BOR	Genes known to be altered in cancer					
		Somatic short variants (% reads, coverage)	Likely somatic short variants (% reads, coverage)	High-level (CN >8) and focal (CN >5) amplifications (CN, exons)	Non-focal lower-level (CN ≤8) amplifications (CN, exons)	HMZ deletions (0, exons)	Likely functional rearrangements (G1_G2_genomic event description_supporting reads)
Arm A							
1	PD	TP53_c.695T>G_p.I232S (0.73,368)	None	PIK3CA amplification (9, exons 20 of 20), SOX2 amplification (9, exons 5 of 5), FGF12 amplification (10, exons 6 of 6)	None	MAP2K4 loss (0, exons 2 of 12)	None
2	PD	ARID1A_c.3826C>T_p.R1276* (0.55,416)	MAP2K4_c.477_477delA_p.Y160fs*37 (0.62,391), TP53_c.782+1G>T_p.splice site 782+1G>T(0.67,333), TSC2_c.3751A>T_p.K1251*(0.64,318)	None	None	None	ARID1A_N/A_truncation_106
3	PD	TP53_c.423C>G_p.C141W (0.36,374)	BRCA1_c.3626_3627insA_p.E1210fs*9 (0.7,762), MLL2_c.4380_4381insC_p.L1461fs*30 (0.24,505)	GNAS amplification (8, exons 15 of 15), ARFRP1 amplification (8, exons 6 of 6), MYC amplification (14, exons 5 of 5)	None	None	None
4	PD	TP53_c.396G>T_p.K132N (0.21,646)	LZTR1_c.1794C>A_p.C598*(0.4,696)	None	None	None	None
5	SD	ATM_c.9022C>T_p.R3008C (0.6,1375), TP53_c.1004_1004delG_p.R335fs*10 (0.56,390)	CTCF_c.489_489delG_p.Q165fs*14 (0.35,792)	ZNF217 amplification (7, exons 4 of 4)	None	None	None
6	PR	TP53_c.578A>G_p.H193R (0.7,345)	BRCA2_c.5616_5620delAGTAA_p.K1872fs*2(0.81,336)	TERC amplification (8, exons 3 of 3), PRKCI amplification (8, exons 18 of 18), PIK3CA amplification (8, exons 20 of 20), SOX2 amplification (8, exons 5 of 5), MYC amplification (12, exons 5 of 5)	None	None	None
7	SD	EPHA6_c.545G>C_p.R182P (0.02,1324), SPEN_c.6995G>A_p.R2332H (0.66,1144), TP53_c.646G>T_p.V216L (0.61,579)	None	ERBB2 amplification (10, exons 27 of 27), CCNE1 amplification (15, exons 10 of 10), AKT2 amplification (9, exons 13 of 13), CCND3 amplification (10, exons 5 of 5)	None	None	PRDM1_N/A_truncation_38

Table S2.3.3 (continued)

#	BOR	Genes known to be altered in cancer					
		Somatic short variants (% reads, coverage)	**Likely somatic short variants (% reads, coverage)**	**High-level (CN >8) and focal (CN >5) amplifications (CN, exons)**	**Non-focal lower-level (CN ≤8) amplifications (CN, exons)**	**HMZ deletions (0, exons)**	**Likely functional rearrangements (G1_G2_genomic event description_ supporting reads)**
8	PD	TP53_c.581T>G_p.L194R (0.66,499)	None	ERBB3 amplification (9, exons 28 of 28), CDK4 amplification (7, exons 7 of 7), GATA6 amplification (7, exons 6 of 6), CCNE1 amplification (13, exons 10 of 10), AKT2 amplification (21, exons 13 of 13), AXL amplification (8, exons 20 of 20), AKT3 amplification (7, exons 14 of 14), FGFR3 amplification (7, exons 17 of 17), RICTOR amplification (7, exons 39 of 39)	None	None	None
Arm B							
1	PD	TP53_c.393_395delCAA_p.N131del(0.53,534)	CDK12_c.1757delC_p.P586fs*24 (0.361,543)	ERBB4 amplification (6, exons 28 of 28), BRAF amplification (7, exons 18 of 18)	None	None	None
2	PD	TP53_c.716A>G_p.N239S (0.36,662)	None	None	None	None	None
3	PD	TP53_c.844C>T_p.R282W (0.1285,1004)	BRCA1_c.5266_5267insC_p.Q1756fs*74(0.5417,1056)	MYC amplification (8, exons 4 of 5)	None	None	None
4	PD	TP53_c.916C>T_p.R306* (0.86,1280)	None	TERC amplification (7, exons 3 of 3), MYC amplification (9, exons 5 of 5)	EMSY amplification (7, exons 20 of 20)	CDKN2A loss (0, exons 5 of 5), CDKN2B loss (0, exons 5 of 5)	None

Table S2.3.3 (continued)

#	BOR	Genes known to be altered in cancer					
		Somatic short variants (% reads, coverage)	Likely somatic short variants (% reads, coverage)	High-level (CN >8) and focal (CN >5) amplifications (CN, exons)	Non-focal lower-level (CN ≤8) amplifica-tions (CN, exons)	HMZ deletions (0, exons)	Likely functional rearrangements (G1_G2_genomic event description_ supporting reads)
5	SD	CDKN2A_c.235_236insA_p.T79fs*41 (0.1113,557), NRAS_c.181C>A_p.Q61K (0.0754,796), TP53_c.602delT_p.L201fs*46 (0.0722,831), BCOR_c.4112C>T_p.S1371L (0.4847,588)	None	None	None	None	None
6	SD	TP53_c.775G>T_p.D259Y(0.64,565)	MLL3_c.9433_9452delGGACCACAGGCCATTCC TCA_p.P3146fs*9(0.72,696)	MCL1 amplification (7, exons 5 of 5)	None	RUNX1 loss (0, exons 9 of 9)	None
7	SD	CREBBP_c.4336C>T_p.R1446C (0.12,537), TP53_c.711G>A_p.M237I (0.13,487)	None	AKT2 amplification (9, exons 13 of 13)	None	None	None
8	PD	TP53_c.548C>G_p.S183* (0.59,572)	None	None	None	PTEN loss (0, exons 9 of 9)	None
9	SD	TP53_c.764T>G_p.I255S (0.04,646), SLIT2_c.826G>A_p.A276T (0.49,911)	None	None	None	None	None
10	SD	PIK3CA_c.317G>T_p.G106V (0.1761,954), KEAP1_c.1085G>A_p.R362Q (0.1806,742)	ARID1A_c.257_274GCGGAGCCGGCAGCGGCG>CT_p.G86fs*10(0.2,235), LZTR1_c.102_123del22_p.C34fs*1 (0.1878,607), ARID1A_c.4270C>T_p.Q1424*(0.1629,792), KEAP1_c.1709-1G>A_p.splice site 1709-1G>A(0.1866,595), FAT1_c.10207-1G>C_p.splice site 10207-1G>C(0.1883,579)	None	None	None	None
11	SD	None	TP53_c.560-1G>T_p.splice site 560-1G>T(0.04,1114)	None	None	None	None

Table S2.3.3 (continued)

#	BOR	Genes known to be altered in cancer					
		Somatic short variants (% reads, coverage)	**Likely somatic short variants (% reads, coverage)**	**High-level (CN >8) and focal (CN >5) amplifications (CN, exons)**	**Non-focal lower-level (CN ≤8) amplifica-tions (CN, exons)**	**HMZ deletions (0, exons)**	**Likely functional rearrangements (G1_G2_genomic event description_ supporting reads)**
12	SD	TP53_c.817C>T_p.R273C (0.3492,799)	BRCA1_c.4189delA_p.R1397fs*8 (0.2887,530)	None	None	None	ETV6_N/A_ truncation_14
13	SD	TP53_c.817C>T_p.R273C (0.58,1044)	None	None	None	None	None
14	SD	KDR_c.2030C>T_p.T677M (0.02,937)	ACVR1B_c.718G>T_p.E240*(0.14,982), ARID1A_c.2402_2403insG_p.Q802fs *15(0.15,554), RAD50_c.2801_2802insA_p.N934fs*10 (0.32,738)	None	None	None	None
15	SD	KDR_c.2030C>T_p.T677M (0.02,937)	ACVR1B_c.718G>T_p.E240*(0.14,982), ARID1A_c.2402_2403insG_p.Q802fs *15(0.15,554), RAD50_c.2801_2802insA_p.N934fs*10 (0.32,738)	None	None	None	None
16	SD	TP53_c.848G>C_p.R283P (0.71,1040)	SPTA1_c.1000delC_p.H334fs*11 (0.39,957), BRCA1_c.3858_3861delTGAG_p.S1286fs *20(0.66,893)	PDGFRA amplification (8, exons 22 of 22), PARP1 amplification (8, exons 23 of 23), MYC amplification (12, exons 5 of 5)	None	PTEN loss (0, exons 2 of 9)	None
17	PR	TP53_c.797G>T_p.G266V (0.5,612)	EP300_c.3459_3459delT_p.I1153fs*4 (0.41,532)	CCNE1 amplification (63, exons 10 of 10), BCL2L1 amplification (11, exons 4 of 4), CRKL amplification (14, exons 5 of 5), TERC amplification (7, exons 3 of 3), PRKCI amplification (7, exons 18 of 18), PIK3CA amplification (7, exons 20 of 20), CCND3 amplification (7, exons 5 of 5)	None	None	None
18	PR	TP53_c.536A>G_p.H179R (0.1634,826)	None	None	None	None	FANCD2_N/A_ truncation_20
19	PR	TP53_c.916C>T_p.R306* (0.03,402)	BRCA2_c.10096_10097insATTATATCTA_p.S336 6fs*4(0.4,652)	None	None	None	None

Table S2.3.3 (continued)

#	BOR	Genes known to be altered in cancer					
		Somatic short variants (% reads, coverage)	Likely somatic short variants (% reads, coverage)	High-level (CN >8) and focal (CN >5) amplifications (CN, exons)	Non-focal lower-level (CN ≤8) amplifications (CN, exons)	HMZ deletions (0, exons)	Likely functional rearrangements (G1_G2_genomic event description_supporting reads)
20	PR	TP53_c.395A>G_p.K132R (0.57,614)	None	CCNE1 amplification (15, exons 10 of 10)	None	None	None
21	PR	TP53_c.902delC_p.P301fs*44 (0.29,1201)	None	PRKCI amplification (8, exons 18 of 18), TERC amplification (8, exons 3 of 3), MYC amplification (8, exons 5 of 5)	None	None	ATM_ATM_duplication_36
22	CR	PIK3CA_c.3140A>G_p.H1047R (0.02,1130)	ARID1A_c.267_295delCAGCGGCGGCGGGCCCGGCGCGGAGCCGG_p.S90fs*11 (0.08,238)	None	None	None	None
23	PR	TP53_c.818G>A_p.R273H (0.015,1132)	None	None	None	None	None
24	PR	TP53_c.524G>A_p.R175H (0.53,190)	None	CCNE1 amplification (11, exons 10 of 10), AKT2 amplification (11, exons 13 of 13)	None	None	None
25	PR	TP53_c.517G>C_p.V173L (0.0182,658)	None	None	None	None	None
26	PR	TP53_c.1024C>T_p.R342* (0.0913,416)	None	None	None	None	None
27	PR	TP53_c.574C>T_p.Q192* (0.1206,887)	None	None	None	None	None
28	SD	None	NF1_c.316G>T_p.E106*(0.09,601), NF2_c.600-1G>C_p.splice site 600-1G>C(0.09,807), TP53_c.674_674delT_p.G226fs*21 (0.07,500)	None	None	None	None

Table S2.3.3. (continued)

#	BOR	Genes known to be altered in cancer					
		Somatic short variants (% reads, coverage)	**Likely somatic short variants (% reads, coverage)**	**High-level (CN >8) and focal (CN >5) amplifications (CN, exons)**	**Non-focal lower-level (CN ≤8) amplifications (CN, exons)**	**HMZ deletions (0, exons)**	**Likely functional rearrangements (G1_G2_genomic event description_supporting reads)**
Arm C							
1	PD	RANBP2_c.3376C>T_p.R1126W (0.28,1101)	TET2_c.4333C>T_p.Q1445*(0.02,529), TP53_c.221_221delC_p.P75fs*48 (0.81,889)	GATA6 amplification (8, exons 6 of 6), NOTCH3 amplification (10, exons 32 of 33), MCL1 amplification (11, exons 5 of 5), BCL2L1 amplification (9, exons 4 of 4), MYCN amplification (8, exons 5 of 5), TERC amplification (9, exons 3 of 3), CCND3 amplification (9, exons 5 of 5), VEGFA amplification (9, exons 8 of 8), MYC amplification (17, exons 5 of 5), LYN amplification (14, exons 12 of 12)	KRAS amplification (8, exons 5 of 5), KDM5A amplification (8, exons 28 of 28), CCND2 amplification (8, exons 5 of 5), FGF23 amplification (8, exons 3 of 3), FGF6 amplification (8, exons 3 of 3), ALK amplification (8, exons 29 of 29)	None	None
2	PD	None	TP53_c.495_495delG_p.Q165fs*5 (0.82,416)	KRAS amplification (24, exons 5 of 5), IRS2 amplification (8, exons 5 of 5), CCNE1 amplification (8, exons 10 of 10), MYCL1 amplification (7, exons 4 of 5), SOX2 amplification (9, exons 5 of 5)	None	None	None
3	SD	None	None	CRKL amplification (16, exons 5 of 5), TERC amplification (7, exons 3 of 3), PRKCI amplification (7, exons 18 of 18), ESR1 amplification (9, exons 8 of 8)	None	None	None

Table S2.3.3 (continued)

#	BOR	Genes known to be altered in cancer					
		Somatic short variants (% reads, coverage)	Likely somatic short variants (% reads, coverage)	High-level (CN >8) and focal (CN >5) amplifications (CN, exons)	Non-focal lower-level (CN ≤8) amplifications (CN, exons)	HMZ deletions (0, exons)	Likely functional rearrangements (G1_G2_genomic event description_supporting reads)
4	SD	TP53_c.528C>G_p.C176W (0.83,842)	PIK3R1_c.1349_1378del30_p.H450_S460>R(0.24,383)	PIK3CB amplification (8, exons 23 of 23)	EPHB1 amplification (8, exons 16 of 16), TERC amplification (8, exons 3 of 3), PRKCI amplification (8, exons 18 of 18), PIK3CA amplification (8, exons 20 of 20)	MAP2K4 loss (0, exons 8 of 12)	None
5	SD	TP53_c.578A>G_p.H193R (0.07,601)	None	None	None	None	None
6	SD	TP53_c.736A>G_p.M246V (0.25,485)	None	CCNE1 amplification (14, exons 10 of 10), MYC amplification (11, exons 5 of 5)	None	None	None
7	SD	TP53_c.857A>G_p.E286G (0.63,1459)	None	CCNE1 amplification (14, exons 10 of 10), TERC amplification (7, exons 3 of 3), PIK3CA amplification (7, exons 20 of 20)	None	None	None
8	SD	TP53_c.796G>T_p.G266* (0.43,719)	None	EMSY_amplification(8, exons 20 of 20)	None	NF1 loss (0, exons 10 of 59)	None
9	SD	TP53_c.844C>T_p.R282W (0.17,901)	None	ERBB2 amplification (24, exons 27 of 27), PIK3C2B amplification (7, exons 32 of 32), MDM4 amplification (7, exons 10 of 10)	None	None	None
10	PR	TP53_c.814G>T_p.V272L (0.73,510)	MAP2K4_c.115+1G>C_p.splice site 115+1G>C(0.43,119)	None	None	PTEN loss (0, exons 9 of 9)	None

Table S2.3.3 (continued)

#	BOR	Genes known to be altered in cancer					
		Somatic short variants (% reads, coverage)	**Likely somatic short variants (% reads, coverage)**	**High-level (CN >8) and focal (CN >5) amplifications (CN, exons)**	**Non-focal lower-level (CN ≤8) amplifications (CN, exons)**	**HMZ deletions (0, exons)**	**Likely functional rearrangements (G1_G2_genomic event description_supporting reads)**
11	PR	None	BRCA2_c.6450_6451insA_p.V2151fs*25 (0.65,671), TP53_c.716_738del23_p.N239fs*17 (0.69,472)	AKT1 amplification (16, exons 13 of 13), TERC amplification (7, exons 3 of 3), PRKCI amplification (7, exons 18 of 18)	None	None	None
12	PR	TP53_c.776A>T_p.D259V (0.43,472)	FBXW7_c.1734_1735delAG_p.G579fs *25(0.11,635)	KRAS amplification (7, exons 5 of 5), CCND2 amplification (7, exons 5 of 5), FGF23 amplification (7, exons 3 of 3), FGF6 amplification (7, exons 3 of 3), AKT2 amplification (9, exons 13 of 13)	None	None	None
13	PR	TP53_c.524G>A_p.R175H (0.62,378)	None	None	None	None	None
14	PR	TP53_c.736A>G_p.M246V (0.43,482)	BRCA1_c.5266_5267insC_p.Q1756fs*74 (0.66,604), PBRM1_c.237-66_238del68_p.splice site 237-66_238del68(0.21,625)	None	None	MAP2K4 loss (0, exons 2 of 12)	MSH6_FBXO11_truncation_11
15	CR	PIK3CA_c.1035T>A_p.N345K (0.4,509)	ARID1A_c.3413C>G_p.S1138*(0.67,199)	ERBB2 amplification (5, exons 27 of 27), ZNF217 amplification (7, exons 4 of 4), MET amplification (6, exons 20 of 20)	None	None	None
Arm C2							
1	SD	TP53_c.584T>C_p.I195T (0.47,997)	None	HGF amplification (7, exons 19 of 19), AKT3 amplification (8, exons 14 of 14), PRKCI amplification (7, exons 18 of 18)	None	MAP2K4 loss (0, exons 12 of 12), LRP1B loss (0, exons 14 of 91)	None
2	SD	TP53_c.467_468GC>CT_p.R156P(0.27,699)	None	None	None	None	None
3	SD	TP53_c.742C>T_p.R248W (0.93,830)	None	None	None	None	NCOR1_NCOR1_duplication_30
4	SD	TP53_c.584T>C_p.I195T (0.41,770)	None	None	None	None	None

Table S2.3.3 (continued)

#	BOR	Genes known to be altered in cancer					
		Somatic short variants (% reads, coverage)	**Likely somatic short variants (% reads, coverage)**	**High-level (CN >8) and focal (CN >5) amplifications (CN, exons)**	**Non-focal lower-level (CN ≤8) amplifications (CN, exons)**	**HMZ deletions (0, exons)**	**Likely functional rearrangements (G1_G2_genomic event description_supporting reads)**
5	PR	PIK3C2B_c.380A>G_p.Y127C (0.65,692), TP53_c.523C>G_p.R175G(0.27,560)	BRCA2_c.8437_8439GGA>TTT_p.G2813F(0.11,557), BRCA2_c.8437G>T_p.G2813*(0.5,557)	KRAS amplification (11, exons 5 of 5), RICTOR amplification (19, exons 39 of 39)	None	None	None
6	PR	TP53_c.722C>T_p.S241F (0.77,819)	TSC1_c.2776C>T_p.Q926*(0.19,776)	CCNE1 amplification (68, exons 10 of 10), KDM5A amplification (7, exons 28 of 28), CCND2 amplification (7, exons 5 of 5), FGF23 amplification (7, exons 3 of 3), KRAS amplification (7, exons 5 of 5), FGF6 amplification (7, exons 3 of 3), EMSY amplification (7, exons 20 of 20), MYC amplification (22, exons 5 of 5)	RICTOR amplification (7, exons 39 of 39), FGF10 amplification (7, exons 3 of 3)	PTPRD loss (0, exons 23 of 37)	TSC1_TSC1_deletion_12
7	PR	TP53_c.488A>G_p.Y163C (0.35,679), GNAS_c.489C>A_p.Y163* (0.14,869), MUTYH_c.1145G>A_p.G382D (0.51,510)	CHD4_c.4730_4737delTAGAAGGA_p.I1577fs*5(0.1,807)	MYC amplification (7, exons 5 of 5), CCNE1 amplification (7, exons 10 of 10), CCND3 amplification (9, exons 5 of 5), VEGFA amplification (9, exons 8 of 8), ROS1 amplification (9, exons 43 of 43)	None	None	None
8	PR	TP53_c.730G>T_p.G244C (0.53,593)	MLL3_c.10760_10761delAA_p.Q3587fs*8(0.38,1338)	None	None	None	None
9	PR	TP53_c.1024delC_p.R342fs*3 (0.22,734)	None	None	None	None	None
10	PR	MUTYH_c.1145G>A_p.G382D (0.31,899), TP53_c.422G>A_p.C141Y (0.43,624)	None	MDM2 amplification (11, exons 11 of 11), FRS2 amplification (11, exons 5 of 5), MCL1 amplification (8, exons 5 of 5), AKT3 amplification (7, exons 14 of 14), BRAF amplification (7, exons 18 of 18), KEL amplification (7, exons 19 of 19), MYC amplification (9, exons 5 of 5)	None	None	None

Table S2.3.3 (continued)

#	BOR	Genes known to be altered in cancer					
		Somatic short variants (% reads, coverage)	**Likely somatic short variants (% reads, coverage)**	**High-level (CN >8) and focal (CN >5) amplifications (CN, exons)**	**Non-focal lower-level (CN ≤8) amplifications (CN, exons)**	**HMZ deletions (0, exons)**	**Likely functional rearrangements (G1_G2_genomic event description_supporting reads)**
11	PR	None	TP53_c.672+1G>T_p.splice site 672+1G>T(0.33,410)	CCNE1 amplification (11, exons 10 of 10), NOTCH3 amplification (8, exons 33 of 33)	None	None	SMARCA4_FBXW9_truncation_115
Arm D							
1	PD	TP53_c.832C>T_p.P278S (0.23,793)	None	ERBB2 amplification (13, exons 27 of 27), AKT2 amplification (9, exons 13 of 13)	None	None	BRD4_N/A_truncation_66, SMARCA4_N/A_truncation_27
2	SD	TP53_c.832C>T_p.P278S (0.23,793)	None	ERBB2 amplification (13, exons 27 of 27), AKT2 amplification (9, exons 13 of 13)	None	None	BRD4_N/A_truncation_66, SMARCA4_N/A_truncation_27
3	PR	TP53_c.544_544delT_p.C182fs*65(0.3,483)	None	None	None	TSC2 loss (0, exons 3 of 41)	RB1_N/A_truncation_27

Table S2.3.3 (continued)

#	BOR	Genes known to be altered in cancer					
		Somatic short variants (% reads, coverage)	**Likely somatic short variants (% reads, coverage)**	**High-level (CN >8) and focal (CN >5) amplifications (CN, exons)**	**Non-focal lower-level (CN ≤8) amplifica-tions (CN, exons)**	**HMZ deletions (0, exons)**	**Likely functional rearrangements (G1_G2_genomic event description_ supporting reads)**
4	PR	CHD4_c.3280G>A_p.E1094K (0.11,799), ERBB3_c.310G>A_p.V104M (0.12,953), FBXW7_c.1513C>T_p.R505C (0.13,838), MLL3_c.8390_8390delA_p.K2797fs*26(0.12,858), MSH6_c.3253_3254insC_p.F1088fs*5(0.1,896), PIK3CA_c.263G>A_p.R88Q (0.12,781), PIK3CA_c.3139C>T_p.H1047Y (0.12,800), PPP2R1A_c.547C>T_p.R183W (0.1,743), PTEN_c.389G>A_p.R130Q (0.13,675), PTEN_c.518G>A_p.R173H (0.12,750), PTPN11_c.1403C>T_p.T468M (0.1,767), TP53_c.817C>T_p.R273C (0.11,1062), TP53_c.524G>A_p.R175H(0.14,817	FUBP1_c.1497-2A>G_p.splice site 1497-2A>G(0.1,834), LRP1B_c.3277C>T_p.R1093*(0.08,777), MSH6_c.2731C>T_p.R911*(0.49,948), SPTA1_c.3139C>T_p.R1047*(0.08,881)	None	None	None	None
Arm D2							
1	SD	TP53_c.817C>T_p.R273C (0.44,291)	None	IGF1R amplification (7, exons 21 of 21)	None	None	CDKN2A_ CDKN2A_deletion_59
2	PD	TP53_c.524G>A_p.R175H (0.09,536)	None	None	None	None	None

Table S2.3.3 (continued)

#	BOR	Genes known to be altered in cancer					
		Somatic short variants (% reads, coverage)	**Likely somatic short variants (% reads, coverage)**	**High-level (CN >8) and focal (CN >5) amplifications (CN, exons)**	**Non-focal lower-level (CN ≤8) amplifica-tions (CN, exons)**	**HMZ deletions (0, exons)**	**Likely functional rearrangements (G1_G2_genomic event description_ supporting reads)**
3	SD	TP53_c.659A>G_p.Y220C (0.47,406)	None	KRAS amplification (8, exons 5 of 5), GATA6 amplification (9, exons 6 of 6), CCNE1 amplification (19, exons 10 of 10), ZNF217 amplification (8, exons 4 of 4), AURKA amplification (8, exons 8 of 8), GNAS amplification (8, exons 15 of 15), MYC amplification (8, exons 5 of 5), FGFR1 amplification (11, exons 18 of 18), ARAF amplification (13, exons 15 of 15)	None	None	None
4	PR	HLA-A_c.684G>A_p.W228*(0.07,197)	NF2_c.361_363+1CAGG>A_p.splice site 361_363+1CAGG>A(0.43,552), TP53_c.920-3_927TAGCACTGCCC>G_p.splice site 920-3_927TAGCACTGCCC>G(0.31,1079)	PIK3C2B amplification (7, exons 32 of 32), MDM4 amplification (7, exons 10 of 10)	None	None	None

Arm A: adavosertib 175 mg qd on days 1–2, 8–9, and 15–16 + gemcitabine 1000 mg/m^2 (subsequently reduced to 800 mg/m^2 following a protocol amendment) IV on days 1, 8, and 15 (every 28 days); arm B: adavosertib 225 mg bid x 5 doses on days 1–3, 8–10, and 15–17 + paclitaxel 80 mg/m^2 IV on days 1, 8, and 15 (every 28 days); arm C: adavosertib 225 mg bid x 5 doses on days 1–3 + carboplatin AUC5 IV on day 1 (every 21 days); arm C2: adavosertib 225 mg bid x 5 doses on days 1–3, 8–10, and 15–17 (weeks 1–3) + carboplatin AUC5 IV on day 1 (every 21 days); arm D and D2: adavosertib 175 or 225 mg bid x 5 doses on days 1–3 + PLD 40 mg/m^2 IV on day 1 (every 28 days). amp, amplifications; AUC5, area under the concentration–time curve, concentration of 5 mg/mL·min; bid, twice daily; BOR, best objective response; CN, copy number; HMZ, homozygous; G1/2, gene 1/2; IV, intravenous; PLD, pegylated liposomal doxorubicin; qd, once daily.

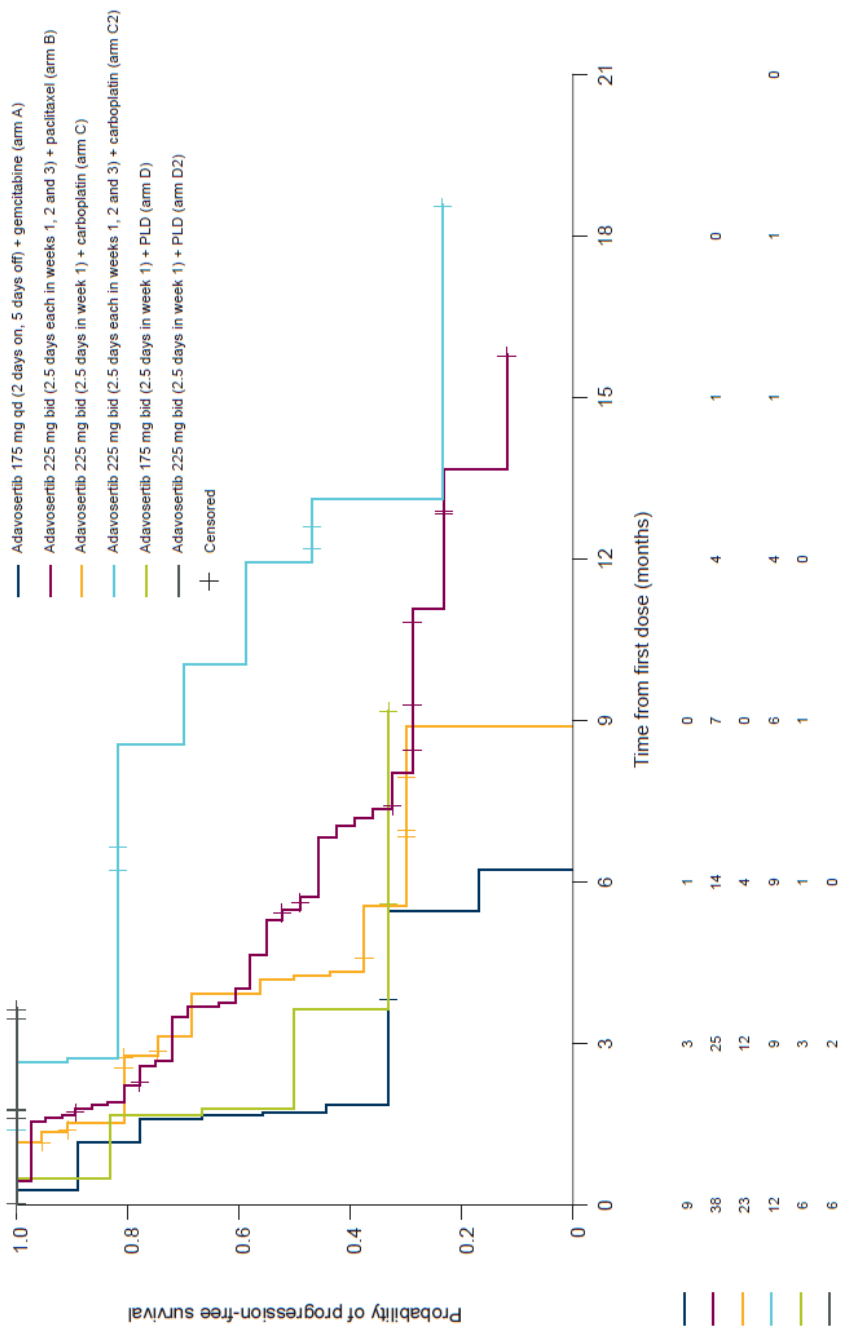
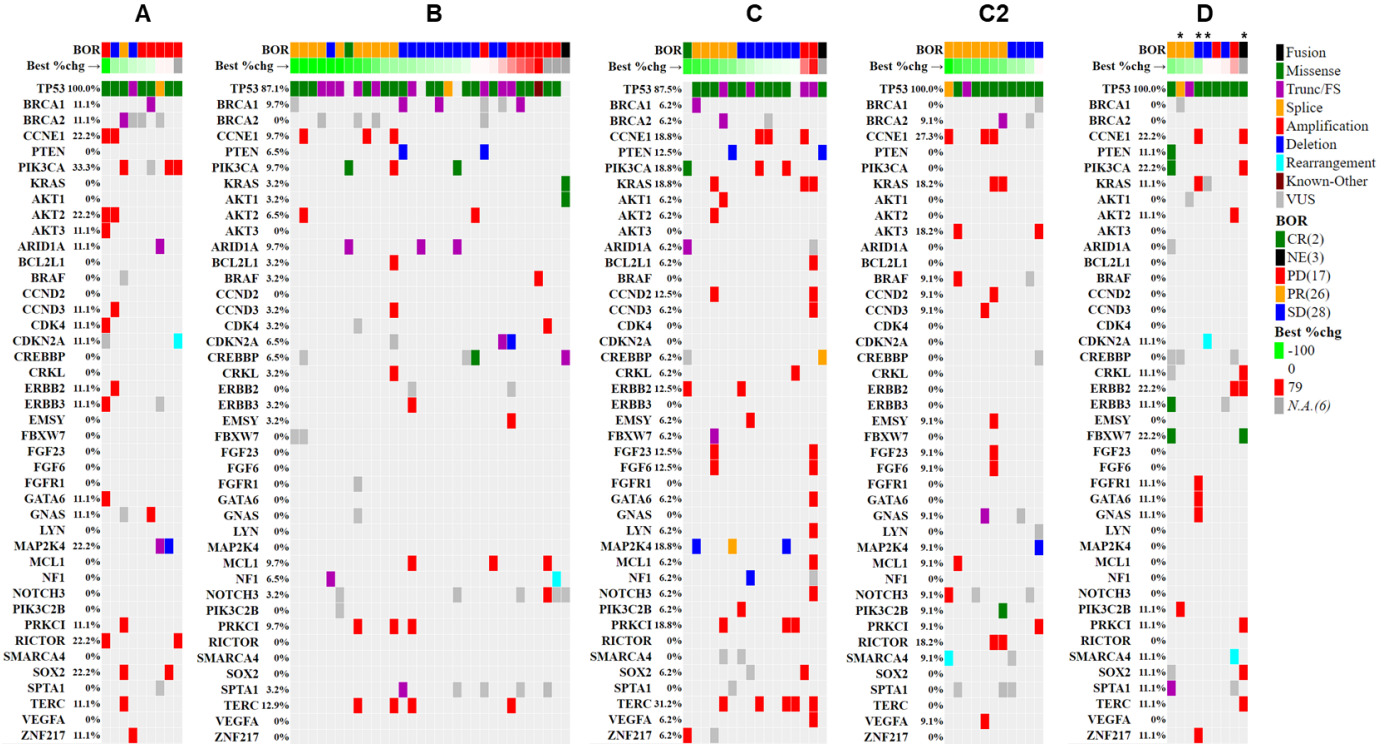
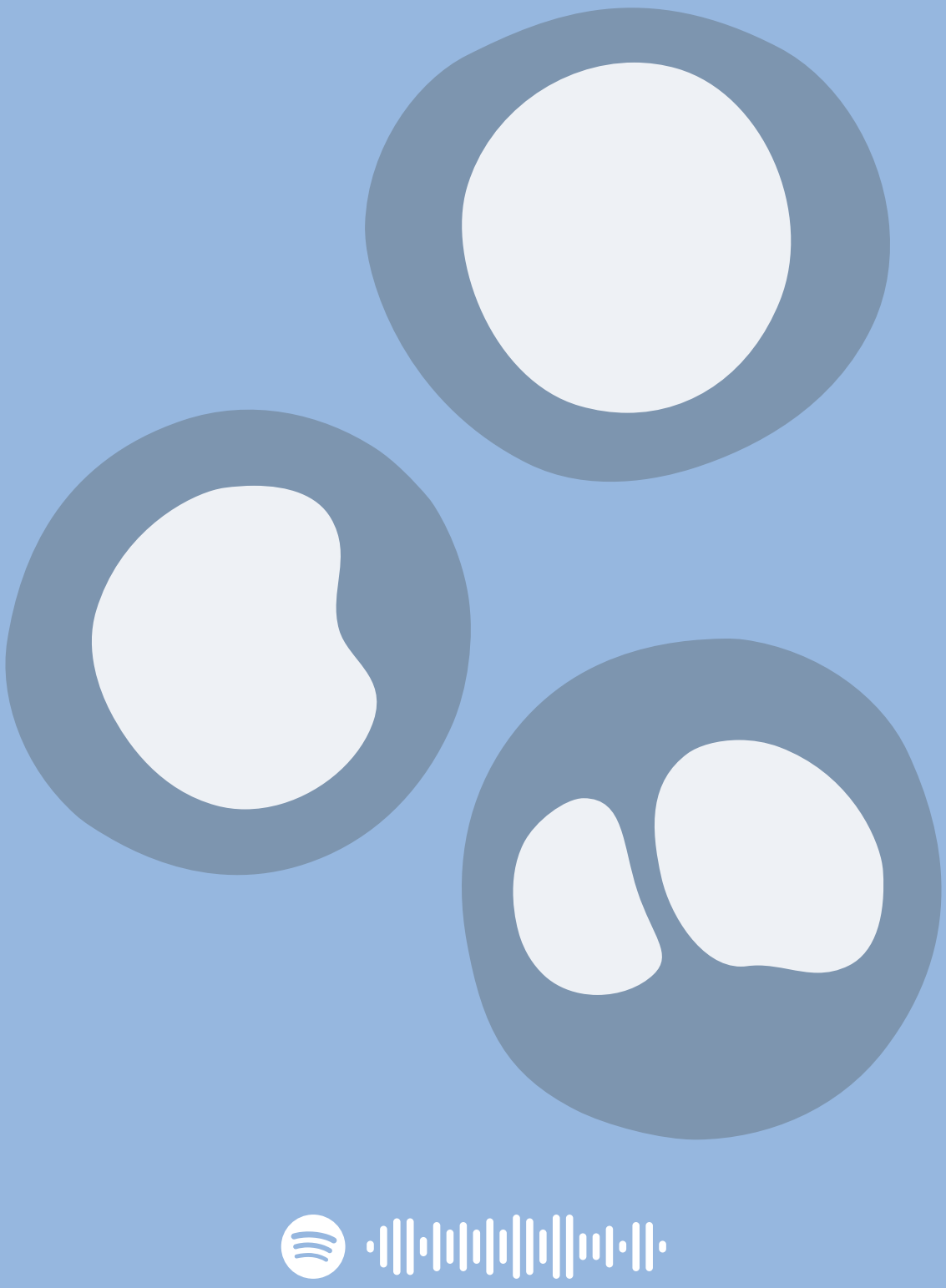


Figure S2.3.1 **Progression-free survival (Kaplan–Meier plot).**



igure S2.3.2 FoundationOne® genomic profiles of all available archival tumor tissue and clinical outcomes across patient cohorts. Arms: (A) 175 mg qd + G (n = 9); (B) 225 mg bid + P (n = 38); (C) 225 mg bid week 1 + C (n = 23); (C2) 225 mg bid weeks 1–3 + C (n = 12); (D) 175 mg bid + PLD and 225 mg bid (denoted by *) + PLD (n = 12). Only patients who had tissues available that were successfully sequenced are shown. bid, twice daily; BOR, best overall response; C, carboplatin; CR, complete response; G, gemcitabine; NA, not applicable; NE, not estimable; P, paclitaxel; PD, progressive disease; PDIAG, primary diagnosis; PLD, pegylated liposomal doxorubicin; qd, once daily; SD, stable disease; Trunc/FS, truncation/frameshift; VUS, variant of unknown significance.



Chapter 3

PD-L1 inhibition

Chapter 3.1

A phase I study to access the safety and tolerability of carboplatin-cyclophosphamide combined with atezolizumab in patients with advanced breast- and gynecologic cancer

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Manuscript in preparation

Summary

Introduction
PD-L1 is an extracellular protein that downregulates immune responses through binding to its two receptors: PD-1 and B7-1. Expression of PD-L1, which suppresses activation of T-cells, can lead to progression of tumors. Atezolizumab is a humanized monoclonal antibody that targets human PD-L1 and inhibits its interaction with its receptors, PD-1 and B7-1, thereby blocking inhibitory signals to T-cells. We hypothesized that combining a bifunctional alkylating agent and a platinum agent with atezolizumab could further improve treatment of breast and gynecologic tumors. Here we investigate the safety and tolerablility of the combination.

Methods
This study was an open label, dose finding, phase Ib clinical study of carboplatin (starting dose: target AUC 5, day 1) and cyclophosphamide (starting dose 600 mg/m^2, day 1) combined with atezolizumab (fixed dose 840 mg, day 1, 15). Patients with histological or cytological proof of advanced breast cancer (M1) or advanced gynaecological (cervix (M1, FIGO IVA/IVB), ovarian (after recurrence on carboplatin and/or paclitaxel) or endometrial (T3-T4, FIGO IVA/IVB)) cancer were enrolled. The primary objective was to determine a safe dose combination of carboplatin and cyclophosphamide combined with atezolizumab fixed dose.

Results
In total 6 patients were included to define the safe dose-level. The median age was 59 years (range 44-59 years). The tumor types included were ovarian (n=2), breast (n=2), endometrial (n=1) and cervical cancer (n=1). The safe dose combination was defined as carboplatin AUC 5 (day 1), cyclophosphamide 600 mg/m^2 (day 1) and atezolizumab 840 mg (day 1,15). Anemia, white blood cell count and platelet count decrease were observed in all patients (6/6). Two patients developed an immune related colitis of whom one patient also suffered from a pneumonitis. Two patients had partial remission (PR) as best response.

Conclusion
The maximum tolerable dose was carboplatin target AUC 5 (day 1), cyclophosphamide 600 mg/m^2 (day 1) and atezolizumab 840 mg fixed dose (day 1/15). No unexpected toxicities occurred, and this combination can be taken forward to investigate which patients with advanced breast and gynecological cancer may benefit from this combination.

Introduction

There are many mechanisms by which tumor cells can escape from destruction by the immune system, including the expression of immune suppressive molecules on their cell surface, secretion of soluble suppressive factors, and the recruitment of other suppressive immune cell populations to the tumor environment[1]. PD-L1 is an inhibitory molecule expressed on T-cells following T-cell activation, which is sustained in states of chronic stimulation such as in chronic infection or cancer. PD-L1 downregulates immune responses primarily in peripheral tissues through binding to its two receptors: PD-1 and B7-1. Many tumors have been found to overexpress PD-L1, which acts to suppress anti-tumor activity[2,3]. Overexpression of PD-L1[4] has been associated with poor outcomes, although context seems to matter[5] as well as cell type that expresses PD-L1[5,6]. Binding of PD-L1 to PD-1 results in cytokine production, inhibition of T-cell proliferation and cytolytic activity, leading to the functional inactivation or exhaustion of T-cells. B7-1 is a molecule expressed on antigen-presenting cells, it activates T-cells. PD-L1 binding to B7-1 on T-cells and antigen presenting cells can mediate downregulation of immune responses, by inhibition of T-cell activation and cytokine production[7]. Overexpression of PD-L1 on tumor cells has been reported to encumber anti-tumor immunity, resulting in immune evasion[8]. Therefore, interrupting the PD-L1/PD-1 and the PD-L1/B7-1 pathways represents an attractive strategy to renew tumor-specific T-cell immunity. Atezolizumab is a humanized monoclonal IgG1 antibody that targets human PD-L1 and inhibits its interaction with its receptors, PD-1 and B7-1, and thereby releasing inhibitory signals to T-cells. In addition to the inhibitory effect on PD-1, atezolizumab also blocks the binding to B7-1 which might further enhance immune responses[9]. Overexpression of PD-L1 has been described in cancers such as ovarian cancer, renal cancer, gastric cancer and esophageal cancer. Anti-tumor activity has also been observed in different tumor types including triple negative breast cancer (TNBC)[10–16].There is increasing evidence that in addition to causing tumor cell death, certain conventional chemotherapies like carboplatin, paclitaxel and cyclophosphamide may have immunogenic effects (e.g. enhancement of NK-cell and T-cell functions)[17]. In addition, killing tumor cells by cytotoxic chemotherapy can be expected to expose the immune system to high levels of tumor antigens, and invigorating tumor-specific T-cell immunity in this setting, by inhibiting PD-L1/PD-1 signaling could result in deeper and more

durable responses compared with chemotherapy alone. TNBC is characterized by genetic instability that can result in formation of immunogenic neo-antigens and increased anti-tumor T-cell infiltration. ThereforeTNBC could be an an attractive type of cancer for treatment with immunomodulary drugs[18]. About 19-27% of TNBC show expression of PD-L1[19]. Besides in TNBC, PD-L1 expression also plays a role in gynecological cancers. Preclinical data show that ovarian cancer affected mice treated with the combination of chemotherapy and an anti-PD-1 antibody had a significantly prolonged survival, with more CD8+ T-cell infiltration into the tumor site[20]. To date, disappointing clinical results of anti-PD-1/PD-1L therapies in patients with relapsed ovarian cancer have been reported[21]. Regarding cervical cancer, recurrent copy number gain of the genes encoding the PD-1 ligands provide a genetic basis for PD-1 expression in a subset of cervical squamous cell carcinoma's and can therefore identify a class of patients that are rational candidates for therapies targeting PD-1[22]. The expression of PD-L1 in tumor samples of endometrial cancer seems to be upregulated. Of the primary endometrial cancers, 72% exhibited expression of PD-L1, suggesting that intervention of the PD-1/PD-L1 axis may be a promising treatment option for patients with endometrial cancer[23]. Carboplatin is the backbone of treatment in advanced cervical and endometrial cancers. To our knowledge the combination carboplatin-cyclophosphamide has not been evaluated as treatment in advanced breast, endometrial and cervical cancer. The schedule is safe and effective in the treatment of ovarian cancer[24-26]. Breast Cancer (BRCA) mutated, BRCA like tumors and tumors with mutations in mismatch repair genes are characterized by genetic instability that can result in formation of immunogenic neo-antigens and increased anti-tumor T-cell infiltration. Combining a bifunctional alkylating agent and a platinum agent with atezolizumab seems therefore a logical step to further improve treatment of these cancers. Because there is no evidence of the addition of atezolizumab to the combination of carboplatin and cyclophosphamide, this phase Ib trial was performed to evaluate the safety and tolerability of this triplet regimen.

Methods

Study design and treatment

This study was an open label, dose finding, phase Ib clinical study of carboplatin (starting dose: target AUC 5, day 1) and cyclophosphamide (starting dose 600 mg/m^2, day 1) combined with atezolizumab (fixed dose 840 mg, day 1, 15). The predefined dose-levels are illustrated in Table 3.1.2. The primary objective was to determine a safe dose combination of carboplatin-cyclophosphamide combined with atezolizumab fixed dose in patients with advanced breast and gynecologic cancer. Secondary objectives were to evaluate the tolerability of this combination therapy and to assess preliminary anti-tumor activity of carboplatin-cyclophosphamide with atezolizumab.

Patient population

This study was performed at the Netherlands Cancer Institute, Antoni van Leeuwenhoek in Amsterdam under a protocol approved by the Institutional Review Board. All participants provided written informed consent before entering the study. We enrolled patients with histological or cytological proof of advanced breast cancer (M1) or advanced cervical (M1, FIGO IVA/IVB), ovarian (after recurrence on carboplatin and/or paclitaxel) or endometrial (T3-T4, FIGO IVA/IVB)) cancer. Patients were not allowed to have received more than one line of systemic chemotherapy in the advanced setting and any line of hormonal therapy for advanced disease. Patients must potentially benefit from the carboplatin-cyclophosphamide-atezolizumab combination. Prior (neo-) adjuvant chemotherapy was accepted and was not counted as one line, since it was administered in early stage of disease. Other inclusion criteria included a WHO performance status of 0 or 1, a life expectancy of ≥3 months, allowing adequate follow up of toxicity evaluation and anti-tumor activity, absolute neutrophil count of ≥1.5 x10^0/L, platelet count of ≥ 100x10^9/L, hemoglobin value of ≥6.2 mmol/L and an adequate hepatic and renal function defined by serum bilirubin ≤1.5 x ULN (or ≤3x ULN in case of known Gilbert syndrome), AST and ALT <2.5x ULN (or <5x ULN in case of liver metastases), serum creatinine ≤1.5 x ULN or creatinine clearance ≥50 mL/min (by Cockcroft-Gault). Patients were excluded if they had been treated with other investigational drugs within 21 days prior to study enrollment, in case of known clinically significant liver

disease (including viral, alcoholic, or other hepatitis), prior treatment with CD137 agonists or immune checkpoint blockade therapies, including anti-CTLA-4, anti-PD-1, or anti PD-L1 therapeutic antibodies. Patients with a history of autoimmune disease or idiopathic pulmonary fibrosis were also excluded.

Maximum tolerable dose and dose-limiting toxicities

The maximum tolerable dose (MTD) was defined as the dose level below the dose level at which 2 of 6 patients experienced drug-related dose limiting toxicity (DLT) in the first cycle (28 days). Hematological dose limiting toxicities were defined as grade ≥4 neutropenia lasting ≥14 days, grade ≥3 febrile neutropenia, grade ≥4 thrombocytopenia lasting ≥14 days and grade ≥3 thrombocytopenia associated with bleeding episodes. Non-hematological toxicities ≥ grade 3 including grade 3 hyperbilirubinemia lasting for >8 hours or any grade 4, grade ≥3 AST/ALT elevations with hyperbilirubinemia or ≥ grade 2, grade 4 AST/ALT elevations. Failure to recovery from any toxicity resulting in a delay of two scheduled administrations of ≥28 days was also considered a DLT. It was not allowed to apply dose modifications for atezolizumab. Dose modifications were allowed for carboplatin and cyclophosphamide. A maximum of two dose reductions were allowed. Patients were not allowed to return to the previous dose-level if a dose reduction had been applied before.

Safety assessments

Safety was assessed by the evaluation of adverse events (AE), DLTs, changes in vital signs, Eastern cooperatieve Oncology Group (ECOG) Performance Status (PS), physical examinations and clinical laboratory tests (including hematology, coagulation, blood chemistry and auto-immune laboratory tests (including TSH, fT4, ACTH, cortisol, E2, FSH and LH)). AEs were graded according to National Cancer Institute (NCI)- Common Terminology Criteria for Adverse Events (CTCAE) version 4.03[19].

Efficacy assessments

Radiologic tumor assessments were made at least every two cycles or more frequently, if indicated. Tumor response was investigator assessed using Response Evaluation Criteria in Solid Tumors (RECIST)1.1. A partial response

(PR) or complete response (CR) must have been confirmed on a second examination performed at least 4 weeks apart in order to be documented as a confirmed response.

Results

Patient characteristics

In total seven patients with advanced breast or gynecologic cancer were enrolled between February 2017 and July 2017 (last patient in). Of the seven patients who received at least one dose of study drug, one patient was replaced because she received by accident a higher dose of carboplatin than necessary (750 mg instead of 510 mg). Six patients were evaluable for safety. The characteristics of the six evaluable female patients who received at least one cycle of carboplatin-cyclophosphamide combined with atezolizumab are shown in Table 3.1.1. The median age was 59 years (range 44-69 years). The tumor types included were ovarian (n=2), breast (n=2), endometrial (n=1), and cervical cancer (n=1). No BRCA mutation was present in all six evaluable patients.

Dose and dose-limiting toxicities

All six evaluable patients were treated at the starting dose-level 0 with carboplatin target AUC 5 (day 1), cyclophosphamide 600 mg/m^2 (day 1) and atezolizumab 840 mg fixed dose (days 1&15). None of the patients experienced a DLT, therefore no lower dose-level was tested. The MTD was carboplatin target AUC 5 (day 1 q 4 weeks), cyclophosphamide 600 mg/m^2 (day 1 q 4 weeks) and atezolizumab 840 mg fixed dose (days 1&15 q 4 weeks).

Safety

The most common adverse events were hematological events: white blood cell count decrease (6/6 patients, 100%), neutrophil count decrease (6/6, 100%), platelet count decrease (6/6, 100%) and anemia (6/6, 100%). Other common adverse events were fatigue (6/6, 100%) and nausea (6/6, 100%). Of the patients with white blood cell count decrease and neutrophil count decrease, the majority of the patients (4/6, 67%) had a grade 3 or 4 adverse event. One patient received four cycles of carboplatin/ cyclophosphamide

and atezolizumab and continued with atezolizumab monotherapy flat dose every three weeks. This was decided because of the severity of the adverse events she sufferend from, including grade 4 thrombocytopenia with petechiae for which she received transfusion and grade 4 neutropenia. No treatment related deaths were reported. Treatment related adverse events of any grade are listed in Table 3.1.3.

Table 3.1.1 Table showing the baseline characteristics for all 6 evaluable patients in this study.

	N	%
Gender		
Female	6	100
Age (median)(range) years	59 (44-69)	-
Tumortype primary disease		
Breast	2	33
Ovarian	2	33
Endometrial	1	17
Cervical	1	17
Stage		
Missing	2	33
Figo IIIC	1	17
Figo IV	2	33
Figo IV B	1	17
Ethnicity		
Caucasian	6	100
WHO performance status		
WHO 0	5	83
WHO 1	1	17
Lines of Rx for advanced disease		
0	4	66
1	2	33

Table 3.1.2 Dose-escalation cohorts for the phase-I part of the PROLOG study including a total of n=6 patients.

Dose-level	Patients (n)	Carboplatin (AUC)	cyclophosphamide (mg/m2)	Atezolizumab (mg)
start	6	5	600	840
-1	0	4.5	500	840
-2	0	4	400	840

Table 3.1.3 Adverse events at least possibly related to study treatment (carboplatin, cyclophosphamide or atezolizumab), graded according to CTCAE version 4.03.

Adverse event	Grade 1/2	Grade 3/4	Total
Hematology			
White blood cell decreased	2 (33%)	4 (67%)	6 (100%)
Neutrophil count decreased	2 (33%)	4 (67%)	6 (100%)
Lymphocyte count decreased	3 (50%)	2 (33%)	5 (83%)
Platelet count decreased	3 (50%)	3 (50%)	6 (100%)
Anemia	4 (67%)	2 (33%)	6 (100%)
Adverse event	Grade 1/2	Grade 3/4	Total
Immunology			
Pneumonitis	0	1 (17%)	1 (17%)
Colitis	2 (33%)	0	2 (33%)
Hypertension	4 (67%)	0	4 (67%)
Hypothyroidism	1 (17%)	0	1 (17%)
Adverse event	Grade 1/2	Grade 3/4	Total
General			
Fatigue	6 (100%)	0	6 (100%)
Nausea	6 (100%)	0	6 (100%)
Diarrhea	4 (67%)	0	4 (67%)
Vomiting	3 (50%)	0	3 (50%)
Alkaline phosphatase increased	3 (50%)	0	3 (50%)
Alanine aminotransferase increased	3 (50%)	0	3 (50%)
Aspartate aminotransferase increased	4 (67%)	1 (17%)	5 (83%)
gGT increased	3 (50%)	0	3 (50%)
Fever	3 (50%)	0	3 (50%)

Immune related toxicity

AEs of special interest were immune related toxicities. One patient developed an immune related hypothyroidism grade 1. Elevated blood pressure grade 1 occurred in four patients (67%) during treatment, for which no blood pressure lowering medication was needed. Two patients developed an immune related colitis. One patient, a 46-year-old female with cervical cancer, developed an immune related colitis after six cycles. She developed mucous diarrhea with high frequency. Feces cultivation showed no causative agent. She received prednisolone 1 mg/kg (60 mg). Shortly after the start of the prednisolone, the frequency of the diarrhea decreased. Phasing out of the prednisolone was started, however during the dose decrease of the prednisolone, the frequency of the diarrhea increased again. It was decided to administer one-time Infliximab 300 mg intravenously. After this administration, the prednisolone dose was successfully gradually reduced. The mucous diarrhea eventually resolved.

The second patient was a 67-year-old female with endometrial cancer, treated in this study with carboplatin, cyclophosphamide and atezolizumab. After cycle four she developed abdominal cramps and diarrhea. She had no fever. Feces cultures showed no cause of infection. An endoscopy was performed, biopsies showed no clear sign of immune related colitis. Few weeks later, the frequency of the diarrhea increased and rectal blood loss was observed. She was treated with prednisolone 1 mg/kg (60 mg) because of the suspicion of an immune related colitis. After three days of treatment, there was only a slight improvement of the complaints. Therefore, the antibiotic ciproxin was added to the treatment. After finishing the ciproxin course, the prednisolone dose was slowly reduced. However, during this reduction, the patient developed severe dyspnea. The differential diagnosis included immune related pneumonitis or infectious pneumonitis of bacterial or viral origin. Therefore, she was treated with a broad range of antibiotics and antiviral medication. A broncho-alveolar lavage (BAL) was performed and cultures were positive for Herpes Simplex virus type I (HSV type I). The patient was treated with both prednisolone 1 mg/kg and acyclovir, because immune related pneumonitis could not be ruled out completely despite the presence of HSV type I. The clinical condition of this patient improved and after two weeks, the acyclovir could be stopped. The prednisolone was slowly reduced. Because of the immune related toxicities, she continued the anti-tumor treatment with carboplatin monotherapy with target AUC 5. The cyclophosfamide and the atezolizumab were stopped.

Efficacy

In six evaluable patients, partial response (PR) was the best response observed in two patients (33%) with endometrial (n=1) and ovarian cancer (n=1). Stable disease (SD) was the best overall response observed in two patients (33%), (breast cancer (n=1), and cervical cancer (n=1)). Three patients (50%) had a prolonged response of >6 months, including one patient with endometrial cancer, one patient with cervical cancer and one patient with ovarian cancer. Two patients with breast cancer and ovarian cancer had progressive disease as best response. The best percentage of target lesions is shown in Figure 3.1.1.

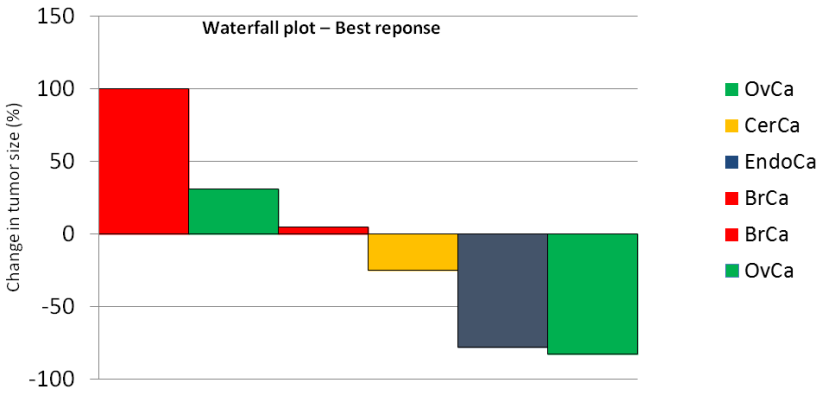

Figure 3.1.1 Waterfall plot showing the maximum change in target lesion diameter sum compared to baseline for the best clinical response observed in 6 PROLOG patients. Bars represent individual patients. Primary tumor origin is indicated.

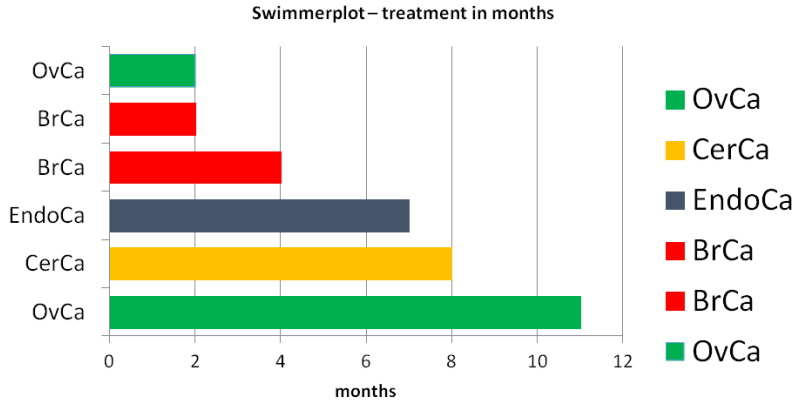

Figure 3.1.2 Swimmerplot showing the duration of treatment for each patient. OvCa: ovarian cancer, BrCA: breast cancer, EndoCa: endometrial cancer, CerCa: cervical cancer.

Table 3.1.4 Best response according to RECIST 1.1.

Best response	Number of patients
Partial response	2 (33%)
Progressive disease	2 (33%)
Stable disease	2 (33%)

Discussion

This phase Ib, dose-finding study evaluated the safety and efficacy of the combination therapy of carboplatin, cyclophosphamide and atezolizumab in advanced breast and gynecologic cancer. The MTD was carboplatin target AUC 5, cyclophosphamide 600 mg/m^2 and atezolizumab 840 mg fixed dose. No DLTs were observed. The most common adverse events were hematologic toxicities with anemia, platelet count decrease, white blood cell count decrease and neutrophil count decreases present in all six evaluable patients. Both low grade (grade 1/2) and high grade (grade 3/4) hematological toxicities occurred, with especially more high-grade white blood cell and neutrophil count decrease. These findings are in line with what we expected based on the knowledge about carboplatin and cyclophosphamide since they can both cause myelosuppression. Atezolizumab is a humanized monoclonal antibody that targets human PD-L1 and therefore inhibits the interaction between PD-L1 and PD-1 and B7-1. It has been approved for the treatment of metastatic non-small cell lung cancer, extensive-stage small cell lung cancer, first line metastatic hepatocellular carcinoma, and for locally advanced or metastatic urothelial cancer. Most common adverse events previously seen with monotherapy treatment with atezolizumab were fatigue, pyrexia, rash, increased aspartate aminotransferase, increased alanine transferase headache and decreased appetite[26,28]. PD-1/PD-L1 inhibitors are associated with a significantly higher risk for immune related adverse events according to a meta-analysis. The incidence of pneumonitis with treatment of PD-1 or PD-L1 inhibitors is on average 3.4%, whereas the incidence of colitis is ranges between 8% and 27%[29]. Compared to conventional chemotherapy, the relative risk for both immune related adverse events is 3.41 and 3.51[29]. In our study, two patients (33%) developed an immune related colitis. Both patients needed additional treatment next to prednisolone because of persistent symptoms. The first patient recovered completely from the immune related colitis. After decrease of the prednisolone to zero, she had no recurrence of diarrhea. The second patient however, developed a severe pneumonitis while decreasing the prednisolone dose. Although microbiological research proved the presence of Herpes Simplex I, an immune related component could not be ruled out completely at that moment. Therefore, both antiviral and systemic steroids were administered to this patient. It will remain unclear what would have happened if only the Herpes Simplex type I was treated,

and no steroids had been administered. Since the presence of an immune related colitis before, the chance of another immune related adverse event was significantly present. Therefore, this two pronged approach was chosen. The incidence of immune related adverse events in our trial seems somewhat higher compared to other trials with PD-L1/PD-1 inhibition monotherapy. New in this trial is the addition of the combination of carboplatin and cyclophosphamide to atezolizumab. The incidence of immune related adverse events is much higher compared to immune related adverse events with the administration of chemotherapy only. That said, immune related adverse events can occur with the administration of chemotherapy[29]. Both carboplatin and cyclophosphamide have immunogenic effects. Carboplatin inhibits the PDL2 expression, whereas cyclophosphamide is immunosuppressive at high doses, induces immunogenic cell death and selectively inhibits T_{reg} cells and restores T cell and natural killer (NK) cell functions[17]. Addition of carboplatin and cyclophosphamide to atezolizumab could therefore increase the immune response from atezolizumab and thus lead to more immune related side effects. A limitation of our study is that the sample size of our study is very small. Therefore, it is impossible to draw firm conclusions about the incidence of specific adverse events. The median time to develop PD-L1/PD-1 immune related adverse events ranges from 1-6 months, but it can vary between different tumor types and different PD-L1/PD-1 inhibitors. The median time to onset of an immune related pneumonitis is approximately 3 months[30]. However when immunotherapy is administered in combination therapy, the onset could be earlier[31]. Both patients with colitis showed first signs after 4 cycles (12 weeks) of treatment. This is in line with the median time to develop PD-L1/PD-1 immune related adverse events as described in literature. Recently the American Society of Clinical Oncology developed a clinical practice guideline for the management of immune related adverse events in patients treated with immune checkpoint inhibitor therapy[32]. Since many patients have been and will be treated with these compounds and awareness of possible adverse events is important, guidelines are indispensable. Two patients (33%) in our study showed partial response as best response. On the other hand, two patients (33%) showed progressive disease with no response on the CT-scan. Since the effects of immunotherapy can be seen after months, question is if you could conclude anything from a CT-scan made after 6 weeks of treatment. In our two patients, there was made a consideration looking not only at the CT-scan

but also at laboratory values, clinical signs and toxicities. It was decided that for both patients there were not enough advantages to continue treatment with carboplatin, cyclophosphamide and atezolizumab. Treatment for another 6 weeks could have been considered if patients had tolerated the treatment better, to assess any late responses. Another limitation of our trial was the lack of any pharmacokinetic measurements. It would have been interesting to have determined pharmacokinetics of carboplatin, cyclophosphamide and atezolizumab in this combination to provide more information about the exposure of each compound in this combination. Especially in combination with the adverse events seen, it might have provided additional information.

In summary, this phase I dose finding study evaluated combination therapy with carboplatin, cyclophosphamide and atezolizumab in advanced breast and gynecologic cancer. This combination could be administered safely, although in two patients immune related toxicities occurred. Preliminary antitumor activity was observed in two out of six patients. It is recommended to perform larger trials with the combination of carboplatin, cyclophosphamide and atezolizumab to gain additional safety and efficacy information.

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Supplemental material

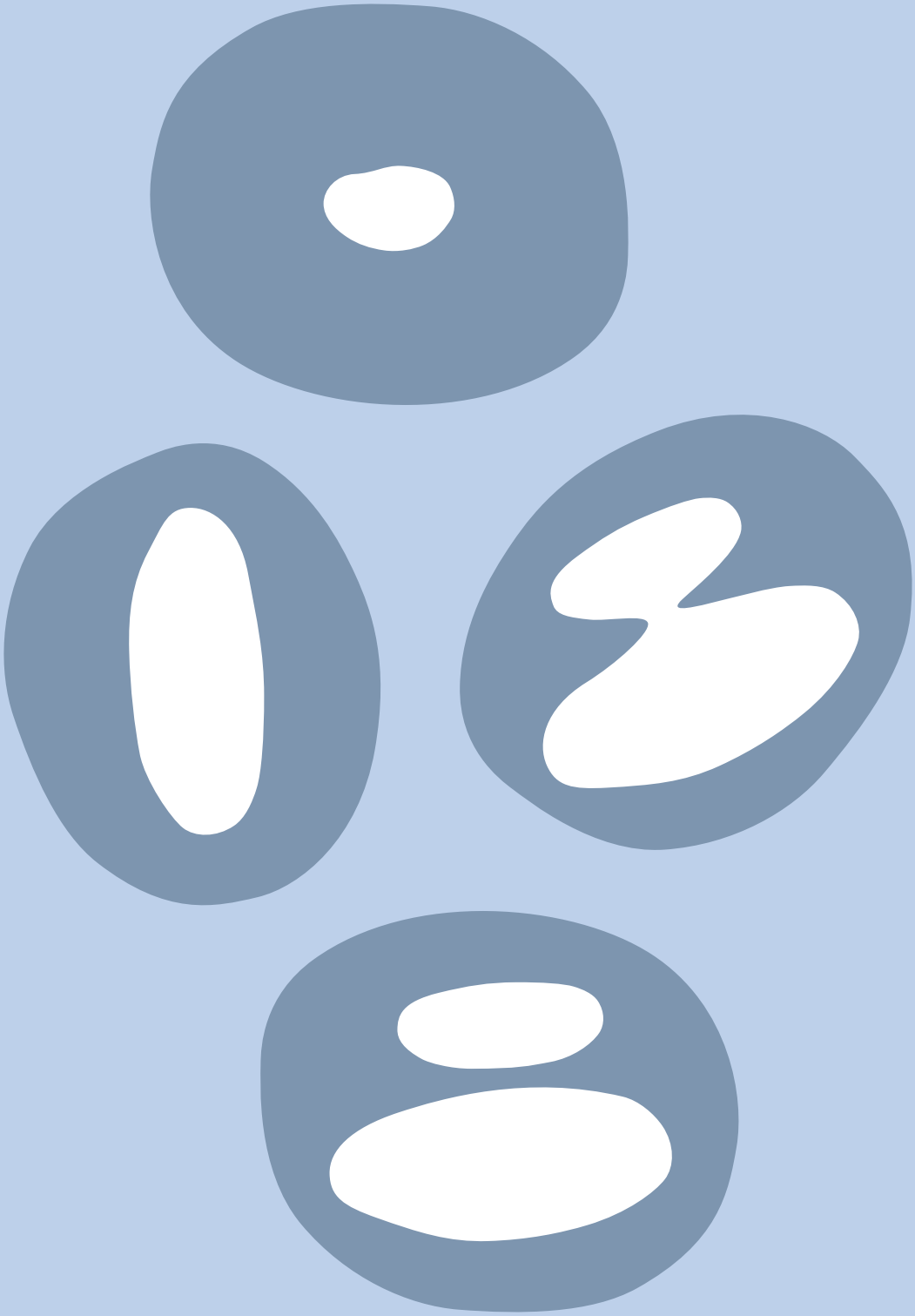
Table S3.1.1 Criteria for defining dose limiting toxicities (DLTs) occurring during the DLT assessment period (3 weeks).

Toxicity	DLT definition*
Hematological toxicity	Grade 4 neutropenia (ANC <0.5 x 109/l) lasting >14 days Grade ≥3 febrile neutropenia Grade 4 thrombocytopenia lasting >14 days Grade ≥3 thrombocytopenia associated with bleeding episodes
Non-hematological toxicity	Any non-hematological toxicity ≥3 including: Grade 3 hyperbilirubinemia lasting for >48 hours or any Grade 4 Grade ≥3 pneumonitis Grade ≥3 colitis Grade ≥3 AST/ALT elevations with hyperbilirubinemia of ≥ grade 2 Grade 4 AST/ALT elevations For patients with Grade 2 AST/ALT, and/or alkaline phosphatase abnormality at baseline, an increase to >10 x the upper limit of normal (ULN) that does not resolve to Grade ≤2 within 48 hours (if symptomatic) or that does not resolve to Grade ≤1 within 3 weeks of onset (if asymptomatic) will be considered a DLT.
Other	Failure to recover from any toxicity which results in a delay of 2 scheduled administrations of ≥28 days is considered a DLT.

*The toxicity or delay should be possibly, probably or definitely related to study drugs ASAT=aspartate aminotransferase, ALAT=alanine aminotransferase, ALP=alkaline phosphatase, yGT=gamma glutamyltransferase, LDH=lactate dehydrogenase, DLT=dose limiting toxicity

Chapter 4

Her2 inhibition



Chapter 4.1

Trastuzumab duocarmazine in locally advanced and metastatic solid tumours and HER2-expressing breast cancer: a phase 1 dose-escalation and dose-expansion study

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Summary

Background
Trastuzumab duocarmazine is a novel HER2-targeting antibody–drug conjugate comprised of trastuzumab covalently bound to a linker drug containing duocarmycin. Preclinical studies showed promising antitumour activity in various models. In this first-in-human study, we assessed the safety and activity of trastuzumab duocarmazine in patients with advanced solid tumours.

Methods
We did a phase 1 dose-escalation and dose-expansion study. The dose-escalation cohort comprised patients aged 18 years or older enrolled from three academic hospitals in Belgium, the Netherlands, and the UK with locally advanced or metastatic solid tumours with variable HER2 status who were refractory to standard cancer treatment. A separate cohort of patients were enrolled to the dose-expansion phase from 15 hospitals in Belgium, the Netherlands, Spain, and the UK. Dose-expansion cohorts included patients aged 18 years or older with breast, gastric, urothelial, or endometrial cancer with at least HER2 immunohistochemistry 1+ expression and measurable disease according to Response Evaluation Criteria in Solid Tumors (RECIST). Trastuzumab duocarmazine was administered intravenously on day 1 of each 3-week cycle. In the dose-escalation phase, trastuzumab duocarmazine was given at doses of 0,3 mg/kg to 2,4 mg/kg (3+3 design) until disease progression or unacceptable toxicity. The primary endpoint of the dose-escalation phase was to assess safety and ascertain the recommended phase 2 dose, which would be the dose used in the dose-expansion phase. The primary endpoint of the dose-expansion phase was the proportion of patients achieving an objective response (complete response or partial response), as assessed by the investigator using RECIST version 1.1. This ongoing study is registered with ClinicalTrials.gov, number NCT02277717, and is fully recruited.

Findings
Between Oct 30, 2014, and April 2, 2018, 39 patients were enrolled and treated in the dose-escalation phase and 146 patients were enrolled and treated in the dose-expansion phase. One dose-limiting toxic effect (death from pneumonitis) occurred at the highest administered dose (2,4 mg/kg) in

the dose-escalation phase. One further death occurred in the dose-escalation phase (1,5 mg/kg cohort) due to disease progression, which was attributed to general physical health decline. Grade 3-4 treatment-related adverse events reported more than once in the dose-escalation phase were keratitis (n=3) and fatigue (n=2). Based on all available data, the recommended phase 2 dose was set at 1,2 mg/kg. In the dose-expansion phase, treatment-related serious adverse events were reported in 16 (11%) of 146 patients, most commonly infusion-related reactions (two [1%]) and dyspnoea (two [1%]). The most common treatment-related adverse events (grades 1–4) were fatigue (48 [33%] of 146 patients), conjunctivitis (45 [31%]), and dry eye (45 [31%]). Most patients (104 [71%] of 146) had at least one ocular adverse event, with grade 3 events reported in ten (7%) of 146 patients. No patients died from treatment-related adverse events and four patients died due to disease progression, which were attributed to hepatic failure (n=1), upper gastrointestinal haemorrhage (n=1), neurological decompensation (n=1), and renal failure (n=1). In the breast cancer dose-expansion cohorts, 16 (33%, 95% CI 20,4-48,4) of 48 assessable patients with HER2-positive breast cancer achieved an objective response (all partial responses) according to RECIST. Nine (28%, 95% CI 13,8-46,8) of 32 patients with HER2-low, hormone receptor- positive breast cancer and six (40%, 16,3-67,6) of 15 patients with HER2-low, hormone receptor-negative breast cancer achieved an objective response (all partial responses). Partial responses were also observed in one (6%, 95% CI 0,2-30,2) of 16 patients with gastric cancer, four (25%, 7·3-52·4) of 16 patients with urothelial cancer, and five (39%, 13,9-68,4) of 13 patients with endometrial cancer.

Interpretation

Trastuzumab duocarmazine shows notable clinical activity in heavily pretreated patients with HER2-expressing metastatic cancer, including HER2-positive trastuzumab emtansine-resistant and HER2-low breast cancer, with a manageable safety profile. Further investigation of trastuzumab duocarmazine for HER2-positive breast cancer is ongoing and trials for HER2-low breast cancer and other HER2-expressing cancers are in preparation.

Introduction

HER2 is a transmembrane tyrosine kinase receptor protein that promotes cell proliferation and inhibits apoptosis. HER2 overexpression, amplification, or both, is seen frequently across different tumour types[1] and is associated with more aggressive disease and lower overall survival compared with cancers without HER2 overexpression[2,3]. During the past two decades, multiple drugs targeting HER2 have been developed for HER2-positive breast cancer, including (bispecific) antibodies, small molecules, vaccines, and antibody–drug conjugates. However, HER2-positive metastatic breast cancer is still incurable and eventual development of resistance to these treatments is almost inevitable[4,5]. Furthermore, trastuzumab did not improve the outcomes of patients with breast cancer expressing low amounts of HER2 (HER2-low), defined as HER2 immunohistochemistry (IHC) 1+ or IHC 2+ and in-situ hybridization (ISH)-negative. Currently, no HER2-targeting drugs are licensed specifically for the treatment of any cancer with low expression of HER2. Therefore, new drugs that also target cancers with low HER2 expression will address an unmet need in several tumour types.

Antibody–drug conjugates are designed for selective delivery of potent cytotoxic drugs to tumour cells by linking the cytotoxins to monoclonal antibodies. Trastuzumab emtansine, a HER2-targeting antibody–drug conjugate that contains trastuzumab covalently linked to a microtubule inhibitor, significantly prolonged progression-free survival and overall survival with acceptable toxicity in patients with HER2-positive metastatic breast cancer[6,7]. Trastuzumab emtansine is currently recommended as second-line treatment for patients with breast cancer who have progressed after at least one line of trastuzumab-based treatment. Several new HER2-targeting antibody–drug conjugates with different linkers and payloads are currently in clinical development for multiple tumour types, with promising results[8,9].

Trastuzumab duocarmazine (also known as SYD985) is a novel HER2-targeting antibody–drug conjugate comprising the monoclonal IgG1 antibody trastuzumab covalently bound to a linker drug containing duocarmycin, with a drug-to-antibody ratio of 2 · 8:1[10–12]. The linker drug contains a cleavable linker and the prodrug *seco*-duocarmycin–hydroxybenzamide–azaindole (*seco*-DUBA). After binding to HER2 and internalization the linker is cleaved in the lysosome by proteases that release the active toxin (DUBA). The active toxin alkylates DNA, resulting in

DNA damage in both dividing and non-dividing cells and ultimately cell death. Additionally, proteases such as cathepsin B can be active extracellularly through secretion by malignant cells[13]. Extracellular cleavage of the linker drug might, therefore, induce a bystander cell-killing effect that is not HER2-mediated[14]. Trastuzumab duocarmazine has shown encouraging preclinical antitumour activity in breast, ovarian, and other cancers with varying (low to high) HER2 expression and was more potent than trastuzumab emtansine[12,15,16].

In this first-in-human study, we assessed the safety, pharmacokinetics, and preliminary antitumour activity of trastuzumab duocarmazine in patients with locally advanced or metastatic solid tumours. Here, we present data for the completed dose-escalation phase and safety and activity data for the fully recruited dose-expansion cohorts.

Methods

Study design and participants

We did a phase 1 dose-escalation and dose-expansion study. Eligible patients were aged 18 years or older, with an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1, an estimated life expectancy of at least 12 weeks, and adequate organ function. For the dose-escalation phase, patients were recruited from three academic hospitals in Belgium, the Netherlands, and the UK. Patients were eligible if they had locally advanced or metastatic solid tumours refractory to standard treatment, regardless of HER2 status. For the dose expansion phase, patients with HER2-expressing breast, gastric (including adenocarcinomas of the gastro-oesophageal junction), urothelial, or endometrial cancer were recruited from 15 hospitals in Belgium, the Netherlands, Spain, and the UK). Eligible patients had to have at least one measurable tumour lesion as defined by Response Evaluation Criteria for Solid Tumours (RECIST) version 1.1, and centrally assessed tumour HER2 expression should have been at least IHC 1+.

Key exclusion criteria for both study phases were anthracycline treatment within the previous 3 months or any other cancer treatment within the previous 4 weeks; a history of infusion-related reaction, hypersensitivity to trastuzumab or trastuzumab emtansine, or both of these; left-ventricular ejection fraction (LVEF) less than 55%; severe uncontrolled systemic disease;

and symptomatic brain metastases or treatment for brain metastases within 4 weeks.
The study protocol, amendments, and informed consent forms were reviewed and approved by local authorities and independent ethics committees at each study site. All patients provided written informed consent before any protocol-related activities started. The study was done in accordance with the Declaration of Helsinki and the International Conference on Harmonisation Good Clinical Practice guidelines.

Procedures

In the dose-escalation phase, trastuzumab duocarmazine (Synthon Biopharmaceuticals, Nijmegen, Netherlands) was administered intravenously on day 1 of each 3-week cycle with a first-in-human starting dose of 0,3 mg/kg. The first infusion was given over 1 h and, if well tolerated, subsequent infusions could be given over 30 min. We used a standard 3+3 dose-escalation design; doses were initially doubled for subsequent dose cohorts if no dose limiting toxic effect was recorded in the first treatment cycle. If a dose-limiting toxic effect was reported in one patient during the first cycle, at least three additional patients were to be treated at that dose level. Intrapatient dose escalation was not permitted, but dose reductions and delays were allowed by protocol. Patients were treated until disease progression or unacceptable toxicity. The highest dose level at which no more than one of six patients had a dose-limiting toxic effect was determined to be the maximum tolerated dose. The recommended phase 2 dose was determined based on all available safety, pharmacokinetic, and activity data. This dose was used in the dose-expansion phase.
Patients in both study phases were assessed for toxicity at least once per week in the first two cycles and once during subsequent cycles, according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) version 4.03. Vital signs, haematology, blood chemistry, 12-lead electrocardiograms (ECGs), cardiac biomarkers, and urinalysis were assessed at each visit. Weight, physical, and ophthalmological examinations, and LVEF assessments were done at each or every other cycle. Blood samples were obtained at each visit for pharmacokinetic assessment and before each infusion for immunogenicity analyses (measurement of antibodies against trastuzumab duocarmazine).

After completion of the dose-escalation phase, several prophylactic measures were introduced, as per an approved protocol amendment (prepared and approved before the start of the dose-expansion phase in 2016), to assess the effect of trastuzumab duocarmazine on ocular toxicity. Lubricating eye drops were to be prescribed to all patients enrolled in the dose-expansion phase. Vasoconstrictive phenylephrine and anti-inflammatory dexamethasone eye drops were to be administered 1 h before the start of the infusion, and the anti-inflammatory dexamethasone eye drops were to be continued up to 2 days after infusion in the HER2-low breast cancer cohorts and in the non-breast cancer cohorts. In the HER2-positive breast cancer expansion cohort, patients were randomly allocated to either 1,2 mg/kg every 3 weeks continuously, 1,2 mg/kg every 3 weeks for four cycles followed by 0,9 mg/kg every 3 weeks, or 1,2 mg/kg every 3 weeks for four cycles followed by 1,2 mg/kg every 6 weeks to investigate the effect of different dosing regimens on ocular toxicity. Randomisation was done in a 1:1:1 ratio using a permutated block design with a block size of three. Allocation of subsequent patients was controlled centrally and communicated by the clinical research organisation (INC Research, Amsterdam, Netherlands) to the investigators, who were to adjust the dosing schedule accordingly (dosing was open-label).

HER2 tumour expression was assessed by IHC and ISH using archival or fresh tissue according to the American Society of Clinical Oncology and College of American Pathologists guidelines for breast and gastric cancer. Fresh tissue was obtained just before the start of study treatment if no archival tissue was available. HER2-positive disease was defined as IHC 3+ or ISH-positive. In the dose-escalation phase, tissue analysis was done by the local site laboratory, whereas in the dose-expansion phase, tissue analysis was done centrally using HER2 IHC and dual ISH assays (Ventana; F Hoffmann-La Roche, Welwyn Garden City, UK). Patients with HER2 IHC 3+ or ISH-positive breast cancer were enrolled into the HER2-positive breast cancer cohort; patients with IHC 2+ or 1+ and ISH-negative breast cancer were enrolled in either the HER2-low hormone receptor positive cohort or the HER2-low hormone receptor negative cohort so that we could assess these populations separately, in view of these patients' substantially different biology, natural history, and treatment recommendations. Non-breast cancer cohorts included patients with HER2-low and HER2-positive tumours. Validated ELISA-based methods were used to measure the plasma concentration of total antibody (irrespective of the amount of conjugated

toxins—i.e., drug-to-antibody ratio ≥0) and conjugated antibody (antibodies that have at least one conjugated toxin—i.e., drug-to-antibody ratio ≥1). A validated liquid chromatography–tandem mass spectrometry method was used for quantification of DUBA (free toxin) in plasma.
Tumour response was assessed by the investigator at baseline and every 6 weeks during treatment according to RECIST version 1.1, using CT, PET-CT, or MRI.

Outcomes

The primary endpoint of the dose-escalation phase was to assess safety and to ascertain the maximum tolerated dose and recommended phase 2 dose for trastuzumab duocarmazine. The primary endpoint of the dose-expansion phase was the proportion of patients who achieved an objective response (defined as either a complete response or a partial response, by RECIST 1.1). Secondary endpoints were safety, pharmacokinetics, immunogenicity, quality of life (data not reported here), and anti-tumour activity. Anti-tumour activity endpoints were best percentage change in target lesion measurements, progression-free survival (defined as the time from first day of treatment to tumour progression or death from any cause), clinical benefit (the proportion of patients with a complete response, partial response, or stable disease for ≥6 months; data not reported here), duration of response (time from first observation of response to disease progression; data not reported here), and overall survival (time from treatment initiation to death from any cause; data not reported here).

Statistical analysis

We estimated that up to 24 patients (three to six patients per dose level) would need to be enrolled in the dose-escalation phase to ascertain the recommended phase 2 dose. In the dose-expansion phase, we initially estimated that up to 128 patients would be enrolled in six cohorts—i.e., 48 patients in the HER2-positive breast cancer cohort and 16 in each of the other five cohorts. A Simon's two stage design was applied to all cohorts except for the HER2-positive breast cancer cohort. The null hypothesis that the true response was 5% or less was to be tested against the one-sided alternative of a response of 20%. In case the null hypothesis could be rejected—i.e., if two or more responders were found in the initial 16 enrolled patients in a cohort—a maximum of 14 additional patients could be enrolled

in that cohort for a total of 30 patients. This design had a type I error of 5% and a power of 80% when the true response was 20%.
Safety and most of the activity endpoints were assessed in the safety population, which was defined as all patients who received at least one dose of study treatment. However, for the tumour response analysis, we excluded patients without measurable disease at baseline or without a post-baseline RECIST assessment. The population for pharmacokinetic analyses included all patients for whom at least one pharmacokinetic variable could be calculated. Descriptive statistics were used to summarise patients' demographics, baseline characteristics, and safety data. Activity proportions were summarised with exact binomial 95% CIs. Progression-free survival was analysed using Kaplan-Meier quartile estimates and two-sided 95% CIs; data were censored either at the date of the last RECIST assessment when no documented date of progression (according to RECIST version 1.1) or death was available or when death or progression occurred after two or more consecutive missed assessments. Actual blood sampling times relative to the time of dose were used to ascertain pharmacokinetic variables. Values lower than the limit of quantification were imputed as zero. Statistical analyses were done with SAS version 9.4. Pharmacokinetic analyses were done with Phoenix WinNonlin version 8.1.
This study is registered with ClinicalTrials.gov, number NCT02277717.

Role of the funding source

The funder contributed to study design, data collection, data analysis, data interpretation, and writing of the report. UB, CMLvH, ECM, NPK, and PA had full access to all the study data. The corresponding author had full access to all data in the study and had final responsibility for the decision to submit for publication.

Results

Between Oct 30, 2014, and April 2, 2018, 39 patients were enrolled and treated in the dose-escalation phase and 146 patients were enrolled and treated in the dose-expansion phase (Figure 4.1.1). In the dose-expansion phase, 50 patients had HER2-positive metastatic breast cancer, 32 had HER2-low hormone receptor-positive metastatic breast cancer, 17 had

HER2-low hormone receptor negative metastatic breast cancer, 17 had gastric cancer, 16 had urothelial cancer, and 14 had endometrial cancer. At data cutoff (July 5, 2018), three patients with HER2-positive breast cancer, two patients with endometrial cancer, and one patient with urothelial cancer were still on treatment.

Median follow-up for all patients was 5 · 0 months (IQR 2,9–7,6) until the final safety assessment (July 5, 2018). Patients' demographics and baseline characteristics are provided in Table 4.1.1. Patients were heavily pretreated with anticancer drugs, with a mean of 5,2 (SD 3,3) previous lines of treatment. In the HER2-positive metastatic breast cancer expansion cohort, 40 (80%) of 50 patients had received previous trastuzumab emtansine.

In the dose-escalation phase, initial doses of trastuzumab duocarmazine were doubled from 0,3 mg/kg up to 2,4 mg/kg because no dose-limiting toxic effects occurred in the first treatment cycle. One of three patients dosed with 2,4 mg/kg trastuzumab duocarmazine developed pneumonitis in cycle 2, which was considered possibly related to the study drug, and after the third infusion this patient died. Although the event did not occur during the first cycle, it was considered a dose-limiting toxic effect. Because of the seriousness of this toxic effect and because promising activity was already seen at the 1,2 mg/kg dose level, the decision was made not to enrol three additional patients at the 2,4 mg/kg dose but to assess lower doses in more detail. Therefore, the protocol-defined maximum tolerated dose of trastuzumab duocarmazine has not been defined. No dose-limiting toxic effects occurred at doses of 1,8 mg/kg (n=12), 1,5 mg/kg (n=12), or 1,2 mg/kg (n=6). The overall median duration of trastuzumab duocarmazine exposure was 3,5 months (IQR 1,4–5,4) and was longest at the 1,2 mg/kg dose (8,1 months [IQR 5,9–9,8]

Treatment-related adverse events are shown in Table 4.1.2 and divided per dose level in the dose-escalation phase. In the dose-escalation phase, grade 3 or grade 4 treatment-related adverse events occurred in 13 (33%) of 39 patients (some patients had more than one event) and events reported more than once were keratitis (n=3) and fatigue (n=2). The most commonly recorded treatment-related adverse events of any grade in the dose-escalation phase were conjunctivitis (12 [31%] of 39 patients), fatigue (11 [28%]), and dry skin (ten [26%]). A reversible decrease in LVEF was reported as an adverse event for two (5%) of 39 patients, and for three (8%) patients, an absolute worst decrease in LVEF from baseline of at least 10% to a value below 50% was measured during treatment. One further patient in

the 1,5 mg/kg cohort died in the dose-escalation phase (attributed to general physical health deterioration), which was related to disease progression. 11 (28%) of 39 patients in the dose-escalation cohorts discontinued the study because of treatment-related toxicity (three patients each in the 1,2 mg/kg and 1,5 mg/kg cohorts, four patients in the 1,8 mg/kg cohort, and one in the 2,4 mg/kg cohort).

Discontinuations were most commonly attributable to ocular adverse events after between five and ten cycles (n=5) or pneumonitis after between two and six cycles (n=4). Ocular toxicity improved after treatment discontinuation and was reported as recovered at the cutoff date for four patients. All four events of pneumonitis occurred at doses of 1,5 mg/kg or higher and three events without respiratory symptoms (of which one was grade 1 and two were grade 2) resolved within 1 month after study discontinuation (the fourth event was the fatal event described). Overall, doses up to 1,8 mg/kg were tolerated well without a clear dose-related occurrence of adverse events, although ocular toxicity seemed to occur earlier at higher doses and increased with augmented exposure (data not shown). Based on these data, in combination with the observed treatment duration and activity data, the recommended phase 2 dose of trastuzumab duocarmazine was set at 1,2 mg/kg, because the 1,5 mg/kg and 1,8 mg/kg doses seemed not to improve the benefit:risk ratio for patients. The 1,2 mg/kg dose was used in the dose-expansion cohorts.

In the dose-expansion cohorts, the mean treatment duration was longest in the cohort with HER2-positive breast cancer (mean 7,1 [SD 4,6] months). Mean treatment durations were similar in the cohorts with HER2-low hormone receptor-positive breast cancer (mean 3,7 [SD 2,3] months), HER2-low hormone receptor negative breast cancer (3,3 [2,2] months), gastric cancer (3,2 [2,1] months), urothelial cancer (3,5 [2,2] months), and endometrial cancer (4,0 [2,8] months).

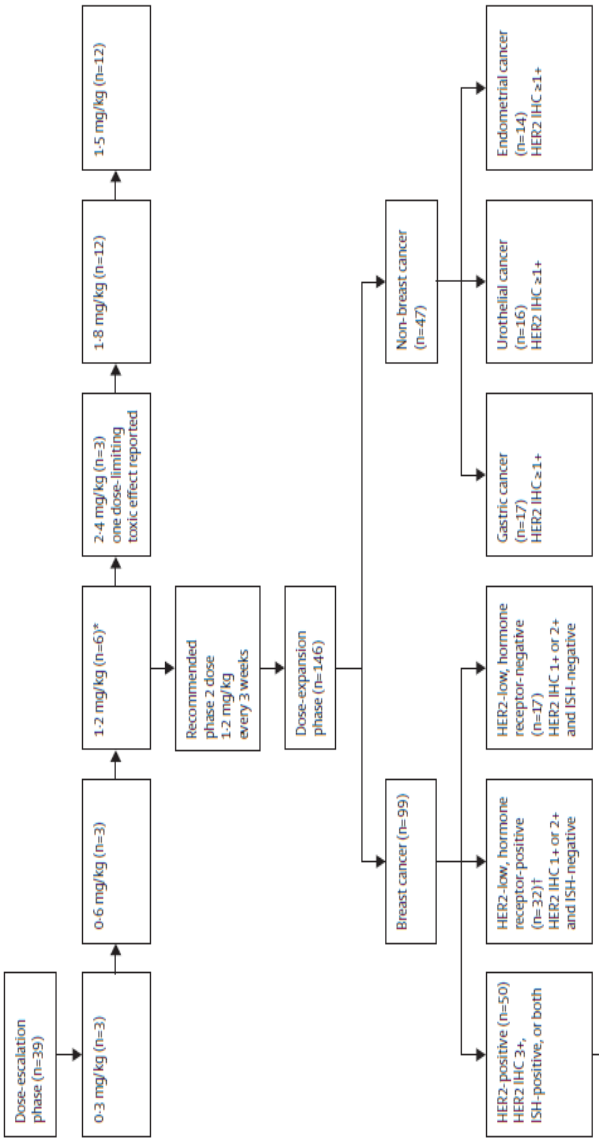

Figure 4.1.1 Trial profile.
IHC=immunohistochemistry, ISH=in-situ hybridisation. *Three patients were added to the 1·2 mg/kg cohort at the same time that enrolment started for the 1·5 mg/kg cohort. †Cohort was expanded from 16 to 32 patients when two of the initial 16 patients had a partial response (Simon two-stage design). ‡Patients with HER2-positive metastatic breast cancer were randomly allocated to one of three different treatment subgroups to investigate the effect of different dosing regimens on ocular toxicity

Table 4.1.1 Baseline characteristics.

	Dose-escalation cohort (n=39)	Dose-expansion cohorts (n=146)	HER2-positive metastatic breast cancer (n=50)	HER2-low, hormone receptor-positive metastatic breast cancer (n=32)	HER2-low, hormone receptor-negative metastatic breast cancer (n=17)	Non-breast cancer expansion cohorts (n=47) *
Demographics						
Age (years)	55 (47-63)	57 (49-65)	54 (47-63)	53 (47-61)	53 (45-62)	64 (54-71)
Sex						
Female	30 (77%)	120 (82%)	50 (100%)	32 (100%)	17 (100%)	21 (45%)
Male	9 (23%)	26 (18%)	0	0	0	26 (55%)
Race						
White	38 (97%)	140 (96%)	47 (94%)	32 (100%)	17 (100%)	44 (94%)
Other	1 (3%)	6 (4%)	3 (6%)	0	0	3 (6%)
Clinical characteristics						
ECOG performance status						
0	22 (56%)	69 (47%)	26 (52%)	19 (59%)	5 (29%)	19 (40%)
1	17 (45%)	77 (53%)	24 (48%)	13 (41%)	12 (71%)	28 (60%)
Time since initial diagnosis (months)	67 (33-143)	53 (27-100)	78 (47-107)	94 (55-136)	43 (23-84)	25 (16-45)
Cancer type						
Breast	26 (67%)	99 (68%)	50 (100%)	32 (100%)	17 (100%)	0
Gastric†	6 (15%)	17 (12%)	0	0	0	17 (36%)
Colorectal	3 (8%)	0	0	0	0	0
Urothelial	0	16 (11%)	0	0	0	16 (34%)
Endometrial	1 (3%)	14 (10%)	0	0	0	14 (30%)
Other	3 (8%)	0	0	0	0	0
Number of metastatic sites	3 (2-4)	3 (2-4)	3 (2-4)	3 (2-3)	3 (2-4)	3 (2-4)
Known brain metastasis	3 (8%)	8 (5%)	5 (10%)	0	0	3 (6%)

Table 4.1.1 (continued)

	Dose-escalation cohort (n=39)	Dose-expansion cohorts (n=146)	HER2-positive metastatic breast cancer (n=50)	HER2-low, hormone receptor-positive metastatic breast cancer (n=32)	HER2-low, hormone receptor-negative metastatic breast cancer (n=17)	Non-breast cancer expansion cohorts (n=47) *
HER2 expression						
Immunohistochemistry 3+	15 (39%)	57 (39%)	41 (82%)	0	0	16 (34%)
Immunohistochemistry 2+	12 (31%)	37 (25%)	3 (6%)	10 (31%)	7 (41%)	17 (36%)
ISH-positive	2 (5%)	6 (4%)	3 (6%)	0	0	3 (6%)
ISH-negative	8 (20%)	27 (18%)	0	10 (31%)	7 (41%)	10 (21%)
ISH-equivocal	1 (3%)	3 (2%)	0	0	0	3 (6%)
ISH-unassessable	1 (3%)	1 (1%)	0	0	0	1 (2%)
Immunohistochemistry 1+	6 (15%)	51 (35%)	5 (10%)‡	22 (69%)	10 (59%)	14 (30%)
Immunohistochemistry 0	4 (10%)	1 (1%)	1 (2%)‡	0	0	0
Missing	2 (5%)	0	0	0	0	0
Previous treatment						
Systemic treatments	6 (2-8)	4 (3-7)	6 (4-8)	7 (5-9)	4 (3-5)	2 (2-3)
1-3	13 (33%)	50 (34%)	5 (10%)	3 (9%)	6 (35%)	36 (77%)
4-6	9 (23%)	55 (38%)	24 (48%)	11 (34%)	9 (53%)	11 (23%)
>6	17 (44%)	41 (28%)	21 (42%)	18 (56%)	2 (12%)	0
HER2 targeting treatment§	20 (51%)	62 (42%)	47 (94%)	6 (19%)	1 (6%)	8 (17%)
Trastuzumab	20 (51%)	61 (42%)	46 (92%)	6 (19%)	1 (6%)	8
Trastuzumab emtansine	16 (41%)	43 (29%)	40 (80%)	3 (9%)	0	0
Lapatinib	9 (23%)	26 (18%)	23 (46%)	2 (6%)	1 (6%)	0
Pertuzumab	2 (5%)	17 (12%)	15 (30%)	2 (6%)	0	0
CDK 4 or CDK6 inhibitors	2 (5%)	5 (3%)	0	5 (16%)	0	0
PD-1 or PD-L1 inhibitors	0	14 (10%)	1 (2%)	2 (6%)	1 (6%)	10 (21%)

Data are median (IQR) or n (%). ECOG=Eastern Cooperative Oncology Group. ISH=in-situ hybridisation. *Baseline characteristics by cancer type are in the appendix (p 2). †Gastric cancer including adenocarcinoma of the gastro-oesophageal junction. ‡Tumour tissue was HER2 ISH-positive for these patients. §In the dose-expansion part of the study, patients with breast cancer were allocated to a cohort based on centrally assessed HER2 status on the most recent available tumour tissue, which in some patients deviated from previous locally assessed HER2 status.

Table 4.1.2 Adverse events considered related to trastuzumab duocarmazine.

Adverse Event	Dose=escalation cohort (n=39)			Dose-expansion cohorts (n=146)		
	Grade 1-2	Grade 3	Grade 4	Grade 1-2	Grade 3	Grade 4
Fatigue	9 (23%)	2 (5%)	0	43 (29%)	5 (3%)	0
Conjunctivitis	11 (28%)	1 (3%)	0	41 (28%)	4 (3%)	0
Dry eye	6 (15%)	0	0	44 (30%)	1 (1%)	0
Lacrimation increased	8 (21%)	0	0	29 (20%)	0	0
Dry skin	10 (26%)	0	0	26 (18%)	0	0
Decreased appetite	6 (15%)	1 (3%)	0	27 (18%)	2 (1%)	0
Keratitis	3 (8%)	3 (8%)	0	25 (17%)	3 (2%)	0
Alopecia	8 (21%)	0	0	26 (18%)	0	0
Nausea	6 (15%)	0	0	27 (18%)	0	0
Stomatitis	8 (21%)	1 (3%)	0	24 (16%)	0	0
Skin hyperpigmentation	5 (13%)	0	0	23 (16%)	0	0
Neutropenia	3 (8%)	1 (3%)	0	14 (10%)	9 (6%)	0
Vomiting	5 (13%)	0	0	17 (12%)	0	0
Anaemia	6 (15%)	1 (3%)	0	13 (9%)	2 (1%)	0
Pyrexia	9 (23%)	0	0	9 (6%)	0	0
Dysgeusia	7 (18%)	0	0	11 (8%)	0	0
Infusion-related reaction	3 (8%)	0	0	13 (9%)	2 (1%)	0
Vision blurred	0	0	0	15 (10%)	1 (1%)	0
LVEF decreased	1 (3%)	1 (3%)	0	10 (7%)	1 (1%)	0
Diarrhoea	1 (3%)	0	0	9 (6%)	1 (1%)	0
Thrombocytopenia	2 (5%)	0	0	7 (5%)	1 (1%)	0
Aspartate aminotransferase increased	1 (3%)	0	0	7 (5%)	1 (1%)	0
Lymphopenia	0	0	0	7 (5%)	2 (1%)	0
Dyspnoea	4 (10%)	0	0	2 (1%)	2 (1%)	0
Asthenia	0	1 (3%)	0	7 (5%)	0	0
Mouth ulceration	1 (3%)	0	0	5 (3%)	1 (1%)	0
Pericardial effusion	0	1 (3%)	0	2 (1%)	2 (1%)	1 (1%)
Gamma-glutamyltransferase increased	0	0	0	4 (3%)	0	2 (1%)
Rash maculo-papular	1 (3%)	0	0	4 (3%)	1 (1%)	0
Blood alkaline phosphatase increased	1 (3%)	0	0	3 (2%)	1 (1%)	0

Table 4.1.2 (continued)

Adverse Event	Dose=escalation cohort (n=39)			Dose-expansion cohorts (n=146)		
	Grade 1-2	Grade 3	Grade 4	Grade 1-2	Grade 3	Grade 4
Pneumonitis	3 (8%)	0	0	0	0	1 (1%)
Aminotransferase enzymes (not otherwise specified) increased	1 (3%)	0	0	3 (2%)	1 (1%)	0
White blood cell count decreased	0	0	0	4 (3%)	1 (1%)	0
Episcleritis	0	1 (3%)	0	4 (3%)	0	0
Lymphocyte count decreased	0	0	0	1 (1%)	3 (2%)	0
Platelet count decreased	0	0	1 (3%)	1 (1%)	2 (1%)	0
Palmar-plantar erythrodysaesthesia syndrome	0	0	0	3 (2%)	1 (1%)	0
Neutrophil count decreased	0	1 (3%)	0	2 (1%)	0	0
Hepatic enzyme increased	0	0	0	1 (1%)	1 (1%)	0
Injection site reaction	0	1 (3%)	0	1 (1%)	0	0
Pleural effusion	0	0	0	1 (1%)	1 (1%)	0
Pancytopenia	0	0	0	0	1 (1%)	0
Corneal toxicity not otherwise specified	0	0	0	0	1 (1%)	0
Retinal haemorrhage	0	0	0	0	1 (1%)	0
Ventricular dysfunction	0	0	0	0	1 (1%)	0
Haemoptysis	0	0	0	0	1 (1%)	0
Conjunctivitis bacterial	0	0	0	0	1 (1%)	0
Pain in extremity	0	0	0	0	1 (1%)	0
Delirium	0	0	0	0	0	1 (1%)
Haematuria	0	0	0	0	1 (1%)	0

Data are presented as n (%). Included in this table are maximum grade adverse events by preferred term deemed related to study drug. Grade 1 and 2 events are presented if they were recorded in at least 10% of all patients, and all grade 3 and 4 events are presented. One patient in the dose-escalation cohort (2·4 mg/kg) died from pneumonitis that was deemed a dose-limiting toxicity; one further patient died (1·5 mg/kg) due to disease progression attributed to general health decline. No patients in the dose-expansion cohorts died from adverse events related to treatment; four patients died due to disease progression, which were attributed to hepatic failure (n=1), upper gastrointestinal haemorrhage (n=1), neurological decompensation (n=1), and renal failure (n=1). LVEF=left-ventricular ejection fraction.

The most common treatment-related adverse events of any grade in the dose-expansion cohorts were fatigue (48 [33%] of 146 patients), conjunctivitis (45 [31%]), and dry eye (45 [31%]; Table 4.1.2). Grade 3 or 4 treatment-related adverse events occurred in 51 (35%) of 146 patients and the most common of these were neutropenia (nine [6%]), fatigue (five [4%]), and conjunctivitis (four [3%]). 104 (71%) of 146 patients had one or more ocular adverse events, with grade 3 events in ten (7%) of 146 patients. Occurrence of ocular toxic effects and their severity generally increased with prolonged exposure (data not shown), with a median time to grade 3 events of 7,6 months (IQR 4,3-8,9). Reduced dosing after four infusions of trastuzumab duocarmazine in patients with HER2-positive breast cancer—i.e., either a decrease to 0,9 mg/kg every 3 weeks or to 1,2 mg/kg every 6 weeks—or use of prophylactic eye drops, or both of these, did not greatly improve tolerability, although several patients seemed to benefit (i.e., toxicity remained stable or improved) from dose delays or dose reduction and could continue with trastuzumab duocarmazine treatment beyond 1 year (Figure 4.1.2). Most ocular events improved or recovered with treatments such as eye drops or ointments, although recovery sometimes took several months. A decrease in LVEF was reported as an adverse event for 11 (8%) of 146 patients, of which eight were reported as resolved before data cutoff. In eight (5%) of 146 patients, an absolute worst decrease in LVEF from baseline of at least 10% to a value below 50% was measured during treatment. Treatment-related serious adverse events were reported in 16 (11%) of 146 patients, most commonly infusion-related reactions (two [1%]) and dyspnoea (two [1%]). Four patients died in the dose-expansion phase, one each from hepatic failure, upper gastrointestinal haemorrhage, neurological decompensation, and renal failure. These deaths were all related to disease progression and were not judged to be related to treatment. Overall, 62 (43%) of 146 patients had at least one treatment-related adverse event leading to one or more dose delays or dose reductions, and 27 (19%) of 146 patients discontinued the study because oftreatment-related toxic effects, of which 15 (10%) were attributable to ocular toxicity. Dyspnoea, decreased LVEF, and decreased appetite were each reported for two (1%) patients as having contributed to treatment discontinuation; other reasons were reported as single events. Most of these events were reported as resolved at data cutoff.

Total antibody, conjugated antibody, and DUBA (free toxin) pharmacokinetics after intravenous infusion of trastuzumab duocarmazine

followed a monophasic log-linear decline and were time independent. Concentrations were generally close to or below the limit of quantification at 3 weeks after treatment at all dose levels, with minimal accumulation across consecutive cycles. Pharmacokinetics of all analytes were dose-proportional within the dose range of 1,2 mg/kg to 2,4 mg/kg. Dose levels below 1,2 mg/kg had faster elimination of total and conjugated antibody, which is indicative of target-mediated drug disposition. Elimination half-life was 2-3 days for conjugated antibody at doses of 1,2 mg/kg or higher. Free toxin exposure was generally 3000 times lower compared with conjugated antibody (on a molar basis) with peak concentrations in the pg/mL range.. No patients were found to have antibodies against trastuzumab duocarmazine at any timepoint.

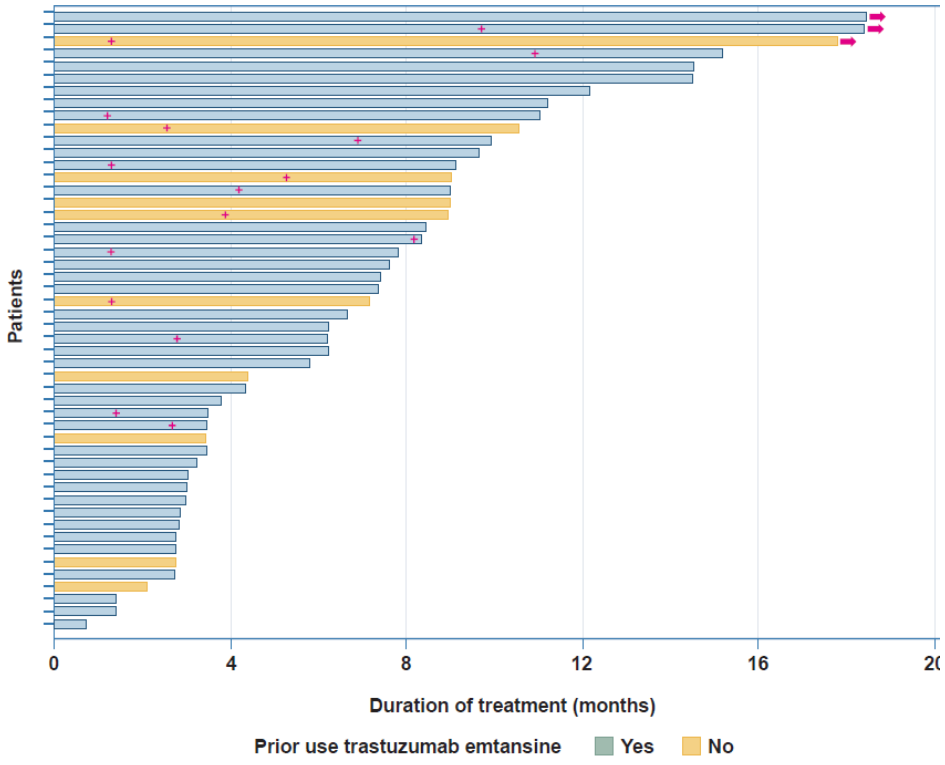

Figure 4.1.2 Duration of treatment for HER2-positive breast cancer expansion cohort.
Cross indicates the time of first partial response. Arrows indicate patients still on treatment at the cutoff date. All patients were treated with 1·2 mg/kg and were randomised to a lower dose (0·9 mg/kg) or longer interval (every 6 weeks) from cycle 5 (about 15 weeks) if dosing was not delayed.

In the dose-escalation phase, five of 39 patients were not assessable because of non-measurable disease (n=3) or a missing post-baseline RECIST assessment (n=2). Of the 34 assessable patients, 11 (32%, 95% CI 17,4–50,5) had a partial response, of whom ten (six confirmed) had breast cancer and one (unconfirmed) had gastric cancer. Responses were seen in both HER2-positive and HER2- low tumours and all occurred at doses of 1,2 mg/kg or higher (Figure 4.1.3A). Two patients (one with breast and one with other [duodenal] cancer) depicted in Figure 4.1.3A had a partial response in target lesions but had progressive disease in non-target lesions at the same assessment.

In the dose-expansion phase, six of 146 patients were not assessable because of a missing post-baseline RECIST assessment (n=5) or non-measurable disease at baseline (n=1). In addition, for five of 146 patients not all target lesions were assessed post baseline: these patients are included in the objective response analysis but are omitted from Figure 4.1.3 on best percentage change in tumour size. In the three breast cancer expansion cohorts, 67 (71%) of 95 assessable patients showed a reduction in target lesions and 31 (33%) had a partial response, of which 23 were confirmed responses. An objective response (all partial responses) was achieved by 16 (33%, 95% CI 20,4-48,4) of 48 patients with HER2-positive breast cancer (Figure 4.1.3B), nine (28%, 13,8-46,8) of 32 patients with HER2-low hormone receptor-positive breast cancer (Figure 4.1.3C), and six (40%, 16,3-67,6) of 15 patients with HER2-low hormone receptor-negative breast cancer (Figure 4.1.3D). Median progression-free survival was 7 · 6 months (95% CI 4,2-10,9) in patients with HER2-positive breast cancer, 4,1 months (2,4-5,4) in patients with HER2-low hormone receptor-positive breast cancer, and 4,9 months (1,2-not estimable [NE]) in patients with HER2-low hormone receptor-negative breast cancer.

In the non-breast cancer expansion cohorts, 25 (57%) of 45 assessable patients had a reduction in target lesions. An objective response (all partial responses) was achieved by one (6%, 95% CI 0,2-30,2) of 16 patients with gastric cancer, four (25%, 7,3-52,4) of 16 patients with urothelial cancer, and five (39%, 13,9-68,4) of 13 patients with endometrial cancer. Median progression-free survival was 3,2 months (95% CI 1,6-5,3) in patients with gastric cancer, 4,0 months (1,3-NE) in patients with urothelial cancer, and 4,3 months (2,4-9,9) in patients with endometrial cancer.

Of 50 patients with HER2-positive breast cancer in the dose-expansion phase, 28 (56%) received trastuzumab duocarmazine for longer than 6

months and seven (14%) were treated for longer than 1 year (Figure 4.1.2). Six of the seven patients with HER2-positive breast cancer treated for longer than 1 year had previously received trastuzumab emtansine.

Figure 4.1.3 Best percentage change in tumour size from baseline in target lesions for assessable patients.
(A) Dose-escalation phase, by cancer type, HER2 expression, and dose (in mg/kg). (B) Dose-expansion phase, HER2-positive breast cancer cohort. (C) Dose-expansion phase, HER2-low hormone receptor-positive breast cancer cohort. (D) Dose-expansion phase, HER2-low hormone receptor-negative breast cancer cohort.

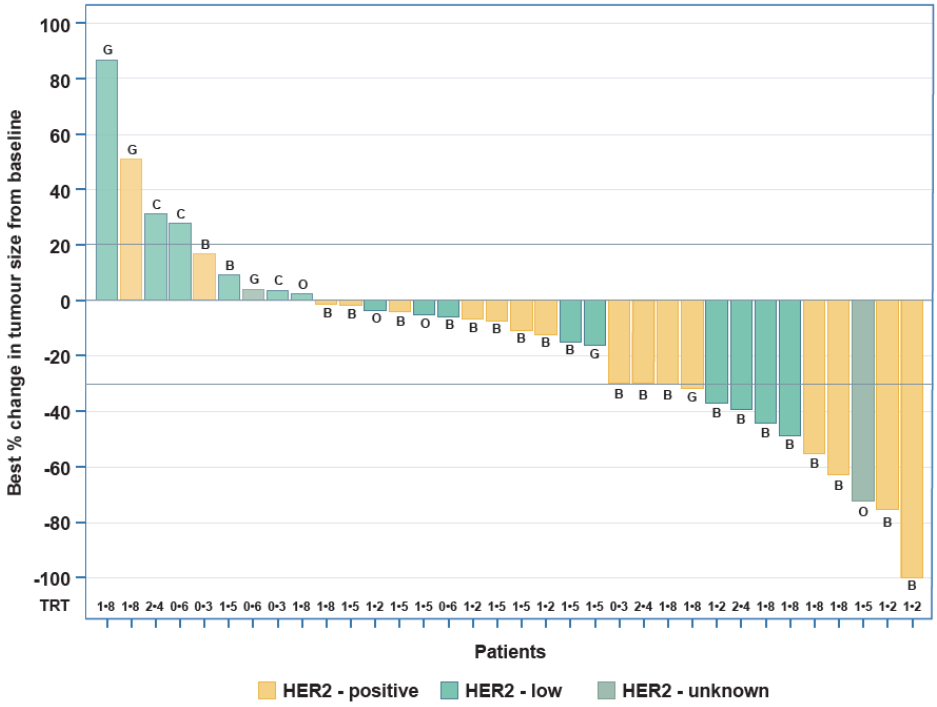

Figure 4.1.3A Dose-escalation phase, by cancer type, HER2 expression, and dose (in mg/kg).

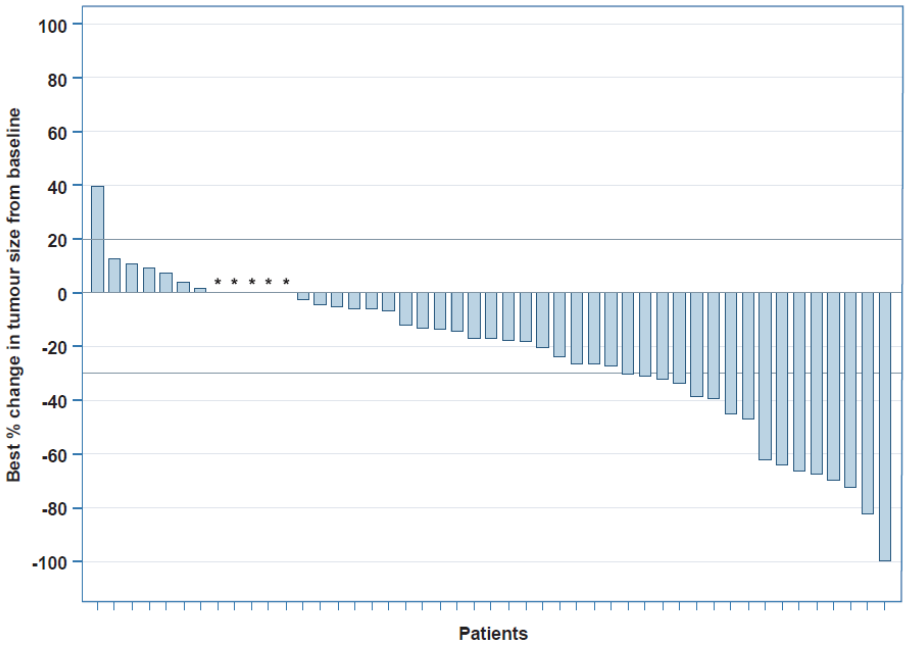

Figure 4.1.3B Dose-expansion phase, HER2-positive breast cancer cohort.

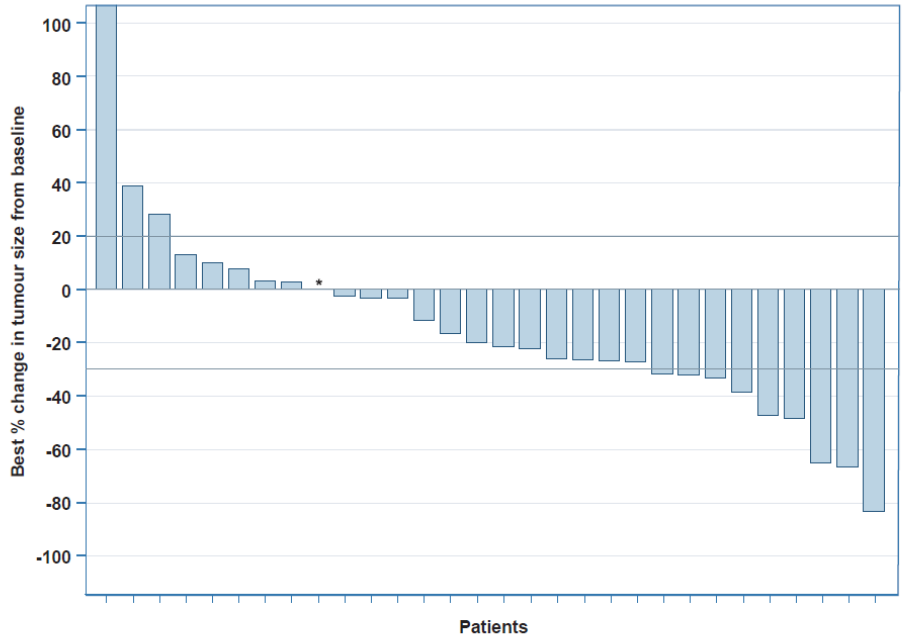

Figure 4.1.3C Dose-expansion phase, HER2-low hormone receptor-positive breast cancer cohort.

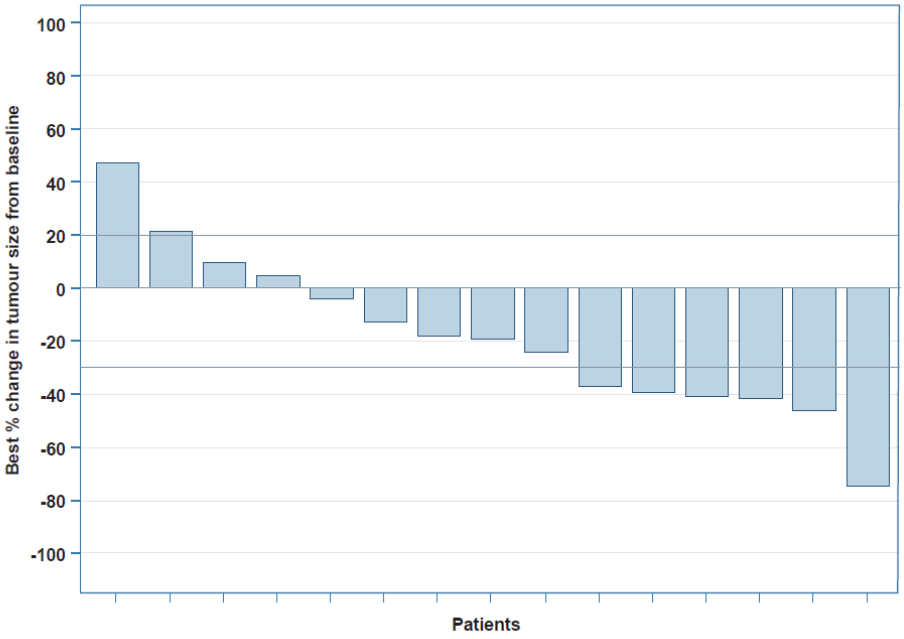

Figure 4.1.3D Dose-expansion phase, HER2-low hormone receptor-negative breast cancer cohort.
Dotted lines reflect 20% increase or 30% reduction in tumour size. Post-baseline target lesion assessment was incomplete for seven patients: three patients in (B), two patients in (C), and two patients in (D). HER2-low=low expression of HER2. G=gastric cancer including adenocarcinoma of the gastro-oesophageal junction. C=colorectal cancer. B=breast cancer. O=other cancer. *Best percentage change was 0%.

Discussion

To our knowledge, our phase 1 study in heavily pretreated patients with locally advanced or metastatic solid tumours is the first to report a novel antibody–drug conjugate with a DNA-alkylating duocarmycin payload. Trastuzumab duocarmazine showed a manageable safety profile with few grade 3 or 4 adverse events. The recommended phase 2 dose was set at 1,2 mg/kg. Responses were noted across all tumour types, not only in HER2-positive tumours but also in tumours expressing lower levels of HER2.
The side-effect profile of trastuzumab duocarmazine has both similarities and differences with other HER2-targeting antibody–drug conjugates. The most common side-effects seen with trastuzumab duocarmazine were

attributable to ocular toxicity. Although such toxic effects have been described with other antibody–drug conjugates, they are less typical in HER2-targeting antibody–drug conjugates, and the pathophysiology of these events is not yet well understood[16]. Planned dose reductions, decreasing the frequency of administration, or the use of prophylactic eye drops did not substantially change long-term tolerability of trastuzumab duocarmazine overall, but several patients were able to continue with study drug beyond 1 year and most ocular events were reported as recovered or improving at data cutoff. However, in view of the relative scarcity of data, additional observations—particularly over a prolonged treatment period—are necessary for drawing more definitive conclusions. Grade 3-4 thrombocytopenia was recorded in fewer than 1% of patients after treatment with trastuzumab duocarmazine, and grade 3-4 neutropenia was noted in 6% of patients—frequencies that are lower than with other HER2-targeting antibody–drug conjugates[7,17]. This finding could be of importance when investigating future combination strategies. Pneumonitis was reported as a dose-limiting toxic effect at the 2,4 mg/kg dose, but risk was diminished at the recommended phase 2 dose of 12 mg/kg. This type of adverse event has also been reported for both trastuzumab emtansine[18] and trastuzumab deruxtecan—a HER2-targeting topoisomerase antibody–drug conjugate that is in development[17,19]. However, the underlying mechanism or risk factor is not yet clear.

The pharmacokinetic profile in combination with the DNA-alkylating mode of action of trastuzumab duocarmazine supports a dosing schedule of once every 3 weeks. Systemically free toxin levels were substantially lower compared with other antibody–drug conjugates such as trastuzumab emtansine[20] and trastuzumab deruxtecan[17]. Amounts of antibody–drug conjugate achieved in patients are consistent with amounts achieved in mice, which showed significant xenograft growth delay with trastuzumab duocarmazine[12].

Our study has some limitations. First, we enrolled patients who had completed several late-line treatment options for metastatic disease. However, pertuzumab was not yet commonly prescribed for patients with HER2-positive breast cancer in Europe when the study started because of pending reimbursement discussions after approval of the drug in 2013, so fewer than half of the patients enrolled were pretreated with pertuzumab. Second, tumour assessments were not assessed centrally. Resulting

estimates should be viewed with this limitation in mind but are nonetheless very encouraging in a phase 1 setting.

Trastuzumab duocarmazine showed meaningful single-agent clinical activity in three areas of unmet need. First, relevant clinical activity was noted in patients with HER2-positive metastatic breast cancer, which is especially important because trastuzumab emtansine therapy is set to move to adjuvant treatment paradigms after the results of the KATHERINE study[21]. Thus, the need for novel treatment options is increased for HER2-positive breast cancer in patients with metastatic disease after progression on trastuzumab emtansine. The TULIP randomised phase 3 study (NCT03262935) comparing trastuzumab duocarmazine with standard-of-care chemotherapy combinations in patients with HER2-positive breast cancer is ongoing. Second, activity of single-agent trastuzumab duocarmazine was also seen in patients with HER2-low (IHC 1+ or IHC 2+ ISH-negative) hormone receptor-negative disease, for whom no HER2-targeted drugs and antibody–drug conjugates are currently approved. These triple-negative breast cancers are a highly diverse group of cancers[22] for which several antibody–drug conjugates targeting different antigens are in development. For example, the anti-TROP2 antibody–drug conjugate sacituzumab govitecan has shown encouraging activity in a phase 1 study[23]. Moreover, the prolonged progression-free survival reported with atezolizumab in combination with nab-paclitaxel in a selective group of triple-negative patients is promising[24]. Nevertheless, there is still a high unmet need to improve outcomes in these patients, and trastuzumab duocarmazine could potentially be of benefit in this setting. Third, trastuzumab duocarmazine showed some activity in non-breast HER2-expressing metastatic cancers, which have few treatment options and poor prognoses (eg, urothelial and endometrial cancers). Several responses were noted in these patients, most of whom had HER2-low tumours. Although HER2 expression data are variable between studies,[1,25] further investigation of HER2-targeting drugs in this subset of patients would be worthwhile.

In conclusion, this phase 1 study of trastuzumab duocarmazine has shown important and relevant clinical activity and a manageable safety profile in heavily pretreated patients with HER2-expressing metastatic cancer, including HER2-positive trastuzumab emtansine-resistant and HER2-low breast cancer. Further investigation of trastuzumab duocarmazine for HER2-positive breast cancer is ongoing in the phase 3 TULIP study to assess the efficacy and safety of trastuzumab duocarmazine compared with

clinician's choice in patients with locally advanced or metastatic breast cancer who progressed during or after at least two previous HER2-targeting treatment regimens or during or after trastuzumab emtansine. Additional studies to further investigate the encouraging signal from our phase 1 trial are in preparation.

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Chapter 5

Conclusions and perspectives

Conclusions and perspectives

The past decades, anticancer therapy has been changed from 'one size fits all' to more personalized treatment, based on the presence of specific tumor characteristics, patient characteristics or patient preferences. The main focus of this thesis is to optimize treatment by using specific patient and tumor characteristics in different tumor types.

Poly ADP-ribose polymerase (PARP) is involved in the repair of single strand breaks by Base Excision Repair (BER). Patients with BReast Cancer (BRCA) mutant cells are sensitive for PARP inhibitors by the concept of synthetic lethality[1]. In the presence of a PARP inhibitor, Single strand breaks (SSBs) cannot be repaired adequately. This results in the formation of Double strand breaks (DSBs). In BRCA mutant cells, DSBs are not being repaired properly because of a defective Homologous recombination (HR). This combination would eventually lead to cell death[2]. In the past PARP inhibitors have been studied both as monotherapy and as combination therapy[3-6]. The most well-known PARP inhibitor is olaparib. In **chapter 1.1** PARP inhibition is the central topic. Between 10-20% of breast cancers has a triple negative profile. Triple negative breast cancer (TNBC) treatment is challenging: aggressive, high recurrence rates and a poor 5-year survival. Basal like TNBC shows similarities with BRCA1 mutated tumors. Therefore PARP-inhibition could be a promising strategy. Also combining PARP inhibitors with cytotoxic chemotherapy for this patient group is currently being studied. In the light of more personalized treatment, PARP inhibition could be measured in tumor cells and PBMSs by using pharmacodynamic (PD) assays[7]. The individual patient PD values could be correlated with the individual PARP-inhibitor drug response, which results in individualization of treatment. However, implementation of a PD assay into regular care is problematic since it is quite labor intensive. Maybe the PD assay could be simplified in the near future, which makes it more accessible for daily routine. Olaparib was first only available in capsule formulation. Additionally, also a tablet formulation was developed. Because of this change in formulation a phase I study was performed to assess the maximum tolerable dose of olaparib in combination with carboplatin in advanced cancer patients. In **chapter 1.2** it was found that the maximum tolerable dose was 75 mg olaparib bidaily (BID) combined with carboplatin area under the curve (AUC) 4. In previous studies it was found that there was a higher exposure of olaparib tablets compared to capsules. In our study that difference was not observed, maybe because of the low dosing

of olaparib in our study compared to the bioequivalence testing dose of 400 miligram (mg) before and the less predominant non-linearity at lower dose-levels. PD analyses were also performed and showed only a slight further reduction in Poly ADP-ribose (PAR) levels with an increasing dose of olaparib. This implicates that there is only limited advantage of higher dosing. Pharmacodynamically-guided reduction of maintenance dosing without compromising treatment response, but with less side effects, could be an interesting topic for future studies. Currently, olaparib is being investigated both as a single drug or in combination with chemotherapy, targeted therapy or immunotherapy. It is investigated in a broad range of tumortypes including castration-resistant prostate cancer, ovarian, fallopian, peritoneal cancer and breast cancer. In our study only patients with metastatic cancer were included, but in the currently recruiting studies also patients who are treated neo-adjuvant are included (NCT03150576). As a result, registration of olaparib might be expanded in the next years. Another interesting topic is the cost effectiveness of olaparib. With the increase of treatment population it is necessary to keep it accessible and affordable, Currently, pharmacokinetic boosting for olaparib is investigated (NCT05078671). A non-therapeutic inhibitor of for example cytochrome p450 enzyme 3A4 (CYP3A4) is added to olaparib, which is metabolized by CYP3A4. Boosting increased the concentration of olaparib and results in lower dosing of the therapeutic drug. In this way, olaparib could be affordable and accessible even when there will hopefully be an increase of treatment population. One patient in our phase I trial also developed brain metastases. Despite ongoing systemic response to olaparib treatment, she developed brain metastases. In **chapter 1.3** treatment of brain metastases with PARP-inhibitors is discussed. Literature shows most evidence of veliparib being the best PARP-inhibitor for treating brain metastases, but the evidence is thin[8]. This is mainly because veliparib is no substrate for P-glycoprotein (P-gp)[9]. For olaparib, which is a substrate for P-gp, there are only a few case reports mentioned with variable outcome. In most clinical trials with PARP-inhibitors and especially olaparib, patients with (symptomatic) brain metastases are excluded from participation. Therefore, gathering enough evidence in this specific patient group is difficult. Currently there are a few studies that investigate stereotactic radiotherapy in combination with olaparib as well as chemotherapy with or without a PARP inhibitor in patients with brain metastases from for example breast cancer (NCT04711824. NCT02595905).

Besides preventing DNA SSB or DSB repair, there are several other mechanisms to target with anticancer therapy. The cell cycle consists of a series of events that take place in a cell as it grows and divides. It is a highly controlled process. In case of DNA damage, cells can modulate progression through the cell cycle in order to provide time to repair damaged DNA before going into mitosis[10]. Normal cells repair DNA damage during the G1 arrest of the cell cycle. However, cancer cells have often a deficient G1-S checkpoint and therefore rely on the G2-M checkpoint[11]. Wee1 kinase plays a crucial role in this checkpoint and is able to arrest the cell cycle in order to repair damaged DNA. Various cancer types have a high expression of Wee1 including breast and ovarian cancers. Targeting the Wee1 kinase by an inhibitor seems therefore to be a logic anticancer treatment. In **chapter 2.1** we discuss the Wee1 kinase in cancer. Preclinical and clinical studies have demonstrated encouraging anti-tumor effects with manageable side effects of the combination of Wee1 inhibition and DNA damaging agents. More recently also the combination of PARP-inhibition and Wee1 inhibition has been studied[12]. The genomic instability caused by Wee1 inhibition could be used to enhance the effect of drugs targeting the DNA repair protein such as PARP-inhibitors[13]. In preclinical data, there is a trend that the combination could be effective. However, no clinical trials have been reported yet. When combining both agents, the occurrence of side effects might be concerning. Both compounds have myelosuppressive and gastro-intestinal side effects so the combination might be too toxic. In **chapter 2.2** the results of an interim analysis of an additional safety and activity cohort of a phase II trial are presented. In absence of a functional p53 gene (and therefore a functional G1 checkpoint), damaged DNA relies on the G2 checkpoint. Inhibition of the G2 checkpoint may therefore make p53 mutant tumor cells more susceptible to anticancer agents. This cohort is an addition to a previous phase II study in which patients with platinum refractory or resistant advanced p53 mutated ovarian cancer were treated with carboplatin and the Wee1 inhibitor AZD1775. In the first part of the study, it was shown that the combination was safe and effective[14]. In the additional cohort we expanded the timeframe for resistant disease from three to six months in line with the definition of the Gynecologic Oncology Group (GOG). Besides that, second line therapy was also allowed, because in previous studies also extensive pretreated patients showed efficacy of the combination of Wee1 inhibition and carboplatin. In line with the previous cohort, we found a significant amount of gastro-intestinal side effects, thrombocytopenia and neutropenia, which also

resulted in multiple dose-reductions. Remarkable however was the duration of response, which was significantly lower in our cohort compared to the previous cohort, probably due to more heavily pretreatment. Regarding the side effects, it would be interesting to see whether lower dosing of carboplatin and AZD1775 would lead to similar antitumor effects, with less side effects. The inclusion of the additional safety cohort has just finished. A total of 32 patients have been enrolled in this cohort, of which 29 patients were evaluable for efficacy. There was an objective response rate of 38% in the intention-to-treat population. To explore genetic determinants of drug resistance and response to AZD1775, tumor biopsies were obtained at three time points. A CCNE1 amplification was found in most patients with stable disease or partial remission. CCNE1 could be a potential predictive marker of response and resistance. This could be an important step forward in individualizing treatment: a predictive marker to select patients who could potentially benefit from this treatment. This could prevent patients from exposure to unnecessary toxicity. (not published yet) In **chapter 2.3** the Wee1 inhibitor adavosertib was combined with chemotherapy in patients with ovarian, fallopian or peritoneal cancer. The most promising combination was adavosertib 225 mg twice daily on days 1-3, 8-10, and 15-17 plus carboplatin every 21 days. However, in this treatment arm the highest rates of adverse events (AEs) were shown. The objective response rate in this arm was 66%, with a durable effect and with a median progression free survival of one year. Interestingly, the responses were not limited to patients with a p53 mutation. Two patients with complete response did not have a p53 mutation. Both patients had a loss of function mutation in *ARID1A* and a hotspot mutation in *PIK3CA*. Hematologic toxicity seems to be a serious point of attention in treating this group, but in both chapter 2.3 and in chapter 2.2 (heavily) pretreated patients have been included. It would be worth investigating the combination also in not (heavily) pretreated patients with more bone marrow reserves in order to explore whether there will be less bone marrow toxicity in this group. Regarding the first and second chapter of this thesis, the combination of adavosertib and olaparib is currently being investigated in patients with recurrent ovarian, peritoneal or fallopian tube cancer (NCT03579316). This could be an interesting combination, however, bone marrow toxicity could be a major issue combining these agents. Adavosertib is also being studied in targeted therapy directed treatment by genetic testing patients with advanced solid tumors (NCT02465060). In this study, patient who have a BRCA1 or BRCA 2 mutation receive adavosertib. Targeted

therapy directed treatment are an important step forward in individualization of therapy.
The past decades the field of immunotherapy as anticancer agent has been expanded enormously. PD-L1 is expressed on T-cells following T-cell activation and it downregulates the immune responses in peripheral tissues through binding to its two receptors PD-1 and B7-1[15,16]. Overexpression of PD-L1 has been found in many tumors such as ovarian cancer and is associated with poor outcome. Certain conventional chemotherapies like cyclophosphamide and carboplatin might have immunogenic effects and combining conventional chemotherapies with inhibition of PD-L1/PD-1 could be effective. Atezolizumab is a humanized monoclonal IgG1 antibody that targets human PD-L1. In **chapter 3.1** results of a phase Ib study with atezolizumab, carboplatin and cyclophosphamide are shown. The safe dose combination was defined in a small cohort of six patients. However, toxicity of the triplet therapy seems to be a point of concern. All patients developed hematological toxicity, with anemia and white blood cell count decrease. Two patients developed also immune related toxicities, both patients suffered from an immune related colitis and one of both also developed a pneumonitis. Both patients had to be treated with prednisolone. In this study the incidence of immune related adverse events was higher compared to studies with PD-L1/PD-1 inhibition alone. The immunogenic effect of carboplatin and cyclophosphamide and as a result the increased effect of atezolizumab could play a role. A limitation of this study is the lack of pharmacokinetic measurements. It would be helpful to explore the exposure of each drug administered at different time points. Especially in patients with adverse events this should have been important information. Whether this triplet combination could have a place in the treatment of advanced ovarian, TNBC, fallopian or peritoneal cancer remains the question. More studies are needed to investigate the safety, efficacy and pharmacokinetics of this triplet. In advanced TNBC the combination of carboplatin-cyclophosphamide versus paclitaxel with or without atezolizumab as first line treatment is currently under investigation in the Triple B study (NCT01898117). In this study they would like to investigate whether there is a specific subgroup of TNBC patients that benefit from treatment with atezolizumab. The use of the BRCA-like test could predict for which type of chemotherapy the tumor is sensitive. An also very important part of this study is the collection of tumor and blood samples of the participating patients. With these samples, the investigators want to investigate if there are other biomarkers present that

can predict sensitivity for chemotherapy. If such biomarkers are found, this results in biomarker driven treatment, which is a big step forward in individualization of therapy.

Besides PARP, Wee1 and PD-L1/PD-1, also Human Epidermal Growth Factor Receptor 2 (HER2) plays an important role as a target for anticancer therapy. HER2 overexpression is seen frequently in many different tumor types, it is a protein that promotes cell proliferation and inhibits apoptosis[17]. The most well-known HER2 targeting drug is trastuzumab. However, in studies it has been shown that trastuzumab has limited effect in patients with low amount of HER2. A relatively new class of drugs are antibody–drug conjugates, which are designed for selective delivery of cytotoxic drugs to tumor cells by linking the cytotoxins to monoclonal antibodies[18]. In **chapter 4.1** results of a first-in-human study with the antibody drug conjugate SYD985 are shown. The study shows that there is clinical activity in heavily pre-treated patients, also in the group of patients with low HER2 expression. Effective novel therapies serve an unmet need in these heavily pretreated patients. Trastuzumab-duocarmazine could possibly be beneficial in this difficult to treat patient group. The TULIP randomized phase III study (NCT0326293) compares trastuzumab-duocarmazine with standard-of-care chemotherapy in patients with HER2 positive breast cancer. This study will provide important information about what the efficacy of the antibody drug conjugate will be compared to standard chemotherapy. In our study it was also shown that the drug was not only effective in breast cancer patients but also showed some activity in HER2 positive non-breast cancers such as urothelial and endometrial cancers. Further investigation in this group is warranted, to expand the treatment population. In the ISPY1 study (NCT04602117), trastuzumab-duocarmazine is being combined with paclitaxel to treat patients with metastatic cancer. In this trial, both HER2 postive and HER2 low patients are being included, because trastuzumab-duocarmazine has proven efficacy in HER2 low tumors as well.
In this thesis we aimed to explore several putative targets for anticancer therapy. We showed effectiveness of pharmacological inhibition of PARP, Wee1, and PD-L1/PD-1 in various patient groups, and preliminary efficacy of targeting HER2 and delivering an anticancer agent specifically at the cancer cell. We looked into their safety, pharmacokinetics and efficacy in patients with advanced cancer. Despite promising effects in different studies, more data should be obtained. Patients that have been treated in these studies

have advanced disease and have received one or multiple lines of therapy before starting with the study medication. Myelosuppression seems to be an overarching problem that occurs in the majority of patients receiving drugs like PARP inhibitors, Wee1 inhibitors, classical DNA-damaging chemotherapy in combination with PD-L1/PD-1 inhibitors or antibody drug conjugates. Since patients have been treated before, their bone marrow reserves may have been exhausted to a greater or lesser extent. That could contribute to the occurrence or timing of hematological side effects during these studies. New drugs are being investigated as monotherapy and in combination with other new or pre-existing drugs. Combining drugs could be a rational choice, however, in this often heavily pre-treated group it could lead to faster and serious side effects. Pharmacodynamic assays, for example to measure the inhibition of PARP, could be used to determine at which dose level an adequate inhibition of PARP is accomplished. This might lead to an individualization of dosing, and therefore hopefully to less side effects and better tolerability. An important step forward in more personalized treatment is the biomarker driven treatment as investigated in the TRIPLE-B study (NCT01898117). Because of the movement to more and more tailored treatment, it is important to also take a closer look at the study designs. Are the study designs as being used for many years still suitable for more personalized treatment or do they need an upgrade? The I-SPY 2 has developed a platform trial, where they use a master protocol that gives the opportunity to investigate several compounds in the same study. The goal of the platform is to identify improved treatment regimens for patient subsets on the basis of molecular characteristics (biomarker signatures) of their disease. At randomization, it gives more weight to arms that have been a greater success in the specific tumor type of the patient. Regimens will be dropped out of the study if they show a low probability of improved efficacy with any biomarker signature. It is also possible to add new drugs. Instead of learning afterwards when analyzing the results, in this trial learning will occur as the trial proceeds. Learning from each patient will inform subsequent treatment assignments. Platform trials like the I-SPY2 are much more suitable for the field of tailored therapy and biomarker driven treatments. It is expected that more trials like the I-SPY platform will be set up.

The DRUP (drug rediscovery protocol) study is also an adaptive, precision-oncology trial which facilitates the expanded use of anticancer drugs[19]. If a potential actionable genetic or molecular variant is found, it can be matched

to one of the drugs available in the study. The design allows for an unlimited amount of parallel cohorts. In this way, patients for who no regular treatment options are left anymore, have the opportunity to receive treatment based on their specific genetic or molecular variants. Early signals of activity can be identified and it creates important knowledge for future decision making.
With this thesis we have shown that in current anticancer treatment there are multiple patient and tumor factors that could be a target for treatment.

So, in the future 'one size fits all' will be abandoned and ‘custom made’ therapy is ahead.

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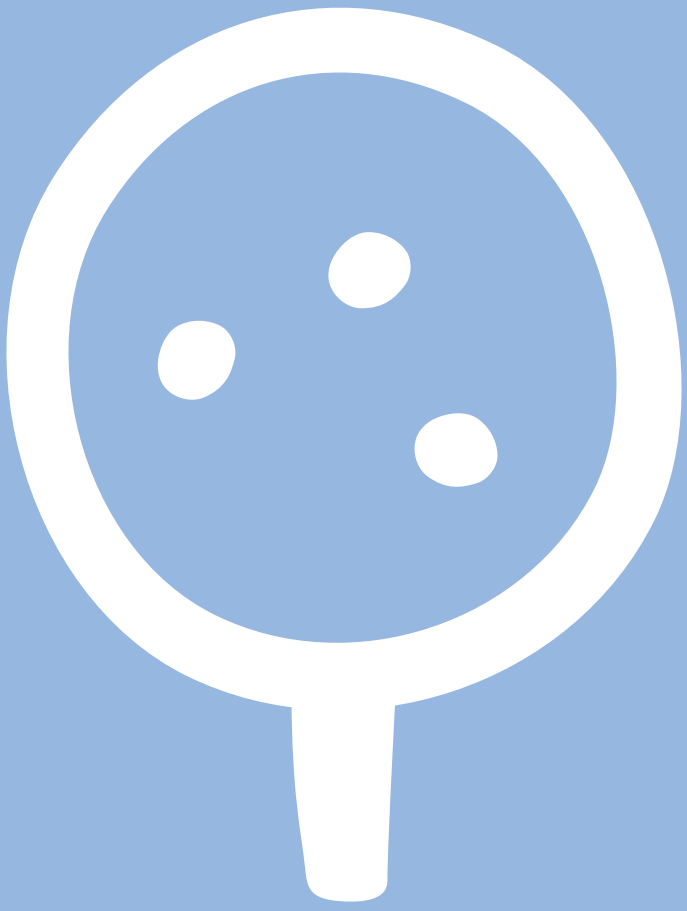

Chapter 6

Summary

Summary

Chapter 1 describes treatment with poly ADP-ribose polymerase (PARP) inhibitors in patients with advanced cancer. Breast cancer is a very heterogeneous disease with a broad range of different phenotypes and morphologic characteristics. In **chapter 1.1** several treatment options for patients with triple negative breast cancer are discussed, with a focus on treatment with PARP inhibitors. We discuss the patient selection, biomarkers, use of combination therapy and pharmacodynamics (PD) assays. Triple negative breast cancer (TNBC) has many similarities with tumors that harbor a BReast Cancer 1 (*BRCA1*) mutation. The basal-like 1 subtype is the most well-known subtype of TNBC and this type has a high incidence of BRCA1 methylation. PARP inhibitors, in combination with a defect in homologous recombination, for example due to a *BRCA* mutation, can lead to synthetic lethality. Individualization of therapy with PARP inhibitors is possible by the use of PD assays. With these assays the activity of PARP can be measured in tumor cells and peripheral blood mononuclear cells (PMBCs). In this way it is possible to individualize dosing based on PARP inhibitor exposure, also toxicity can be part of this consideration. PD assays for PARP inhibitors are not used in daily practice yet, additional prospective clinical validation is needed. Combining PARP inhibition with Wee1 inhibition could also be a potentially beneficial combination. In **chapter 1.2** the results of a phase I 3+3 dose escalation study with olaparib and carboplatin are discussed. The primary endpoint was to determine the maximum tolerable dose of the combination of olaparib and carboplatin in patients with advanced cancer. Important was to look into the toxicity profile of this combination. In total, 24 patients were included, of which most patients suffered from breast cancer. The maximum tolerable dose was olaparib 75 mg bidaily (BID) in combination with carboplatin target area under the curve (AUC) 5. The toxicity profile showed mainly hematological toxicity and gastro-intestinal side effects like nausea and vomiting. Fourteen patients had a partial response as best outcome. The systemic exposure to olaparib tablets was comparable with the previous capsule formulation. PARP levels in PBMCs decreased with 98.7% on day 8 compared to day 1 in dose level -3, which means there is a strong almost complete PARP inhibition. In this study, one patient was included with advanced breast cancer who was treated successfully with olaparib and carboplatin. Despite an ongoing systemic response, she developed brain metastases during the maintenance

treatment with olaparib. In **chapter 1.3** this case has been described, followed by a review of the literature regarding the blood/brain barrier, the occurrence of brain metastases in patients with breast- and ovarian cancer and the use of PARP inhibitors in the treatment of brain metastases. Best evidence for the treatment of brain metastases seems to be for veliparib. In contrast to other PARP inhibitors, veliparib is no substrate for p-glycoprotein (P-gp), which is the most probable explanation for the difference in effect observed between the PARP inhibitors. However, evidence is thin and literature is contradictory. Patients with (symptomatic) brain metastases are often excluded from clinical trials, this makes it difficult to gather more evidence regarding this topic.

Another target for anticancer therapy that has been studied in this thesis is Wee1. This is a protein that is involved in the cell cycle process. It regulates the G2 checkpoint of the cell cycle, which can result in cell cycle arrest and time for DNA repair before entry into mitosis. In **chapter 2** the Wee1 protein and the inhibition of Wee1 as anticancer treatment has been discussed. In a review in **chapter 2.1** the cell cycle, the role of Wee1 and Wee1 inhibition as target for anti-cancer therapy are being discussed. AZD1775 is a small molecule inhibitor of Wee1 kinase. In preclinical studies activity has been shown of Wee1 inhibitors as well in patients with a p53 mutation and in p53 wild type. An explanation for this observation could be that this shows no proof for the functionality of the entire p53 pathway. An alternative reason for the observed effect in p53 wild type could be the existence of an alternative mechanism for synthetic lethality caused by p53 mutation and Wee1 inhibition. Preclinical studies show that the combination of chemotherapy and a Wee1 inhibitor could be effective. The rationale behind this is when drug induced DNA damage occurs, the DNA damage cannot be fully repaired in the occurrence of Wee1 inhibition because of the lack of cell cycle arrest. In clinical studies, different dosing schedules and combination therapies have been investigated. AZD1775 has a relatively short half-life. Besides this, studies showed that multiple doses per week leads to an increased anti-tumor effect. As a result, the dosing schedule of 2.5 day per week was introduced. AZD1775 as monotherapy was also investigated in multiple studies, which also showed some efficacy. The most reported side effects are fatigue, nausea, diarrhea and thrombocytopenia. Apart from combining AZD1775 with chemotherapy, also combination with PARP inhibition could be effective. Although the question arises if toxicity will be

manageable. Future studies are necessary to investigate different combination strategies, and their efficacy and toxicity profile. In **chapter 2.2** an interim analysis with the Wee1 inhibitor AZD1775 in combination with carboplatin is discussed. Patients with advanced ovarian cancer who are refractory or resistant to platinum containing therapy are being treated with carboplatin and AZD1775. The interim analyses discusses the results of part of an additional cohort to a previously performed phase II study. In this additional cohort the efficacy and toxicity of this combination has been further explored. The Gynecologic Oncology Group (GOG) defined platinum resistance as relapse within six months after the last platinum therapy. Therefore, we expanded the timeframe for resistant disease from 3 to 6 month. Another difference between this cohort and the original cohort was that patients could be included that had received a maximum of two lines of therapy (non-platinum containing) instead of a maximum of one line in the previous cohort. Patients received carboplatin with a target AUC 5 mg/ml.min in combination with AZD1775 225 mg bidaily during 2.5 days in a 21 day cycle. This was similar as in the previous cohort. The interim analysis showed comparable toxicity with mainly hematological toxicity, nausea, vomiting and fatigue. There were 6 doses reductions applied in the AZD1775 dose and 3 in the carboplatin dose. Of the 8 evaluable patients, 5 patients had a partial response as best response. It was remarkable that the duration of the response was shorter than in the previous cohort. In **chapter 2.3** the results are presented of a phase II study combining the Wee1 inhibitor adavosertib with chemotherapy in patients with advanced ovarian, fallopian and peritoneal cancer. The study had four treatment arms where patients were treated with adavosertib in combination with gemcitabine, paclitaxel, Pegylated liposomal doxorubicin (PDL) of a combination of these, in different dosing schedules. The responses observed were highest in the carboplatin with weekly adavosertib group, 225 mg bidaily on day 1-3, 8-10 and 15-17. Relatively much hematological toxicity was observed, with almost 50% of the patients with neutropenia grade 3 or higher.

Chapter 3 shows the results of this Ib study in patients with advanced ovarian, fallopian, endometrial, cervical and breast-cancer. Patients received the combination of cyclophosphamide, carboplatin and atezolizumab. The primary end point of this study was to determine a safe dose combination of carboplatin and cyclophosphamide with atezolizumab fixed dose. In total, 6 patients were included. The safe dose was carboplatin target AUC

5 mg/ml.min, cyclophosphamide 600mg/m2 on day 1 and atezolizumab 840 mg on day 1 and day 15. Most common toxicities were hematological. Two patients developed immune related toxicity. Both patients developed an immune related colitis, one of them also suffered from a pneumonitis.

In **chapter 4** the results of a dose escalation study are discussed, in which patients received trastuzumab-duocarmazine, an antibody drug conjugate. The dose escalation part of the study, included patients with advanced cancer with variable Her2 status, who were refractory for standard therapy. In the expansion part, patients with breast, gastric, urothelial or endometrial cancer with at least a Her2 score of 1+ were treated with trastuzumab-duocarmazine. Based on this study the recommended phase II dose was 1.2 mg/kg. Ocular toxicity was common, with 71% of the patients with at least one ocular event, with a grade 3 toxicity in 7% of the patients. The symptoms were mainly conjunctivitis and dry eyes. In the dose expansion cohorts with breast cancer patients, 33% of the patients had a partial response. Of them, 68% of the patients had a Her2 status describes as 'low'. Future studies have to investigate the efficacy of trastuzumab-duocarmazine in Her2 low tumors.

Finally, conclusions, future perspectives and challenges were discussed in **chapter 5.**

Nederlandse samenvatting

Hoofdstuk 1 beschrijft de behandeling met Poly ADP-ribose polymerase (PARP) remmers in patiënten met gemetastaseerde kanker. Borstkanker is een zeer heterogene ziekte met een breed pallet aan verschillende fenotypes en morfologische eigenschappen. In **hoofdstuk 1.1** worden de behandelopties besproken van triple negatieve borstkanker, met een focus op behandeling met PARP remmers. We bespreken de patiënt selectie, biomarkers, het gebruik van combinatietherapie en farmacodynamische (PD) metingen. Triple negatieve borstkanker heeft veel overeenkomsten met tumoren die een *BRCA1* mutatie hebben. Het 'basal-like 1' is het meest bekende subtype van triple negatieve borstkanker en dit heeft een hoge incidentie van BRCA1 methylering. PARP remmers, in combinatie met een defect in homologe recombinatie zoals bijvoorbeeld bij een *BRCA* mutatie, kunnen leiden tot synthetische lethaliteit. Individualisatie van therapie met PARP remmers is mogelijk door gebruik van PD metingen. Daarbij wordt de activiteit van PARP gemeten in tumorcellen en perifere bloed mononucleaire cellen (PBMCs). Op deze manier kan individueel worden bepaald of de dosis moet worden aangepast op basis van de mate van PARP remming. Daarnaast kan dit ook worden afgezet tegen toxiciteit. PD bepalingen voor PARP remmers worden nog niet in de dagelijkse klinische praktijk gebruikt; onderzoek met prospectieve klinische validatie is hier nog voor nodig. Ook de combinatie van PARP remmers met Wee1 remmers (zoals besproken in hoofdstuk 2) is potentieel een effectieve combinatie. In **hoofdstuk 1.2** worden de resultaten besproken van een fase I 3+3 dosisescalatie studie met olaparib en carboplatine. Het primaire doel van de studie was om de maximaal tolereerbare dosis te bepalen van de combinatie van olaparib en carboplatine in patiënten met gevorderde kanker. Belangrijk was te kijken naar het toxiciteitsprofiel van deze middelen. In totaal werden 24 patiënten geïncludeerd, waarvan de meeste borstkanker patiënten betroffen. De maximaal tolereerbare dosering bleek olaparib 75 mg tweemaal daags in combinatie met carboplatine target AUC 4. Het toxiciteitsprofiel toonde met name hematologische toxiciteit en gastro-intestinale bijwerkingen in de vorm van misselijkheid en braken. Veertien patiënten hadden een partiële respons als beste resultaat. De systemische blootstelling aan olaparib was vergelijkbaar met de eerdere capsule formulering. PARP levels in PBMCs daalden met 98.7% op dag 8 in vergelijking met dag 1 in dosis level -3, wat betekent dat een sterke, bijna volledige PARP remming wordt bereikt. In

deze studie was een patiënte geïncludeerd met een gemetastaseerd mamacarcinoom die succesvol kon worden behandeld met olaparib en carboplatine. Ondanks een goede systemische respons, ontwikkelde zij hersenmetastasen gedurende de onderhoudsbehandeling met olaparib. In **hoofdstuk 1.3** wordt deze casus beschreven, gevolgd door een review van de literatuur over de bloed/hersenbarrière, het voorkomen van hersenmetastasen bij patiënten met borst- en eierstokkanker en het gebruik van PARP remmers bij hersenmetastasen. Er lijkt het beste bewijs te zijn voor veliparib als PARP remmer voor behandeling van hersenmetastasen. Veliparib is in tegenstelling tot de andere PARP remmers geen substraat voor P-glycoproteine (P-gp), wat de meest waarschijnlijk oorzaak is voor het verschil dat werd waargenomen. Het bewijs is echter dun en de resultaten van studies spreken elkaar tegen. Lastig is, dat veel patiënten met (symptomatische) hersenmetastasen worden uitgesloten van deelname aan klinische studies. Dat belemmert het verkrijgen van gedegen bewijs.

Een ander target voor antikanker behandeling dat onderzocht wordt in dit proefschrift is Wee1. Dit is een eiwit dat betrokken is bij de celcyclus. Het reguleert het G2 checkpoint van de celcyclus en het zorgt voor remming van de cyclus waardoor er DNA reparatie kan plaatsvinden voordat de cel in mitose gaat. In **hoofdstuk 2** wordt er ingegaan op dit eiwit en remming van dit eiwit door een Wee1 remmer. In een review wordt in **hoofdstuk 2.1** ingegaan op de celcyclus, de rol van Wee1 en Wee1 remming als therapie voor maligniteiten. AZD1775 is een 'klein-molecuul' remmer van Wee1 kinase. In preklinische studies is er activiteit aangetoond van Wee1 remmers zowel in patiënten met een p53 mutatie als ook in wildtype p53. Verklaring hiervoor zou kunnen zijn dat er met het bepalen van de p53 status geen zicht is op de functie van het gehele p53 systeem. Alternatief voor het waargenomen effect in p53 wildtypen zou kunnen zijn dat er nog een alternatief mechanisme bestaat voor synthetische letaliteit veroorzaakt door p53 mutatie en Wee1 remming. Preklinische studies laten zien dat de combinatie van chemotherapie met een Wee1 remmer effectief zou kunnen zijn. De rationale hierachter is dat wanneer er DNA schade optreedt door geneesmiddelen die DNA schade veroorzaken, in aanwezigheid van Wee1 remming de schade niet volledig gerepareerd kan worden door gebrek aan cel cyclus arrest. In klinische studies zijn er diverse doseerschema's en combinatietherapieën met verschillende chemotherapeutica onderzocht. AZD1775 heeft een relatief korte halfwaardetijd, daarnaast bleek uit studies

dat enkele doseringen per week leidt tot een toegenomen anti-tumor effect. Het resultaat was dat een doseerschema van 2.5 dag per week werd geïntroduceerd. AZD1775 als monotherapie werd ook in meerdere studies onderzocht, waarbij er ook bij monotherapie effectiviteit werd gezien. De meest voorkomende bijwerkingen zijn vermoeidheid, misselijkheid, diarree en trombocytopenie. Naast combinatie met chemotherapie, zou ook een combinatie van AZD1775 met PARP remmers effectief kunnen zijn, hoewel de vraag is of de toxiciteit acceptabel blijft. Vervolgstudies zijn nodig om de combinatietherapieën te onderzoeken, hun effectiviteit en het toxiciteitsprofiel. In **hoofdstuk 2.2** wordt een interim analyse met de Wee1 remmer AZD1775 in combinatie met carboplatine besproken. Patiënten met gemetastaseerd ovariumcarcinoom die refractair of resistent zijn voor platinum bevattende therapie worden behandeld met carboplatine met AZD1775. Deze interim analyse bespreekt de resultaten van een deel van een vervolgcohort op een eerder uitgevoerde fase II studie. In dit vervolgcohort wordt de effectiviteit en toxiciteit van deze combinatie verder in kaart gebracht. De richtlijn van de Gynecologic Oncology Group (GOG) hanteert als definitie voor resistente ziekte terugkeer van ziekte binnen 6 maanden na het staken van de platinum bevattende therapie. Daarom werd in dit aanvullende cohort de definitie van resistente ziekte uitgebreid van 3 naar 6 maanden. Een andere aanpassing in dit nieuwe cohort was, dat patiënten geïncludeerd konden worden die tweedelijns therapie (niet platinum bevattend) hadden ontvangen, in plaats van een maximum van eerste lijns therapie als voorbehandeling. Patiënten kregen carboplatine met een target AUC van 5 mg/ml.min in combinatie met AZD1775 225 mg tweemaal per dag gedurende 2.5 dag in een 21 daagse cyclus. Deze dosering en toediencyclus was ongewijzigd ten opzichte van het gepubliceerde cohort. De interim analyse toont vergelijkbare toxiciteit met eerder waarbij met name beenmergtoxiciteit, misselijkheid, braken en vermoeidheid voorop staan. Er waren 6 dosis reducties nodig van AZD1775 en 3 van carboplatine in het kader van graad 3 of 4 toxiciteit. Van de acht evalueerbare patiënten, hadden 5 patiënten een partiele respons als beste respons. Wel viel op dat de duur van respons significant korter bleek dan in het voorgaande cohort. **Hoofdstuk 2.3** bespreekt de resultaten van een fase II studie waarin de Wee1 remmer adavosertib wordt gecombineerd met chemotherapie in patiënten met gemetastaseerd ovarium, eileider en peritoneaal kanker. De studie bestond uit vier armen waarbij patiënten naast adavosertib, gemcitabine, paclitaxel, Pegylated Liposmal doxorubicine (PDL) of een combinatie kregen,

alles in diverse doseerschema's. De mate van respons was het hoogst in de carboplatine met wekelijks adavosertib groep, 225 mg tweemaal daags op de dagen 1–3, 8–10, en 15–17. Wel werd er relatief veel hematologische toxiciteit gezien met in bijna 50% van de gevallen neutropenie graad 3 of hoger.

Hoofdstuk 3 beschrijft een fase Ib studie met immunotherapie in patiënten met gemetastaseerde borst- en gynaecologische kanker. **Hoofdstuk 3.1** laat de resultaten zien van een fase Ib studie in patiënten met gemetastaseerd ovarium, endometrium, cervix en mamacarcinoom, waarbij zij de combinatie van cyclofosfamide, carboplatine en atezolizumab kregen. Het primaire doel van de studie was om een veilige dosis te bepalen van carboplatine en cyclofosfamide met atezolizumab in een vastgestelde dosering. In totaal werden 6 patiënten geïncludeerd. De veilige dosis werd beschouwd als carboplatine target AUC 5, cyclofosfamide 600 mg/m2 op dag 1 en atezolizumab 840 mg op dag 1 en dag 15. De meest voorkomende bijwerkingen waren hematologisch van aard. Twee patiënten ontwikkelden immuun gerelateerde toxiciteit: beiden kregen zij een immuun gerelateerde colitis en een van beiden ontwikkelde daarnaast ook een pneumonitis.

Hoofdstuk 4

In hoofdstuk 4.1 worden de resultaten van een fase I dosisescalatie studie besproken waarin patiënten trastuzumab-duocarmazine, 'antibody-drug conjugate' krijgen toegediend. Het dosis escalatiedeel van de studie includeerde patiënten met gemetastaseerde maligniteit met variabele Her2 status, die refractair waren voor standaard therapie. In het expansiedeel werden patiënten met borst, maag, urotheelcel of endometriumcarcinoom met tenminste een Her2 score van 1+ behandeld met trastuzumab-duocarmazine. Op basis van deze studie werd de aanbevolen dosering van 1.2 mg/kg bepaald. Oculaire toxiciteit kwam frequent voor, 71% van de patiënten had tenminste een oculair event. Een graad 3 toxiciteit werd gemeld in 7% van de patiënten. Klachten bestonden met name uit conjunctivitis en droge ogen. In de dosis expansie cohorten met mamacarcinoom patiënten, kreeg 33% van de patiënten een partiele respons. Daarvan had 68% van de patiënten een Her2 status beschreven als 'low'. Vervolgonderzoeken naar de effectiviteit van trastuzumab-duocarmazine in Her2 low tumoren zal volgen.

Tenslotte worden de conclusies, toekomstige perspectieven en uitdagingen bediscussieerd in **hoofdstuk 5.**

Chapter 7

Appendix

List of publications

Articles

Jill J J Geenen, Gwen M H E Dackus, Philip C Schouten, Dick Pluim, Serena Marchetti, Gabe S Sonke, Katarzyna Jóźwiak, Alwin D R Huitema, Jos H Beijnen, Jan H M Schellens, Sabine C Linn.
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Kathleen N Moore, Setsuko K Chambers, Erika P Hamilton, Lee-May Chen, Amit M Oza, Sharad A Ghamande, Gottfried E Konecny, Steven C Plaxe, Daniel L Spitz, **Jill J J Geenen**, Tiffany A Troso-Sandoval, Janiel M Cragun, Esteban Rodrigo Imedio, Sanjeev Kumar, Ganesh M Mugundu, Zhongwu Lai, Juliann Chmielecki, Suzanne F Jones, David R Spigel, Karen A Cadoo.
Adavosertib with Chemotherapy in Patients with Primary Platinum-Resistant Ovarian, Fallopian Tube, or Peritoneal Cancer: an Open-Label, Four-Arm, Phase II Study.
Clinical Cancer Research. 2022;28:36-44.

In preparation

Jill J J Geenen, Marta I. Lopez, Ingrid Mandjes, Sabine C Linn.
A phase I study to access the safety and tolerability of carboplatin-cyclophosphamide combined with atezolizumab in patients with advanced breast- and gynecologic cancer.

Jill J J Geenen, Jos H Beijnen, Sabine C Linn.
PARP-inhibitors in the treatment of brain metastases.

Interim analysis

Jill J J Geenen, Frans Opdam, Jos H Beijnen.
Wee-1 inhibitor AZD1775 in advanced p53 mutated ovarian cancer.

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Jill Geenen
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Curriculum vitae

Jill J.J. Geenen was born on March 6th 1988 in Geleen. She grew up in Geleen, in the south part of the Netherlands. After completing the Gymnasium at the Graaf Huyn College te Geleen, she moved to Amsterdam to study medicine. In September 2012 she graduated *cum laude* from the University of Amsterdam. In 2012 she started as a resident internal medicine at the Flevo Hospital in Almere. After one year, she entered the training program to become an internist. In 2015 she interrupted the training program to enter a PhD traject at the Netherlands Cancer Institute. She performed phase I and early phase II clinical trials in patients with advanced solid tumors. In addition to her PhD project, Jill also followed the clinical pharmacology training of the Dutch Society for Clinical Pharmacology and Biopharmacy. She finished this study program successfully in August 2019. After this, she continued the training to become an internist. In February 2023 Jill will start a fellowship hematology at the Amsterdam University Medical Centre.