Trilaciclib plus chemotherapy versus chemotherapy alone in patients with metastatic triple-negative breast cancer: a multicentre, randomised, open-label, phase 2 trial

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Summary

Background Trilaciclib is an intravenous cell-cycle inhibitor that transiently maintains immune cells and haemopoietic stem and progenitor cells in G1 arrest. By protecting the immune cells and bone marrow from chemotherapy-induced damage, trilaciclib has the potential to optimise antitumour activity while minimising myelotoxicity. We report safety and activity data for trilaciclib plus gemcitabine and carboplatin chemotherapy in patients with metastatic triple-negative breast cancer.

Methods In this randomised, open-label, multicentre, phase 2 study, adult patients (aged ≥18 years) with evaluable, biopsy-confirmed, locally recurrent or metastatic triple-negative breast cancer who had no more than two previous lines of chemotherapy were recruited from 26 sites in the USA, three in Serbia, two in North Macedonia, one in Croatia, and one in Bulgaria; sites were academic and community hospitals. Availability of diagnostic samples of tumour tissue confirming triple-negative breast cancer was a prerequisite for enrolment. Eligible patients were randomly assigned (1:1:1) by an interactive web-response system, stratified by number of previous lines of systemic therapy and the presence of liver metastases, to receive intravenous gemcitabine 1000 mg/m² and intravenous carboplatin (area under the concentration-time curve 2 μg×h/mL) on days 1 and 8 (group 1), gemcitabine and carboplatin plus intravenous trilaciclib 240 mg/m² on days 1 and 8 (group 2), or gemcitabine and carboplatin on days 2 and 9 plus trilaciclib on days 1, 2, 8, and 9 (group 3) of 21-day cycles. Patients continued treatment until disease progression, unacceptable toxicity, withdrawal of consent, or discontinuation by the investigator. The primary objective was to assess the safety and tolerability of combining trilaciclib with gemcitabine and carboplatin chemotherapy. The primary endpoints were duration of severe neutropenia during cycle 1 and the occurrence of severe neutropenia during the treatment period. Overall survival was included as a key secondary endpoint. Analyses were in the intention-to-treat population. Safety was assessed in all patients who received at least one dose of study treatment. This study is registered with EudraCT, 2016-004466-26, and ClinicalTrials.gov, NCT02978716, and is ongoing but closed to accrual.

Findings Between Feb 7, 2017, and May 15, 2018, 142 patients were assessed for eligibility and 102 were randomly assigned to group 1 (n=34), group 2 (n=33), or group 3 (n=35). Of all patients, 38 (37%) had received one or two lines of previous chemotherapy in the metastatic setting. Median follow-up was 8·4 months (IQR 3·8–13·6) for group 1, 12·7 months (5·5–17·4) for group 2, and 12·9 months (6·7–16·8) for group 3. Data cutoff for myelosuppression endpoints was July 30, 2018, and for antitumour activity endpoints was May 17, 2019. During cycle 1, mean duration of severe neutropenia was 0·8 day (SD 2·4) in group 1, 1·5 days (3·5) in group 2, and 1·0 day (2·6) in group 3 (group 3 *vs* group 1 one-sided adjusted p=0·70). Severe neutropenia occurred in nine (26%) of 34 patients in group 1, 12 (36%) of 33 patients in group 2, and eight (23%) of 35 patients in group 3 (p=0·70). Overall survival was 12·6 months (IQR 5·8–15·6) in group 1, 20·1 months (9·4–not reached) in group 2, and 17·8 months (8·8–not reached) in group 3 (group 3 *vs* group 1 two-sided p=0·0023). The most common treatment-emergent adverse events were anaemia (22 [73%] of 34), neutropenia (21 [70%]), and thrombocytopenia (18 [60%]) in group 1; neutropenia (27 [82%] of 33), thrombocytopenia (18 [55%]) and anaemia (17 [52%]) in group 2; and neutropenia (23 [66%] of 35), thrombocytopenia (22 [63%]), and nausea (17 [49%]) in group 3. There were no treatment-related deaths.

Interpretation No significant differences were observed in myelosuppression endpoints with trilaciclib plus gemcitabine and carboplatin in patients with metastatic triple-negative breast cancer; however, the regimen was generally well tolerated and overall survival results were encouraging. Further studies of trilaciclib in this setting are warranted.

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Research in context

Evidence before this study

We searched PubMed for publications in English and congress abstracts from the annual meetings of the American Association of Cancer Research, the American Society of Clinical Oncology, the European Society of Medical Oncology, and the San Antonio Breast Cancer Symposium for entries and publications between June 1, 2010, and June 30, 2019, using the terms "triple-negative breast cancer", "chemotherapy", "myelosuppression OR myeloprotection OR myelopreservation", "CDK4/6 inhibition", "trilaciclib OR G1T28", and "antitumour immunity". The results showed that multilineage myelosuppression is an acute toxic side-effect of cytotoxic chemotherapy that often leads to haematological toxicities and subsequent dose reductions and delays. Emerging data from early phase clinical trials in patients with small-cell-lung cancer suggest that due to its first-in-class mechanism of action, trilaciclib given before chemotherapy has the potential to protect the immune system and bone marrow from the cytotoxic effects of chemotherapy, resulting in improved benefit-to-risk ratios for patients.

Added value of this study

Patients with metastatic triple-negative breast cancer have few treatment options beyond standard chemotherapy. Trilaciclib inhibits an established pathway (CDK4/6 inhibition) but has a unique delivery schedule and pharmacokinetic properties to target a novel cell population (haemopoietic stem and progenitor cells and lymphocytes). Combining trilaciclib with chemotherapy has the potential to preserve host immunity and improve treatment outcomes in patients with metastatic triple-negative breast cancer without adding clinically significant toxicity.

Implications of all the available evidence

The preliminary antitumour activity reported without substantial added toxicity in this phase 2 trial supports the further assessment of trilaciclib in patients with metastatic triple-negative breast cancer and other chemotherapy-treated tumour types.

Introduction

Triple-negative breast cancer is associated with several aggressive clinicopathological features compared with other types of breast cancer, including younger age of onset and large high-grade tumours.[1–3] Although triple-negative breast cancer accounts only for approximately 15–20% of breast cancer diagnoses, more than a third of patients with triple-negative breast cancer develop metastatic disease.[1] Recurrences generally occur early and are typically visceral.[3]

Despite substantial improvements in clinical outcomes in the field of breast cancer over the past 50 years, the triple-negative breast cancer subtype remains an area of unmet clinical need. Chemotherapy is the cornerstone of treatment for patients with metastatic triple-negative breast cancer;[4] however, chemotherapy-induced myelosuppression commonly leads to dose reductions that can restrict therapeutic dose intensity.[5] By depleting the number and function of lymphocytes, off-target chemotherapy-induced cytotoxicity might also restrict the ability of the patient's immune system to mount an effective response against the cancer.[6,7] Introducing therapy that can protect the immune cells and bone marrow from the cytotoxic effects of chemotherapy has the potential to optimise antitumour activity while minimising myelotoxicity.[8–10]

Trilaciclib (G1T28) is a highly potent and selective inhibitor of cyclin-dependent kinases-4/6 (CDK4/6) that enhances antitumour immunity and preserves haemopoietic stem and progenitor cells during chemotherapy (myelopreservation). Both haemopoietic stem and progenitor cells and lymphocyte populations are dependent on CDK4/6 activity[11,12] for proliferation and become arrested in the G1 phase of the cell cycle on exposure to trilaciclib.[9] This transient, trilaciclib-induced cell cycle arrest has been shown to provide resistance to chemotherapy-induced cell damage by preventing haemopoietic stem and progenitor cells from proliferating in the presence of cytotoxic chemotherapy and favourably altering the tumour immune microenvironment through transient T-cell inhibition when combined with chemotherapy.[8–10,13] Therefore, trilaciclib administered before chemotherapy has the potential to enhance immune activity in patients with triple-negative breast cancer, while protecting bone marrow from the cytotoxic effects of chemotherapy, potentially improving both antitumour activity and safety. Trilaciclib differs from approved CDK4/6 inhibitors (palbociclib, ribociclib, and abemaciclib) in its route of administration, dosing schedule, short half-life, and intended use. Approved oral CDK4/6 inhibitors are dosed chronically to inhibit CDK4/6-dependent tumour proliferation, whereas trilaciclib is administered intravenously and in association with chemotherapy to target normal haemopoietic stem and progenitor cells and lymphocyte populations.[9]

Here, we report safety and activity data for patients with metastatic triple-negative breast cancer who received standard chemotherapy (gemcitabine and carboplatin) with or without trilaciclib. The objectives of the study were to assess the safety and tolerability of combining trilaciclib with gemcitabine and carboplatin, assess the effect of trilaciclib on chemotherapy-induced myelosuppression, and assess the antitumour activity of trilaciclib plus gemcitabine and carboplatin in terms of tumour reduction and survival.

Methods

Study design and participants

In this multicentre, randomised, open-label, phase 2 study, we investigated use of trilaciclib (G1 Therapeutics, manufactured by University of Iowa Pharmaceuticals, Iowa City, IA, USA) in combination with commercially available gemcitabine and carboplatin for patients with metastatic triple-negative breast cancer. Patients were recruited from 26 sites in the USA, three in Serbia, two in North Macedonia, one in Croatia, and one in Bulgaria (appendix p 1). These sites were a mixture of academic and community hospitals.

Patients were eligible for inclusion if they were aged 18 years and older with evaluable, biopsy-confirmed, locally recurrent or metastatic triple-negative breast cancer, provided that the tumours were oestrogen and progesterone receptor negative by immunohistochemistry assessment (defined as <10% nuclei staining) and HER2 negative, according to American Society of Clinical Oncology and College of American Pathologists Clinical Practice Guidelines (ie, non-overexpressing by local assessment of immunohistochemistry [0 or 1+] or fluorescence in-situ hybridisation [HER2/chromosome enumeration probe 17 ratio <2·0] or had an average HER2 gene copy number of <4 signals per cell by local assessment). Availability of diagnostic samples of tumour tissue confirming triple-negative breast cancer was a prerequisite for enrolment. Further eligibility criteria were haemoglobin concentrations of 9·0 g/dL or higher in the absence of red blood cell transfusion within 14 days before the first dose of trilaciclib; absolute neutrophil count of $1·5 \times 10^9$ cells per L or higher; a platelet count of 100×10^9 per L or higher; an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1; and adequate kidney and liver function, as determined by laboratory tests of serum creatinine (≤1·5 mg/dL or creatinine clearance ≥60 mL/min), total bilirubin concentration of 1·5×upper limit of normal (ULN) or lower, and aspartate aminotransferase and alanine aminotransferase concentrations of 2·5×ULN or lower (or ≤5×ULN in the presence of liver metastases). With the exception of alopecia, resolution of non-haematological toxicities from previous treatment to grade 1 or lower was required. Patients were not eligible for inclusion if they had received more than two previous cytotoxic chemotherapy regimens for locally recurrent or metastatic triple-negative breast cancer. Initially, patients were not eligible for inclusion if they had received more than one line of therapy for locally recurrent or metastatic triple-negative breast cancer or if they had rapid progression (less than 12 months between last chemotherapy and first recurrence). However, after a protocol amendment on Aug 31, 2017, the exclusion criteria were updated to increase the number of previous lines of therapy allowable in the locally recurrent or metastatic triple-negative breast cancer setting from one to two, and to include a specific definition as to how to count lines of previous therapy for locally recurrent or metastatic triple-negative breast cancer. Additionally, patients with rapid progression were eligible for inclusion. This change was made to increase the eligible patient population without compromising study objectives, while ensuring a more consistent interpretation of the eligibility criteria by investigators. The number of previous lines of therapy was determined by investigator sites, however, because the number determined did not always agree with that determined by internal clinical review, both datasets were collected and are reported here. Chemotherapy administered in the neoadjuvant or adjuvant setting was considered a line of therapy when fewer than 12 months had elapsed between the last treatment and disease recurrence. Furthermore, patients were not eligible for inclusion if they had malignancies other than triple-negative breast cancer within the 3 years before randomisation, central nervous system metastases or leptomeningeal disease requiring immediate treatment, uncontrolled ischaemic heart disease or symptomatic congestive heart failure, known history of stroke or cerebrovascular accident within 6 months before the first dose of trilaciclib, known serious active infection, or any other uncontrolled serious chronic disease or psychiatric condition that could affect patient safety, compliance, or follow-up. Before study entry, a 2-week washout period was required for previous radiotherapy and a 3-week washout period was required for previous cytotoxic chemotherapy.

See Online for appendix

The study was designed and undertaken in compliance with the principles of the Declaration of Helsinki and the Good Clinical Practice guidelines of the International Council for Harmonisation. The study protocol (appendix pp 17–122) and all associated amendments and study-related materials were approved by the institutional review board or independent ethics committee of each investigational site. Written, informed consent was obtained from each patient before initiation of study procedures. An external, independent Data Monitoring Committee assessed accumulating safety data from the point when the first 20 patients had been enrolled and completed at least one cycle, with interim reviews approximately every 4 months during the treatment phase.

Randomisation and masking

Patients were randomly assigned (1:1:1) by an interactive web-response system to one of three treatment groups. Initially, randomisation was stratified by the presence of liver metastases (yes or no) and ECOG performance status (0 or 1). After an approved protocol amendment (Aug 31, 2017), in which the exclusion criterion restricting previous lines of therapy was refined, stratification factors were changed to the number of previous lines of systemic therapy (zero *vs* one or two) and the presence of liver metastases (yes or no). This study was open-label; therefore, patients, study staff, and investigators were not masked to assignment.

Procedures

Patients were randomly assigned to the following treatments given in 21-day cycles: group 1 was given gemcitabine and carboplatin on days 1 and 8 (chemotherapy only), group 2 was given trilaciclib before gemcitabine and carboplatin on days 1 and 8 (trilaciclib plus chemotherapy days 1 and 8), and group 3 was given only trilaciclib on days 1 and 8, and trilaciclib before gemcitabine and carboplatin on days 2 and 9 (trilaciclib days 1 and 8, trilaciclib plus chemotherapy days 2 and 9). Group 3 was included to test the hypothesis that a second dose of trilaciclib before chemotherapy could increase the proportion of haemopoietic stem and progenitor cells in transient arrest at the time of chemotherapy administration, thereby improving myelosuppression outcomes. Gemcitabine was administered at 1000 mg/m² and carboplatin at area under the concentration-time curve (AUC) 2 μg×h/mL, both as intravenous infusions. Trilaciclib 240 mg/m² was given as an intravenous infusion over 30 min (allowable range 25–35 min) before gemcitabine and carboplatin treatment. No dose modifications of trilaciclib were allowed.

Treatment cycles occurred consecutively without interruption, except when necessary to manage toxicities. If dose reductions were required for chemotherapy they ocurred in the following order: first, the gemcitabine dose was reduced from 1000 mg/m² to 800 mg/m²; second, the carboplatin dose was reduced from AUC 2 μg×h/mL to AUC 1·5 μg×h/mL; third, either carboplatin or gemcitabine was discontinued and the other drug continued at the reduced dose; and finally, all study drugs were permanently discontinued. Dose reductions were allowed only once per cycle and were permanent.

Trilaciclib was administered only if gemcitabine or carboplatin therapy, or both, was administered. For group 3 , the interval between first dose of trilaciclib and chemotherapy on sucessive days was no more than 28 h. If administration of chemotherapy was delayed or discontinued, trilaciclib was also delayed or discontinued. Study drug administration was continued until disease progression, unacceptable toxicity, withdrawal of consent, or discontinuation by the investigator, whichever occurred first.

Per protocol, samples were collected for haematological laboratory assessment on days 1, 8, and 15 of each 21-day cycle, regardless of treatment group. If the start of a subsequent cycle was delayed, laboratory assessments were done weekly (eg, days 22, 29, 36, and so on) until the patient was able to start the next cycle or discontinued chemotherapy permanently. Unscheduled laboratory assessments were permitted as clinically indicated. The use of prophylactic growth factors, including granulocyte-colony stimulating factor (G-CSF) and granulocyte-macrophage colony-stimulating factor, was not permitted during cycle 1. From cycle 2 onward, growth factors were administered per the American Society of Clinical Oncology guidelines for neutropenia if patients had an absolute neutrophil count of less than $1·0×10^9$ cells per L on day 1 or 2, or had skipped dosing on day 8 or 9 of a previous cycle due to an absolute neutrophil count of less than $1·0×10^9$ cells per L. Otherwise, supportive care, including transfusions, was allowed as needed throughout the treatment period. Platelets were transfused at a threshold of 10 000 per μL or less, or at a threshold of less than 50 000 per μL (100 000 per μL for CNS or ocular bleeding) in any patient with bleeding related to thrombocytopenia. Patients with a haemoglobin concentration of less than 8·0 g/dL or with symptomatic anaemia could be treated with red blood cell transfusions at the investigator's discretion. The percentage of patients receiving red blood cell transfusions and the number of red blood cell transfusions received over time was analysed from on or after week 5 and from day 1 on study as part of a sensitivity analysis.

Assessment of antitumour response was done by the investigator according to Response Evaluation Criteria in Solid Tumors version 1.1. For tumour assessment, CT or MRI was done at screening and at protocol-specified intervals (every 9 weeks for the first 6 months, then every 12 weeks thereafter) until disease progression, withdrawal of consent, or receipt of subsequent anticancer therapy. Bone scans were required at screening; alternative imaging methods could be used for follow-up assessments of bone lesions. After a protocol amendment (Aug 31, 2017), brain scans were also required at screening. If brain metastases were present at screening, brain scans were repeated with each tumour assessment.

To assess the ability of T cells to produce cytokines, whole blood was stimulated with 5 μg/mL staphylococcal enterotoxin B overnight (15–18 h) in the presence of Brefeldin A. Cells were processed and labelled with fluorophore-labelled antibodies against IFN-γ, IL-17A, CD3, and CD8 and assessed by flow cytometry (BD FACSCalibur and FACSCanto II clinical cell analysers; BD Biosciences, Franklin Lakes, NJ, USA) by Covance Central Laboratory Services (Indianapolis, IN, USA and Geneva, Switzerland). Flow cytometry data were analysed by Fios Genomics (appendix p 2).

Using two established signatures (Prosigna Breast Cancer Prognostic Gene Signature Assay [PAM50][14] and Lehmann triple-negative breast cancer type 1–4[15,16]), patient tumours were characterised as CDK4/6 independent, dependent, or indeterminate. Because triple-negative breast cancer is predominantly a functionally CDK4/6-independent disease, despite a genomic retinoblastoma inactivation rate of only 20%, these signatures were chosen to provide a more comprehensive analysis of CDK4/6 sensitivity. Using the PAM50 signature, CDK4/6 independence correlates with basal-like tumours. Because their reliance on the CDK4/6 pathway for proliferation is either unknown or heterogeneous, the remaining PAM50 signature groups (including HER2, normal-like, luminal A, and luminal B) are categorised as

CDK4/6 indeterminate.[14] Conversely, using the Lehmann signature, CDK4/6 dependence is closely correlated with luminal-androgen receptor tumours, whereas the remaining Lehmann signature groups (including basal-like and mesenchymal) are categorised as CDK4/6 indeterminate for the same reasoning as outlined for the PAM50 signature.[15,16] Additional details of the prespecified T-cell activation analyses and CDK4/6 dependence subtyping are in the appendix (p 2).

Safety was monitored continuously throughout the study, from provision of informed consent until 30 days after the last dose of study treatment. Safety assessments included analyses of treatment duration and dose modifications, assessments of treatment-emergent adverse events and serious treatment-emergent adverse events, infusion-related reactions, laboratory safety assessments, vital signs, physical examination, and electrocardiography. Treatment-emergent adverse events were summarised according to grade (Common Terminology Criteria for Adverse Events [CTCAE] version 4.03) and association with study drug.

Since the primary toxicity of chemotherapy is myelosuppression (which trilaciclib was hypothesised to reduce), several haematological parameters were assessed across multiple haemopoietic lineages, including the incidence and severity of haematological adverse events, laboratory values (absolute neutrophil count, haemoglobin concentration, and platelet count), supportive care interventions (red blood cell and platelet transfusions, use of G-CSF), and dose intensity and incidence of gemcitabine and carboplatin dose reductions. Full details of all parameters are included in the appendix (p 2).

Outcomes

The primary objective was to assess the safety and tolerability of trilaciclib given with chemotherapy. The primary endpoints were defined in the statistical analysis plan (Sept 28, 2018; prior to database lock) as the duration duration of severe neutropenia (severe neutropenia is defined as CTCAE grade 4, absolute neutrophil count $<0\cdot5\times10^9$ cells per L) in cycle 1 and occurrence of severe neutropenia during the treatment period. Duration of severe neutropenia in cycle 1 was defined as the number of days from the date of the first absolute neutrophil count value of less than $0\cdot5\times10^9$ cells per L to the date of the first absolute neutrophil count value of $0\cdot5\times10^9$ cells per L or higher without observing absolute neutrophil count values of less than $0\cdot5\times10^9$ cells per L until the end of the cycle. Duration of severe neutropenia was set to zero days for patients who did not have severe neutropenia in cycle 1. The occurrence of severe neutropenia was a binary endpoint defined as those having one or more readings of absolute neutrophil count below $0\cdot5\times10^9$ cells per L during the treatment period. Both scheduled and unscheduled haematological laboratory results were included in the analysis of both primary endpoints. A clinically relevant level of $0\cdot5\times10^9$ cells per L was chosen for the primary analysis on the basis of the clinical link between severe neutropenia and an increased risk of infection and morbidity and mortality.

Key secondary endpoints included the occurrence of red blood cell transfusions on or after week 5, G-CSF administrations, platelet transfusions, all-cause dose reductions, and overall survival. The exclusion of red blood cell transfusions before week 5 on study was based on the half-life of red blood cells (approximately 8–9 weeks) and to ensure that analyses of potential benefit were not confounded by the residual effect of previous treatment. Occurrence of red blood cell and platelet transfusions was a binary endpoint (yes or no) and the total number of transfusions was a count endpoint (number of transfusions with a unique start date). Overall survival was calculated as the time (in months) from the date of randomisation to the date of death due to any cause.

Supportive secondary antitumour activity endpoints that are reported here are the proportion of patients who achieved an objective response (defined as a confirmed complete or partial response), and progression-free survival. We calculated clinical benefit rate using data from any patient who had a complete or partial response at any time after treatment or stable disease for 24 weeks or longer; if a patient did not have a complete or partial response and duration of stable disease was indeterminate, they were considered not evaluable. Progression-free survival was defined as the time (in months) from the date of randomisation until the date of radiologically confirmed disease progression or death due to any cause, whichever came first. A full list of supportive secondary and exploratory endpoints, as defined in the statistical analysis plan, is in the appendix (p 2) and will be reported elsewhere.

Statistical analysis

We originally determined the sample size for the assessment of safety and tolerability of trilaciclib when administered in combination with gemcitabine and carboplatin. In conjunction with regional regulatory input, we subsequently refined analyses of specific endpoints across the trilaciclib development programme and prespecified in the statistical analysis plan to show superiority of group 3 over group 1 with 90% power for at least one primary endpoint (either duration of severe neutropenia in cycle 1 or occurrence of severe neutropenia). We used an equally-weighted Bonferroni procedure to maintain the overall two-sided type I error rate at 0·05 and calculated that 64 patients (32 per group) were needed to detect a 3 day reduction of duration of severe neutropenia in cycle 1 with a common SD of 2·5 days or a 41 percentage point absolute reduction in the proportion of patients with severe neutropenia (ie, 45% for group 1 and 4% for group 3). Assuming a 5% attrition rate, we needed 102 patients in total (34 per group).

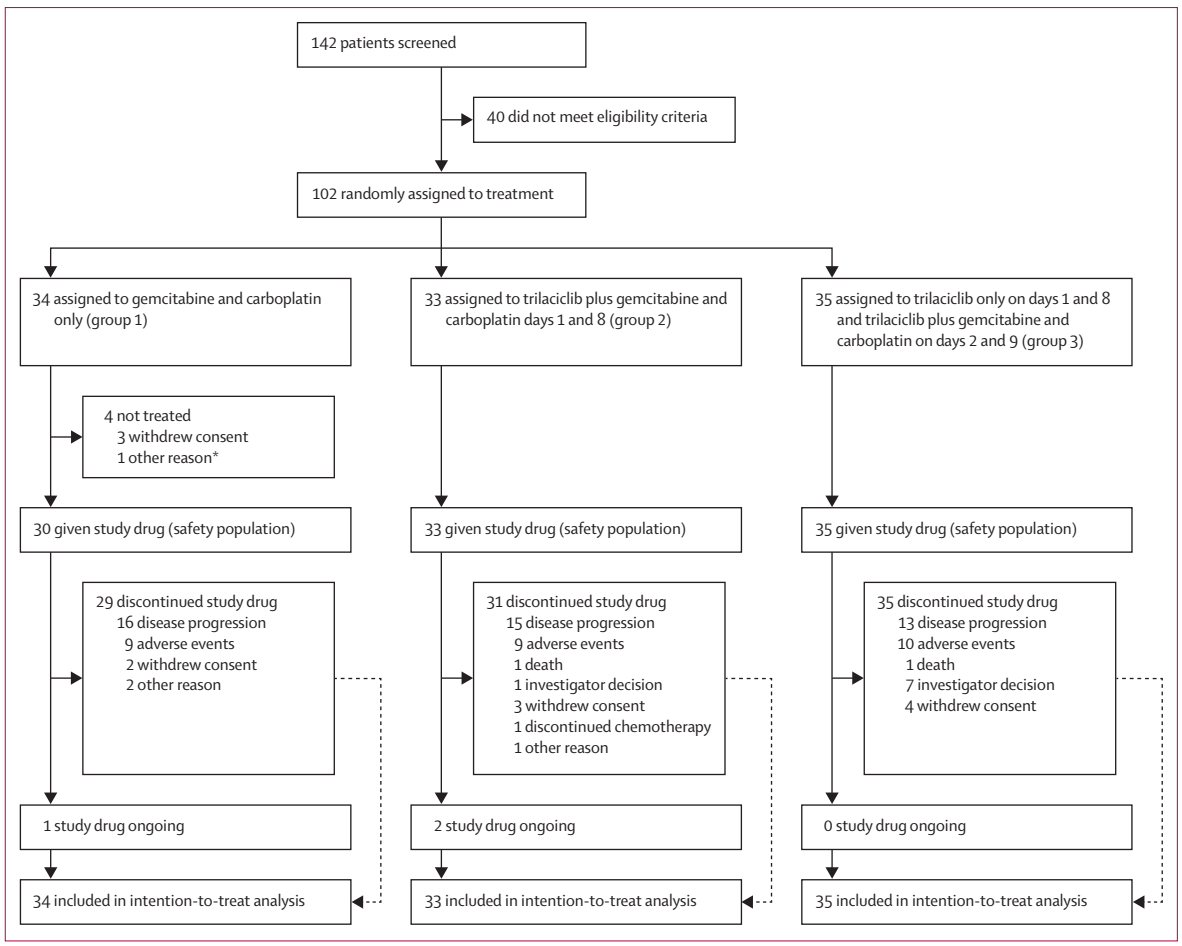

Figure 1: **Trial profile**
Data correct as of May 17, 2018. *Patient's screening tests expired and they did not meet eligibility criteria upon re-screening.

We used a non-parametric analysis of covariance to assess treatment group differences for duration of severe neutropenia in cycle 1 using stratification factors and treatment as fixed effects, with baseline absolute neutrophil count value as a covariate. For occurrence of severe neutropenia, G-CSF administration, red blood cell transfusions on or after week 5, and platelet transfusions, we used a modified Poisson regression model[17] to assess the treatment effect. The model included the same fixed terms as used for duration of severe neutropenia, with baseline absolute neutrophil count as the covariate for severe neutropenia and G-CSF administration analyses, baseline haemoglobin concentration as the covariate for red blood cell transfusion analysis and baseline platelet count as the covariate for platelet transfusion analysis. Duration of treatment (in weeks) was adjusted in this model. From day 1 on study, we analysed the percentage of patients receiving red blood cell transfusions and the number of red blood cell transfusions over time as part of a sensitivity analysis. For the number of red blood cell transfusions on or after week 5 and platelet transfusions, we used a negative binomial regression model to assess treatment effect. The model included the same fixed terms as used for duration of severe neutropenia, with baseline haemoglobin as the covariate for red blood cell transfusion analysis and baseline platelet count as the covariate for platelet transfusion analysis. Duration of treatment (in weeks) was adjusted in this model. We also analysed the number of all-cause dose reductions using a negative binomial regression model, which included only stratification factors and treatment as fixed effects, and was adjusted for the number of cycles. We controlled the family-wise type I error rate of 0·025 (one-sided) across the primary and key secondary myelosuppression endpoints using a Hochberg-based gatekeeping procedure.[18] We report model-based point estimates along with 95% CIs.

We analysed treatment group differences in objective response by use of an exact Cochran-Mantel-Haenszel method accounting for the stratification factors. We calculated the 95% CIs for the proportion of patients who achieved an objective response using the exact Clopper-Pearson method. For time-to-event variables,

such as duration of response, progression-free survival, and overall survival, we used the Kaplan-Meier method to estimate the median time and its 95% CI. We tested treatment group differences using the stratified log-rank test to account for the stratification factors. We calculated hazard ratios (HRs) and their associated 95% CIs from the Cox proportional hazards model, with treatment and stratification factors as fixed effects.

The statistical analysis plan prespecified the primary statistical comparison for the primary and key secondary endpoints to be between group 3 and group 1, and prespecified secondary comparisons were to be between group 2 and group 1, and between the combined trilaciclib groups and group 1.

We undertook activity analyses using the intention-to-treat (ITT) population on the basis of assigned treatment for myelosuppression and antitumour activity endpoints, with the exception of tumour response endpoints (objective response and clinical benefit), which were analysed in patients who received at least one dose of study drug, had measurable target lesions at the baseline tumour assessment, and either had at least one tumour assessment after treatment (or no tumour assessment after treatment but had clinical progression as noted by the investigator) or had died due to disease progression before their first tumour scan after treatment (response evaluable population). Duration of survival follow-up was calculated from date of randomisation to date of death or the last contact date as of the antitumour activity data cutoff on May 17, 2019. Safety analyses included all patients who received at least one dose of study medication. We adjusted the stratification factors (number of previous lines of systemic therapy and liver involvement) in statistical models.

We did additional prespecified analyses assess T-cell function. We report the mean frequency of the IFN-γ positive T-cell population after ex-vivo stimulation by group along with 95% CIs.

We did prespecified subgroup analyses for progression-free survival and overall survival to assess consistency of treatment effect (ie, age group, race, liver involvement, country, ECOG performance status, number of previous lines of therapy, BRCA classification, and histological triple-negative breast cancer classification).

To address the theoretical risk that trilaciclib could decrease antitumour activity in patients with CDK4/6 dependent tumours by arresting CDK4/6-dependent tumour cells during chemotherapy, we did an additional prespecified subgroup analysis of the antitumour activity endpoints (objective response, progression-free survival, and overall survival) using two established signatures (PAM50[14] and Lehmann triple-negative breast cancer type 1–4[15,16]) to characterise patient tumours as CDK4/6 independent, dependent, or indeterminate (appendix p 2).

We also did a post-hoc analysis of antitumour activity endpoints (objective response, progression-free survival, and overall survival) based on the overall median number of cycles patients received during the study. The analysis was done in patients grouped according to whether they had received 1–7 cycles or more than 7 cycles.

	Chemotherapy only group (group 1; n=34)	Trilaciclib plus chemotherapy (day 1 and 8) group (group 2; n=33)	Trilaciclib (day 1 and 8), trilaciclib plus chemotherapy (day 2 and 9) group (group 3; n=35)
Sex			
Female	34 (100%)	32 (97%)	35 (100%)
Male	0	1 (3%)*	0
Age, years			
Median	55 (43–64)	55 (47–66)	58 (49–65)
18 to <65	26 (76%)	24 (73%)	26 (74%)
65 to 75	6 (18%)	7 (21%)	9 (26%)
>75	2 (6%)	2 (6%)	0
Race			
White	28 (82%)	22 (67%)	28 (80%)
Black or African American	5 (15%)	7 (21%)	2 (6%)
Asian	0	2 (6%)	4 (11%)
Other	1 (3%)	2 (6%)	1 (3%)
Country			
USA	28 (82%)	28 (85%)	27 (77%)
Outside of USA	6 (18%)	5 (15%)	8 (23%)
Hormone receptor status			
Oestrogen			
<1%	31 (91%)	30 (91%)	33 (94%)
1–10%	2 (6%)	2 (6%)	2 (6%)
Oestrogen negative, other†	1 (3%)	1 (3%)	0
Progesterone			
<1%	29 (85%)	29 (88%)	34 (97%)
1–10%	4 (12%)	3 (9%)	1 (3%)
Progesterone negative, other†	1 (3%)	1 (3%)	0
ECOG performance status			
0	15 (44%)	17 (52%)	21 (60%)
1	19 (56%)	16 (48%)	14 (40%)
Liver involvement	8 (24%)	8 (24%)	10 (29%)
Received neoadjuvant or adjuvant chemotherapy	24 (71%)	23 (70%)	25 (71%)
Previous lines of therapy			
Interactive web-response system			
0	21 (62%)	22 (67%)	21 (60%)
1 or 2	13 (38%)	11 (33%)	14 (40%)
Clinical review			
0	18 (53%)	19 (58%)	17 (49%)
1	11 (32%)	11 (33%)	14 (40%)
2	5 (15%)	3 (9%)	4 (11%)

Data are n (%) or median (IQR). ECOG=Eastern Cooperative Oncology Group. *One male patient was enrolled, but was later found to have an incorrect diagnosis of triple-negative breast cancer; his disease was pathologically confirmed as metastatic renal cell carcinoma. †Patients were determined by treating physician to have a diagnosis of triple-negative breast cancer, but not all pathology reports were available for review; includes one patient in group 1 who was randomly assigned, but not given study treatment.

Table 1: **Baseline demographic and clinical characteristics**

	Chemotherapy only group (group 1; n=34)	Trilaciclib plus chemotherapy (day 1 and 8) group (group 2; n=33)	Trilaciclib (day 1 and 8), trilaciclib plus chemotherapy (day 2 and 9) group (group 3; n=35)	p value*
Duration of severe neutropenia in cycle 1, days†‡	0·8 (2·4)	1·5 (3·5)	1·0 (2·6)	0·70§
Patients with severe neutropenia†¶	9 (26%)	12 (36%)	8 (23%)	0·70§
All-cause dose reductions, n per 100 cycles‖**	14·1	11·8	13·3	0·98
Patients with G-CSF administration¶	16 (47%)	21 (64%)	14 (40%)	0·14
Patients with red blood cell transfusion on or after week 5¶	12 (35%)	11 (33%)	8 (23%)	0·075
Red blood cell transfusions on or after week 5, n per 100 weeks**	4·6	1·9	1·6	0·020
Patients with platelet transfusions¶	4 (12%)	3 (9%)	6 (17%)	0·98
Platelet transfusions, n per 100 weeks**	1·9	0·4	1·2	0·61

Data are mean (SD), n, or n (%). Based on data as of July 30, 2018. G-CSF=granulocyte-colony stimulating factor. *For comparison between groups 3 and 1. †Primary endpoint. ‡p value obtained from a non-parametric analysis of covariance. §Multiplicity adjusted one-sided p value, all other p values are two-sided. ¶p value obtained from a modified Poisson model. ‖Total number of all-cause dose reductions was the number of cycles with at least one dose reduction—if a patient did not have any dose reduction, they were assigned a value of 0. **p value was obtained from a negative binomial model.

Table 2: **Myelosuppression endpoints summary**

We did all statistical analyses using SAS software (version 9.4). This study is registered with EudraCT, 2016-004466-26 and ClinicalTrials.gov, NCT02978716.

Role of the funding source

The funder had a role in study design, data collection, data analysis, data interpretation, and writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Between Feb 7, 2017, and May 15, 2018, 142 patients were screened, and 102 eligible patients were randomly assigned to the chemotherapy alone group (group 1; n=34), the trilaciclib plus chemotherapy group (group 2; n=33), or in the trilaciclib (day 1 and 8), trilaciclib plus chemotherapy (day 2 and 9) group (group 3; n=35; ITT population). 98 (96%) received at least one dose of study drug (safety analysis population; figure 1). Baseline demographic characteristics were similar between the treatment groups (table 1). 38 (37%) of 102 patients had received one or two previous lines of chemotherapy, and 26 (25%) had liver metastases. Median follow-up was 8·4 months (IQR 3·8–13·6) for group 1, 12·7 months (5·5–17·4) for group 2, and 12·9 months (6·7–16·8) for group 3.

The addition of trilaciclib to gemcitabine and carboplatin did not result in significant improvements in the predetermined, primary myelosuppression endpoints. During cycle 1, mean duration of severe neutropenia was 0·8 day (SD 2·4) in group 1, 1·5 days (3·5) in group 2, and 1·0 day (2·6) in group 3 (group 3 *vs* group 1 one-sided adjusted p=0·70). Severe neutropenia occurred in nine (26%) of 34 patients in group 1, 12 (36%) of 33 patients in group 2, and eight (23%) of 35 patients in group 3 (p=0·70; table 2). The number of red blood cell transfusions on or after week 5 per 100 weeks decreased in both trilaciclib groups (4·6 in group 1 *vs* 1·9 in group 2 and 1·6 in group 3; p=0·020). The red blood cell transfusion data collected from day 1 (sensitivity analysis) on study was similar to that observed when data before week 5 were excluded (appendix p 7). We found no significant differences in the number of patients who were administered G-CSF or undergoing platelet transfusions (table 2). Data cutoff for the primary endpoints and key secondary myelosuppression endpoints was July 30, 2018, when data supporting these endpoints were considered final.

Data cutoff for antitumour activity (objective response, overall survival, progression-free survival), safety, and drug exposure (ie, dose reductions), was May 17, 2019. The number of patients with at least one carboplatin dose reduction was ten (33%) in group 1, 13 (39%) in group 2, and 15 (43%) in group 3. For gemcitabine, 13 (43%) patients in group 1, 20 (61%) patients in group 2, and 17 (49%) patients in group 3 had at least one dose reduction. Adding trilaciclib to gemcitabine and carboplatin increased the duration of exposure and cumulative dose of gemcitabine and carboplatin compared with patients treated with gemcitabine and carboplatin alone. Median duration of treatment was 101 days (IQR 63–203 [median of four cycles]) in group 1, 161 days (77–287 [median of seven cycles]) in group 2, and 168 days (91–217 [median of eight cycles]) in group 3. The median cumulative dose of carboplatin was AUC 15 µg×h/mL (IQR 8–28) in group 1 versus AUC 24 µg×h/mL (10–40) in group 2 and AUC 22 µg×h/mL (15–34) in group 3. For gemcitabine, the median cumulative dose increased to 7306·2 mg/m² (4020·1–15 138·9) in group 1, to 12 000·0 mg/m² (5029·4–21 882·7) in group 2 and 11 800·1 mg/m² (7000·0–17 446·9) in group 3. Despite a longer duration of gemcitabine and carboplatin for patients who received trilaciclib, haematological treatment-emergent adverse events occurred as frequently or less frequently in the trilaciclib groups as in the chemotherapy alone group (table 3).

All but one patient (in group 3) had one or more treatment-emergent adverse event. For most patients, treatment-emergent adverse events were considered to be drug related. The most common treatment-emergent adverse events were anaemia (22 [73%] of 34), neutropenia (21 [70%]), and thrombocytopenia (18 [60%]) in group 1; neutropenia (27 [82%]) of 33), thrombocytopenia (18 [55%]) and anaemia (17 [52%]) in group 2;

	Chemotherapy only group (group 1; n=30)			Trilaciclib plus chemotherapy (day 1 and 8) group (group 2; n=33)			Trilaciclib (day 1 and 8), trilaciclib plus chemotherapy (day 2 and 9) group (group 3; n=35)		
	Grade 1 and 2	Grade 3	Grade 4	Grade 1 and 2	Grade 3	Grade 4	Grade 1 and 2	Grade 3	Grade 4
Anaemia*	8 (27%)	14 (47%)	0	9 (27%)	8 (24%)	0	4 (11%)	11 (31%)	0
Neutropenia†	1 (3%)	12 (40%)	8 (27%)	1 (3%)	14 (42%)	12 (36%)	3 (9%)	13 (37%)	7 (20%)
Thrombocytopenia‡	3 (10%)	8 (27%)	7 (23%)	9 (27%)	3 (9%)	6 (18%)	7 (20%)	9 (26%)	6 (17%)
Fatigue	10 (33%)	1 (3%)	0	13 (39%)	1 (3%)	0	13 (37%)	2 (6%)	0
Vomiting	7 (23%)	1 (3%)	0	6 (18%)	1 (3%)	0	11 (31%)	0	0
Nausea	7 (23%)	0	0	14 (42%)	0	0	16 (46%)	1 (3%)	0
Leucopenia§	2 (7%)	4 (13%)	1 (3%)	2 (6%)	5 (15%)	0	2 (6%)	1 (3%)	0
Dizziness	6 (20%)	0	0	4 (12%)	0	0	6 (17%)	0	0
Headache	6 (20%)	0	0	9 (27%)	0	0	13 (37%)	1 (3%)	0
Constipation	5 (17%)	0	0	8 (24%)	0	0	9 (26%)	0	0
Diarrhoea	3 (10%)	1 (3%)	0	8 (24%)	0	0	5 (14%)	0	0
Pain	2 (7%)	2 (7%)	0	2 (6%)	0	0	3 (9%)	0	0
Urinary tract infection	4 (13%)	0	0	2 (6%)	1 (3%)	0	3 (9%)	0	0
Alanine aminotransferase increased	3 (10%)	0	0	2 (6%)	2 (6%)	0	3 (9%)	1 (3%)	0
Arthralgia	3 (10%)	0	0	6 (18%)	0	0	2 (6%)	1 (3%)	0
Aspartate aminotransferase increased	3 (10%)	0	0	2 (6%)	2 (6%)	0	3 (9%)	1 (3%)	0
Back pain	3 (10%)	0	0	6 (18%)	1 (3%)	0	3 (9%)	0	0
Breast pain	3 (10%)	0	0	0	1 (3%)	0	0	0	0
Depression	3 (10%)	0	0	5 (15%)	0	0	0	1 (3%)	0
Dyspnoea	2 (7%)	1 (3%)	0	7 (21%)	1 (3%)	0	5 (14%)	1 (3%)	0
Hypokalaemia	3 (10%)	0	0	3 (9%)	1 (3%)	0	3 (9%)	1 (3%)	0
Cellulitis	0	2 (7%)	0	0	0	0	0	0	0
Cough	2 (7%)	0	0	7 (21%)	0	0	7 (20%)	0	0
Hypertension	1 (3%)	1 (3%)	0	1 (3%)	0	0	0	1 (3%)	0
Musculoskeletal chest pain	1 (3%)	1 (3%)	0	2 (6%)	0	0	3 (9%)	0	0
Pain in extremity	1 (3%)	1 (3%)	0	3 (9%)	1 (3%)	0	3 (9%)	0	0
Abdominal pain	1 (3%)	0	0	2 (6%)	1 (3%)	0	1 (3%)	1 (3%)	0
Abdominal pain upper	1 (3%)	0	0	3 (9%)	0	0	2 (6%)	1 (3%)	0
Acute myocardial infarction	0	1 (3%)	0	0	0	0	0	0	0
Ascites	0	1 (3%)	0	0	0	0	0	0	0
Chronic obstructive pulmonary disease	1 (3%)	0	0	0	1 (3%)	0	0	0	0
Diabetic ketoacidosis	0	1 (3%)	0	0	0	0	0	0	0
Enteritis	0	1 (3%)	0	0	0	0	0	0	0
Faecaloma	0	1 (3%)	0	0	0	0	0	0	0
Febrile neutropenia	0	1 (3%)	0	0	1 (3%)	0	0	0	0
Granulocytopenia	0	1 (3%)	0	0	0	0	0	0	0
Hyperglycaemic hyperosmolar nonketotic syndrome	0	0	1 (3%)	0	0	0	0	0	0
Hypocalcaemia	0	1 (3%)	0	0	1 (3%)	0	0	0	0
Hyponatremia	0	1 (3%)	0	0	1 (3%)	1 (3%)	1 (3%)	1 (3%)	0
Hypotension	0	1 (3%)	0	0	0	0	2 (6%)	0	0
Lymphopenia¶	0	1 (3%)	0	0	1 (3%)	0	1 (3%)	0	0
Mental status change	0	1 (3%)	0	0	0	0	1 (3%)	0	0
Nasal congestion	1 (3%)	0	0	1 (3%)	1 (3%)	0	4 (11%)	0	0
Pneumonia	0	1 (3%)	0	2 (6%)	0	0	0	0	0
Pulmonary embolism	1 (3%)	0	0	0	1 (3%)	1 (3%)	0	0	0

(Table 3 continues on next page)

	Chemotherapy only group (group 1; n=30)			Trilaciclib plus chemotherapy (day 1 and 8) group (group 2; n=33)			Trilaciclib (day 1 and 8), trilaciclib plus chemotherapy (day 2 and 9) group (group 3; n=35)		
	Grade 1 and 2	Grade 3	Grade 4	Grade 1 and 2	Grade 3	Grade 4	Grade 1 and 2	Grade 3	Grade 4
(Continued from previous page)									
Septic shock	0	0	1 (3%)	0	0	0	0	0	0
Varices oesophageal	0	1 (3%)	0	0	0	0	0	0	0
Acute respiratory failure	0	0	0	0	0	1 (3%)	0	0	0
Bacteraemia	0	0	0	0	1 (3%)	0	0	0	0
Cardiac arrest	0	0	0	0	0	0	0	0	1 (3%)
Embolism	0	0	0	0	1 (3%)	0	0	0	0
Flank pain	0	0	0	1 (3%)	1 (3%)	0	0	1 (3%)	0
Haemoglobin decreased	0	0	0	1 (3%)	1 (3%)	0	0	0	0
Hypercalcaemia	0	0	0	0	0	0	0	1 (3%)	0
Hypophosphatemia	0	0	0	2 (6%)	1 (3%)	0	0	3 (9%)	0
Lumbar vertebral fracture	0	0	0	0	1 (3%)	0	0	0	0
Myositis	0	0	0	0	1 (3%)	0	0	0	0
Non-cardiac chest pain	0	0	0	1 (3%)	0	0	1 (3%)	1 (3%)	0
Normochromic normocytic anaemia	0	0	0	0	1 (3%)	0	0	0	0
Oesophageal stenosis	0	0	0	0	1 (3%)	0	0	0	0
Pericardial effusion	0	0	0	0	1 (3%)	0	0	0	0
Pleural effusion	0	0	0	2 (6%)	1 (3%)	0	0	0	0
Pleuritic pain	0	0	0	1 (3%)	0	0	0	1 (3%)	0
Subdural haematoma	0	0	0	0	0	0	0	1 (3%)	0
Suicidal ideation	0	0	0	0	0	0	0	1 (3%)	0
Supraventricular tachycardia	0	0	0	0	1 (3%)	0	1 (3%)	0	0
Syncope	0	0	0	0	1 (3%)	0	0	0	0

Data are n (%) and treatment-emergent adverse events are ordered from highest frequency to lowest frequency in group 1. Safety analysis population, based on data cut-off of May 17, 2019; treatment-emergent adverse events (all grades) reported in 20% or more of patients overall and all grade 3 and 4 treatment-emergent adverse events are included; one death due to a treatment-emergent adverse event was reported in group 1 (right ventricular failure). *Includes anaemia, anaemia macrocytic, decreased red blood cell count, and decreased haemoglobin concentration. †Includes neutropenia and decreased neutrophil count. ‡Includes thrombocytopenia and decreased platelet count. §Includes leucopenia and decreased white blood cell count. ¶Includes lymphopenia and decreased lymphocyte count.

Table 3: **Summary of treatment-emergent adverse events**

and neutropenia (23 [66%] of 35), thrombocytopenia (22 [63%]), and nausea (17 [49%]) in group 3 (table 3; appendix pp 8–10). Febrile neutropenia occurred in one patient in group 1 and one patient in group 2 (table 3). Serious treatment-emergent adverse events were reported in ten (33%) patients in group 1 and 11 (33%) in group 2, and four (11%) in group 3. All serious treatment-emergent adverse events occurred in two or fewer patients (appendix pp 11–13). No serious treatment-emergent adverse events or treatment-emergent adverse events leading to permanent discontinuation were deemed to be related to trilaciclib. There were 45 deaths; 20 in group 1 (disease progression [n=17], treatment-emergent adverse event [n=1; right ventricular failure deemed unrelated to treatment], other [n=2]), 11 in group 2 (disease progression [n=9], other [n=2]), and 14 in group 3 (disease progression [n=13], other [n=1]). Similar numbers of patients in each group reported a treatment-emergent adverse event that led to discontinuation of any study drug: ten (33%) in group 1, 14 (42%) in group 2, and 11 (31%) in group 3. Most treatment-emergent adverse events leading to discontinuation occurred in two or fewer patients, with the exception of thrombocytopenia, which resulted in the discontinuation of any study drug in eight (27%) patients in group 1, five (15%) in group 2, and nine (26%) in group 3, and neutropenia, which led to discontinuation in two (7%) patients in group 1, nine (27%) in group 2, and four (11%) in group 3.

Among patients evaluable for response, the proportion who achieved an objective response was eight (33%) of 24 in group 1 versus 15 (50%) of 30 in group 2 and 11 (37%) of 30 in group 3 (table 4). Progression-free survival is shown in table 4 and figure 2A. Patients enrolled in both trilaciclib groups had significantly longer overall survival than those enrolled in group 1 (table 4; figure 2B). The results of pooled subgroup analyses showed that the observed progression-free survival and overall survival benefits were consistent across subgroups (figure 3).

	Chemotherapy only group (group 1; n=34)	Trilaciclib plus chemotherapy (day 1 and 8) group (group 2; n=33)	Trilaciclib (day 1 and 8), trilaciclib plus chemotherapy (day 2 and 9) group (group 3; n=35)	Combined trilaciclib groups (n=68)
Best overall response				
Response evaluable population	24	30	30	60
Complete response	1 (4%)	0	0	0
Partial response	7 (29%)	15 (50%)	11 (37%)	26 (43%)
Stable disease	10 (42%)	9 (30%)	15 (50%)	24 (40%)
Progressive disease	6 (25%)	5 (17%)	3 (10%)	8 (13%)
Not evaluable*	0	1 (3%)	1 (3%)	2 (3%)
Clinical benefit for 24 weeks	9 (38%)	17 (57%)	13 (43%)	30 (50%)
Not evaluable†	5 (21%)	7 (23%)	11 (37%)	18 (30%)
Proportion of patients with an objective response (95% CI)	8 (33%, 15·6–55·3)	15 (50%, 31·3–68·7)	11 (37%, 19·9–56·1)	26 (43%, 30·6–56·8)
p value‡	..	0·23	0·68	0·37
Progression-free survival				
Patients with events	18 (53%)	19 (58%)	18 (51%)	37 (54%)
Median progression-free survival, months (95% CI)	5·7 (3·4–9·2)	9·4 (6·1–13·0)	7·3 (6·2–12·9)	8·8 (6·4–10·9)
Hazard ratio (95% CI)	..	0·60 (0·30–1·18)	0·59 (0·30–1·16)	0·59 (0·33–1·05)
p value§	..	0·13	0·12	0·063
Overall survival				
Number who died	20 (59%)	11 (33%)	14 (40%)	25 (37%)
Median overall survival, months (95% CI)	12·6 (6·3–15·6)	20·1 (10·2–not reached)	17·8 (12·9–not reached)	20·1 (15·3–not reached)
Hazard ratio (95% CI)	..	0·33 (0·15–0·74)	0·34 (0·16–0·70)	0·36 (0·19–0·67)
p value§	..	0·028	0·0023	0·0015
Median duration of follow-up, months (IQR)	8·4 (3·8–13·6)	12·7 (5·5–17·4)	12·9 (6·7–16·8)	12·7 (6·4–17·1)

Data are median (IQR), n (%), or point estimate with 95% CI in parentheses. Based on data as of May 17, 2019. *Includes patients who withdrew consent or did not have a tumour assessment during the study. †If a patient did not have a complete or partial response and duration stable disease was indeterminate, they were considered not evaluable. ‡The two-sided p-value was calculated using stratified exact Cochran-Mantel-Haenszel method to account for the stratification factors. §Two-sided p value was calculated using the stratified log-rank test to account for stratification factors.

Table 4: **Antitumour activity results**

A prespecified assessment of objective response, progression-free survival, and overall survival across and within groups 1, 2, and 3 for tumours categorised as CDK4/6 independent, dependent, or indeterminate did not reveal any results favouring one tumour subtype over another (appendix p 16).

Although the addition of trilaciclib to gemcitabine and carboplatin did not preserve lymphocyte counts or enhance T-cell activation (appendix p 4–5), in a prespecified analysis we saw a higher frequency of CD8+ T cells producing IFN-γ after ex-vivo stimulation in patients treated with gemcitabine and carboplatin plus trilaciclib than in patients treated with chemotherapy alone (ie, group 1; appendix p 6).

Results from a post-hoc analysis of antitumour effects according to median number of cycles (1–7 *vs* >7 cycles) are provided in the appendix (pp 14–15).

Discussion

The addition of trilaciclib did not result in a significant improvement in the prespecified primary endpoints of duration and occurrence of severe neutropenia. However, we saw a clinically meaningful improvement in overall survival with trilaciclib (both dosing schedules) compared with gemcitabine and carboplatin alone. The median overall survival for gemcitabine and carboplatin alone is consistent with published literature for patients with metastatic triple-negative breast cancer treated in a similar setting.[5] In a phase 3 study of iniparib plus gemcitabine and carboplatin versus gemcitabine and carboplatin alone in patients who had received zero, one, or two previous chemotherapy regimens for metastatic disease, median overall survival among 258 patients treated with gemcitabine and carboplatin alone was 11·1 months.[5] Similarly, in a study of combination chemotherapy for the first-line treatment of patients with metastatic triple-negative breast cancer, median overall survival was 12·1 months with gemcitabine and carboplatin.[19]

Transient trilaciclib-induced cell cycle arrest has been shown to provide resistance to chemotherapy-induced cell damage by preventing haemopoietic stem and progenitor cells from proliferating in the presence of cytotoxic chemotherapy and favourably altering the tumour microenvironment when combined with

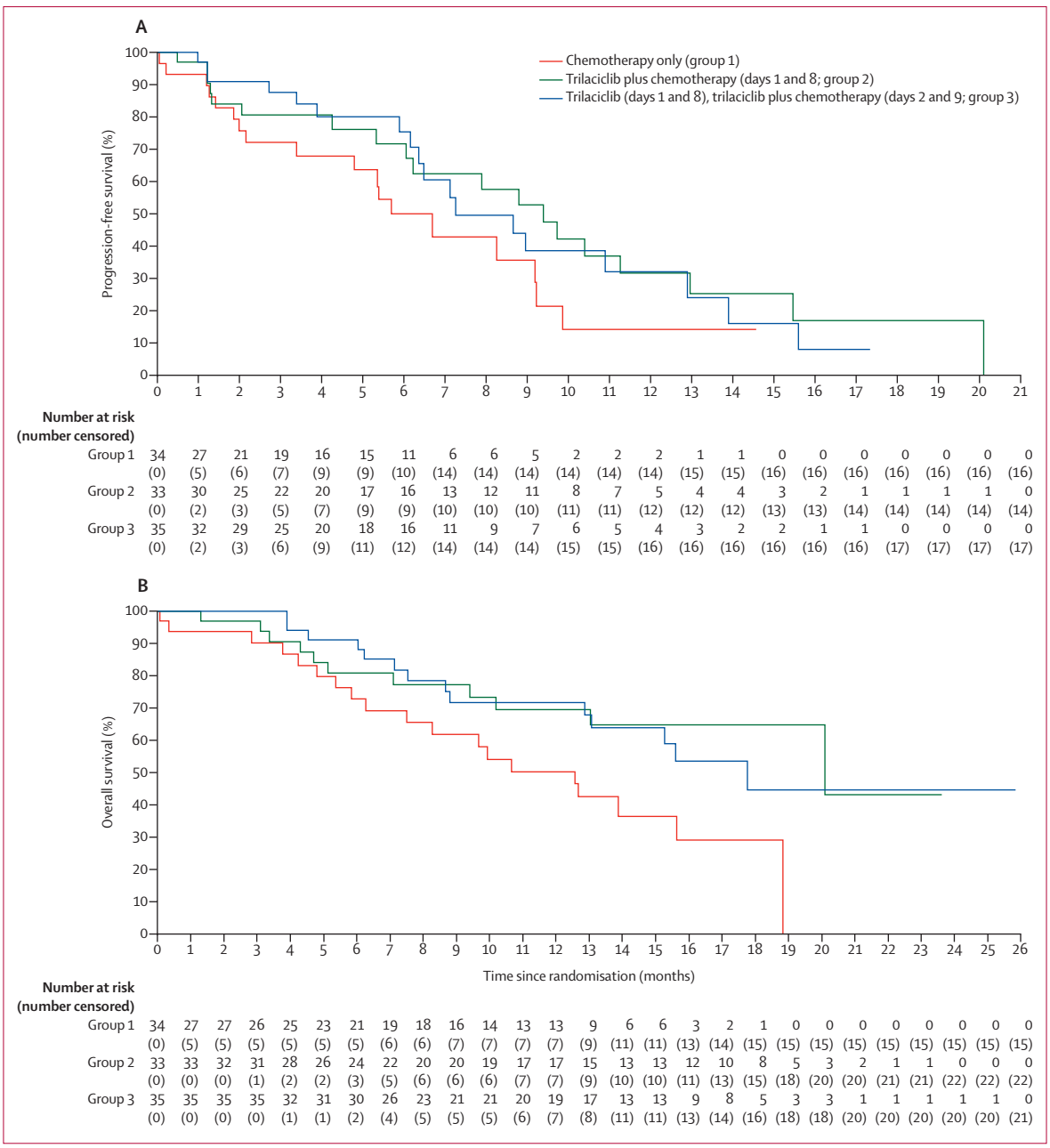

Figure 2: **Kaplan-Meier curves for progression-free survival (A) and overall survival (B)**

chemotherapy.[20] Emerging clinical data from the present study, combined with results from the assessment of trilaciclib in patients with small-cell lung cancer,[20] suggest that the consequences of CDK4/6 inhibition by trilaciclib might depend on the type of chemotherapy, the schedule of chemotherapy, and tumour type. In some clinical situations, the benefits of trilaciclib might manifest as either antitumour activity, decreased chemotherapy-induced myelosuppression, or both of these effects.

Non-clinical and clinical data[10,13,20] suggest that trilaciclib antitumour activity benefit is an immune-mediated event, during which both the type of chemotherapy and tumour are likely to be relevant (ie, chemotherapy that induces immunogenic cell death, and tumours with a micro-environment more favourable to immune modulation would be predicted to support trilaciclib antitumour activity). For example, although small-cell lung cancer is characterised by a high degree of genomic instability and smoking-associated mutational profile, small-cell lung cancer tumours have reduced levels of both major histocompatibility complex class I and class II, a known method of escaping antitumour immunity.[21] Therefore,

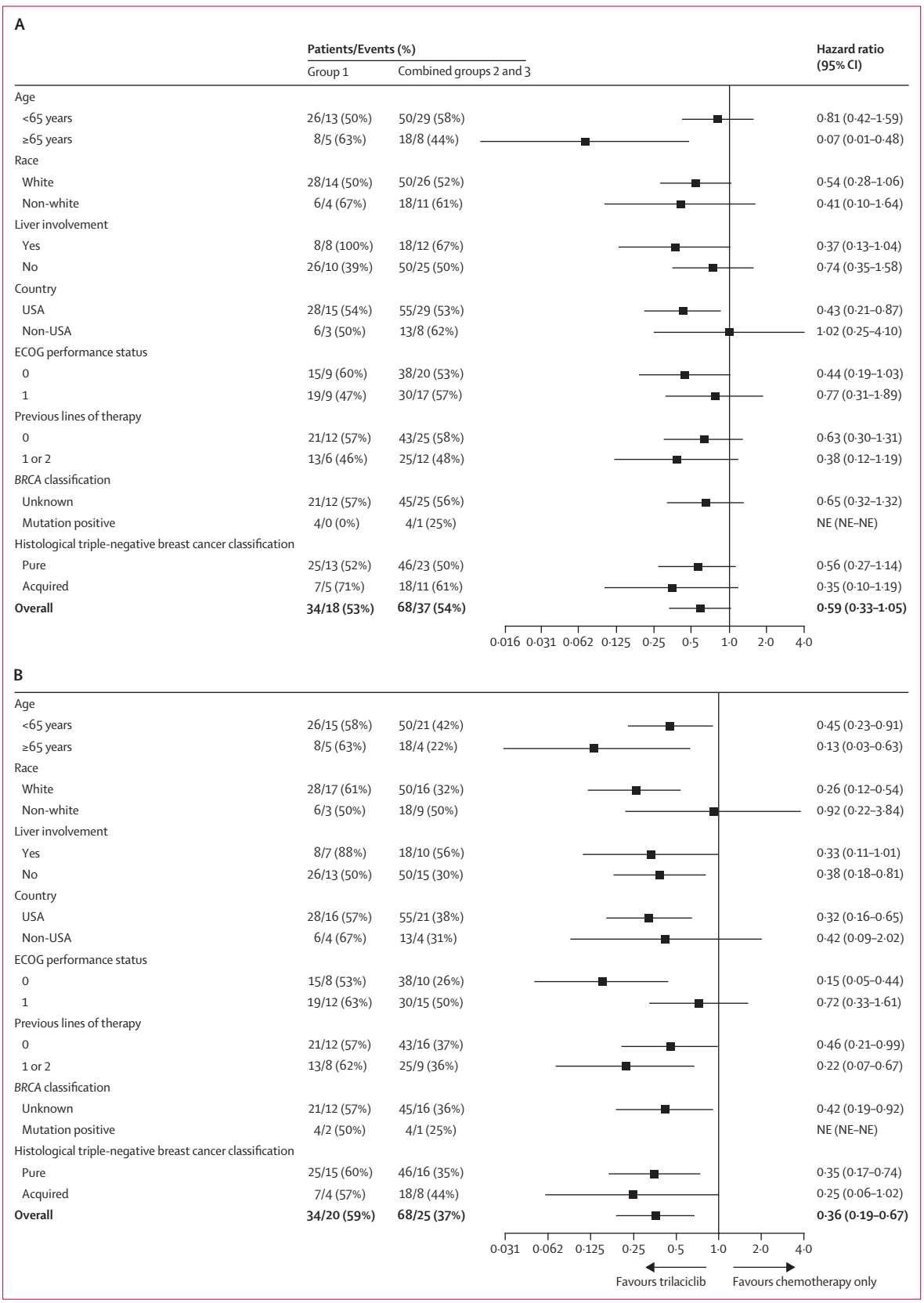

Figure 3: **Forest plots for progression-free survival (A) and overall survival (B)**
Data are from the intention-to-treat population, and data from trilaciclib groups 2 and 3 were pooled for prespecified subgroup analysis. Acquired triple-negative breast cancer refers to a patient with any previous biopsy showing oestrogen and progesterone receptor or HER2 positivity. ECOG=Eastern Cooperative Oncology Group. NE=not estimable.

trilaciclib benefits appear to manifest more robustly as a reduction in chemotherapy-induced myelosuppression than improved antitumour activity. By contrast, triple-negative breast cancer is considered genomically unstable and the tumour microenvironment is moderately immunogenic;[22] a state that could be potentially enhanced by trilaciclib in combination with chemotherapy, leading to improved antitumour activity.

Trilaciclib enhances immune activation and promotes antitumour immunity by differentially arresting cytotoxic and regulatory T-cell subsets followed by a faster recovery of cytotoxic T lymphocytes than regulatory T cells in tumours.[10] This differential alteration of cell cycle kinetics between cytotoxic T lymphocytes and regulatory T cells results in a higher proportion of cytotoxic T lymphocytes than regulatory T cells, enhancement of T-cell activation, and a decrease in regulatory T cell-mediated immunosuppressive functions.[23–26] Together, these events promote the cytotoxic T lymphocyte-mediated clearance of tumour cells. Therefore, the antitumour effects of trilaciclib might result from the transient proliferative arrest of T cells (protecting them from chemotherapy-induced damage), followed by activation of cytotoxic T lymphocytes in the context of fewer regulatory T cells.

In addition to the immune-activating effects of transient CDK4/6 inhibition, retinoblastoma-independent effects have also been observed. CDK4/6 inhibition in preclinical models of bladder cancer resulted in enhanced antitumour activity of cisplatin through inhibition of FOXM1 phosphorylation and activation, independent of *RB1* gene status.[27] FOXM1, which is consistently overexpressed in metastatic triple-negative breast cancer tissue,[28] mediates proliferation, survival, migration and invasion, progression, and tumourgenesis of breast cancer cells.[29] Therefore, whether trilaciclib decreased the activity of FOXM1 in this trial, leading to increased sensitivity to gemcitabine and carboplatin chemotherapy, should be further investigated.

Although triple-negative breast cancer is predominantly a functionally CDK4/6-independent disease a subset of patients with tumours that were CDK4/6 dependent could not be excluded. Based on observations from a preclinical study, in which palbociclib was given in combination with carboplatin in a retinoblastoma-competent murine model,[30] inducing G1 arrest could theoretically reduce the proliferation of tumour cells and negatively affect the activity of chemotherapy in CDK4/6-dependent tumours. However, preclinical studies of trilaciclib given concurrently with a variety of chemotherapy drugs and in multiple CDK4/6 dependent murine models,[31] together with clinical data from the present study using established signatures of CDK4/6 dependence, do not suggest that trilaciclib negatively affects the antitumour activity of chemotherapy.

Overall, addition of trilaciclib to gemcitabine and carboplatin was well tolerated. The most frequently reported treatment-emergent adverse events that occurred more frequently in the trilaciclib groups were grade 1 and 2 nausea, headache, dyspnoea, and cough. Of these, only headache has previously been associated with trilaciclib;[20] however, events to date have been mild-to-moderate in severity and self-limited. Fewer patients had anaemia and fewer needed red blood cell transfusions on and after week 5, a procedure that adds to the burden and risk of patients treated with chemotherapy,[32] in the groups with trilaciclib than among those in the gemcitabine and carboplatin only group.

Limitations of this study include the small sample size and open-label nature of the study design. Furthermore, antitumour activity outcomes were not the primary study endpoints and the overall survival data are not yet mature (final analysis of overall survival will take place once 70% of events have occurred). Use of a doublet chemotherapy backbone might also restrict extrapolation to the metastatic triple-negative breast cancer population receiving single-agent chemotherapy. Finally, the hypothesised immune effects of trilaciclib are not yet fully understood and will require further study in additional clinical trials.

In summary, the results of this randomised phase 2 trial did not show an improvement in myelosuppression endpoints with the addition of trilaciclib to gemcitabine and carboplatin in patients with metastatic triple-negative breast cancer. However, an improvement in antitumour activity was observed with both trilaciclib administration schedules (across multiple endpoints) and was consistent across patient subgroups; no increase in toxicity was observed with the addition of trilaciclib. Together with the safety profile reported, the clinically meaningful improvements in overall survival support further studies of trilaciclib in patients with metastatic triple-negative breast cancer.

Contributors

ARTa, JO, SRM, JMA, and ZY contributed to the study design. ARTa, GSW, ARTh, MAD, LP, TJP, HSH, ŽV, NV, LM, DAR, STW, DM, and JO contributed to the collection and assembly of data. SRM, ZY, and JMA were responsible for data analysis. All authors contributed to the interpretation of data, preparation and writing of the manuscript, and approved the final manuscript for submission.

Declaration of interests

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Data sharing

All data requests should be submitted to the corresponding author for consideration. After publication, access to anonymised data that underlie the results reported in this Article (text, tables, figures, and appendices) might be granted following review.

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References

1 Dent R, Trudeau M, Pritchard KI, et al. Triple-negative breast cancer: clinical features and patterns of recurrence. *Clin Cancer Res* 2007; **13:** 4429–34.

2 Cheang MC, Voduc D, Bajdik C, et al. Basal-like breast cancer defined by five biomarkers has superior prognostic value than triple-negative phenotype. *Clin Cancer Res* 2008; **14:** 1368–76.

3 Foulkes WD, Smith IE, Reis-Filho JS. Triple-negative breast cancer. *N Engl J Med* 2010; **363:** 1938–48.

4 Twelves C, Jove M, Gombos A, Awada A. Cytotoxic chemotherapy: still the mainstay of clinical practice for all subtypes metastatic breast cancer. *Crit Rev Oncol Hematol* 2016; **100:** 74–87.

5 O'Shaughnessy J, Schwartzberg L, Danso MA, et al. Phase III study of iniparib plus gemcitabine and carboplatin versus gemcitabine and carboplatin in patients with metastatic triple-negative breast cancer. *J Clin Oncol* 2014; **32:** 3840–47.

6 Lyman GH. Chemotherapy dose intensity and quality cancer care. *Oncology (Williston Park)* 2006; **20** (suppl 9)**:** 16–25.

7 Smith RE. Trends in recommendations for myelosuppressive chemotherapy for the treatment of solid tumors. *J Natl Compr Canc Netw* 2006; **4:** 649–58.

8 Bisi JE, Sorrentino JA, Roberts PJ, Tavares FX, Strum JC. Preclinical characterization of G1T28: a novel CDK4/6 inhibitor for reduction of chemotherapy-induced myelosuppression. *Mol Cancer Ther* 2016; **15:** 783–93.

9 He S, Roberts PJ, Sorrentino JA, et al. Transient CDK4/6 inhibition protects hematopoietic stem cells from chemotherapy-induced exhaustion. *Sci Transl Med* 2017; **9:** eaal3986.

10 Lai AY, Sorrentino JA, Strum JC, Roberts PJ. Transient exposure to trilaciclib, a CDK4/6 inhibitor, modulates gene expression in tumor immune infiltrates and promotes a pro-inflammatory tumor microenvironment. American Association for Cancer Research Annual Meeting 2018; Chicago, IL; April 14–18, 2018 (abstr 1752). https://www.g1therapeutics.com/file.cfm/34/docs/tr-G1_AACR2018_Lai.pdf (accessed Sept 5, 2019).

11 Malumbres M, Sotillo R, Santamaría D, et al. Mammalian cells cycle without the D-type cyclin-dependent kinases CDK4 and CDK6. *Cell* 2004; **118:** 493–504.

12 Ramsey MR, Krishnamurthy J, Pei XH, et al. Expression of p16Ink4a compensates for p18Ink4c loss in cyclin-dependent kinase 4/6-dependent tumors and tissues. *Cancer Res* 2007; **67:** 4732–41.

13 Sorrentino JA, Lai AY, Strum JC, Roberts PJ. Trilaciclib (G1T28), a CDK4/6 inhibitor, enhances the efficacy of combination chemotherapy and immune checkpoint inhibitor treatment in preclinical models. American Association for Cancer Research Annual Meeting 2017; Washington, DC; April 1–5, 2017 (abstr 5628). https://www.g1therapeutics.com/file.cfm/34/docs/tr-G1_AACR2017_Sorrentino.pdf (accessed Sept 5, 2019).

14 Prat A, Bianchini G, Thomas M, et al. Research-based PAM50 subtype predictor identifies higher responses and improved survival outcomes in HER2-positive breast cancer in the NOAH study. *Clin Cancer Res* 2014; **20:** 511–21.

15 Lehmann BD, Jovanović B, Chen X, et al. Refinement of triple-negative breast cancer molecular subtypes: implications for neoadjuvant chemotherapy selection. *PLoS One* 2016; **11:** e0157368.

16 Asghar US, Barr AR, Cutts R, et al. Single-cell dynamics determines response to CDK4/6 inhibition in triple-negative breast cancer. *Clin Cancer Res* 2017; **23:** 5561–72.

17 Zou G. A modified poisson regression approach to prospective studies with binary data. *Am J Epidemiol* 2004; **159:** 702–06.

18 Dmitrienko A, Tamhane AC. Mixtures of multiple testing procedures for gatekeeping applications in clinical trials. *Stat Med* 2011; **30:** 1473–88.

19 Yardley DA, Coleman R, Conte P, et al. nab-Paclitaxel plus carboplatin or gemcitabine versus gemcitabine plus carboplatin as first-line treatment of patients with triple-negative metastatic breast cancer: results from the tnAcity trial. *Ann Oncol* 2018; **29:** 1763–70.

20 Weiss JM, Csoszi T, Maglakelidze M, et al. Myelopreservation with the CDK4/6 inhibitor trilaciclib in patients with small cell lung cancer receiving 1st-line chemotherapy: a phase 1b/randomized phase 2 trial. *Ann Oncol* 2019; published online Aug 27. DOI:10.1093/annonc/mdz278.

21 Semenova EA, Nagel R, Berns A. Origins, genetic landscape, and emerging therapies of small cell lung cancer. *Genes Dev* 2015; **29:** 1447–62.

22 Park JH, Ahn JH, Kim SB. How shall we treat early triple-negative breast cancer (TNBC): from the current standard to upcoming immuno-molecular strategies. *ESMO Open* 2018; **3** (suppl 1)**:** e000357.

23 Chaikovsky AC, Sage J. Beyond the cell cycle: enhancing the immune surveillance of tumors via CDK4/6 inhibition. *Mol Cancer Res* 2018; **16:** 1454–57.

24 Deng J, Wang ES, Jenkins RW, et al. CDK4/6 inhibition augments antitumor immunity by enhancing T-cell activation. *Cancer Discov* 2018; **8:** 216–33.

25 Goel S, DeCristo MJ, Watt AC, et al. CDK4/6 inhibition triggers anti-tumour immunity. *Nature* 2017; **548:** 471–75.

26 Schaer DA, Beckmann RP, Dempsey JA, et al. The CDK4/6 inhibitor abemaciclib induces a T cell inflamed tumor microenvironment and enhances the efficacy of PD-L1 checkpoint blockade. *Cell Rep* 2018; **22:** 2978–94.

27 Rubio C, Martínez-Fernández M, Segovia C, et al. CDK4/6 inhibitor as a novel therapeutic approach for advanced bladder cancer independently of *RB1* status. *Clin Cancer Res* 2019; **25:** 390–402.

28 Craig DW, O'Shaughnessy JA, Kiefer JA, et al. Genome and transcriptome sequencing in prospective metastatic triple-negative breast cancer uncovers therapeutic vulnerabilities. *Mol Cancer Ther* 2013; **12:** 104–16.

29 Hamurcu Z, Ashour A, Kahraman N, Ozpolat B. FOXM1 regulates expression of eukaryotic elongation factor 2 kinase and promotes proliferation, invasion and tumorgenesis of human triple negative breast cancer cells. *Oncotarget* 2016; **7:** 16619–35.

30 Roberts PJ, Bisi JE, Strum JC, et al. Multiple roles of cyclin-dependent kinase 4/6 inhibitors in cancer therapy. *J Natl Cancer Inst* 2012; **104:** 476–87.

31 Sorrentino JA, Bisi JE, Thompson D, et al. Trilaciclib, a CDK4/6 inhibitor, does not impair the efficacy of chemotherapy in CDK4/6-dependent tumor models. 30th European Organisation for Research and Treatment of Cancer, National Cancer Institute, and American Association for Cancer Research Symposium; Dublin, Ireland; Nov 13–16 2018 (abstr 377). https://www.g1therapeutics.com/file.cfm/34/docs/tr-G1_ENA2018_Sorrentino.pdf (accessed Sept 18, 2019).

32 Corey-Lisle PK, Desrosiers MP, Collins H, et al. Transfusions and patient burden in chemotherapy-induced anaemia in France. *Ther Adv Med Oncol* 2014; **6:** 146–53.