neoMono

Title Page

Protocol Title:

An adaptive randomized neoadjuvant two arm trial in triple-negative breast cancer comparing a mono Atezolizumab window followed by a Atezolizumab - CTX therapy with Atezolizumab – CTX therapy (neoMono)

Protocol Number:	Phaon 1
Protocol Version:	7.0
EudraCT Number:	2020-001651-40
Short Title:	neoMono

Testing Objective:

Pathological complete response in TNBC neoadjuvant treatment with PD-L1 inhibitor (Atezolizumab) monotherapy window of two weeks followed by Atezolizumab in combination with CTX versus neoadjuvant CTX with Atezolizumab.

Sponsor Name and Legal Registered Address:

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NCT04770272

neoMono

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2023-10-26

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Data Safety and Monitoring Board (DSMB)

The DSMB includes the following 5 experts in, or representatives of the fields of:

Gynecological Oncology:

[redacted]

Gynecological Oncology:

[redacted]

Hematology / Medical Oncology / Clinical Immunology:

[redacted]

Drug Safety:

[redacted]

Biostatistics:

[redacted]

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1. Synopsis

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Institution	Phaon scientific GmbH Taunusstraße 5a 65183 Wiesbaden Germany
Sponsor	Palleos healthcare GmbH Taunusstraße 5a 65183 Wiesbaden Germany
Full Study Title	An adaptive randomized neoadjuvant two arm trial in triple-negative breast cancer comparing a mono Atezolizumab window followed by a Atezolizumab-CTX therapy with Atezolizumab-CTX therapy (neoMono)
Study Type	Clinical Trial
Study Short Title	neoMono
Study ID	Phaon1
Trial registration	Clinicaltrials.gov, clinicaltrialsregister.eu
Study design	Randomized, open-label, adaptive, two arm, multicenter, Phase II trial
Clinical Phase	Phase II
Background and Rationale	*Triple negative breast cancer (TNBC)* Breast cancer is a heterogeneous disease consisting of several disease subtypes that differ with regard to molecular phenotype, treatability and prognosis (Sørlie et al., 2001). As one of these subtypes, TNBC is defined by both absence of immunostaining for estrogen receptor (ER), progesterone receptor (PR), and lack of overexpression/amplification of human epidermal growth factor receptor 2 (HER2/neu) (Dent et al., 2007). TNBC accounts for approximately 15% - 20% of breast cancer cases (Bauer et al., 2007) and is associated with younger age at diagnosis, premenopausal status, more advanced disease stage, higher grade, higher mitotic indices, family history of breast cancer, germline mutations in breast cancer (BRCA), and more aggressive clinical course than other breast cancer subtypes (Bauer et al., 2007). Improvement of systemic treatment of TNBC represents an unmet medical need. Whereas former clinical studies defined the complete lack of ER, PR and HER2/neu as triple negative, current recommendations such as the German AGO recommendations are considering ER/PR low tumors (i.e. < 10% positive cells on Immunohistochemistry (IHC)) as belonging to the same subgroup (Ditsch et al., 2019). The ER low-positive group is characterized molecularly by having features of triple-negative cancer in the majority of cases (Iwamoto et al., 2012). This includes basal-like phenotype, high incidence of germline BRCA mutation, and high-risk score by OncotypeDX. Also, distant-disease-free survival is similar to TNBC in these cases. Therefore, a low threshold of 1% for ER positivity, may lead to the false

exclusion of biologically ER negative tumors from correctly targeted strategies and instead to the categorization of these tumors as ER positive with consecutive treatment recommendations (Deyarmin et al., 2013; Dixon et al., 2019; Sanford et al., 2015; Yi et al., 2014). The effect of endocrine therapy on survival outcomes in these patients remains unclear (Raghav et al., 2012). These data are justifying the inclusion of ER/PR low patients in trials on TNBC. However, after neoadjuvant therapy endocrine therapy has to be discussed as an option.

Choice of Chemotherapy (CTX) in TNBC

Currently, poly-CTX is the only systemic treatment option for patients with early TNBC. Fortunately, patients with TNBC carry an increased chance of pathological complete remission (pCR) compared to patients with non-TNBC (Cortazar et al., 2014; Houssami et al., 2012) and in case of pCR prognosis is excellent (Liedtke et al., 2008). However, prognosis is unfavorable compared to other breast cancer subtypes, in case of non-pCR.

There is an accumulating body of evidence suggesting that platinum salts should be added to Anthracycline/Taxane CTX in case of TNBC. In addition to historical data suggesting that platinum-containing CTX may be particularly beneficial for patients with TNBC (O. Gluz et al., 2009) the GeparSixto trial (Untch et al., 2016) and the CALGB 40603 trial (Sikov et al., 2015) have provided prospective evidence supporting the use of platinum-salts among patients with TNBC. Consequently, an Anthracycline/Taxane/Carboplatin-containing poly-CTX regimen is regarded as standard-of-care (SOC) for neoadjuvant treatment of patients with TNBC in Germany and is recommended in the current AGO recommendations (Ditsch et al., 2019). Furthermore, current and recent clinical trials such as Keynote-522 and NeoTRIP (see below) include a platinum-containing poly-CTX regimen.

Immunotherapy

Recently, targeted therapy of regulatory immune pathways has become an important tool in clinical applications. In contrast to conventional chemotherapeutics, immune checkpoint inhibitors do not target the tumor cells themselves but molecules which are part of the T-cell regulatory cascade. The main focus lies on the removal of inhibitory mechanisms by which the tumor escapes from a T-cell response (Pardoll, 2012). Atezolizumab (Trade name: Tecentriq®) is a humanized antibody against programmed cell death 1 ligand 1 (PD-L1) that has shown tolerability and tumor responses in patients with advanced malignancies. PD1 is localized on T-cells, B-cells, tumor-infiltrating lymphocytes (TILs), dendritic cells (DCs), natural killer T-cells and activated monocytes. Its ligands PD-L1 and -L2 are expressed on a variety of cancer types, e.g. breast, ovary, lung, colon, melanoma, kidney and bladder (Alme et al., 2016). For some cancer types PD-L1 and -L2 expression could be associated with tumor progression and poor prognosis (Thompson et al., 2007). Recently combination therapies consisting of conventional agents and immune checkpoint inhibitors showed a high clinical relevance in cancer therapy (Sharma & Allison, 2015). Treatment of tumor tissues which lack an immunologic microenvironment with markers like CD8+ T-cells, CD4+ T-cells or PD-L1, could be improved by combination therapies. Conventional cancer therapies, e.g. CTX, radiation, surgery, anti-angiogenetic or hormonal, can induce tumor cell death, resulting in a release of antigens (Crittenden et al., 2015; Slovin et al., 2013).

Immunotherapy in TNBC

Among breast cancer subtypes, TNBC is a preferable target for immunotherapy. Hence, there is a large and increasing body of evidence for use of immunotherapy in TNBC (Marra et al., 2019). Consequently, and in particular based on the results of the IMpassion130 trial (Peter Schmid, 2018), Atezolizumab has been approved for PD-L1 (IHC) positive metastatic TNBC (mTNBC) in the first line of therapy in combination with nab-Paclitaxel.

In the neoadjuvant setting, data regarding the use of immunotherapy are heterogeneous. In the Keynote-522 trial, among 602 patients Pembrolizumab in combination with 4 cycles of

	Paclitaxel + Carboplatin followed by 4 cycles of Doxorubicin or Epirubicin + Cyclophosphamide showed a statistically significant improvement in pCR (ypT0/Tis, ypN0) vs. placebo in combination with CTX: 64.8% (95% CI, 59.9 - 69.5) vs. 51.2% (95% CI, 44.1 - 58.3), p = 0.00055 regardless of PD-L1 IC-status (P. Schmid et al., 2019). Another neoadjuvant trial in TNBC, the NeoTRIP study presented at the SABCS 2019, investigated Atezolizumab 1200 mg q3w in combination with 8 cycles of Carboplatin and nab-Paclitaxel versus the same neoadjuvant CTX alone. pCR rates between both groups did not differ significantly. After surgery patients received 4 cycles of Epirubicin and Cyclophosphamide, results of the 5 year follow-up are awaited in 2024 (Gianni et al., 2019). Compared to Keynote-522 trial the results of the NeoTRIP trial are implying the conclusion that neoadjuvant therapy schedules investigating checkpoint inhibitors in TNBC should include an Anthracycline. This is in line with previously published data pointing to a synergistic effect of Anthracyclines and checkpoint inhibitors (Matsushita & Kawaguchi, 2018). *Immunotherapy window before neoadjuvant combined immune- and poly-CTX* In the GeparNuevo trial a significant effect of the checkpoint inhibitor Durvalumab in combination with neoadjuvant CTX compared to CTX alone could only be demonstrated in a subgroup of patients (n = 117) treated with a pre-therapeutic (i.e. before initiation of poly-CTX) 2-week Durvalumab-monotherapy-window (Loibl et al., 2019). An increase in pCR in association with Durvalumab was seen only in patients treated with the pre-therapeutic durvalumab monotherapy (pCR 61.0% vs 41.4%, OR = 2.22, 95% CI 1.06 - 4.64, p = 0.035; interaction p = 0.048). Among patients without pre-therapeutic window (n = 57), pCR rates were 37.9 vs. 50.0%. A biological rationale for the window effect can be found in the systematic biomarker analysis of the GeparNuevo trial. Patients received a re-biopsy after the 2-week window in both arms. The change of intra-tumoral TILs (iTILs) between baseline and after the window-phase significantly predicted achieving a pCR with Durvalumab in univariate (OR = 5.15, 95% CI 1.10 – 24.05, p = 0.037) and multivariate regression analysis (OR = 9.36, 95% CI 1.26 – 69.65, p = 0.029). In the placebo Arm, the change of iTILs did not predict pCR (univariate analysis OR = 1.19, 95% CI 0.65 – 2.17, p = 0.581 and multivariate analysis OR = 1.22, 95% CI 0.65 – 2.27, p = 0.540). An increase of iTILs in post-window samples compared with pre-therapeutic samples was predictive of pCR specifically in the Durvalumab arm. Probably due to the small sample size the interaction test did not formally meet statistical significance (p = 0.085). However, these data support the hypothesis that checkpoint inhibitors induce a modulation of the immune-microenvironment by stimulating lymphocytes to migrate from the stroma into the tumor-cell nests before introduction of cytotoxic CTX. This increased infiltration of immune cells into the tumor cell nests might be an indicator of a response to the checkpoint inhibitor. Promoting this initial immune response by addition of a poly-CTX could lead to an immunogenic cell death, possibly explaining the increased pCR rate in the window arm. Based on the results of the GeparNuevo trial (and particularly given the size of the effect) a single arm neoadjuvant trial combining poly-CTX with Pembrolizumab, the NeoImmunoBoost trial was amended for the inclusion of a 2-week immunotherapy window at the beginning of the therapy (ClinicalTrials.gov Identifier: NCT03289819). The investigation of the effect of a 2-week immunotherapy monotherapy window before combination immune- and poly-CTX in a randomized prospective clinical trial has the potential to define a new treatment standard. *Dosing of immunotherapy* In contrast to the approved dose in the metastatic setting of ongoing trials, in the neoadjuvant setting Atezolizumab is applied in a 3-weekly dose of 1200 mg in combination with carboplatin and paclitaxel (https://clinicaltrials.gov/ct2/show/NCT03281954;

https://clinicaltrials.gov/ct2/show/NCT03595592). Due to the investigational nature of the monotherapy window we chose the approved 2-weekly dose of 840 mg for the 2-week window phase and the approved 3-weekly dose of 1200 mg for the neoadjuvant phase.

Duration of immunotherapy

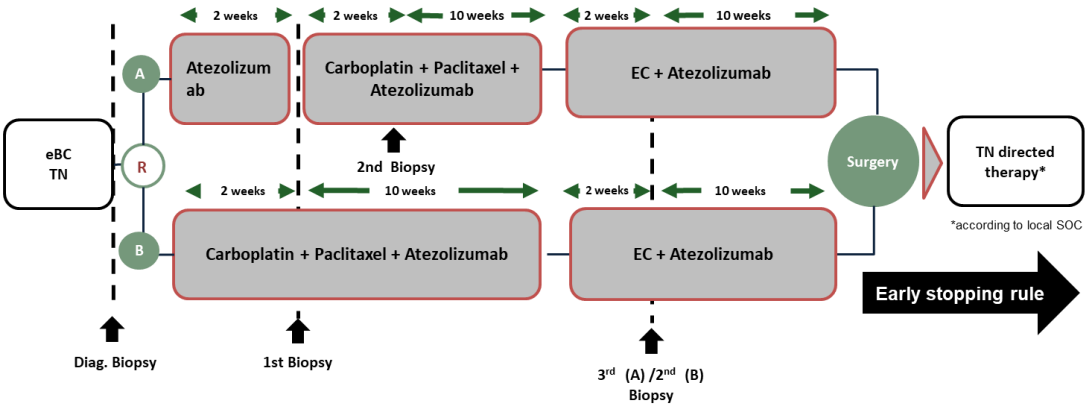
The question, if it is sufficient to add immunotherapy to the neoadjuvant phase of the treatment in TNBC or if an adjuvant application is necessary, is still unclear and study designs are heterogeneous. There are trials like the NeoTRIP trial (Gianni et al., 2019) and the NeoImmunoBoost trial (ClinicalTrials.gov Identifier: NCT03289819) adding the checkpoint inhibitor only to the neoadjuvant phase, whereas in Keynote-522 (P. Schmid et al., 2019) the immunotherapy agent was both added to the neoadjuvant phase continued as adjuvant treatment and after surgery. The results regarding the follow-up of this trial are still immature. A large adjuvant trial, IMpassion030 is investigating the effect of Atezolizumab in the adjuvant setting only (ClinicalTrials.gov Identifier: NCT03498716), however, results are not expected to be published before 2023. Whereas the neoadjuvant application of immunotherapy in combination with poly-CTX is already a new treatment paradigm, this is much less clear for the adjuvant use of these drugs. Therefore, it has to be considered adequate for current study designs in TNBC to include immunotherapy in the neoadjuvant treatment phase only. However, studies should anticipate a potential change regarding adjuvant immunotherapy and prepare to amend study protocols to incorporate adjuvant immunotherapy after robust results of the studies discussed above are being published.

Neoadjuvant therapy and window-of-opportunity trials in breast cancer

Neoadjuvant CTX trials include a population with therapy naïve tumor. Data from window-of-opportunity trials including repeat tumor biopsies allow the creation of prediction models for clinical response based on the results of early biopsies (Tan et al., 2018). The biomarkers used in these models have been tested in different tumor-biologies in the neoadjuvant setting. For instance, the IMPACT trial and the POETIC trial have demonstrated that short-term changes in proliferation by Ki-67 expression in the neoadjuvant setting may be able to predict outcome (Dowsett et al., 2005, 2011).
Furthermore, among patients treated as part of the triple negative subprotocol of the ADAPT Trial (NCT01815242), low-cellularity (< 500 vital tumor cells) at week 3 was strongly associated with response to therapy. Higher levels of TILs were associated with pCR, both at baseline and after 3 weeks of neoadjuvant CTX. Ki-67 expression after 3 weeks was potentially associated with pCR (Oleg Gluz et al., 2015; Liedtke et al., 2018).
Due to the fact that many patients had less than 500 tumor cells in the re-biopsy after 3 weeks, which made an evaluation impossible, a 2-week approach is preferred. Accordingly, the Combined score based on tumor cellularity and TILs (CelTIL)-Score (Nuciforo et al., 2017), integrates cellularity and TILs and thus avoids the problem of non-evaluable patients. Its predictive value regarding CTX response in a neoadjuvant therapy setting has been demonstrated.
Recently, it has also been demonstrated that TILs after 3 weeks are significantly associated with response to checkpoint inhibitors (Loi et al., 2019).
The inconsistent clinical data regarding neoadjuvant checkpoint inhibitor therapy with and without Anthracyclines (Gianni et al., 2019; P. Schmid et al., 2019) make it mandatory to investigate the biological effects described above not only of CTX in general in combination with Atezolizumab but also separately according to CTX regimen, e.g. Carboplatin and Paclitaxel versus Epirubicin and Cyclophosphamide in the setting of a sequential use.

Follow-up duration

TNBC is known to show early recurrence in case of disease relapse. For instance, in an analysis among 1,118 patients who received neoadjuvant CTX at M.D. Anderson Cancer Center for stage I-III breast cancer from 1985 to 2004, recurrence and death rates were higher for TNBC only in the first 3 years. In fact, hazard functions for disease recurrence

	among patients with TNBC compared with non-TNBC have been demonstrated to cross at 2.5 years of follow-up, demonstrating lower incidences of disease recurrence among patients with TNBC compared to other breast cancer subtypes thereafter (Liedtke et al., 2008). In a preplanned interim analysis performed after 100 pCR events, patients with TNBC did not show a significant benefit from an atezolizumab monotherapy window suggesting that the primary endpoint would not be reached. Based on these results and in accordance with the study stopping rules, the patient recruitment was permanently stopped in August 2022. However, patients in both treatment arms demonstrated the highest pCR rates ever reported in phase II/III trials with TNBC patients treated with neoadjuvant immune checkpoint inhibitors (Kolberg et al., 2023, compare with Loibl et al. 2019; Schmid et al., 2020; Foldi et al., 2022; Huober et al., 2022). Given that novel post-neoadjuvant treatment options (on and off-trial) have emerged such as pembrolizumab, the post-neoadjuvant treatment in the follow-up phase of this study will most certainly be highly heterogeneous. Thus, completion of follow-up to 3 years is no longer justified. Therefore, follow-up will be limited to 2 years and only clinically significant survival signals may be observed. **Study goal and medical need** The goal of this study is: a) Compare efficacy of neoadjuvant CTX with PD-L1-inhibition (Atezolizumab) and Atezolizumab 2-week window to CTX with PD-L1-inhibition (Atezolizumab) b) to identify biomarkers predicting (early) response to or resistance against Atezolizumab (alone and with CTX)) allowing patients stratification in future clinical trials.
Investigational Medicinal Product (IMP) / Intervention	Atezolizumab: 840 mg day 1 for 2 weeks in Arm A Atezolizumab mono-window, then 1200 mg day 1 every 3 weeks plus Carboplatin, Paclitaxel, Epirubicin and Cyclophosphamide in both, Arm A & B
Study Population	The study will include patients with primary, treatment naïve triple negative early breast cancer.
Study Design	 After primary local diagnosis the patients will undergo screening which includes a centralized confirmation of TNBC subtypes. Patients will be randomized to Arm A and B. Randomization will be stratified by PD-L1 IC-status and anatomic tumor stage (AJCC 8th edition Anatomic Stage Groups I, II and III). Patients in Arm A will be treated for a total of 2 weeks with an Atezolizumab mono-therapy of 840 mg day 1 for 2 weeks before undergoing a biopsy after the 2-week cycle has ended. They will then continue with a 12-week therapy with a combination of Paclitaxel 80 mg/m² IV weekly x 12 doses + Carboplatin AUC of 2 IV weekly x 12 doses + Atezolizumab 1200 mg day 1 every 3 weeks for 4 doses. Every 3-week-interval is considered 1 cycle, therefore

	Carboplatin/Paclitaxel/Atezolizumab therapy will be applied for four 3-week cycles (12 weeks total). This will be followed by Epirubicin 90 mg/m² + Cyclophosphamide 600 mg/ m² every 3 weeks for 4 cycles + Atezolizumab 1200 mg day 1 every 3 weeks for 4 doses. Patients in Arm B will be treated with a 12-week regimen of Paclitaxel 80 mg/m² IV weekly x 12 doses + Carboplatin AUC of 2 IV weekly x 12 doses + Atezolizumab 1200 mg Day 1 every 3 weeks for 4 doses without a mono-therapy window. Every 3-week-interval is considered 1 cycle, therefore Carboplatin/Paclitaxel/Atezolizumab therapy will be applied for four 3-week cycles (12 weeks total). This will be followed by Epirubicin 90 mg/m² + Cyclophosphamide 600 mg/m² every 3 weeks for 4 cycles + Atezolizumab 1200 mg day 1 every 3 weeks for 4 doses. Both groups, A and B will undergo a biopsy after 2 weeks of Carboplatin + Paclitaxel + Atezolizumab therapy. Furthermore, in both arms, for patients with a tumor size greater 10 mm in diameter, which have not achieved a 50% decrease in tumor volume (or if not assessable a decrease by 50% in diameter), another biopsy (the third in Arm A, the second in Arm B) will be performed after 2 weeks of Epirubicin + Cyclophosphamide + Atezolizumab therapy. Patients in both arms will undergo surgery after 29 - 30 weeks therapy in total for Arm A and 27 - 28 weeks therapy in total for Arm B (3 - 4 weeks after last dose of neoadjuvant therapy) and move on to a treatment by local SOC. Further considerations with regards to immunological treatment in the adjuvant setting will be considered when further results are published. Safety and toxicities under therapy will be supervised *via* regular DSMB meetings. After surgery, patients will be treated according to the local TNBC directed therapy SOC. Thereafter the patients will be followed-up until 24 months after baseline. It is planned to perform up to 4 efficacy interim analyses in blocks of 40 patients after 100, 140, 180 and 220 patients evaluable for the primary endpoint pCR, assuming an equal sample size in both arms. At each interim analysis, decision rules based on predictive probabilities (*PP*) of trial success will be evaluated by the sponsor to determine whether the trial is to continue with patient recruitment, or whether to stop early for futility or success respectively. The DSMB will provide an independent review of these decisions and the interim efficacy results.
Objective(s)	**Primary Objective:** 1. Compare efficacy in terms of pCR in TNBC with Atezolizumab 2-week monotherapy window followed by neoadjuvant CTX with Atezolizumab (Arm A) to neoadjuvant CTX with Atezolizumab (Arm B). **Secondary Objectives:** 1. Assess and compare safety of Atezolizumab monotherapy window of 2 weeks followed by neoadjuvant treatment with Atezolizumab in combination with CTX (Arm A) to neoadjuvant CTX with Atezolizumab (Arm B). 2. Assess and compare efficacy of Atezolizumab mono-therapy window of 2 weeks followed by neoadjuvant treatment with Atezolizumab in combination with CTX (Arm A) to neoadjuvant CTX with Atezolizumab (Arm B) in patients with an ER/PR expression of < 1% and an ER/PR expression of 1% to 10%. 3. Compare efficacy of Atezolizumab mono-therapy window of 2 weeks followed by neoadjuvant treatment with Atezolizumab in combination with CTX (Arm A) to neoadjuvant CTX with Atezolizumab (Arm B) per alternative pCR definitions. 4. Compare early biological response (2 weeks in both arms) of Atezolizumab mono-therapy window of 2 weeks followed by neoadjuvant treatment with Atezolizumab in combination with CTX (Arm A) to neoadjuvant CTX with Atezolizumab (Arm B), as

	measured by Complete Cell Cycle Arrest (CCCA), decrease of Ki-67 expression (≥ 30%), low cellularity and TILs (≥ 60%), or a combined early response. 5. Compare early biological response (2 weeks in Arm B vs. 4 weeks in Arm A) of Atezolizumab mono-therapy window of 2 weeks followed by neoadjuvant treatment with Atezolizumab in combination with CTX (Arm A) to neoadjuvant CTX with Atezolizumab (Arm B), as measured by CCCA, decrease of Ki-67 expression (≥ 30%), low cellularity and TILs (≥ 60%), or a combined early response. 6. Assess and compare the prognostic and predictive values of the biomarkers (measured after 2 weeks and 4 weeks): CCCA, decrease of Ki-67 expression (≥ 30% and continuous), low cellularity and TILs (≥ 60% and continuous), or a combined early response, with respect to the outcome pCR. 7. Compare the efficacy of Atezolizumab mono-therapy window of 2 weeks followed by neoadjuvant treatment with Atezolizumab in combination with CTX (Arm A) to neoadjuvant CTX with Atezolizumab (Arm B), as measured by disease free survival (DFS). 8. Compare the efficacy of Atezolizumab mono-therapy window of 2 weeks followed by neoadjuvant treatment with Atezolizumab in combination with CTX (Arm A) to neoadjuvant CTX with Atezolizumab (Arm B), as measured by overall survival (OS). 9. Compare the efficacy of Atezolizumab mono-therapy window of 2 weeks followed by neoadjuvant treatment with Atezolizumab in combination with CTX (Arm A) to neoadjuvant CTX with Atezolizumab (Arm B), as measured by event free survival (EFS).
	Translational objectives: 1. Identify candidate genes for response/resistance to Atezolizumab (Sokolenko & Imyanitov, 2017). 2. Analyze the influence of immune markers (e.g. PD-1/L1) *via* circulating tumor deoxyribonucleic acid (ctDNA) as predictor for response to Atezolizumab (Appendix B) (Raja et al., 2018; Saliou et al., 2016). 3. Evaluate the influence of intrinsic subtype on response to Atezolizumab (Llombart-Cussac et al., 2017). 4. Assess the continuous ER/PR/HER2 expression (*via* polymerase chain reaction) as a predictive factor for response/resistance to Atezolizumab (Park et al., 2014). 5. Assess a specific DNA panel (Appendix A) *via* ctDNA as a predictive factor for response/resistance to Atezolizumab (Keup et al., 2019). 6. Evaluate the influence of polymorphisms *via* ctDNA on response to Atezolizumab (Mcardle et al., 2018). 7. Compare immune markers, polymorphisms, DNA panel activity, and ctDNA levels (Raja et al., 2018) after 14/28 days (+/- 2 days) of treatment between mono- and non-mono-therapy patients. 8. Evaluate T-Cell influence (CD 3/4/8) on the response to Atezolizumab. 9. Identify immune markers as candidate genes for response/resistance to Atezolizumab (Appendix C) (Oleg Gluz et al., 2019; Lee et al., 2015; Sokolenko & Imyanitov, 2017; Yang et al., 2018). 10. Assess and compare the prognostic and predictive values of the biomarkers: CCCA, Ki-67, low cellularity, TILs and CelTIL score after switch of the CTX to Epirubicin and Cyclophosphamide with respect to the outcome pCR in patients with less than 50% tumor shrinkage after 4 cycles of Carboplatin/Paclitaxel/Atezolizumab +/- the 2-week Atezolizumab window.

	11. Develop a new single drug RNA-based biomarker signature to predict response/resistance to Atezolizumab. 12. Assess and compare the prognostic and predictive values of the biomarkers (measured after 2 weeks and 4 weeks): CCCA, decrease of Ki-67 expression (≥ 30% and continuous), low cellularity and TILs (≥ 60% and continuous) with respect to the outcomes DFS and OS. 13. Assess optimal cut-offs for decrease of Ki-67 expression, TILs and the CelTIL score with respect to prediction of pCR, DFS and OS and compare with existing cut-offs.
Hypotheses	**Primary Hypothesis:** 1. Atezolizumab mono-therapy window of 2 weeks followed by neoadjuvant treatment with Atezolizumab in combination with CTX (Arm A) is superior to neoadjuvant CTX with Atezolizumab (Arm B). **Secondary Hypothesis:** 1. A monotherapy Atezolizumab window of 2 weeks followed by neoadjuvant treatment with Atezolizumab in combination with CTX has an acceptable toxicity profile. 2. There is an increased response rate in the subgroup of patients with ER/PR expression of < 1% as compared to patients with ER/PR expression of 1% to 10%. 3. Atezolizumab mono-therapy window of 2 weeks followed by neoadjuvant treatment with Atezolizumab in combination with CTX (Arm A) is superior to neoadjuvant CTX with Atezolizumab (Arm B) per alternative pCR definitions. 4. Early biological response (2 weeks in both arms), as measured by CCCA, decrease of Ki-67 expression (≥ 30%), low cellularity and TILs (≥ 60%), or a combined early response, is increased in Arm A with Atezolizumab mono-therapy window of 2 weeks followed by neoadjuvant treatment with Atezolizumab in combination with CTX. 5. Early biological response (2 weeks in Arm B vs. 4 weeks in Arm A), as measured by CCCA, decrease of Ki-67 expression (≥ 30%), low cellularity and TILs (≥60%), or a combined early response, is increased in Arm A with Atezolizumab mono-therapy window of 2 weeks followed by neoadjuvant treatment with Atezolizumab in combination with CTX. 6. The following biomarkers, measured after 2 and 4 weeks respectively, are prognostic and predictive with respect to outcome pCR: CCCA, decrease of Ki-67 expression (≥ 30% and continuous), low cellularity and TILs (≥ 60% and continuous), or a combined early response. 7. There is an increased efficacy in Arm A with Atezolizumab mono-therapy window of 2 weeks followed by neoadjuvant treatment with Atezolizumab in combination with CTX, as measured by DFS. 8. There is an increased efficacy in Arm A with Atezolizumab mono-therapy window of 2 weeks followed by neoadjuvant treatment with Atezolizumab in combination with CTX, as measured by OS. 9. There is an increased efficacy in Arm A with Atezolizumab mono-therapy window of 2 weeks followed by neoadjuvant treatment with Atezolizumab in combination with CTX, as measured by EFS.

Translational hypothesis:

1. There are candidate genes for response/resistance to Atezolizumab.
2. Immune markers (e.g. PD-1/L1) *via* ctDNA serve as predictor for response to Atezolizumab (Raja et al., 2018; Saliou et al., 2016).
3. Intrinsic subtype has an influence on response to Atezolizumab (Llombart-Cussac et al., 2017).
4. The continuous ER/PR/HER2 expression (*via* polymerase chain reaction) is a predictive factor for response/resistance to Atezolizumab (Park et al., 2014).
5. A specific DNA panel *via* ctDNA is a predictive factor for response/resistance to Atezolizumab (Keup et al., 2019).
6. Polymorphisms *via* ctDNA influence the response to Atezolizumab (Mcardle et al., 2018).
7. Immune markers, polymorphisms, DNA panel activity, and ctDNA levels (Raja et al., 2018) after 14/28 days (+/- 2 days) of treatment between mono- and non-mono-therapy patients show different influences on the response.
8. T-Cells (CD 3/4/8) influence the response to Atezolizumab.
9. There are immune markers for response/resistance to Atezolizumab.
10. Biological response measured by CCCA, Ki-67, low cellularity, TILs and CelTIL score after switch of the CTX to Epirubicin and Cyclophosphamide is predictive and prognostic with respect to the outcome pCR in patients with less than 50% tumor shrinkage after 4 cycles of Carboplatin/Paclitaxel/Atezolizumab +/- the two-week Atezolizumab window.
11. There is an RNA-based biomarker signature to predict response/resistance to Atezolizumab.
12. Biomarkers measured after 2 or 4 weeks respectively are prognostic and predictive with respect to the outcomes DFS and OS.
13. Optimal cut-offs can be found for decrease of Ki-67 expression, TILs and the CelTIL score with respect to prediction of pCR, DFS and OS.

Endpoints/ Outcome(s)

Objective		Endpoints
Primary	1.	a
Secondary	1.	b
	2.	c
	3.	d, e, f
	4.	i, j, k, l, m
	5.	i, j, k, l, m
	6.	a
	7.	n
	8.	o
	9.	p
Translational	1.	a, z
	2.	a, r
	3.	a, s
	4.	a, s
	5.	a, t
	6.	a, t
	7.	t, r
	8.	a, r
	9.	a, r
	10.	a, u, v, w, x, y
	11.	a, z

	12.	n, o, i, j, k, l
	13.	a, n, o, g, h, q

Primary Endpoint:

a) pCR defined as no residual invasive tumor cells in the breast and in the lymph nodes (ypT0/is, ypN0)

Secondary Endpoints:

b) Safety (incidence, relationship, seriousness, and severity of all AEs, SAEs, AESIs coded by medical dictionary for regulatory activities (MedDRA), summarized by Preferred Term and System Organ Class and graded according to common terminology criteria of adverse events (CTCAE) V5.0)
c) pCR defined as no residual invasive tumor cells in the breast and in the lymph nodes (ypT0/is, ypN0) in patients with an ER/PR expression of < 1% and an ER/PR expression of 1% to 10%
d) pCR defined as no tumor cells (invasive or non-invasive) in the breast but also in the lymph nodes (ypN0, ypT0)
e) Near pCR defined as residual tumor < 5 mm in the breast irrespective of *in situ* and lymph nodes status
f) pCR defined as no invasive tumor in the breast, irrespective of lymph node status
g) Decrease of Ki-67 expression versus baseline after 14/28 days (+/- 2 days) of treatment as continuous predictor
h) TILs after 14/28 days (+/- 2 days) of treatment as continuous predictor
i) CCCA: Ki-67 expression ≤ 2.7% after 14/28 days (+/- 2 days) of treatment
j) Low cellularity: < 500 tumor cells after 14/28 days (+/- 2 days) of treatment
k) Decrease of Ki-67 expression versus baseline by 30% or more after 14/28 days (+/- 2 days) of treatment
l) TILs ≥ 60% after 14/28 days (+/- 2 days) of treatment
m) Combined early response defined by
    - CCCA (Ki-67 expression ≤ 2.7%) or
    - low cellularity or
    - decrease of Ki-67 expression (versus baseline) by 30% or more or
    - TILs ≥ 60%
n) DFS[1] defined as time from the first date of no disease [i.e. date of surgery] to the first occurrence of disease recurrence or death from any cause
o) OS defined as length of time from randomization to death from any cause
p) EFS defined as length of time after randomization till death from any cause, failure to achieve remission after induction therapy, relapse in any site, or second malignancy.

Additional translational endpoints

q) CelTIL score as defined by (Nuciforo et al., 2017)
r) Immune markers (e.g. PD-1/L1) *via* ctDNA
s) Intrinsic subtype continuous ER/PR/HER2 expression
t) Specific DNA panel
u) Ki-67 expression as a continuous variable after 14 days (+/- 2 days) of treatment with Epirubicin and Cyclophosphamide in patients with less than 50% tumor shrinkage after 4 cycles of Carboplatin/Paclitaxel/Atezolizumab +/- the 2-week Atezolizumab window measured by tumor volume (or if not assessable by volume a decrease by 50% in diameter) through sonographic assessment

	v) TILs as a continuous variable after 14 days (+/- 2 days) of treatment with Epirubicin and Cyclophosphamide in patients with less than 50% tumor shrinkage after 4 cycles of Carboplatin/Paclitaxel/Atezolizumab +/- the 2-week Atezolizumab window w) CCCA: Ki-67 expression ≤ 2.7% after 14 days (+/- 2 days) of treatment with Epirubicin and Cyclophosphamide in patients with less than 50% tumor shrinkage after 4 cycles of Carboplatin/Paclitaxel/Atezolizumab +/- the 2-week Atezolizumab window x) Low cellularity: < 500 tumor cells after 14 days (+/- 2 days) of treatment with Epirubicin and Cyclophosphamide in patients with less than 50% tumor shrinkage after 4 cycles of Carboplatin/Paclitaxel/Atezolizumab +/- the 2-week Atezolizumab window y) CelTIL score as defined by (Nuciforo et al., 2017) after 14 days (+/- 2 days) of treatment with Epirubicin and Cyclophosphamide in patients with less than 50% tumor shrinkage after 4 cycles of Carboplatin/Paclitaxel/Atezolizumab +/- the 2-week Atezolizumab window z) Genome-wide gene expression analysis for RNA-based biomarker signature related to response/resistance to Atezolizumab
Key Inclusion Criteria	**Key Inclusion Criteria:** ● Female and male patients, age at diagnosis 18 years and above ● Written informed consent prior to admission to this study ● Histologically confirmed unilateral primary invasive carcinoma of the breast ● Clinical T1c – T4d ● Stage N0-N3 until 21 patients (5%) with stage N3 are randomized, thereafter N0 - N2 ● TNBC defined by and confirmed by central pathology: ○ ER negative (< 10% positive cells in IHC) and PR negative (<10% positive cells on IHC) ○ HER2 negative breast cancer: ▪ Either defined by IHC: IHC scores of 0 - 1 or an IHC score of 2 in combination with a negative *in-situ*-hybridization (ISH) ▪ Or defined by ISH: negative ISH ● Identifiable PD-L1 IC-status by central pathology (positive or negative) by means of VENTANA PD-L1 (SP142) Assay; positive status is defined by PD-L1 expression on IC on ≥ 1% of the tumor area, negative status is defined by PD-L1 expression on IC on < 1% of the tumor area ● No clinical evidence for distant metastasis (cM0) ● Tumor block available for translational research ● Performance Status Eastern Cooperative Oncology Group (ECOG) ≤ 1 or KI ≥ 80% ● Negative pregnancy test (urine or serum) within 7 days prior to screening in premenopausal patients ● Women of childbearing potential and male patients with partners of childbearing potential must accept to implement a highly effective (less than 1% failure rate according to Pearl index) including at least one non-hormonal contraceptive measures during the study treatment and for 5 months following the last dose of study treatment such as: ○ Intrauterine device (IUD) ○ bilateral tubal occlusion ○ vasectomised partner ○ sexual abstinence ● The patient must be accessible for treatment and follow-up ● Normal cardiac function: ○ Normal electrocardiogram (ECG) (within 6 weeks prior to screening) ○ Normal left ventricular ejection fraction (LVEF) on echocardiography ● Normal thyroid function ○ Normal TSH and FT4

	• Blood counts within 14 days prior screening:
	  ○ ANC must be ≥1,500/mm^3
	  ○ Platelet count must be ≥ 100,000 / mm^3
	  ○ Hemoglobin must be ≥ 10 g/dl
	• Hepatic functions:
	  ○ Total bilirubin must be ≤ 1 upper limit of normal (ULN) for the lab unless the patient has a bilirubin elevation > 1 x ULN to 1.5 x ULN due to Gilbert's disease or similar syndrome involving slow conjugation of bilirubin
	  ○ Alkaline phosphatase (ALK) must be ≤ 2.5 x ULN for the lab
	  ○ Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) must be ≤ 1.5 x ULN for the lab.
	  ○ Patients with AST and ALT or ALK > 1 x ULN are eligible for inclusion if liver imaging (computerized tomography (CT), magnetic resonance imaging (MRI), positron emission tomography (PET)-CT, or PET scan) performed within 3 months prior to randomization (and part of SOC) does not demonstrate metastatic disease and the requirements in criterion (just above) are met
	  ○ Patients with ALK that is > 1 x ULN but less than or equal to 2.5 x ULN or with unexplained bone pain are eligible if bone imaging does not demonstrate metastatic disease.
	  ○ Creatinine clearance ≥ 40 ml/min performed 28 days prior to screening

Key Exclusion Criteria	**Key Exclusion Criteria:**
	
	• Previous history of malign diseases, non-melanoma skin cancer and carcinoma of the cervix are allowed if treated with curative intent
	• Any other disease, metabolic dysfunction, physical examination finding, or clinical laboratory finding that, in the investigator's opinion, gives reasonable suspicion of a disease or condition that contraindicates the use of Paclitaxel, Carboplatin, Epirubicin, Cyclophosphamide or Atezolizumab
	• Psychological, familial, sociological or geographical conditions that do not permit compliance with the study protocol
	• Concurrent treatment with other drugs that are contraindicating the use of the study drugs
	• Existing pregnancy
	• Breastfeeding
	• Sequential breast cancer
	• Concurrent treatment with other experimental drugs and participation in another clinical trial or clinical research project (except registry study) within 30 days prior to study entry
	• Severe and relevant co-morbidity that would interact with the application of cytotoxic agents or the participation in the study including but not confined to:
	  ○ Uncompensated chronic heart failure or systolic dysfunction (LVEF < 55%, congestive heart failure (CHF) New York Heart Association (NYHA) classes II-IV),
	  ○ unstable arrhythmias requiring treatment i.e., atrial tachycardia with a heart rate ≥ 100/min at rest, significant ventricular arrhythmia (ventricular tachycardia) or higher-grade AV-block,
	  ○ Angina pectoris within the last 6 months requiring anti-anginal medication,
	  ○ Clinically significant valvular heart disease,
	  ○ Evidence of myocardial infarction on ECG,
	  ○ Poorly controlled hypertension (e.g., systolic > 180 mmHg or diastolic > 100 mmHg).
	• Inadequate organ function including but not confined to:
	  ○ hepatic impairment as defined by bilirubin > 1.5 x ULN
	  ○ pulmonary disease (severe dyspnea at rest requiring oxygen therapy)
	• Abnormal blood values:
	  ○ Platelet count below 100,000/mm^3
	  ○ AST/ALT > 1.5 x ULN

	○ Hypokalaemia > CTCAE grade 1 ○ Neutropenia > CTCAE grade 1 ○ Anaemia > CTCAE grade 1 ● Administration of a live, attenuated vaccine within 4 weeks before cycle 1 day 1 or anticipation that such a vaccine will be required during the study ● Treatment with systemic immunosuppressive medications (including but not limited to interferons, IL-2) within 28 days or 5 half-lives of the drug, whichever is longer, prior to randomization ● Treatment with systemic immunosuppressive medications (including but not limited to Prednisone, Cyclophosphamide, Azathioprine, Methotrexate, Thalidomide, and anti-tumor necrosis factor (anti-TNF) agents) within 14 days prior to screening or anticipation of need for systemic immunosuppressive medications during the study ● Patients with prior allogeneic stem cell or solid organ transplantation ● Active or history of autoimmune disease or immune deficiency, including but not limited to myasthenia gravis, myositis, autoimmune hepatitis, systemic lupus erythematosus, rheumatoid arthritis, inflammatory bowel disease, antiphospholipid syndrome, Wegener granulomatosis, Sjögren syndrome, Guillain-Barré syndrome, or multiple sclerosis with the following exceptions: ○ Patients with a history of autoimmune-related hypothyroidism on a stable dose of thyroid replacement hormone may be eligible for this study. ○ Patients with controlled Type 1 diabetes mellitus on a stable dose of insulin regimen may be eligible for this study. ○ Patients with eczema, psoriasis, lichen simplex chronicus, or vitiligo with dermatologic manifestations only (e.g., patients with psoriatic arthritis are excluded) are permitted provided all of following conditions are met: Rash must cover < 10% of body surface area; Disease is well controlled at baseline and requires only low-potency topical corticosteroids; No occurrence of acute exacerbations of the underlying condition requiring psoralen plus ultraviolet A radiation, Methotrexate, retinoids, biologic agents, oral calcineurin inhibitors, or high-potency or oral Corticosteroids within the previous 12 months. ● History of idiopathic pulmonary fibrosis, organizing pneumonia (e.g., bronchiolitis obliterans), drug-induced pneumonitis, idiopathic pneumonitis, or evidence of active pneumonitis on screening chest CT scan ● History of HIV infection, hepatitis B or hepatitis C infection ● Patients with significant cardiovascular disease ● Patients with inadequate hematological and end-organ function ● Patients receiving therapeutic anti-coagulants ● Stage N3, as soon as 21 patients with stage N3 are randomized
Statistical Rationale and Sample Size Calculation	neoMono adapts the idea of a proof-of-concept trial that uses Bayesian posterior and predictive probabilities for inference about the primary hypothesis. Up to 4 planned efficacy interim analyses provide decision points for early stopping for success or futility, the results of which will be independently reviewed by the DSMB. *A detailed explanation of statistical methods and the design with literature sources is provided in an appendix to this document.* The primary analysis is based on non-informative uniform (beta) priors for the pCR rates $p_A$and p_B in Arms A (experimental) and B (control) respectively. The proof-of-concept trial uses a **dual criterion** to *simultaneously test for significant and relevant superiority* at different levels of certainty by requiring posterior probabilities, conditional on observed response counts x_A, x_B respectively in the two arms, to exceed the following thresholds: $$\underset{\textit{significance}}{P(p_A > p_B \mid x_A, x_B) \geq 0.975} \;\wedge\; \underset{\textit{relevance}}{P(p_A - p_B > \delta \mid x_A, x_B) \geq 0.85},$$ with a clinically meaningful difference of $\delta = 0.05$.

<table>
<tr><td></td><td>
The trial is planned to have a maximal sample size of $N_{max} = 370$ evaluable patients, with up to 4 planned interim analyses in order to assess early futility or success of the trial based on posterior predictive probabilities PP for trial success. That is, the probability of claiming superiority in terms of the dual criterion if the trial were to continue to the maximal sample size N_{max}, conditional on the responses observed in the trial so far (see statistical appendix for mathematical details).

During the trial, up to 4 interim analyses are to be performed and reviewed by the DSMB after 100, 140, 180 and 220 patients evaluable for the primary endpoint. The following decision rules will be implemented at each interim analysis point:

• If $PP < 0.025$ the trial is stopped for futility
• If $PP > 0.975$ the trial is stopped for success

The maximal sample size $N_{max}$was determined by Monte Carlo simulation of the full adaptive trial with the parameters above using 10^8 repetitions and calculating the global operating characteristics (OC, power and type I error) in different scenarios of interest.

$N_{max} = 370$ is the smallest maximal sample size for the trial to reach at least 80% power to rightly claim superiority in the scenario $p_A = 60\%, p_B = 45\%$ and at most a 2.5% type I error rate to wrongly claim superiority in the scenario $p_A = 45\%, p_B = 45\%$. (see tables below; see statistical appendix for further details and a justification of pCR assumptions).

We assume an analysis dropout rate of 10% for the primary objective, where an analysis dropout is defined as any patient for whom a critical analysis-enabling covariate or the primary endpoint is not measurable for any reason, thus requiring 412 patients to be randomized (206 per arm with 1:1 randomization). In addition, we account for a 10% screening failure rate. As a result, the expected number of patients to be recruited is set to 458.
<table>
<tr><th>Scenario</th><th>Operating Characteristics</th><th>P(correct early stop)</th><th>$\mathbb{E}$[sample size]</th></tr>
<tr><td>H₀ significance:
$p_A = 45\%,\ p_B = 45\%$</td><td>type 1 error: 2.4%</td><td>68.5 %</td><td>296</td></tr>
<tr><td>H₀ relevance:
$p_A = 49\%,\ p_B = 45\%$</td><td>type 1 error: 10.9%</td><td>46.3%</td><td>321</td></tr>
<tr><td>H₁: $p_A = 60\%, p_B = 45\%$</td><td>power: 80.1%</td><td>34.9%</td><td>332</td></tr>
</table>
</td></tr>
<tr><td>Number of study centers</td><td>40 sites in Germany</td></tr>
<tr><td>Study Duration:</td><td>First patient in (FPI): Mar 2021
Last patient in (LPI): Aug 2022 (due to recruitment stop)
Last patient last visit (LPLV): Aug 2024
Recruitment period: 17 months
Expected recruitment rate: 0.5 patients/site/month</td></tr>
<tr><td>GCP Statement:</td><td>This study will be conducted in compliance with the protocol, the current version of the Declaration of Helsinki, the ICH-GCP as well as all national legal and regulatory requirements.</td></tr>
</table>

- 1 **DFS is defined as:** time from surgery to: Ipsilateral invasive breast tumor recurrence (i.e., an invasive breast cancer involving the same breast parenchyma as the original primary lesion)
- Ipsilateral local-regional invasive breast cancer recurrence (i.e., an invasive breast cancer in the axilla, regional lymph nodes, chest wall, and/or skin of the ipsilateral breast)
- Distant recurrence (i.e., evidence of breast cancer in any anatomic site – other than the two abovementioned sites – that has either been histologically confirmed or clinically diagnosed as recurrent invasive breast cancer)
- contralateral invasive breast cancer
- Ipsilateral or contralateral ductal carcinoma *in situ* (DCIS)
- Second primary non-breast invasive cancer (with the exception of non-melanoma skin cancers and *in situ* carcinoma of any site)
- Death attributable to any cause including breast cancer, non-breast cancer, or unknown cause (but cause of death should be specified if possible)

2. Schedule of Activities (SOA)

The SOA table provides an overview of the protocol visits and procedures.

Due to logistical reasons, it may be difficult for participating sites to carry out all screening assessments in the period indicated. Therefore, specific timelines will be given for the different tests to confirm disease status.

Since treatment times in Arm A and B are different (26 weeks in Arm A; 24 weeks in Arm B) and the fact that there is a shift in time between the 24 weeks treatment of Atezolizumab associated with Carboplatin, Paclitaxel, Epirubicin and Cyclophosphamide between Arm A and B, the SOA table is presented separately for Arm A and B.

Table 1: Schedule of Activities (on the following pages)

Table 1a: Arm A

Table 1b: Arm B

Table 1a: Schedule of Activity ARM A

Schedule and assesssment	Screening visit	Randomisation (no patient visit)	Baseline visit : day 1 treatment		Visit 1 after baseline **	Visit 2 **	Visit 3 **	Visit 4 **	Visit 5 **	Visit 6 **	Visit 7**	Visit 8 **	Visit 9 **	Visit 10 **	Visit 11 **	Visit 12**	Visit 13 **	Visit 14 **	Visit 15**	Visit 16 **	Visit 17**	Visit 18**	Visit 19**	Visit 20 **	Visit 21 **	Visit 22 **	Visit 23**	Visit 24**	EOT Visit 3 weeks after last dose chemo**	Surgery: 3-4 weeks after last dose chemo	Follow up every 6 months after surgery (if no surgery will be performed: 6 months after EOT)*** (j)	EOS visit: week 104 or month 24*** (j)
week			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	wk 29	wk 29-30	until year 2 after baseline	
day*	-17	-10 to -3	1	8	15	22	29	36	43	50	57	64	71	78	85	92	99	106	113	120	127	134	141	148	155	162	169	176				
Atezolizumab 840 mg IV, day 1			X																												
Atezolizumab 1200 mg IV Day 1 every 3 weeks for 4 doses					X			X			X			X			X			X			X			X					
Paclitaxel 80 mg/m2 IV weekly X 12 doses					X	X	X	X	X	X	X	X	X	X	X	X																
Carboplatin AUC of 2 IV day 1 every week X 12 doses					X	X	X	X	X	X	X	X	X	X	X	X																
Epirubicin 90 mg/m² every 3 weeks for 4 cycles																	X			X			X			X						
Cyclophosphamide 600 mg/m² every 3 weeks for 4 cycles																	X			X			X			X						
Study Procedures																																
ICF (before any study procedure starts)	X																															
Medical history	X																															
Core Biopsy (a)	X SOC				X		X												X													
physical examination (b)	X		X		X			X			X			X			X			X			X			X			X		SOC	SOC
Ultrasound (breast, lymph nodes, SoC) (c)	X										X						X												X		SOC	SOC
Other imaging: Mammography, chest X Ray or CT thorax; bone scan; liver imaging (SOC) (d)	X																														SOC	SOC
central pathology (e)	X				X		X												X										X			
clinical assessment	X		X		X			X			X			X			X			X			X			X			X		X	X
pregnancy test and check of adequate contraception measures (f)	X		(X)				X				X				X				X				X				X		X		every 4 weeks until month 5 after last chemo	
LVEF (g)	X																X												X		SOC	
ECG (h)	X																X												X		SOC	
Laboratory (hematology, biochemistry) (i)	X		X		X			X			X			X			X			X			X			X			X		SOC	
Laboratory (hematology)						X	X		X	X		X	X		X	X		X	X		X	X		X	X		X	X				
blood sample for translational research			X		X		X												X										X			
Randomization****		X																														
surgery																														X		
start of adjuvant CTX / Follow up (SoC)																															SOC	
(Serious) Adverse Event, AESIs, pregnancies	continuosly																															
Concomittant medication	continuosly																															

* timing different depending on test (see below); ** time of visits with a window of 2 days; ***time of visits with a window of +/-14 days

**** Randomisation : performed by investigator once all I/E criteria are fullfilled. Atezolizumab is ordered at Roche; chemo agents are ordered at central pharmacy; patient is contacted for baseline visit (no patient visit for randomisation)

(a): SOC core biopsy available prior to randomization

(b): physical examination at screening should be done within 7 days of screening

(c, d): Ultrasound is performed within 4 weeks prior to screening, other imaging procedures are performed within 3 months before randomization. After that they are performed as SOC .

(e): central pathology results need to be available before randomisation. In case central pathology does not confirm local pathology results for TNBC, the patient will not be randomised and considered as screen failure.

(f): pregnancy test at screening should not be older than 7 days, otherwise it has to be redone. In addition, if at baseline the pregnancy test is older than 10 days, it has to be redone. During treatment and FU, pregnancy tests have to be performed every 4 weeks runtil 5 months after last dose of neoadjuvant treatment

(g): LEVF at screening within 6 weeks before screening visit; then after 4 cycles and at EOT

(h): ECG at screening within 6 weeks before screening visit; then after 4 cycles and at EOT

(i): laboratory analyses at screening should be done within 14 days before screening visit

(j): Follow-up visits could also be performed at a local gynecologist/oncologist. In this case, site contacts patients by phone.

EOT visit should not be later than 3 weeks from last neoadjuvant treatment

surgery is recommended to be performed within 3-4 weeks after completion of neoadjuvant treatment

Table 1b: Schedule of Activity B																														
Schedule and assesssment	**Screening visit**	**Randomisation (no patient visit)**	**Baseline visit : day 1 treatment**	**Visit 1 after baseline ******	**Visit 2 ******	**Visit 3 ******	**Visit 4 ******	**Visit 5 ******	**Visit 6 ******	**Visit 7 ******	**Visit 8 ******	**Visit 9 ******	**Visit 10 ******	**Visit 11 ******	**Visit 12 ******	**Visit 13 ******	**Visit 14 ******	**Visit 15 ******	**Visit 16 ******	**Visit 17 ******	**Visit 18** **	**Visit 19 ******	**Visit 20 ******	**Visit 21** **	**Visit 22 ******	**Visit 23 ******	**EOT Visit 3 weeks after last dose chemo****	**Surgery: 3-4 weeks after last dose chemo**	**Follow up every 6 months after surgery (if no surgery will be performed: 6 months after EOT)*** (j)**	**EOS visit: week 104 or month 24*** (j)**
week			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	wk 27	wk 27-28	until year 2 after baseline	
day*	-17	-10 to -3	1	8	15	22	29	36	43	50	57	64	71	78	85	92	99	106	113	120	127	134	141	148	155	162				
Atezolizumab 1200 mg IV Day 1 every 3 weeks for 4 doses			X			X			X			X			X			X			X			X					
Paclitaxel 80 mg/m2 IV weekly X 12 doses			X	X	X	X	X	X	X	X	X	X	X	X																
Carboplatin AUC of 2 IV day 1 every week X 12 doses			X	X	X	X	X	X	X	X	X	X	X	X																
Epirubicin 90 mg/m² every 3 weeks for 4 cycles															X			X			X			X						
Cyclophosphamide 600 mg/m² every 3 weeks for 4 cycles															X			X			X			X						
Study Procedures																														
ICF (before any study procedure starts)	X																													
Medical history	X																													
Core Biopsy (a)	X SOC				X												X													
physical examination (b)	X		X			X			X			X			X			X			X			X			X		SOC	SOC
Ultrasound (breast, lymph nodes, SoC) (c)	X								X						X												X		SOC	SOC
Other imaging: Mammography, chest X Ray or CT thorax; bone scan; liver imaging (SOC) : (d)	X																												SOC	SOC
central pathology (e)	X				X												X											X		
clinical assessment	X		X			X			X			X			X			X			X			X			X		SOC	X
pregnancy test and check of adequate contraception measures (f)	X		(X)				X				X				X				X				X				X		every 4 weeks until month 5 after last chemo	
LVEF (g)	X														X												X		SOC	
ECG (h)	X														X												X		SOC	
Laboratory (hematology, biochemistry) (i)	X		X			X			X			X			X			X			X			X			X		SOC	
Laboratory (hematology)				X	X		X	X		X	X		X	X		X	X		X	X		X	X		X	X				
blood sample for translational research			X		X												X										X			
Randomization****		X																												
surgery																												X		
start of adjuvant CTX / Follow up (SoC)																													SOC	
(Serious) Adverse Event, AESIs, pregnancies	continuosly																													
Concomittant medication	continuosly																													

* timing different depending on test (see below); ** time of visits with a window of 2 days; ***time of visits with a window of +/-14 days

**** Randomisation : performed by investigator once all I/E criteria are fullfilled. Atezolizumab is ordered at Roche; chemo agents are ordered at central pharmacy; patient is contacted for baseline visit (no patient visit for randomisation)

(a): SOC core biopsy available prior to randomization

(b): physical examination at screening should be done within 7 days of screening

(c, d): Ultrasound is performed within 4 weeks prior to screening, other imaging procedures are performed within 3 months before randomization. After that they are performed as SOC .

(e): central pathology results need to be available before randomisation. In case central pathology does not confirm local pathology results for TNBC, the patient will not be randomised and considered as screen failure.

(f): pregnancy test at screening should not be older than 7 days, otherwise it has to be redone. In addition, if at baseline the pregnancy test is older than 10 days, it has to be redone. During treatment and FU, pregnancy tests have to be performed every 4 weeks until 5 months after last dose of neoadjuvant treatment

(g): LEVF at screening within 6 weeks before screening visit; then after 4 cycles and at EOT

(h): ECG at screening within 6 weeks before screening visit; then after 4 cycles and at EOT

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EOT visit should not be later than 3 weeks from last neoadjuvant treatment

surgery is recommended to be performed within 3-4 weeks after completion of neoadjuvant treatment

3. Introduction

3.1. Study rationale and background

TNBC

Breast cancer is a heterogeneous disease consisting of several disease subtypes that differ with regard to molecular phenotype, treatability and prognosis (Sørlie et al., 2001). As one of these subtypes, TNBC is defined by both absence of immunostaining for ER, PR, and lack of overexpression/amplification of HER2/neu (Dent et al., 2007). TNBC accounts for approximately 15% - 20% of breast cancer cases (Bauer et al., 2007) and is associated with younger age at diagnosis, premenopausal status, more advanced disease stage, higher grade, higher mitotic indices, family history of breast cancer, germline mutations in BRCA, and more aggressive clinical course than other breast cancer subtypes (Bauer et al., 2007). Improvement of systemic treatment of TNBC represents an unmet medical need.

Whereas former clinical studies defined the complete lack of ER, PR and HER2/neu as triple negative, current recommendations such as the German AGO recommendations are considering ER/PR low tumors (i.e. < 10% positive cells on IHC) as belonging to the same subgroup (Ditsch et al., 2019). The ER low-positive group is characterized molecularly by having features of triple-negative cancer in the majority of cases (Iwamoto et al., 2012). This includes basal-like phenotype, high incidence of germline BRCA mutation, and high-risk score by OncotypeDX. Also, distant-disease-free survival is similar to TNBC in these cases. Therefore, a low threshold of 1% for ER positivity, may lead to the false exclusion of biologically ER negative tumors from correctly targeted strategies and instead to the categorization of these tumors as ER positive with consecutive treatment recommendations (Deyarmin et al., 2013; Dixon et al., 2019; Sanford et al., 2015; Yi et al., 2014). The effect of endocrine therapy on survival outcomes in these patients remains unclear (Raghav et al., 2012). These data are justifying the inclusion of ER/PR low patients in trials on TNBC. However, after neoadjuvant therapy endocrine therapy has to be discussed as an option.

Choice of CTX in TNBC

Currently, poly-CTX is the only systemic treatment option for patients with early TNBC. Fortunately, patients with TNBC carry an increased chance of pCR compared to patients with non-TNBC (Cortazar et al., 2014; Houssami et al., 2012) and in case of pCR prognosis is excellent (Liedtke et al., 2008). However, prognosis is unfavorable compared to other breast cancer subtypes, in case of non-pCR.

There is an accumulating body of evidence suggesting that platinum salts should be added to Anthracycline/Taxane CTX in case of TNBC. In addition to historical data suggesting that platinum-containing CTX may be particularly beneficial for patients with TNBC (O. Gluz et al., 2009) the GeparSixto trial (Untch et al., 2016) and the CALGB 40603 trial (Sikov et al., 2015) have provided prospective evidence supporting the use of platinum-salts among patients with TNBC. Consequently, an Anthracycline/Taxane/Carboplatin-containing poly-CTX regimen is regarded as SOC for neoadjuvant treatment of patients with TNBC in Germany and is recommended in the current AGO recommendations (Ditsch et al., 2019). Furthermore, current and recent clinical trials such as Keynote-522 and NeoTRIP (see below) include a platinum-containing poly-CTX regimen.

Immunotherapy

Recently, targeted therapy of regulatory immune pathways has become an important tool in clinical applications. In contrast to conventional chemotherapeutics, immune checkpoint inhibitors do not target the tumor cells themselves but molecules which are part of the T-cell regulatory

cascade. The main focus lies on the removal of inhibitory mechanisms by which the tumor escapes from a T-cell response (Pardoll, 2012). Atezolizumab (trade name: Tecentriq®) is a humanized antibody against PD-L1 that has shown tolerability and tumor responses in patients with advanced malignancies. PD1 is localized on T-cells, B-cells, TILs, DCs, natural killer T-cells and activated monocytes. Its ligands PD-L1 and -L2 are expressed on a variety of cancer types, e.g. breast, ovary, lung, colon, melanoma, kidney and bladder (Alme et al., 2016). For some cancer types PD-L1 and -L2 expression could be associated with tumor progression and poor prognosis (Thompson et al., 2007). Recently combination therapies consisting of conventional agents and immune checkpoint inhibitors showed a high clinical relevance in cancer therapy (Sharma & Allison, 2015). Treatment of tumor tissues which lack an immunologic microenvironment with markers like CD8+ T-cells, CD4+ T-cells or PD-L1, could be improved by combination therapies. Conventional cancer therapies, e.g. CTX, radiation, surgery, anti-angiogenetic or hormonal, can induce tumor cell death, resulting in a release of antigens (Crittenden et al., 2015; Slovin et al., 2013).

Immunotherapy in TNBC

Among breast cancer subtypes, TNBC is a preferable target for immunotherapy. Hence, there is a large and increasing body of evidence for use of immunotherapy in TNBC (Marra et al., 2019). Consequently, and in particular based on the results of the IMpassion130 trial (Peter Schmid, 2018), Atezolizumab has been approved for PD-L1 IC-positive mTNBC in the first line of therapy in combination with nab-Paclitaxel.

In the neoadjuvant setting, data regarding the use of immunotherapy are heterogeneous. In the Keynote-522 trial, among 602 patients Pembrolizumab in combination with 4 cycles of Paclitaxel + Carboplatin followed by 4 cycles of Doxorubicin or Epirubicin + Cyclophosphamide showed a statistically significant improvement in pCR (ypT0/Tis, ypN0) vs. placebo in combination with CTX: 64.8% (95% CI, 59.9 - 69.5) vs. 51.2% (95% CI, 44.1 - 58.3), P = 0.00055 regardless of PD-L1 IC-status (P. Schmid et al., 2019).

Another neoadjuvant trial in TNBC, the NeoTRIP study presented at the SABCS 2019, investigated Atezolizumab 1200 mg q3w in combination with 8 cycles of Carboplatin and nab-Paclitaxel versus the same neoadjuvant CTX alone. pCR rates between both groups did not differ significantly. After surgery patients received 4 cycles of Epirubicin and Cyclophosphamide, results of the 5 year follow-up are awaited in 2024 (Gianni et al., 2019). Compared to Keynote-522 trial the results of the NeoTRIP trial are implying the conclusion that neoadjuvant therapy schedules investigating checkpoint inhibitors in TNBC should include an Anthracycline. This is in line with previously published data pointing to a synergistic effect of Anthracyclines and checkpoint inhibitors (Matsushita & Kawaguchi, 2018).

Immunotherapy window before neoadjuvant combined immune- and poly-CTX

In the GeparNuevo trial a significant effect of the checkpoint inhibitor Durvalumab in combination with neoadjuvant CTX compared to CTX alone could only be demonstrated in a subgroup of patients (n = 117) treated with a pre-therapeutic (i.e. before initiation of poly-CTX) two-week Durvalumab-mono-therapy-window (Loibl et al., 2019). An increase in pCR in association with Durvalumab was seen only in patients treated with the pre-therapeutic Durvalumab mono-therapy (pCR 61.0% vs. 41.4%, OR = 2.22, 95%CI 1.06 - 4.64, p = 0.035; interaction p = 0.048). Among patients without pre-therapeutic window (n = 57), pCR rates were 37.9% vs. 50.0%.
A biological rationale for the window effect can be found in the systematic biomarker analysis of the GeparNuevo trial. Patients received a re-biopsy after the 2-week window in both arms. The change of iTILs between baseline and after the window-phase significantly predicted achieving a pCR with Durvalumab in univariate (OR = 5.15, 95% CI 1.10 – 24.05, p = 0.037) and multivariate regression analysis (OR = 9.36, 95% CI 1.26 – 69.65, p = 0.029). In the placebo arm, the change of iTILs did not predict pCR (univariate analysis OR = 1.19, 95% CI 0.65 – 2.17, p = 0.581 and multivariate analysis OR = 1.22, 95% CI 0.65 – 2.27, p = 0.540). An increase of iTILs in post-

window samples compared with pre-therapeutic samples was predictive of pCR specifically in the Durvalumab arm. Probably due to the small sample size the interaction test did not formally meet statistical significance (p = 0.085). However, these data support the hypothesis that checkpoint inhibitors induce a modulation of the immune-microenvironment by stimulating lymphocytes to migrate from the stroma into the tumor-cell nests before introduction of cytotoxic CTX. This increased infiltration of immune cells into the tumor cell nests might be an indicator of a response to the checkpoint inhibitor. Promoting this initial immune response by addition of a poly-CTX could lead to an immunogenic cell death, possibly explaining the increased pCR rate in the window arm. Based on the results of the GeparNuevo trial (and particularly given the size of the effect) a single arm neoadjuvant trial combining poly-CTX with Pembrolizumab, the NeoImmunoBoost trial was amended for the inclusion of a 2-week immunotherapy window (ClinicalTrials.gov Identifier: NCT03289819).

The investigation of the effect of a 2-week immunotherapy mono-therapy window before combination immune- und poly-CTX in a randomized prospective clinical trial has the potential to define a new treatment standard.

Dosing of immunotherapy

In contrast to the approved dose in the metastatic setting of ongoing trials, in the neoadjuvant setting Atezolizumab is applied in a 3-weekly dose of 1200 mg in combination with Carboplatin and Paclitaxel (https://clinicaltrials.gov/ct2/show/NCT03281954; https://clinicaltrials.gov/ct2/show/NCT03595592). Due to the investigational nature of the mono-therapy window we chose the approved 2-weekly dose of 840 mg for the 2-week window phase and the approved 3-weekly dose of 1200 mg for the neoadjuvant phase.

Duration of immunotherapy

The question, if it is sufficient to add immunotherapy to the neoadjuvant phase of the treatment in TNBC or if an adjuvant application is necessary, is still unclear and study designs are heterogeneous. There are trials like the NeoTRIP trial (Gianni et al., 2019) and the NeoImmunoBoost trial (ClinicalTrials.gov Identifier: NCT03289819) adding the checkpoint inhibitor only to the neoadjuvant phase, whereas in Keynote-522 (P. Schmid et al., 2019) the immunotherapy agent was both added to the neoadjuvant phase continued as adjuvant treatment and after surgery. The results regarding the follow-up of this trial are still immature. A large adjuvant trial, IMpassion030 is investigating the effect of Atezolizumab in the adjuvant setting only (ClinicalTrials.gov Identifier: NCT03498716), however, results are not expected to be published before 2023. Whereas the neoadjuvant application of immunotherapy in combination with poly-CTX is already a new treatment paradigm, this is much less clear for the adjuvant use of these drugs. Therefore, it has to be considered adequate for current study designs in TNBC to include immunotherapy in the neoadjuvant treatment phase only. However, studies should anticipate a potential change regarding adjuvant immunotherapy and prepare to amend study protocols to incorporate adjuvant immunotherapy after robust results of the studies discussed above are being published.

Neoadjuvant therapy and window-of-opportunity trials in breast cancer

Neoadjuvant CTX trials include a population with therapy naïve tumor. Data from window-of-opportunity trials including repeat tumor biopsies allow the creation of prediction models for clinical response based on the results of early biopsies (Tan et al., 2018). The biomarkers used in these models have been tested in different tumor-biologies in the neoadjuvant setting. For instance, the IMPACT trial and the POETIC trial have demonstrated that short-term changes in proliferation by

Ki-67 expression in the neoadjuvant setting may be able to predict outcome (Dowsett et al., 2005, 2011).

Furthermore, among patients treated as part of the triple negative subprotocol of the ADAPT Trial (NCT01815242), low-cellularity (< 500 vital tumor cells) at week 3 was strongly associated with response to therapy. Higher levels of TILs were associated with pCR, both at baseline and after 3 weeks of neoadjuvant CTX. Ki-67 expression after three weeks was potentially associated with pCR (Oleg Gluz et al., 2015; Liedtke et al., 2018).

Due to the fact that many patients had less than 500 tumor cells in the re-biopsy after 3 weeks, which made an evaluation impossible, a 2-week approach is preferred. Accordingly, the CelTIL-Score (Nuciforo et al., 2017), integrates cellularity and TILs and thus avoids the problem of non-evaluable patients. Its predictive value regarding CTX response in a neoadjuvant therapy setting has been demonstrated.

Recently, it has also been demonstrated that TILs after 3 weeks are significantly associated with response to checkpoint inhibitors (Loi et al., 2019).

The inconsistent clinical data regarding neoadjuvant checkpoint inhibitor therapy with and without Anthracyclines (Gianni et al., 2019; P. Schmid et al., 2019) make it mandatory to investigate the biological effects described above not only of CTX in general in combination with Atezolizumab but also separately according to CTX regimen, e.g. Carboplatin and Paclitaxel versus Epirubicin and Cyclophosphamide in the setting of a sequential use.

Follow-up duration

TNBC is known to show early recurrence in case of disease relapse. For instance, in an analysis among 1,118 patients who received neoadjuvant CTX at M.D. Anderson Cancer Center for stage I-III breast cancer from 1985 to 2004, recurrence and death rates were higher for TNBC only in the first 3 years. In fact, hazard functions for disease recurrence among patients with TNBC compared with non-TNBC have been demonstrated to cross at 2.5 years of follow up, demonstrating lower incidences of disease recurrence among patients with TNBC compared to other breast cancer subtypes thereafter (Liedtke et al., 2008). In a preplanned interim analysis performed after 100 pCR events, patients with TNBC did not show a significant benefit from an atezolizumab monotherapy window suggesting that the primary endpoint would not be reached. Based on these results and in accordance with the study stopping rules, the patient recruitment was permanently stopped in August 2022. However, patients in both treatment arms demonstrated the highest pCR rates ever reported in phase II/III trials with TNBC patients treated with neoadjuvant immune checkpoint inhibitors (Kolberg et al, 2023, compare with Loibl et al. 2019; Schmid et al., 2020; Foldi et al., 2022; Huober etal., 2022). Given that novel post-neoadjuvant treatment options (on and off-trial) have emerged such as pembrolizumab, the post-neoadjuvant treatment in the follow-up phase of this study will most certainly be highly heterogeneous. Thus, completion of follow-up to 3 years is no longer justified. Therefore, follow-up will be limited to 2 years and only clinically significant survival signals may be observed. If patients perform follow-up care at their local gynecologist/oncologist, the study follow-up visits will be performed by the site staff by phone.

3.2. Study goal and medical need

The goal of this study is:

- Compare efficacy of PD-L1-inhibition (Atezolizumab) 2-week mono-therapy window followed by neoadjuvant CTX with Atezolizumab to CTX with PD-L1-inhibition (Atezolizumab).

- To identify biomarkers predicting (early) response to or resistance against Atezolizumab (alone and with CTX) allowing patients stratification in future clinical trials.

3.3. Benefit/Risk Assessment

Based on preclinical and clinical data, treatment of Atezolizumab in combination with CTX including Carboplatin, Paclitaxel, Epirubicin and Cyclophosphamide is expected to be tolerable, and toxicities of the treatment are expected to be manageable and reversible upon dose reduction, treatment interruption, or discontinuation. Patients in this study will be carefully monitored for key toxicities that have been observed with Atezolizumab or CTX with Carboplatin, Paclitaxel, Epirubicin or Cyclophosphamide (see respective SmPCs). Risk will be further minimized by adherence to inclusion/exclusion criteria (see section 6), avoidance of prohibited medication (see section 7.8), close safety monitoring (see section 9.9) and dose-adjustment guidelines (see section 7.4).
An independent data safety monitoring board (DSMB) (see section 9.9.11.1.) will be constituted and will monitor safety as outlined in the protocol and in the DSMB Charter. In addition, the DSMB will review scheduled interim efficacy analyses and decision rules for stopping the trial early.

Immunotherapy with Atezolizumab used in combination with nab-Paclitaxel has been licensed for mTNBC and is currently being investigated in the curative/neoadjuvant setting; results of the first studies (KEYNOTE-522) with immunotherapy in this setting (P. Schmid et al., 2019) have demonstrated a significant and clinically relevant benefit of 15% regarding pCR in TNBC. The recently published “Dear Investigator Letter” for study MO39196 /IMpassion131 indicated a lack of efficacy for the combination of Atezolizumab and Paclitaxel in first-line patients with PD-L1 IC positive mTNBC. While in MO39196 a mono-CTX backbone (i.e. Paclitaxel) is used in combination with immune checkpoint blockade in mTNBC, the aforementioned KEYNOTE-522 study is using a combination of Pembrolizumab with a poly-CTX backbone consisting of Paclitaxel, Carboplatin, Epirubicine and Cyclophosphamide in early TNBC. Since the neoMono study is using the same CTX backbone in the same population, the KEYNOTE-522 study is considered to a greater extend indicative for the outcome of the neoMono study than the IMpassion131 study.

The start of neoadjuvant CTX in Arm A of the study will be delayed by 2 weeks due to the 2-week immunotherapy window. This delay is not expected to have any impact of the short-term or long-term outcomes of study patients. In a pooled analysis, Loibl et al. investigated the impact of the interval between the time of biopsy and the start of CTX in a meta-analysis of 6 neoadjuvant trials. The time between biopsy and CTX did neither influence the pCR overall rate nor in subgroups. In multivariable logistic regression analysis length of this interval did also not independently predict pCR or influence DFS or OS, neither in all patients nor in subgroups (Loibl et al., 2017).
Furthermore, recent data suggests, that an immunotherapy mono-therapy-window before immunotherapy/CTX combination therapy might even further enhance efficacy of immunotherapy/CTX combination therapy for patients with TNBC.

Patients taking part in the study may therefore benefit by receiving immunotherapy in both arms (Atezolizumab alone or in combination with CTX). This is perceived as the future SOC**.**

4. Objectives

4.1. Primary Objectives

1. Compare efficacy in terms of pCR in TNBC with Atezolizumab 2-week monotherapy window followed by neoadjuvant CTX with Atezolizumab (Arm A) to neoadjuvant CTX with Atezolizumab (Arm B).

4.2. Secondary Objectives

1. Assess and compare safety of Atezolizumab mono-therapy window of 2 weeks followed by neoadjuvant treatment with Atezolizumab in combination with CTX (Arm A) to neoadjuvant CTX with Atezolizumab (Arm B).
2. Assess and compare efficacy of Atezolizumab mono-therapy window of 2 weeks followed by neoadjuvant treatment with Atezolizumab in combination with CTX (Arm A) to neoadjuvant CTX with Atezolizumab (Arm B) in patients with an ER/PR expression of < 1% and an ER/PR expression of 1% to 10%.
3. Compare efficacy of Atezolizumab mono-therapy window of 2 weeks followed by neoadjuvant treatment with Atezolizumab in combination with CTX (Arm A) to neoadjuvant CTX with Atezolizumab (Arm B) per alternative pCR definitions.
4. Compare early biological response (2 weeks in both arms) of Atezolizumab mono-therapy window of 2 weeks followed by neoadjuvant treatment with Atezolizumab in combination with CTX (Arm A) to neoadjuvant CTX with Atezolizumab (Arm B), as measured by CCCA, decrease of Ki-67 expression (≥ 30%), low cellularity and TILs (≥60%), or a combined early response.
5. Compare early biological response (2 weeks in Arm B vs 4 weeks in Arm A) of Atezolizumab mono-therapy window of 2 weeks followed by neoadjuvant treatment with Atezolizumab in combination with CTX (Arm A) to neoadjuvant CTX with Atezolizumab (Arm B), as measured by CCCA, decrease of Ki-67 expression (≥ 30%), low cellularity and TILs (≥60%), or a combined early response.
6. Assess and compare the prognostic and predictive values of the biomarkers (measured after 2 weeks and 4 weeks): CCCA, decrease of Ki-67 expression (≥ 30% and continuous), low cellularity and TILs (≥ 60% and continuous), or a combined early response, with respect to the outcome pCR.
7. Compare the efficacy of Atezolizumab mono-therapy window of 2 weeks followed by neoadjuvant treatment with Atezolizumab in combination with CTX (Arm A) to neoadjuvant CTX with Atezolizumab (Arm B), as measured by disease free survival (DFS).
8. Compare the efficacy of Atezolizumab mono-therapy window of 2 weeks followed by neoadjuvant treatment with Atezolizumab in combination with CTX (Arm A) to neoadjuvant CTX with Atezolizumab (Arm B), as measured by OS.
9. Compare the efficacy of Atezolizumab mono-therapy window of 2 weeks followed by neoadjuvant treatment with Atezolizumab in combination with CTX (Arm A) to neoadjuvant CTX with Atezolizumab (Arm B), as measured by event free survival (EFS).

4.3. Translational Research objectives

1. Identify candidate genes for response/resistance to Atezolizumab (Sokolenko & Imyanitov, 2017).
2. Analyze the influence of immune markers (e.g. PD-1/L1) *via* ctDNA as predictor for response to Atezolizumab (Appendix B) (Raja et al., 2018; Saliou et al., 2016).
3. Evaluate the influence of intrinsic subtype on response to Atezolizumab (Llombart-Cussac et al., 2017).
4. Assess the continuous ER/PR/HER2 expression (*via* polymerase chain reaction) as a predictive factor for response/resistance to Atezolizumab (Park et al., 2014).
5. Assess a specific DNA panel (Appendix A) *via* ctDNA as a predictive factor for response/resistance to Atezolizumab (Keup et al., 2019).
6. Evaluate the influence of polymorphisms *via* ctDNA on response to Atezolizumab (Mcardle et al., 2018).
7. Compare immune markers, polymorphisms, DNA panel activity, and ctDNA levels (Raja et al., 2018) after 14/28 days (+/- 2 days) of treatment between mono- and non-mono-therapy patients.
8. Evaluate T-Cell influence (CD 3/4/8) on the response to Atezolizumab.
9. Identify immune markers as candidate genes for response/resistance to Atezolizumab (Appendix C) (Oleg Gluz et al., 2019; Lee et al., 2015; Sokolenko & Imyanitov, 2017; Yang et al., 2018).
10. Assess and compare the prognostic and predictive values of the biomarkers: CCCA, Ki-67, low cellularity, TILs and CelTIL score after switch of the CTX to Epirubicin and Cyclophosphamide with respect to the outcome pCR in patients with less than 50% tumor shrinkage after 4 cycles of Carboplatin/Paclitaxel/Atezolizumab +/- the 2 week Atezolizumab window.
11. Develop a new single drug RNA-based biomarker signature to predict response/resistance to Atezolizumab.
12. Assess and compare the prognostic and predictive values of the biomarkers (measured after 2 weeks and 4 weeks): CCCA, decrease of Ki-67 expression (≥30% and continuous), low cellularity and TILs (≥ 60% and continuous) with respect to the outcomes DFS and OS.
13. Assess optimal cut-offs for decrease of Ki-67 expression, TILs and the CelTIL score with respect to prediction of pCR, DFS and OS and compare with existing cut-offs.

5. Study design

5.1. Overall design

This is a randomized, open-label, adaptive, two arm, multicenter phase II trial comparing a pre-surgical combination of Atezolizumab 2 weeks before biopsy followed by Carboplatin + Paclitaxel + Atezolizumab and then by Epirubicin + Cyclophosphamide + Atezolizumab (Arm A) with a direct treatment consisting of Carboplatin + Paclitaxel + Atezolizumab and then Epirubicin + Cyclophosphamide + Atezolizumab (Arm B) in patients with operable TNBC breast cancer.

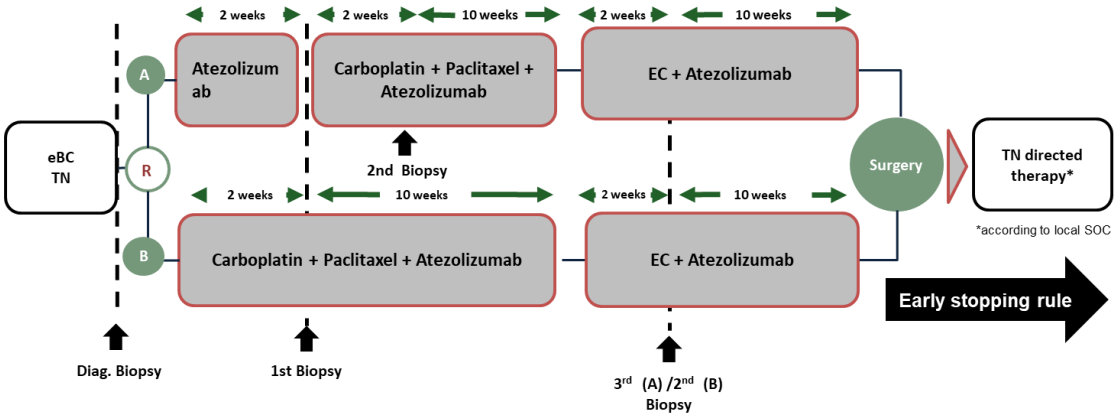

Figure 1: Overall Design.

After primary local diagnosis the patients will undergo screening which includes a centralized confirmation of TNBC subtypes (including triple-negative like subtype (ER/PR < 10%)).
Patients will be randomized to Arm A and B. Randomization will be stratified by PD-L1 IC-status and anatomic tumor stage (AJCC 8th edition Anatomic Stage Groups I, II and III). Patients in Arm A will be treated for a total of 2 weeks with 840 mg Atezolizumab mono-therapy before undergoing a biopsy after the 2-week cycle has ended. They will then continue with a 12-week therapy with a combination of Paclitaxel 80 mg/m² IV weekly x 12 doses + Carboplatin AUC of 2 IV weekly x 12 doses + Atezolizumab 1200 mg day 1 every 3 weeks for 4 doses. Every 3-week-interval is considered 1 cycle, therefore Carboplatin/Paclitaxel/Atezolizumab therapy will be applied for four 3-week cycles (12 weeks total). This will be followed by Epirubicin 90 mg/m² + Cyclophosphamide 600 mg/m² every 3 weeks for 4 cycles + Atezolizumab 1200 mg day 1 every 3 weeks for 4 doses. Patients in Arm B will be treated with a 12-week regimen of Paclitaxel 80 mg/m² IV weekly x 12 doses + Carboplatin AUC of 2 IV weekly x 12 doses + Atezolizumab 1200 mg day 1 every 3 weeks for 4 doses without a mono-therapy window. Every 3-week-interval is considered one cycle, therefore Carboplatin/Paclitaxel/Atezolizumab therapy will be applied for four 3-week cycles (12 weeks total). This will be followed by Epirubicin 90 mg/m² + Cyclophosphamide 600 mg/m² every 3 weeks for 4 cycles + Atezolizumab 1200 mg day 1 every 3 weeks for 4 doses.
Both groups, A and B will undergo a biopsy after 2 weeks of Carboplatin + Paclitaxel + Atezolizumab therapy. Furthermore, in both arms, for patients with a tumor size greater 10 mm in diameter, which have not achieved a 50% decrease in tumor volume (or if not assessable a decrease by 50% in diameter), another biopsy (the third in Arm A, the second in Arm B) will be performed after 2 weeks of Epirubicin + Cyclophosphamide + Atezolizumab therapy.
Patients in both arms will undergo surgery after 29 - 30 weeks therapy in total for Arm A and 27 - 28 weeks therapy in total for Arm B (3 - 4 weeks after last dose of neoadjuvant therapy) and move on to a treatment by local SOC. Further considerations with regards to immunological treatment in the adjuvant setting will be considered when further results of those regimes are more mature and published. Safety and toxicities under therapy will be supervised *via* regular DSMB meetings. After surgery, patients will be treated according to the local TNBC directed therapy SOC. Thereafter the patients will be followed-up until 24 months after baseline.

It is planned to perform up to 4 efficacy interim analyses in blocks of 40 patients after 100, 140, 180 and 220 patients evaluable for the primary endpoint pCR, assuming an equal sample size in both arms. At each interim analysis, decision rules based on predictive probabilities of trial success (see section 5.4) will be evaluated by the sponsor to determine whether the trial is to continue with patient recruitment, or whether to stop early for futility or success respectively. The DSMB will provide an independent review of these decisions and the interim efficacy results.

5.2. End of treatment (EOT)

EOT is defined as 21 days after the last dose of neoadjuvant therapy and prior to surgery.
The EOT visit will be performed no later than 4 weeks from the last dose of neoadjuvant therapy.
For details on procedures to be performed at the EOT visit, see the SOA table 1.

5.3. Surgery

After completion of 24 weeks (Arm B) or 26 weeks (Arm A) of neoadjuvant treatment, surgery is planned for all patients. Surgery is recommended to be performed within 3 - 4 weeks from last dose of neoadjuvant therapy in both arms. This would be at week 29 - 30 for Arm A and week 27 - 28 for Arm B.
In case of disease progression or toxicity of study treatment systemic treatment will be stopped prematurely and surgery or switch to non-cross resistant therapy will be performed immediately.

5.4. Early stopping Rules

During the trial, up to 4 interim analyses are to be performed after 100, 140, 180 and 220 patients evaluable for the primary endpoint in an intention-to-treat (ITT) collective. Analyses of pCR rates are based on predictive probabilities *PP* for trial success. That is, the probability of claiming superiority in terms of a dual criterion if the trial was to continue to the maximal sample size, conditional on the responses observed in the trial so far (see section 11 and statistical appendix 9 for details).

The following decision rules will be implemented at each interim analysis point:

- If $PP < 0.025$ the trial recruitment is stopped early for futility
- If $PP > 0.975$ the trial recruitment is stopped early for success

Interim analyses will be carried out and reported by the sponsor statistician**.** Based on these reports, the sponsor will carry out decisions regarding the continuation of recruitment according to the decision rules above. At each interim analysis timepoint, the DSMB will provide an independent review of interim efficacy results and trial design execution.

5.5. Follow up Treatment

Patients will receive a TNBC directed therapy according to local SOC: defined by S3-guideline and recommendation of the AGO Mamma in its latest version. The duration of the follow-up period of 2 years in this study is explained in section 3.1. If the patients are receiving follow-up care at their local oncologist/gynecologist, the study site may perform the neoMono follow-up visits by phone regarding the parameters listed below on the scheduled dates (see SOA tables).

Further considerations with regards to immunological treatment in the adjuvant setting will be considered when further results of those regimes are more mature and published.

Patient data will be collected every 6 months starting from surgery (week 29 - 30 after baseline for Arm A and week 27 - 28 after baseline for Arm B) until year 2 (or month 24) or until relapse to document:

- Invasive disease-free survival

- OS
- Further therapy
- Long term toxicities
- Relapse (local relapse)
- 2nd primary malignancy
- First treatment for metastatic breast cancer or 2nd primary malignancy
- Results for biopsy of distant metastases (if feasible)

In addition, pregnancy test and check of highly effective contraceptive measures will be performed every 4 weeks until 5 months following the last dose of CTX + Atezolizumab.

Blood tests will be performed according to SOC.
Physical examination and clinical assessment are performed according to SOC.

Patients who relapse or suffer from 2nd primary malignancy will only be followed for survival. Any distant metastasis occurring should be biopsied and the result should be reported in the case report form (CRF).

5.6. End of Study (EOS) definition

A patient is considered to have completed the study when month 24 or 2 years after baseline is reached.

The EOS is defined as the date of the last visit of the last participant in the study or last scheduled procedure shown in the SOA for the last participant in the trial globally (2 years after baseline).

5.7. Participants and study completion

This is an adaptive trial that uses Bayesian posterior and predictive probabilities to make inference about the primary hypothesis. Regular interim analyses allow for continuous learning during the trial and provide opportunities for adaptation, in particular for early stopping for success or futility. This has the potential to reduce the trial length, sample size and costs.

- Drug dosing

  Arm A: Atezolizumab 840 mg day 1 mono-therapy over a 2-week cycle and then Atezolizumab 1200 mg IV on day 1 on 3-week cycle (4 cycles) in combination with Paclitaxel 80 mg/m^2 IV weekly x 12 doses and Carboplatin AUC of 2 IV day 1 weekly for 12 doses (12 weeks). This will be followed by Epirubicin 90 mg/m^2 plus Cyclophosphamide 600 mg/m^2 every 3 weeks for 4 cycles (12 weeks) with a total of 26 weeks treatment.

  Arm B: Atezolizumab 1200 mg IV on day 1 on 3-week cycle (4 cycles) in combination with Paclitaxel 80 mg/m^2 x 12 doses and Carboplatin AUC of 2 IV weekly x 12 doses. This will be followed by Epirubicin 90 mg/m^2 plus Cyclophosphamide 600 mg/m^2 every 3 weeks for 4 cycles (12 weeks) with a total of 24 weeks treatment.

- Approximately 458 participants will be screened in 40 sites in Germany to achieve approximately 412 patients randomly assigned (1:1 randomization, 206 patients in each arm) to study treatment (the screen failure rate is estimated at 10%).

- After randomization, the analysis drop-out rate (see section 10.1 for definition) is estimated at 10% and therefore a total number of 370 patients will be evaluable (185 evaluable patients per treatment group).
- Planned Timelines:
    - First patient in (FPI): March 2021
    - Recruitment end: last patient in (LPI): January 2023 (Recruitment period of 23 months). Planned recruitment was stopped in August 2022 due to interim analysis results (resulting in shortened recruitment period of 17 months).
    - Study end: last patient last visit (LPLV): August 2024
    - Maximum number of patients per site: 10% of all randomized patients (n = 42 patients). This number may be increased in sites that show good quality of data based on key performance indexes used in the study.

5.8. Justification for dose

5.8.1. Atezolizumab 840 mg

Atezolizumab (Tecentriq®) in combination with nab-Paclitaxel is indicated for the treatment of adult patients with unresectable locally advanced or mTNBC whose tumors have PD-L1 expression ≥ 1% and who have not received prior CTX for metastatic disease.

More details can be found in the Investigator's Brochure (IB) of Atezolizumab.

5.8.2. Atezolizumab 1200 mg

In contrast to the approved dose in the metastatic setting ongoing trials, in the neoadjuvant setting Atezolizumab is applied in a dose of 1200 mg in combination with Carboplatin and Paclitaxel (https://clinicaltrials.gov/ct2/show/NCT03281954; https://clinicaltrials.gov/ct2/show/NCT03595592). Due to the investigational nature of the mono-therapy window we chose the approved 2-weekly dose of 840 mg for the 2-week window phase and the approved 3-weekly dose of 1200 mg for the neoadjuvant phase.

5.8.3. Carboplatin, Paclitaxel, Epirubicin and Cyclophosphamide

These CTX agents are approved by the competent authorities in Germany. All details can be found in the Summary of Product Characteristics (SmPCs) of the respective study drugs.

6. Study population

The study will include patients with primary, treatment naïve TNBC.

Patient eligibility must be reviewed and documented by an appropriate member (principal investigator (PI) or delegated sub-investigator registered for the study) of the investigator's study team before patients are included in the study.

Prospective approval of protocol deviations to recruitment and enrollment criteria, also known as protocol waivers or exemptions, is not permitted, according to “No waiver policy” (ICH-GCP).

Following the diagnostic core biopsy and identification of a TNBC tumor, and after informed consent is obtained the patients meeting the inclusion/exclusion criteria will be randomized.

6.1. Inclusion criteria

- Female and male patients, age at diagnosis 18 years and above
- Written informed consent prior to admission to this study
- Histologically confirmed unilateral primary invasive carcinoma of the breast
- Clinical T1c – T4d *
- Stage N0 - N3 until 21 patients (5%) with stage N3 are randomized, thereafter N0 - N2
- TNBC defined by and confirmed by central pathology:
    - ER negative (< 10% positive cells in IHC) and PR negative (< 10% positive cells on IHC)
    - HER2 negative breast cancer:
        - Either defined by IHC: ICH scores of 0 - 1 or an ICH score of 2 in combination with a negative ISH
        - Or defined by ISH: negative ISH
- Identifiable PD-L1 IC-status by central pathology (positive or negative) by means of VENTANA PD-L1 (SP142) assay; positive status is defined by PD-L1 expression on IC on ≥ 1% of the tumor area, negative status is defined by PD-L1 expression on IC on < 1% of the tumor area
- No clinical evidence for distant metastasis (cM0)
- Tumor block available for translational research
- Performance Status ECOG ≤ 1 or KI ≥ 80%
- Negative pregnancy test (urine or serum) within 7 days prior to screening in premenopausal patients
- Women of childbearing potential and male patients with partners of childbearing potential must accept to implement a highly effective (less than 1% failure rate according to Pearl index) including at least one non-hormonal contraceptive measures during the study treatment and for 5 months following the last dose of study treatment such as:
    - IUD
    - bilateral tubal occlusion
    - vasectomized partner
    - sexual abstinence
- The patient must be accessible for treatment and follow-up
- Normal cardiac function:
    - Normal ECG (within 6 weeks prior to screening)
    - Normal LVEF on ECG
- Normal thyroid function
    - Normal TSH and FT4
- Blood counts within 14 days prior screening:
    - absolute neutrophile count (ANC) must be ≥ 1,500/mm^3
    - Platelet count must be ≥ 100,000/mm^3
    - Hemoglobin must be ≥ 10 g/dl
- Hepatic functions:
    - Total bilirubin must be ≤ 1 upper limit of normal (ULN) for the lab unless the patient has a bilirubin elevation > 1 x ULN to 1.5 x ULN due to Gilbert’s disease or similar syndrome involving slow conjugation of bilirubin
    - ALK must be ≤ 2.5 x ULN for the lab
    - AST and ALT must be ≤1.5 x ULN for the lab.

- Patients with AST and ALT or ALK > 1 x ULN are eligible for inclusion if liver imaging (CT, MRI, PET-CT, or PET scan) performed within 3 months prior to randomization (and part of SOC) does not demonstrate metastatic disease and the requirements in criterion (just above) are met
- Patients with ALK that is > 1 x ULN but less than or equal to 2.5 x ULN or with unexplained bone pain are eligible if bone imaging does not demonstrate metastatic disease.
- Creatinine clearance ≥ 40ml/min performed 28 days prior to screening

**TNM staging according to the Union for International Cancer Control (UICC) classification, see Appendix 1*

6.2. Exclusion criteria

- Previous history of malign diseases, non-melanoma skin cancer and carcinoma of the cervix are allowed if treated with curative intent
- Any other disease, metabolic dysfunction, physical examination finding, or clinical laboratory finding that, in the investigator's opinion, gives reasonable suspicion of a disease or condition that contraindicates the use of Paclitaxel, Carboplatin, Epirubicin, Cyclophosphamide or Atezolizumab
- Psychological, familial, sociological or geographical conditions that do not permit compliance with the study protocol
- Concurrent treatment with other drugs that are contraindicating the use of the study drugs
- Existing pregnancy
- Breastfeeding
- Sequential breast cancer
- Concurrent treatment with other experimental drugs and participation in another clinical trial or clinical research project (except registry study) within 30 days prior to study entry
- Severe and relevant co-morbidity that would interact with the application of cytotoxic agents or the participation in the study including but not confined to:
  - Uncompensated chronic heart failure or systolic dysfunction (LVEF < 55%, CHF NYHA classes II-IV),
  - unstable arrhythmias requiring treatment i.e., atrial tachycardia with a heart rate ≥ 100 bpm at rest, significant ventricular arrhythmia (ventricular tachycardia) or higher-grade AV-block,
  - Angina pectoris within the last 6 months requiring anti-anginal medication,
  - Clinically significant valvular heart disease,
  - Evidence of myocardial infarction on ECG,
  - Poorly controlled hypertension (e.g., systolic > 180 mmHg or diastolic > 100 mmHg).
- Inadequate organ function including but not confined to:
  - hepatic impairment as defined by bilirubin > 1.5 x ULN
  - pulmonary disease (severe dyspnea at rest requiring oxygen therapy)
- Abnormal blood values:
  - Platelet count below 100,000/mm^3
  - AST/ALT > 1.5 x ULN
  - Hypokalaemia > CTCAE grade 1
  - Neutropenia > CTCAE grade 1
  - Anaemia > CTCAE grade 1
- Administration of a live, attenuated vaccine within 4 weeks before cycle 1 day 1 or anticipation that such a vaccine will be required during the study
- Treatment with systemic immunosuppressive medications (including but not limited to interferons, IL-2) within 28 days or 5 half-lives of the drug, whichever is longer, prior to randomization

- Treatment with systemic immunosuppressive medications (including but not limited to Prednisone, Cyclophosphamide, Azathioprine, Methotrexate, Thalidomide, and anti-TNF factor agents) within 14 days prior to screening or anticipation of need for systemic immunosuppressive medications during the study
- Patients with prior allogeneic stem cell or solid organ transplantation
- Active or history of autoimmune disease or immune deficiency, including but not limited to myasthenia gravis, myositis, autoimmune hepatitis, systemic lupus erythematosus, rheumatoid arthritis, inflammatory bowel disease, antiphospholipid syndrome, Wegener granulomatosis, Sjögren syndrome, Guillain-Barré syndrome, or multiple sclerosis with the following exceptions:
    - Patients with a history of autoimmune-related hypothyroidism on a stable dose of thyroid replacement hormone may be eligible for this study.
    - Patients with controlled Type 1 diabetes mellitus on a stable dose of insulin regimen may be eligible for this study.
    - Patients with eczema, psoriasis, lichen simplex chronicus, or vitiligo with dermatologic manifestations only (e.g., patients with psoriatic arthritis are excluded) are permitted provided all of following conditions are met: Rash must cover < 10% of body surface area; Disease is well controlled at baseline and requires only low-potency topical corticosteroids; No occurrence of acute exacerbations of the underlying condition requiring psoralen plus ultraviolet A radiation, Methotrexate, retinoids, biologic agents, oral calcineurin inhibitors, or high-potency or oral corticosteroids within the previous 12 months.
- History of idiopathic pulmonary fibrosis, organizing pneumonia (e.g., bronchiolitis obliterans), drug-induced pneumonitis, idiopathic pneumonitis, or evidence of active pneumonitis on screening chest CT scan
- History of HIV infection, hepatitis B or hepatitis C infection.
- Patients with significant cardiovascular disease
- Patients with inadequate hematological and end-organ function
- Patients receiving therapeutic anti-coagulants
- Stage N3, as soon as 21 patients with stage N3 are randomized

7. Treatments

7.1. Definition of study treatment: Investigational Medicinal Product (IMP)

Study treatment is defined as neoadjuvant therapy i.e.: Atezolizumab, Carboplatin, Paclitaxel, Epirubicin and Cyclophosphamide are considered IMPs.

After surgery adjuvant therapy is administered as per SOC TNBC and is not considered as study treatment.

7.2. Treatments administered

Table 2: Schedule of administration of study drugs

Treatment scheme ARM A																									
week	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
Atezolizumab 840 mg IV, day 1	X																									
Atezolizumab 1200 mg IV Day 1 every 3 weeks for 4 doses			X			X			X			X			X			X			X			X		
Paclitaxel 80 mg/m2 IV weekly X 12 doses			X	X	X	X	X	X	X	X	X	X	X	X												
Carboplatin AUC of 2 IV day 1 every week X 12 doses			X	X	X	X	X	X	X	X	X	X	X	X												
Epirubicin 90 mg/m² every 3 weeks for 4 cycles															X			X			X			X		
Cyclophosphamide 600 mg/m² every 3 weeks for 4 cycles															X			X			X			X		
Treatment scheme ARM B																										
week	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24		
Atezolizumab 1200 mg IV Day 1 every 3 weeks for 4 doses	X			X			X			X			X			X			X			X				
Paclitaxel 80 mg/m2 IV weekly X 12 doses	X	X	X	X	X	X	X	X	X	X	X	X														
Carboplatin AUC of 2 IV day 1 every week X 12 doses	X	X	X	X	X	X	X	X	X	X	X	X														
Epirubicin 90 mg/m² every 3 weeks for 4 cycles													X			X			X			X				
Cyclophosphamide 600 mg/m² every 3 weeks for 4 cycles													X			X			X			X				

7.2.1. Atezolizumab

For patients randomized in Arm A and B in the neoadjuvant treatment phase, Roche will provide the study sites with Atezolizumab labeled study-specifically.

In Arm A: Roche will provide 206 treatments of Atezolizumab 840 mg and 1648 treatments of Atezolizumab (206 x 8 cycles) 1200 mg.

In Arm B: Roche will provide 1648 treatments of Atezolizumab (206 x 8 cycles) 1200 mg.

This includes the number of Atezolizumab treatments needed for 206 patients randomized in each treatment arm, including the 10% potential analysis drop outs.

Documentation of preparation and distribution of Atezolizumab has to be recorded in accordance with the SmPC (prescribing information/ "Fachinformation").

7.2.2. Carboplatin, Paclitaxel, Epirubicin and Cyclophosphamide

These chemotherapeutic agents in Arm A and in Arm B are considered IMPs in the neoadjuvant phase of the study. They will be provided to sites and labeled study-specifically. As these drugs are approved in Germany, they will be used according to currently valid SmPC.

7.2.3. TNBC directed therapy in adjuvant phase

TNBC directed therapy in the adjuvant phase (Arm A and Arm B) is used according to German SOC defined by S3-guideline and recommendation of the AGO Mamma (in its latest version) and will not be provided to sites nor be labeled study-specifically (use according to currently valid SmPC).

Further considerations with regards to immunological treatment in the adjuvant setting will be considered when further results of those regimes are more mature and published.

7.3. Treatment plans

7.3.1. Biopsies and overall plan

- Tumor evaluation is done by a core biopsy as SOC in breast cancer. Evaluation of the tumor entity from the first (diagnostic) core biopsy will be done by the study site's local pathologist. For comparison of local and central pathological results, a sample of the diagnostic core biopsy has to be shipped to the central pathology laboratory.
- Other biopsies will be performed in Arm A and B as follows:
    - Patients in Arm A will undergo a biopsy two weeks after Baseline visit.
    - Patients of Arm A and B will undergo a biopsy after two weeks of Carboplatin + Paclitaxel + Atezolizumab therapy.
    - Furthermore, in both arms, for patients with a tumor size greater than 10 mm in diameter, which have not achieved a 50% decrease in tumor volume (or if not assessable a decrease by 50% in diameter), another biopsy (the third in Arm A, the second in Arm B) will be performed after 2 weeks of Epirubicin + Cyclophosphamide + Atezolizumab therapy.
    - Even if previous biopsies or cycles were omitted, the following biopsies should always be performed in the designated time window according to SOA (Table 1).

- In addition to the above-mentioned biopsies, a sample of the breast tissue collected at surgery must be sent to the central pathology laboratory for analysis.
- All the biopsies performed during the neoadjuvant phase and sample tissues from surgery (all paraffin embedded), will be shipped to:

Universitätsklinikum Erlangen
Pathologisches Institut
[redacted]

- The following tests will be performed:
    - Hormone receptor status (ER and PR)
    - HER2 expression
    - PD-L1 IC-status
    - Histology
    - Tumor grade
    - Proliferation index Ki-67
    - Low cellularity
    - TILs
    - Further molecular markers for translational research

Preferably and where appropriate, tissue microarrays (TMA) will be constructed to evaluate the tumors of study participants. Where TMAs are an inappropriate approach for evaluation other

approaches are taken (e.g. PD-L1 IC-Status will be performed by means of large surface sections).
After construction of TMAs or execution of other approaches, the remaining tumor blocks will be stored at the Institute of Pathology of the University Clinic Erlangen.

- TNBC status: ER negative (< 10% positive cells in IHC), PR negative (< 10% positive cells on IHC) and HER2 negative: either defined by IHC: ICH scores of 0 - 1 or an ICH score of 2 in combination with a negative ISH; or defined by ISH: negative ISH.

- In case the central pathology results does not confirm the local laboratory assessment of TNBC, the patient will be considered as a screening failure and will not be randomized.

- PD-L1 IC-status: Patients with TNBC whose tumors have PD-L1 expression ≥ 1% (PD-L1 expression on IC with the VENTANA PD-L1 (SP142) assay)) and patients with non-determinable PD-L1 expression will be identified. Randomization of patients will be stratified by PD-L1 IC-status and anatomic tumor stage (AJCC 8th edition Anatomic Stage Groups I, II and III). Positive status is defined by PD-L1 expression on IC on ≥ 1% of the tumor area, negative status is defined by PD-L1 expression on IC on < 1% of the tumor area.

- Following the diagnostic core biopsy and confirmed identification of a TNBC tumor by the central pathology laboratory, the patients meeting the inclusion/exclusion criteria after informed consent was obtained will be subsequently randomized to the neoadjuvant therapy:

    - Arm A: Atezolizumab for 2 weeks followed by Carboplatin + Paclitaxel + Atezolizumab for 12 weeks and then followed by Epirubicin + Cyclophosphamide + Atezolizumab for 12 weeks (total treatment phase of 26 weeks).
    - Arm B: directly randomized to Carboplatin + Paclitaxel + Atezolizumab (12 weeks) and then followed by Epirubicin + Cyclophosphamide + Atezolizumab for 12 weeks, for a total treatment phase of 24 weeks.

- Core Biopsies will be taken as described above.

- After completion of 24 - 26 weeks of targeted therapy within either of the two treatment arms, the patients will undergo surgery and pCR will be assessed by local pathology. All patients will be treated according to local SOC after surgery.

- Definitive surgical treatment must be performed according to current Arbeitsgemeinschaft Gynäkologische Onkologie e.V (AGO) guidelines for operable breast cancer (T1c – 4d, stage N0-N3, cM0). Margins of the resected specimen from definitive surgery must be histologically-free of invasive carcinoma and/or ductal carcinoma in situ. Lobular carcinoma *in-situ* (except LN3) will not be considered a positive margin.

- TMA of all core biopsies and surgery samples will be used for translational research (Nanostring Ncounter: PAM 50, Appendix C) and genome-wide gene expression analysis for RNA-based biomarker signature related to response/resistance to Atezolizumab using ArrayXS microarrays (Appendix D).

7.3.2. Atezolizumab (Tecentriq®)

- Dose: 1200 mg concentrate for solution for infusion. After dilution (according to SmPc), 1 mL of solution contains approximately 4.4 mg of Atezolizumab. The recommended dose of Tecentriq® is 1200 mg administered intravenously every three weeks. Tecentriq® is for intravenous use. The infusions must not be administered as an intravenous push or bolus.

- Dose of 840 mg concentrate: 14 mL of Tecentriq® concentrate should be withdrawn from the vial and diluted into a 250 mL polyvinyl chloride (PVC), polyolefin (PO), polyethylene (PE), or polypropylene (PP) infusion bag containing sodium chloride 9 mg/mL (0.9%) solution for injection. After dilution, 1 mL of solution should contain approximately 3.2 mg of Tecentriq® (840 mg/264 mL).

- The initial dose of Tecentriq® (both dosages) must be administered over 60 minutes. If the first infusion is well tolerated, all subsequent infusions may be administered over 30 minutes.

- Instructions on dilution and handling of the medicinal product before administration: chemical and physical in-use stability has been demonstrated for up to 24 hours at ≤ 30 °C and for up to 30 days at 2 °C to 8 °C from the time of preparation.

- From a microbiological point of view, the prepared solution for infusion should be used immediately. If not used immediately, in-use storage times and conditions prior to use are the responsibility of the user and would normally not be longer than 24 hours at 2 °C to 8 °C or 8 hours at ambient temperature (≤ 25 °C) unless dilution has taken place in controlled and validated aseptic conditions.

- Route: Intravenous infusion for both dosages.

- Schedule: The 840 mg will be administered once at day 1 of a 2-week cycle. The 1200 mg dose will be administered every 3 weeks over 4 cycles.

- Traceability: In order to improve the traceability of biological medicinal products, the trade name and the batch number of the administered product should be clearly recorded in the patient file.

- Mechanism of action: Atezolizumab is an Fc-engineered, humanized IgG1 anti-programmed death-ligand 1 (PD-L1) monoclonal antibody produced in Chinese hamster ovary cells by recombinant DNA technology.

7.3.3. Carboplatin

- Dose: AUC of 2 IV weekly X 12 doses
- Route: Intravenous infusion. The solution for infusion is given as a short IV infusion over 15 - 60 minutes.
- Schedule: every week for 12 weeks (12 cycles).

7.3.4. Paclitaxel

- Dose: 80 mg/m^2, day 1
- Route: 1-hour intravenous infusion. During the first 5 minutes, the infusion must be done drop by drop in order to reduce the incidence of acute hypersensitivity reaction.
- Schedule: every week for 12 weeks (12 cycles).

7.3.5. Epirubicin

- Dose: 90 mg/m^2, day 1
- Route: IV over 3 - 5 min or according to local standard.
- Schedule: 4 cycles (1 cycle = 3 weeks) over 12 weeks.

7.3.6. Cyclophosphamide

- Dose: 600 mg/m^2, day 1
- Route: IV from 30 min to 2 hours or according to local standard.
- Schedule: 4 cycles (1 cycle = 3 weeks) over 12 weeks.

7.3.7. TNBC directed therapies during adjuvant phase

Standard TNBC directed therapies will be used as according to German SOC as defined through S3-guideline and recommendations of the AGO Mamma (in its latest version) during adjuvant phase.

For approved drugs, refer to the current version of the respective SmPC provided by the manufacturers.

7.3.8. Medication error

In the neoadjuvant phase, all medication is administered at the hospital. Refer to the SmPC or IB for the respective drugs administered.

7.4. Dose modification and treatment delays

Adverse Events will be graded using the National Cancer Institiute Common Toxicity Criteria (NCI CTC), version 5.0. For modification of dose and treatment in case of immune-related side effects refer to the section 6.7 of the current IB of Tecentriq® (Atezolizumab). Dose reduction is planned for the CTX containing treatment arm in case of severe hematological and/or non-hematological toxicities. Dose adjustments are to be made according to the organ system showing the greatest degree of toxicity. In case of several toxicities in one patient and conflicting recommendations, the most conservative dose adjustment has to be followed. Doses which have been reduced for toxicity must not be re-escalated with the exception of liver function tests that improve within ranges given. In case of persistent toxicity attributed to CTX, the possibility of a dose reduction in accordance with drug-specific recommendations as given below should always be considered. It should be noted that once a dose reduction of CTX has been carried out, it must be adhered to for all subsequent cycles. A second dose reduction step for Paclitaxel/Carboplatin and

Epirubicin/Cyclophosphamide during the study is not intended (7.4.3.2, Table 3 and 7.4.4, Table 5).

If an AE occurs during therapy with an already reduced dose, the investigator has to consider the pausing of the causative substance. If the criteria for resumption of therapy (retreatment criteria) are met, the therapy should be resumed at the already reduced dose. Alternatively, it is at the discretion of the investigator to terminate the treatment with the causative substance.

To ensure adequate monitoring and toxicity management during administration of study treatment, the administration of study drugs will be performed in a monitored setting where there is immediate access to trained personnel and adequate equipment and medicine to manage potentially serious reactions.

7.4.1. Atezolizumab

7.4.1.1. Administration of Atezolizumab

First infusion:

- No premedication is administered
- Record patient's vital signs (heart rate, respiratory rate, blood pressure, and temperature) at the following time points:
  - Within 60 minutes prior to infusion
  - During infusion or after infusion, if clinically indicated
- Infuse Atezolizumab (1 vial in 250 mL NaCl solution) over 60 (± 15) min
- Patients will be informed about the possibility of delayed post-infusion symptoms and instructed to contact their study physician if they develop such symptoms.

Subsequent infusion:

- If the patient tolerated the 1st infusion well without infusion-associated adverse events, the 2nd infusion will be delivered over 30 (± 10) min; record patient vital signs (heart rate, respiratory rate, blood pressure, and temperature) as clinically indicated.
- If patient experienced infusion-related reaction during any previous infusion premedication may be administered for cycles ≥ 2 at the discretion of the treating physician (see Table 48 of the current IB of Tecentriq® (Atezolizumab).
- If the patient had an infusion-related reaction during the previous infusion, the subsequent infusion must be delivered over 60 (± 15) min.
- Record patient's vital signs (heart rate, respiratory rate, blood pressure, and temperature) at the following time points:
  - Within 60 min prior to infusion
  - During infusion or after infusion, if clinically indicated
- If no reaction occurs, continue subsequent infusions over 30 (± 10) min with the same schedule for recording vital signs as above (within 60 min prior to infusion, during infusion or after infusion, if clinically indicated).

7.4.1.2. Atezolizumab dose modification

For modification of dose and treatment in case of immune-related side effects refer to the section 6.7 of the current IB of Tecentriq® (Atezolizumab).

7.4.1.3. Management of Atezolizumab-specific Adverse Events

The current version of the IB of Tecentriq® (Atezolizumab) provides detail information about Adverse Events (AEs) experienced by patients.

Side effects associated or possibly associated with Atezolizumab treatment should be managed according to standard medical practice. Additional tests, such as autoimmune serology or biopsies, should be used to evaluate for a possible immunogenic aetiology.

For organ-specific management guidelines of immune-related adverse events (irAEs) see section 6.7 of the current IB of Tecentriq® (Atezolizumab) including management guidelines for pulmonary events, pneumonitis, hepatic events, gastrointestinal events (diarrhea or colitis), endocrine events, ocular events, immune-mediated myocarditis, infusion-related reactions, pancreatic events, pancreatitis, dermatologic events, neurologic disorders, immune-mediated meningoencephalitis, renal events, immune-mediated myositis. Recent data have reported that immune-mediated pericardial disorders including pericarditis, pericardial effusion and cardiac tamponade are associated with use of immune checkpoint inhibitors as a class of medications [source Dear Investigator Letter (DIL), Identified Risk: Immune-Mediated Pericardial Disorders with Tecentriq® (atezolizumab) use; 27-Jul-2022]. Based on these data, immune-mediated pericardial disorders are now considered to be an identified risk for Atezolizumab. Atezolizumab should be withheld for patients with suspected immune-mediated pericardial disorders. Caution should be used when considering the use of Atezolizumab in a patient who has previously experienced a pericardial disorder on prior treatment with other immune-stimulatory anticancer agents.

Further recently identified important risks associated with use of Atezolizumab are immune-mediated myelitis and immune-mediated facial paresis [source Dear Investigator Letter (DIL), Identified Risks: Immune-Mediated Myelitis and Immune-mediated Facial Paresis with Tecentriq® (atezolizumab) use; 21-Nov-2022]. Atezolizumab should be withheld for patients with grade 1 or 2 immune-mediated facial paresis. Patients should be monitored for clinical signs and symptoms that are suggestive of myelitis and may present with signs and symptoms of sensory and/or motor neuropathy regarding facial paresis. Diagnostic workup is essential for an accurate characterization to differentiate between alternative etiologies. Consider referring patient to neurologist.

Although most irAEs observed with immunomodulatory agents have been mild and self-limiting, such events should be recognized early and treated promptly to avoid potential major complications. Discontinuation of Atezolizumab may not have an immediate therapeutic effect, and in severe cases, immune-related/ immune–mediated toxicities may require acute management with topical corticosteroids, systemic corticosteroids, or other immunosuppressive agents.

The investigator should consider the benefit-risk balance a given patient may be experiencing prior to further administration of Atezolizumab. In patients who have met the criteria for permanent discontinuation, resumption of Atezolizumab may be considered if the patient is deriving benefit and has fully recovered from the immune-related event. Patients can be re-challenged with Atezolizumab only after the approval has been documented by both the investigator (or an appropriate delegate) and the sponsor. According to DIL (Identified Risk: Immune-Mediated Pericardial Disorders with Tecentriq® (atezolizumab) use; 27-Jul-2022), Atezolizumab should be permanently withdrawn for any grade confirmed immune-mediated pericardial disorders. According to recent DIL (Identified Risks: Immune-Mediated Myelitis and Immune-mediated Facial Paresis with Tecentriq® (atezolizumab) use; 21-Nov-2022), Atezolizumab should be permanently withdrawn for ≥ grade 2 immune-mediated myelitis as well as for ≥ grade 3 immune-mediated facial paresis.

For suspected immune-related adverse reactions, thorough evaluation to confirm aetiology or exclude other causes should be performed. Based on the severity of the adverse reaction, Atezolizumab should be withheld, and corticosteroids administered. Upon improvement to grade ≤ 1, corticosteroid should be tapered over ≥ 1 month. Based on limited data from clinical studies in patients whose immune-related adverse reactions could not be controlled with systemic corticosteroid use, administration of other systemic immunosuppressants may be considered.
Atezolizumab must be permanently discontinued for any grade 3 immune-related adverse reaction that recurs and for any grade 4 immune-related adverse reactions, except for endocrinopathies that are controlled with replacement hormones. According to DIL (Identified Risk: Immune-Mediated Pericardial Disorders with Tecentriq® (atezolizumab) use; 27-Jul-2022), Atezolizumab should be permanently withdrawn for any grade confirmed immune-mediated pericardial disorders. According to recent DIL (Identified Risks: Immune-Mediated Myelitis and Immune-mediated Facial Paresis with Tecentriq® (atezolizumab) use; 21-Nov-2022), Atezolizumab should be permanently withdrawn for ≥ grade 2 immune-mediated myelitis as well as for ≥ grade 3 immune-mediated facial paresis.

For further recommendations for the management of irAEs please refer to the ASCO Clinical Practice Guideline: “Management of Immune-Related Adverse events in Patients Treated with Immune Checkpoint Inhibitor Therapy”.

7.4.2. Delays in cycles

If therapy cycles are partially or completely delayed due to an adverse event, a strict hierarchy applies. Atezolizumab, as the leading substance, determines the sequence and duration of the therapy cycles. Specifically, this means:

1. If a **CTX side effect** is anticipated, Atezolizumab therapy may be continued, and CTX must be modified according to the drug-specific information given below. Regarding the modification of CTX administration, please note:
    i. If a dose modification of a chemotherapeutic agent has been carried out, the dose modification does apply to the entire further course of therapy.
    ii. If on the day of therapy administration (day 1, 8 or 15), a toxicity does not allow the administration of the causative chemotherapeutic substance, the administration of this substance is cancelled on this day. If the criteria for resuming the therapy are met on the next day of therapy administration (day 1, 8 or 15) the therapy may be resumed in a modified form (see below for details).
    iii. If CTX (either Paclitaxel/Carboplatin or Epirubicin/Cyclophosphamide) had been interrupted (and Atezolizumab therapy continued), the CTX doses (day 1, 8 or 15) must not be made up and thereby extending the length of an Atezolizumab cycle beyond day 21. Rather, the following cycle will resume Atezolizumab combination therapy with either Paclitaxel/Carboplatin or Epirubicin/Cyclophosphamide on day 1.

2. If a **side effect of Atezolizumab** is assumed (i.e., in particular an immunologic side effect) and therapy with Atezolizumab is interrupted (see current IB of Tecentriq®), Atezolizumab is omitted as the leading substance and CTX is to be continued independently. In this case, the duration and sequence of therapy shall be based on 12 weekly doses of "Paclitaxel/Carboplatin" or four 3-weekly doses of "Epirubicin/Cyclophosphamide".

In the event, that the start of a new cycle is delayed due to treatment-related toxicity, procedures required on day 1 (as per SOA) of the given cycle will be performed when either Atezolizumab or

CTX is resumed. New cycle day 1 procedures that were performed prior to knowing the need to delay the start of the cycle do not need to be repeated:

- if not required to determine whether study drug may be resumed and
- If performed within 7 days prior to study drug resumption.

If the AE that led to the treatment interruption recovers within the same cycle, then re-dosing in that cycle is allowed. Doses omitted for toxicity are not replaced within the same cycle.

In the event of a treatment interruption for reasons other than treatment-related toxicity (e.g., non-cancer related surgery) lasting > 3 weeks, treatment resumption will be decided in consultation with the sponsor (Medical Monitor (MM)).

7.4.3. Paclitaxel and Carboplatin Dosage and Modifications

7.4.3.1. Carboplatin dosage

Carboplatin dosage is based on Area under the curve (AUC). AUC can be calculated using the following mathematical formula, which includes renal function. This will reduce the risk of overdosing or under dosing because of individual differences in renal function.

-
- Formula according to Calvert:

  Total dose (mg) = (target AUC*) x (GFR {glomerular filtration rate} + 25)

  For more information, refer to the SmPC.

7.4.3.2. Dose reductions/modifications of Paclitaxel and Carboplatin

Treatment should be delayed for at least 1 week for an absolute neutrophil count less than 1.0 x 10^9/L and /or a platelet count less than 100 x 10^9/L. For resumption of Paclitaxel/Carboplatin therapy, absolute neutrophile count has to be ≥ 1.5 x 10^9/L and platelets ≥ 100 x 10^9/L and other treatment-related hematological and treatment-related non-hematological toxicity need to be resolved to ≤ grade 1 (except for alopecia and fatigue for which resolution is not required). If Paclitaxel/Carboplatin is delayed for at least 1 week due to treatment-related toxicity, dose reduction of the presumably causative medication may be considered. Should neutrophil count and/or platelet count persist below the value of absolute neutrophil count less than 1.0 x 10^9/L and/or a platelet count less than 100 x 10^9/L for more than 14 days, please contact the sponsor (MM).

If dose reductions of either of the two drugs are indicated, the dose should be reduced by one dose-level. The following dosing levels are applicable (see table 3 below):

Table 3: Dosing level reduction for Paclitaxel and Carboplatin*

	Paclitaxel (mg/m^2)	Carboplatin (AUC)
Level 0 (initial dose):	80	2.0
Level -1	64	1.6

* See also general information given for persistent CTX related toxicity under 7.4.

If toxicity does not resolve during a 14-day monitoring interval, drug exposure should be interrupted with continued monitoring for an additional 14 days.

If treatment is being interrupted for more than 21 days, the investigator must contact the sponsor and the subject's condition needs to be reviewed before therapy may be resumed.

Detailed recommendations for dose interruptions/modifications in case of specific treatment-emergent AEs are provided in the following sections.

Subjects experiencing any of the following toxicities during the previous cycle should have their CTX reduced for all subsequent cycles by 1 dose level as outlined in table 4 (below):

Table 4: Dose modifications for Paclitaxel and/or Carboplatin

Dose Modifications for Carboplatin and Paclitaxel (a)	
Toxicity	**Adjustment for treatment component believed to be associated with specific toxicity, continue other treatment component per protocol**
ANC < 0.5 x 10^9/L for ≥ 5 days	Decrease 1 level (b)
Febrile neutropenia (≥ 38.5 °C) associated with ANC < 1.0 x 10^9/L	Decrease 1 level (b)
≥ Grade 3 thrombocytopenia or in presence of significant bleeding or requiring blood transfusion ***at first occurrence***	Decrease 1 level (b)
Grade 2 sensory neuropathy lasting > 7 days	Withheld Paclitaxel till neuropathy improves to ≤ grade 1 and decrease 1 level
Grade 3 sensory neuropathy	Withheld Paclitaxel. Treatment may be resumed at a reduction of 1 level in subsequent cycles after neuropathy improves to ≤ grade 1
Grade 4 sensory neuropathy	Withheld Paclitaxel. Treatment may be resumed at a reduction of 1 level in subsequent cycles after neuropathy improves to ≤ grade 1. If neuropathy does not improve to ≤ grade 1 within 6 weeks, discontinue treatment
Abnormal Bilirubin value: Grade 1 Grade 2 Grade 3 or 4	 Re-test bilirubin value every week, continue study treatment Hold treatment until improvement to grade 1. Restart treatment at a lower dose level Discontinue treatment
Abnormal AST/ALT values (c): Grade 1 Grade 2 Grade 3 or 4	 Continue study treatment Hold Paclitaxel until improvement to Grade 1. Restart Paclitaxel at a lower dose level Discontinue Paclitaxel
Renal toxicity ≥ 2 (serum creatinine > 1.5 x ULN)	Recalculate Carboplatin dose to AUC 1.6
Weight change ≥ 10% from baseline	Recalculate Carboplatin dose
Other grade ≥ 3 toxicities (d)	Adjust dose or discontinue therapy as medically indicated after discussion with sponsor

(a): Despite adequate/maximal medical intervention and/or prophylaxis
(b): platelets have to recover to ≥ 100 x 10^9/L (and neutrophils have to be ≥ 1.5 x 10^9/L) before the start of the next cycle. If platelets have not recovered at day 35, discontinue treatment.

(c): In the case of **liver toxicity under Paclitaxel**, it must be examined in individual cases, depending on the clinical situation, whether further laboratory tests or, if necessary, invasive diagnostics (liver biopsy) can exclude other triggering factors of an increase in transaminases.
(d): Except grade 3 fatigue, transient joint or muscle pain for which no dose modifications are required.

7.4.3.3. Hypersensitivity reactions to Paclitaxel

If hypersensitivity reactions occur, minor symptoms (flushing, skin reactions, lower back pain, hypotension, tachycardia) might require temporary interruption of application. In case of severe reactions (hypotension/dyspnea/requiring medication, angioedema, generalized urticaria) immediate discontinuation of study drug administration is required.

In case of severe hypersensitivity reactions, Paclitaxel should not be re-challenged.

7.4.4. Epirubicin and Cyclophosphamide

Treatment should be delayed for at least 1 week for an absolute neutrophil count less than 1.0 x 10^9/L and /or a platelet count less than 100 x 10^9/L. For resumption of therapy, the absolute neutrophile count has to be ≥ 1.5 x10^9/L and treatment-related non-hematological toxicity has to be resolved to ≤ grade 1 (except for alopecia and fatigue for which resolution is not required).). Should neutrophil count and/or platelet count persist below the above-mentioned value for more than 14 days, please contact the sponsor.
If dose reductions of either of the two drugs are indicated, the dose should be reduced by one dose-level. The following dosing levels are applicable:

Table 5: Dosing level reduction for Epirubicin and Cyclophosphamide*

	Epirubicin (mg/m^2)	Cyclophosphamide (mg/m^2)
Level 0 (initial dose):	90	600
Level -1	75	450

* See also general information given for persistent CTX related toxicity under 7.4.

If toxicity does not resolve during a 14-day monitoring interval, drug exposure should be interrupted with continued monitoring for an additional 14 days.

If treatment is being interrupted for more than 21 days, the investigator must contact the sponsor and the subject's condition needs to be reviewed before therapy may be resumed.

Detailed recommendations for dose interruptions/modifications in case of specific treatment-emergent AEs are provided in the following sections. If subjects experience any of the following toxicities during the previous cycle should have their CTX reduced for all subsequent cycles by 1 dose level as outlined in table 6 (below):

Table 6: Dose modifications for Epirubicin/Cyclophosphamide

Dose Modifications for Epirubicin and Cyclophosphamide (a)	
Toxicity	**Adjustment for treatment component believed to be associated with specific toxicity, continue other treatment component per protocol**
ANC < 0.5 x 10^9/L for ≥ 5 days	Decrease 1 level (b)
Febrile neutropenia (≥ 38,5 °C) associated with ANC < 1.0 x 10^9/L	Decrease 1 level (b)
≥ Grade 3 thrombocytopenia or in presence of significant bleeding or requiring blood transfusion ***at first occurrence***	Decrease 1 level (b)
Abnormal Bilirubin value: Grade 1 Grade 2 Grade 3 or 4	 Re-test bilirubin value every week, continue study treatment Hold treatment until improvement to grade 1. Restart treatment at a lower dose level Repeat biochemical tests every two days; liver ultrasound should be performed immediately. Call the sponsor to discuss the case
Abnormal AST/ALT values: Grade 1 Grade 2 Grade 3 or 4	 Continue study treatment Hold treatment until improvement to grade 1. Restart treatment at a lower dose level Discontinue treatment
Mucositis and dysphagia	May occur with Epirubicin administration. Temporary withhold Epirubicin if these side effects are moderate to severe (≥ grade 2), but re-institute full dose once they resolve
Cystitis	May occur with Cyclophosphamide administration. Temporary withhold Cyclophosphamide if cystitis is moderate to severe. Encourage the patient to drink large amounts of water; if urine culture is positive, antibiotics will be given
Cardiac toxicity	The maximum cumulative dose of Epirubicin is 900 mg/m^2. The maximum cumulative dose planned in the neoMono trial is 360 mg/m^2. At this cumulative dose cardiac effects are infrequent. Epirubicin will be discontinued if: 1. CHF appears; 2. Persistent arrhythmia (including sinus tachycardia with no demonstrable cause) appears;

	3. Asymptomatic decrease of LVEF to below 45% Call the sponsor to discuss the case
Other grade ≥ 3 toxicities (c)	Adjust dose or discontinue therapy as medically indicated after discussion with sponsor

(a): Despite adequate/maximal medical intervention and/or prophylaxis
(b): platelets have to recover to ≥ 100 x 10^9/L (and neutrophils have to be ≥ 1.5 x 10^9L) before the start of the next cycle. If platelets have not recovered at day 35, discontinue treatment.
(c): Except grade 3 fatigue, transient joint or muscle pain for which no dose modifications are required.

7.4.5 QT corrected interval (QTc) prolongation management

In the event of QTc prolongation of > 480 and ≤ 500 ms, possible reversible causes such as serum electrolytes abnormalities, or usage of concomitant medications with the potential to prolong the QTc interval should be evaluated. If such reversible causes are identified, then they should be corrected accordingly (i.e. correction of electrolyte abnormalities with supplements to within normal limits and/or discontinuation - if possible - of concomitant medications with the potential to prolong the QT interval).

If the QTc remains > 480 ms and ≤ 500 ms for more than 2 cycles, or if grade 2 QTc prolongation recurs in the absence of other alternative causes or despite correction of alternative causes, discontinuation should be considered in consultation with a cardiologist and the study Medical Monitor.

7.5. Method of treatment assignment

Pre-Coded central Randomization

Upon successful screening, participants will be assigned a unique number (subject ID) in ascending numerical order and will be randomized centrally to one of the 2 arms of the study with a randomization ratio of 1:1, according to the randomization schedule generated prior to the study by the Statistics Department at palleos healthcare GmbH. Randomization will be realized by permuted block design, stratified by PD-L1 IC-status and anatomic tumor stage (AJCC 8th edition Anatomic Stage Groups I, II and III).

7.6. Blinding

This is an open-label study with no blinding at site level. Potential bias will be reduced by organizing a central randomization as described in section 7.5.

7.7. Preparation/Handling/Storage/Accountability

Refer to section 7.1 for the definition of IMP.

7.7.1. Drug packaging, labeling, dispensing and storage

7.7.1.1. Packaging and labeling

The Roche-provided Atezolizumab will be labeled study-specific for the neoadjuvant therapy phase in patients randomized to Arm A (2-week-window of Atezolizumab mono-therapy followed by 12 weeks of Atezolizumab + Carboplatin + Paclitaxel) and then by 12 weeks of Atezolizumab + Epirubicin + Cyclophosphamide and for Arm B (12 weeks of Atezolizumab + Carboplatin + Paclitaxel) and then by 12 weeks of Atezolizumab + Epirubicin + Cyclophosphamide. It will be labelled at Roche and sent directly to the study site.

Carboplatin, Paclitaxel, Epirubicin and Cyclophosphamide administered during the neoadjuvant phase are considered as IMPs. Therefore, these commercial drugs will be labelled study-specifically by the central pharmacy and provided to the study sites.

7.7.1.2. Dispensing and storage

For preparation of the CTX, which is commercial product, solutions and storage, please refer to the SmPCs of the drugs.

For Atezolizumab preparation, see the local prescription information and section 7.3.2.

- Keep the vial in the outer carton in order to protect from light. Vials of Atezolizumab are shipped at a temperature ranging from 2°C - 8°C and must be placed in a refrigerator (at the same temperature range) immediately upon receipt and should remain refrigerated until immediately prior to use. Temperature logs must be maintained on the refrigerator (in accordance with local pharmacy practice).
- If a temperature deviates from the allowed 2 - 8°C range either during shipment or storage, contact the sponsor to determine if the drug is still appropriate for use. The vials must not be frozen or shaken. Store the vials within the outer carton and protect them from light. The medication must not be used beyond the use by date provided on the outer carton.

The following general rules will be applied for all study medication:

- Storage and dispensation of study medication must be carefully documented by the investigator.
- The investigator will confirm receipt of the first and all subsequent batches of study drugs in writing to the sponsor. The investigator or designee must confirm appropriate temperature conditions have been maintained during transit for all study treatment received and any discrepancies are reported and resolved before use of the study treatment.
- All drug supplies must be stored in accordance with the manufacturer's instructions, and separately from normal clinic stocks present at the study site. The investigator is responsible for assurance of adequate storage, protected from exposure to any environmental changes. Moreover, the study medication must be stored in a lockable room or locker, so that only the investigator and specifically designated study personnel can have access.
- Only participants enrolled in the study may receive study treatment and only authorized site staff may supply or administer study treatment.
- Documentation of preparation and distribution of the study medication has to be documented in accordance with the SmPCs (prescribing information/ "Fachinformation").

7.7.2. Drug accountability and compliance

- Atezolizumab as well as the standard CTX agents will be administered by the study staff during patient's visits and accountability will be documented and recorded in the CRF.
- Accountability will be assessed by maintaining adequate drug dispensing and return records for all study treatments. Any dose modification must be recorded.
- The drug records must contain documentation of drug shipments received by the sponsor (date and quantity received).
- The drug dispensing log must be current and contain:
    - The study number of the patient to whom this drug was administered
    - The date(s) and quantity of the study medication administered to the patient.
- Copies of the dispensing and inventory logs must be available for inspection by the monitor.
- All used and partially used Atezolizumab vials and chemotherapeutic agents must be destroyed either on-site or per site's specific procedures for handling and disposing of hazardous drugs. The specific procedures for destructions of IMPs are to be provided to the monitor for review.
- Unused Atezolizumab vials will be disposed only according to the process approved by the provider (Roche). Vials that are not opened will be returned according to the process established by Roche. Partially used vials may only be destroyed upon written approval from the provider Roche. The release of Atezolizumab in the environment should be minimized. Any unused medicinal product or waste material should be disposed also in accordance with local requirements.
- Written documentation of destruction must contain the following:
    - Identity (batch number, patient number) of investigational products destroyed
    - Quantity of investigational products destroyed
    - Date of destruction
    - Method of destruction
    - Name and signature of the responsible person who discarded the investigational product.

7.8. Concomitant therapy

- Any existing concomitant medication not compatible with study medication has to be checked and excluded during the neoadjuvant phase where study medication is administered.
- Any medication or vaccine (including over-the-counter or prescription medicines, vitamins, and/or herbal supplements) that the participant is receiving at the time of enrollment or receives during the study must be recorded along with:
    - Reason for use
    - Dates of administration including start and end dates
    - Dosage information including dose and frequency

The MM should be contacted if there are any questions regarding concomitant or prior therapy.

- Concomitant therapy during targeted treatment for permitted prophylactic premedication: premedication for nausea and infusion reactions (e.g., acetaminophen or other analgesics, anti-histamines such as diphenhydramine or corticosteroids) may be given at the investigator's discretion.

- Ancillary treatments will be given as medically indicated. Any concomitant medication must be documented in the CRF.

7.8.1. Interaction with other medicinal products and other forms of interaction

- No formal pharmacokinetic drug interaction studies have been conducted with Atezolizumab. Since Atezolizumab is cleared from the circulation through catabolism, no metabolic drug-drug interactions are expected.

- The use of systemic corticosteroids or immunosuppressant before starting Atezolizumab should be avoided because of their potential interference with the pharmacodynamic activity and efficacy of Atezolizumab. However, the use of Paclitaxel in the study necessitates the use of Dexamethasone at 8 mg and this will be allowed (has to be below 10 mg). Systemic corticosteroids or other immunosuppressant can be used to treat immune-related adverse reactions after starting Atezolizumab.

7.8.2. Prophylactic premedication regimen for treatment with chemotherapeutic agents

Proposed premedication regimens for the administration of chemotherapeutic agents should be applied according to local SOC.

7.8.3. Use of prophylactic antibiotics and Granulocyte colony stimulating factor (G-CSF) with CTX

Primary/secondary G-CSF and antibiotics prophylaxis should be given according to SOC as defined by S3-guideline and recommendation of the AGO Mamma (in its latest version). No primary G-CSF prophylaxis is indicated in association with Atezolizumab.

7.8.4. Radiation

Patients will be treated according to local SOC.

7.8.5. Other anti-cancer treatments

Administration of further antitumor therapy (i.e. in case of disease progression or persistence of residual tumor after neoadjuvant CTX) after study medication is completed or stopped is at the discretion of the investigator. Therapy should be documented in the CRF accordingly.
In case of relapse after surgery (i.e. during follow-up) further treatment again is at the discretion of the investigator and should be documented in the CRF.

8. Discontinuation/Withdrawal criteria

8.1. Discontinuation of study treatment

- The term "interruption" refers to a patient stopping the study treatment during the course of the study, but then re-starting it at a later time in the study. The reason for dosing interruption will be collected on the appropriate CRF.
- The term "discontinuation" or withdrawal refers to a patient's withdrawal from the study treatment after baseline visit and administration to study drugs in Arm A or B until the planned end of the study (up to 24 months after baseline).
- The reason for discontinuation from treatment will be collected on the appropriate CRF.
- Patients may withdraw from the study at any time at their own request, or they may be withdrawn at any time at the discretion of the investigator or sponsor for safety or behavioral reasons, or the inability of the patient to comply with the protocol-required schedule of study visits or procedures at a given study site.
- Patients must be withdrawn from the active treatment phase in case of:
    - Disease progression
    - Symptomatic deterioration (i.e., global deterioration of health status without objective evidence of disease progression)
    - Need for new or additional anticancer therapy not specified in the protocol
    - Unacceptable toxicity
    - Investigator’s conclusion that discontinuing therapy is in the patient’s best interest
    - Lost to follow-up
    - Withdrawal of patient consent (follow-up permitted by patient or request of cessation of follow-up)
    - Death
- The investigator should inquire about the reason for withdrawal, request that the patient returns for a final visit, if applicable, and follow-up with the patient regarding any unresolved AEs.
- Patients who discontinue the active treatment phase (neoadjuvant treatment phase) should have end of treatment/withdrawal evaluations performed as soon as possible but no later than 4 weeks from the last dose of investigational products and prior to initiation of any new anticancer therapy. Data to be collected for the end of study treatment/withdrawal visit are described in the SOA tables. Unless a patient actively withdraws consent, every effort should be made to continue collecting further endpoints and any auxiliary variables that may be informative with regard to missing values, if this is feasible in any way. These data are required to apply the intention-to-treat principle and to handle missing values in the statistical analysis.

- If a patient opts to discontinue from the active treatment phase as a result of an unacceptable adverse drug reaction, "withdrawal of consent" should not be the reason for discontinuation. Instead, the reason for discontinuation of active treatment phase must be recorded as "Unacceptable toxicity".
- If the patient withdraws from the study, and also withdraws consent for disclosure of future information, no further evaluations should be performed, and no additional data should be collected. The sponsor may retain and continue to use any data collected before such withdrawal of consent.

8.2. Lost to follow-up

If a patient does not return for a scheduled visit, every effort should be made to contact the patient. After three unsuccessful attempts to contact the patient, the patient should be considered “lost to follow-up”.

The following actions must be taken if a participant fails to return to the clinic for a required study visit:

- The site must attempt to contact the patient and reschedule the missed visit as soon as possible and counsel the patient on the importance of maintaining the assigned visit schedule and ascertain whether or not the patient wishes to and/or should continue in the study.
- Before a patient is deemed lost to follow up, the investigator or designee must make every effort to regain contact with the patient (where possible, 3 telephone calls and, if necessary, a certified letter to the patient’s last known mailing address or local equivalent methods). These contact attempts should be documented in the patient’s medical record.

9. Study assessments and procedures

9.1. Screening

Table 7: Study assessments during screening

	INVESTIGATIONS	**TIMING**
Patient informed consent	Obtained	Prior to study entry[1]
Diagnostic core biopsy	Obtained	Prior to randomization
TNBC status	✔	Prior randomization
Anatomic tumor stage group I, II, III	✔	Within 4 weeks prior to screening
Ultrasound status	✔	Within 4 weeks prior to screening
Demographic information, Medical	History including: • Diagnosis of unilateral primary invasive breast cancer from core biopsy	Within 7 days prior to screening

History and physical examination	• Assessment of cN and cT status • Receptor status (Triple negative) at diagnosis • Menopausal status and highly effective contraceptive measures • General medical history including cardiac history and allergy • Concurrent illness and existing signs and symptoms • Concomitant medications and their indication used within one month prior to study entry Physical examination as defined in section 9.10.1	
Imaging	Mandatory for all patients (as per SOC defined by S3-guideline and recommendations of the AGO Mamma). • Contralateral mammography and ultrasound (Breast, lymph nodes) • CT scans • Bone scan; additional bone X-ray in case of hot spots in bone scan in high risk patients • Liver imaging (CT) Other instrumental examinations as indicated by radiologist.	≤ 3 months prior to randomization and part of SOC
Laboratory	Hematology and Biochemistry tests as defined in appendix 2 Blood sample for translational research (refer to section 9.11)	≤ 14 days prior to screening
	Pregnancy test urine or serum (if applicable)	≤ 7 days prior to screening
Clinical Assessment	Includes palpation and inspection of the breast	At screening visit
Check for adequate contraception measures (if applicable)	As defined in section 6.1	
ECG	ECG	≤ 6 weeks prior to screening
LVEF	Echocardiography	≤ 6 weeks prior to screening

- [1]Voluntary, dated, and signed informed consent must be obtained before any study specific procedures are performed (except certain imaging assessments, see this section below).
- All screening evaluations must be completed and reviewed to confirm that potential participants meet all eligibility criteria.
- The investigator will maintain a screening log to record details of all participants screened and to confirm eligibility or record reasons for screening failure, as applicable. Procedures conducted as part of the participant's routine clinical management (e.g. blood count, imaging,

etc.) and obtained before signing of the ICF may be utilized for screening or baseline purposes provided the procedures met the protocol-specified criteria and were performed within the time frame defined in the SOA. For timing of baseline examinations and examinations during treatment please refer to table 1.

- Physical examination will include:
  - ✓ Complete physical examination as defined in section 9.10.1
  - ✓ Height and Weight
  - ✓ Vital signs (heart rate, respiratory rate, blood pressure, and temperature)
  - ✓ ECOG or Karnofsky index (KI) for performance status. The score used at screening has to be used during the whole study for one patient
  - ✓ Clinical tumor assessment

- Laboratory work-up will include hematology and biochemistry as defined in appendix 2.
- Blood sample for translational research as defined in 9.11.
- Radiographic tumor assessments that were performed before signing the informed consent form (ICF) as routine procedures (but within 3 months prior to screening) do not need to be repeated and may be used as baseline assessments.
- The windows permitted for the various procedures are described in the table 1.
- A postmenopausal state is defined as no menses for 12 months without an alternative medical cause. A high follicle stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a post-menopausal state in women not using hormonal contraception or hormonal replacement therapy. However, in the absence of 12 months of amenorrhea, a single FSH measurement is insufficient.
- Adequate contraceptive measures will be checked and documented in premenopausal patients:
  - IUD
  - bilateral tubal occlusion
  - vasectomised partner
  - sexual abstinence

- Pregnancy testing:

  For women of childbearing potential, a serum or urine pregnancy test will be performed at screening visit within 7 days prior that visit. A negative pregnancy result is required before the patient may receive the study treatment (baseline visit, day 1 of treatment). If at baseline visit, the pregnancy test is older than 10 days, it has to be redone. No routine pregnancy test will be carried out in postmenopausal. Pregnancy tests will also be done whenever a potential pregnancy is suspected. In the case of a positive human choriongonadotropin (hCG) test, the patient will be withdrawn from study treatment but will remain on study for follow up until birth.

9.2. Screen failures

- Screen failures are defined as participants who consent to participate in the clinical study but are not subsequently randomly assigned to study treatment. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants to meet the Consolidated Standards of Reporting Trials publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any serious adverse event (SAE).
- In this protocol the screen failure rate is estimated to be 10%.
- Patients for whom the TNBC diagnosis performed by the local laboratory is not confirmed by the centralized pathology laboratory with be considered as screen failures and will not be randomized.
- Individuals who do not meet the criteria for participation in this study (screen failure) cannot be rescreened.

9.3. Randomization and active treatment phase

9.3.1. Randomization

The principal investigator will check all inclusion/exclusion criteria and if all inclusion/exclusion criteria are met, the patient can be randomized to Arm A or B through the electronic data capture (EDC) system. Randomization will be stratified by PD-L1 IC-status and anatomic tumor stage (AJCC 8th edition Anatomic Stage Groups I, II and III).

The investigator will then order the relevant treatment as described below and in parallel the investigator will contact the patient for the baseline visit:

- Atezolizumab will be ordered by the study site to Roche directly as per SOA.

- The CTX agents (Paclitaxel, Carboplatin, Epirubicin and Cyclophosphamide) will be sent to the central pharmacy and dispatched to the investigator upon request.

9.3.2. Baseline visit: day 1 of treatment

- Day 1 of therapy: Arm A: Atezolizumab 840 mg and Arm B: Atezolizumab 1200 mg + Paclitaxel 80 mg/m^2 + Carboplatin AUC of 2 IV
- Physical examination
- Clinical assessment
- Pregnancy test (should not be older than 10 days, otherwise it has to be redone)
- Blood sample for hematology and biochemistry and for translational research
- Safety assessment & Concomitant medication

9.3.3. Neoadjuvant visits after baseline

Patients randomized to Arm A will have 24 visits after baseline; patients randomized to Arm B will have 23 visits after baseline. Refer to tables 1a and 1b SOA tables for Arm A and Arm B for details of assessments.

9.3.4. End of treatment (EOT) visit in the neoadjuvant phase:

The EOT visit will be performed 3 weeks after last dose of CTX (Epirubicin-Cyclophosphamide), i.e.; week 29 in Arm A and at week 27 in Arm B.

The following procedures will be performed:

- Physical examination
- Ultrasound (breast, lymph nodes)
- Clinical assessment
- Pregnancy test
- LVEF
- ECG
- Laboratory (hematology, biochemistry)
- Blood sample for translational research
- Safety assessment & concomitant medication

Please note that Safety assessment and concomitant medication are checked at every patient visit. For details on procedures during the active treatment phase, see SOA table 1.

9.4. Ultrasound assessment

The tumor (marker lesion) is measured in all three dimensions. The two longest diameters must be documented. Progressive disease (PD) is defined as ≥ 20% increase of at least 5 mm in the sum of the longest diameters of the target lesions compared with the smallest sum of the longest diameters recorded. In case of PD, the therapy should be changed or surgery performed at discretion of the investigator.

Response will be evaluated by clinical and ultrasound assessment as SOC throughout the study. The tumor needs to be marked with a clip before the first cycle of CTX to be able to reliably identify the region of the former tumor at the time of surgery.

9.5. Surgery

Surgery will be performed 3 - 4 weeks after last dose of the neoadjuvant treatment (week 29 - 30 for Arm A and week 27 - 28 for Arm B). Refer to section 5.3. A surgery sample of the breast (paraffin embedded) will be sent to the central pathology for analysis.

9.6. Adjuvant therapy and Follow-up visits

- All visits will be performed as SOC. During these visits, the following will be assessed: physical examination, clinical assessment, ultrasound, (breast, lymph nodes), routine blood tests.
- In addition, for childbearing potential women, pregnancy test will be performed every 4 weeks until 5 months following the last dose of Atezolizumab:

    - The investigator will prescribe at the EOT visit of the neoadjuvant therapy phase, a monthly pregnancy test for 5 months.
    - The patient will perform the pregnancy test as prescribed and communicate monthly the result to the investigational site staff.
    - The investigational site staff will ensure that the information is received monthly and will also question the patient on the maintenance of the adequate contraceptive measures. The pregnancy test result and the information on the maintenance of the adequate contraceptive measures will be documented in the patient records and in the CRF.

- If patients are receiving follow-up care at their local gynecologist/oncologist, the study follow-up visits will be performed by the study site staff by phone (see 5.5).

Safety assessment, adjuvant therapy and concomitant medication will be assessed at all visits.

9.7. End of Study visit

EOS visit will be performed at month 24 (week 104) after randomization or earlier in case of study discontinuation. The following procedures will be performed as SOC:

- Physical examination
- Ultrasound (breast, lymph nodes)
- Clinical assessment
- Safety assessment and concomitant medication

If the end-of-study examination according to SOC take place at the patient's local oncologist/ gynecologist after 24 months, the site will contact the patients by phone to document the parameter listed under Section 5.5.

9.8. Efficacy assessments: primary, secondary and translational efficacy parameters

Mapping of endpoints, as defined below, to objectives:

Table 8: Mapping Objectives to Endpoints

Objective		Endpoints
Primary	1.	a
Secondary	1.	b
	2.	c
	3.	d,e,f
	4.	i , j, k, l, m
	5.	i , j, k, l, m
	6.	a
	7.	n
	8.	o
	9.	p
Translational	1.	a, z
	2.	a, r
	3.	a, s
	4.	a, s
	5.	a, t
	6.	a, t
	7.	t, r
	8.	a, r
	9.	a, r
	10.	a, u, v, w, x, y
	11.	a, z
	12.	n, o, i, j, k, l
	13.	a, n, o, g, h, q

9.8.1. Primary endpoint: Pathological complete response

a) pCR defined as no residual invasive tumor cells in the breast and in the lymph nodes (ypT0/is, ypN0)

9.8.2. Secondary Endpoints

b) Safety (incidence, relationship, seriousness, and severity of all AEs, SAEs, adverse events of special interest (AESIs) coded by MedDRA, summarized by Preferred Term and System Organ Class and graded according to CTCAE 5.0)
c) pCR defined as no residual invasive tumor cells in the breast and in the lymph nodes (ypT0/is, ypN0) in patients with an ER/PR expression of < 1% and an ER/PR expression of 1% to 10%.
d) pCR defined as no tumor cells (invasive or non-invasive) in the breast and in the lymph nodes (ypN0, ypT0)
e) Near pCR defined as residual tumor < 5 mm in the breast irrespective of *in-situ* and lymph nodes status
f) pCR defined as no invasive tumor in the breast, irrespective of lymph node status

g) Decrease of Ki-67 expression versus baseline after 14/28 days (+/- 2 days) of treatment as continuous predictor
h) TILs after 14/28 days (+/- 2 days) of treatment as continuous predictor
i) CCCA: Ki-67 expression ≤ 2.7% after 14/28 days (+/- 2 days) of treatment
j) Low cellularity: < 500 tumor cells after 14/28 days (+/- 2 days) of treatment
k) Decrease of Ki-67 expression versus baseline by 30% or more after 14/28 days (+/- 2 days) of treatment
l) TILs ≥ 60% after 14/28 days (+/- 2 days) of treatment
m) Combined early response defined by
    - CCCA Ki-67 expression ≤ 2.7% or
    - low cellularity or
    - decrease of Ki-67 expression (versus baseline) by 30% or more or
    - TILs ≥ 60%
n) DFS[1] defined as time from the first date of no disease [i.e. date of surgery] to the first occurrence of disease recurrence or death from any cause
o) OS defined as length of time from randomization to death from any cause
p) EFS defined as length of time after randomization till death from any cause, failure to achieve remission after induction therapy, relapse in any site, or second malignancy

1 DFS is defined as time from surgery to:

- Ipsilateral invasive breast tumor recurrence (i.e., an invasive breast cancer involving the same breast parenchyma as the original primary lesion)
- Ipsilateral local-regional invasive breast cancer recurrence (i.e., an invasive breast cancer in the axilla, regional lymph nodes, chest wall, and/or skin of the ipsilateral breast)
- Distant recurrence (i.e., evidence of breast cancer in any anatomic site – other than the two abovementioned sites – that has either been histologically confirmed or clinically diagnosed as recurrent invasive breast cancer)
- contralateral invasive breast cancer
- Ipsilateral or contralateral DCIS
- Second primary non-breast invasive cancer (with the exception of non-melanoma skin cancers and *in-situ* carcinoma of any site)
- Death attributable to any cause including breast cancer, non-breast cancer, or unknown cause (but cause of death should be specified if at all possible)

9.8.3. Additional Translational Endpoints

q) CelTIL score as defined by (Nuciforo et al., 2017)
r) Immune markers (e.g. PD-1/L1) *via* ctDNA
s) Intrinsic subtype continuous ER/PR/HER2 expression
t) Specific DNA panel
u) Ki-67 expression as a continuous variable after 14 days (+/- 2 days) of treatment with Epirubicin and Cyclophosphamide in patients with less than 50% tumor shrinkage after 4 cycles of Carboplatin/Paclitaxel/Atezolizumab +/- the 2-week Atezolizumab window measured by tumor volume (or if not assessable by volume a decrease by 50% in diameter) through sonographic assessment

v) TILs as a continuous variable after 14 days (+/- 2 days) of treatment with Epirubicin and Cyclophosphamide in patients with less than 50% tumor shrinkage after 4 cycles of Carboplatin/Paclitaxel/Atezolizumab +/- the 2-week Atezolizumab window
w) CCCA: Ki-67 expression ≤ 2.7% after 14 days (+/- 2 days) of treatment with Epirubicin and Cyclophosphamide in patients with less than 50% tumor shrinkage after 4 cycles of Carboplatin/Paclitaxel/Atezolizumab +/- the 2-week Atezolizumab window
x) Low cellularity: < 500 tumor cells after 14 days (+/- 2 days) of treatment with Epirubicin and Cyclophosphamide in patients with less than 50% tumor shrinkage after 4 cycles of Carboplatin/Paclitaxel/Atezolizumab +/- the 2-week Atezolizumab window
y) CelTIL score as defined by (Nuciforo et al., 2017) after 14 days (+/- 2 days) of treatment with Epirubicin and Cyclophosphamide in patients with less than 50% tumor shrinkage after 4 cycles of Carboplatin/Paclitaxel/Atezolizumab +/- the 2-week Atezolizumab window
z) Genome-wide gene expression analysis for RNA-based biomarker signature related to response/resistance to Atezolizumab

9.9. Adverse Events

The definitions of an AE or SAE can be found in Appendix 8.

AEs will be reported by the participant (or, when appropriate, by a caregiver, surrogate, or the participant's legally authorized representative) to the investigator.

The investigator and any designees are responsible for detecting, documenting, and recording events that meet the definition of an AE or SAE and remain responsible for following up all AEs and SAEs regardless of the event is serious or considered related to the study treatment or study procedures. For all AEs, sufficient information should be obtained by the investigator to determine the causality of the AE.

Subjects must be carefully monitored for AEs. This monitoring includes clinical laboratory tests. AEs should be assessed in terms of their seriousness, intensity, and relationship to the study drug and reported according to the NCI CTCAE v5.0.

All AEs have to be recorded in the patient’s medical record and on the AE CRF. Investigators should use correct medical terminology/concepts when recording AEs on the AE CRF and avoid colloquialisms and abbreviations. Only one AE term should be recorded in the event field on the AE CRF. All AE terms will be coded with the most recent MedDRA version by the sponsor.

As part of ongoing safety reviews conducted by the sponsor, any non-SAE that is determined by the sponsor to be serious will be reported by the investigator as an SAE. To assist in the determination of event seriousness further information may be requested from the investigator to provide clarity and understanding of the event in the context of the clinical study.

An isolated laboratory abnormality that is assigned grade 4, according to CTCAE v5.0 definition, is not reportable as SAE unless the investigator assesses that the event meets standard ICH criteria for an SAE.

The criteria for determining whether an abnormal test finding should be reported as an AE are as follows:

- The test result is associated with accompanying symptoms, and/or
- The test result requires additional diagnostic testing or medical/surgical intervention, and/or

- The test result leads to a change in study drug dosing or discontinuation from the study, and/or
- The test result leads to significant additional concomitant drug treatment, or other therapy, and/or
- The test result is considered to be an AE by the investigator or sponsor.

Merely repeating an abnormal test, in the absence of any of the above conditions, does not constitute an AE. Any abnormal test result that is determined to be an error does not require reporting as an AE.

If the clinically significant laboratory abnormality is a sign of a disease or syndrome (e.g. ALK and bilirubin 5 x the upper limit of normal associated with cholecystitis), only the diagnosis (e.g., cholecystitis) needs to be recorded on the AE CRF.

If the clinically significant laboratory abnormality is not a sign of a disease or syndrome (e.g. ALK and bilirubin 5 x ULN associated with cholestasis), the abnormality itself (i.e., cholestasis) should be recorded as an AE or SAE on the CRF.

If the laboratory abnormality can be characterized by a precise clinical term, the clinical term should be recorded as the AE or SAE. For example, an elevated serum potassium level of 5.2 mmol/L should be recorded as “hyperkalemia”.

AEs occurring secondary to other events (e.g., cascade events or clinical sequelae) should be identified by their primary cause, with the exception of severe or serious secondary events. A medically significant secondary AE that is separated in time from the initiating event should be recorded as an independent event on the AE CRF. For example:

- If diarrhea results in mild dehydration with no additional treatment required, it is sufficient to record only diarrhea in the AE CRF.
- If diarrhea results in severe dehydration, both events should be recorded separately on the CRF.
- If a severe gastrointestinal hemorrhage leads to a renal failure, both events should be recorded separately on the eCRF.
- If dizziness leads to a fall and a consequent fracture, all three events should be recorded separately on the eCRF.

However, medically significant AEs occurring secondary to an initiating event that are separated in time should be recorded as independent events on the CRF. For example, if a severe gastrointestinal hemorrhage leads to renal failure, both events should be recorded separately on the CRF.

AE without resolution between patient evaluation time points should only be recorded once in the eCRF with the highest CTC Grade occurring. A persistent AE is one that extends continuously for longer than one sequence of CTX (three month sequence of Carboplatin/Paclitaxel/ Atezolizumab or Epirubicin/Cyclophosphamide/Atezolizumab), without resolution between patient evaluation time points. Such events should only be recorded once in the eCRF, except if the common terminology criteria (CTC) grade changes after start of the next sequence of CTX. In such cases, an AE with a certain CTC grade must be completed with end date and outcome as soon as the CTC grade changes. Subsequently a new AE with the same event term and the current CTC grade should be documented.

A recurrent AE is one that occurs and resolves between patient evaluation time points and subsequently recurs. Each recurrence of an AE should be recorded as a separate event on the AE CRF.

9.9.1. Time Period and Frequency for Collecting AE and SAE Information

A preexisting medical condition that is present at the screening visit for this study should be recorded on the Medical History CRF.

A preexisting medical condition should be recorded as an AE only if the frequency, severity or character of the condition worsens during the study. When recording such events on the AE CRF, it is important to convey the concept that the preexisting condition has changed by including applicable descriptors (e.g., “more frequent headaches”).

After informed consent has been obtained **but prior to initiation of study treatment**, only SAEs caused by a protocol-mandated intervention (e.g., invasive procedures such as biopsies, discontinuation of medications) should be reported.

After initiation of study treatment, non-SAEs will be recorded until 30 days after the last dose of study treatment or until initiation of new anti-cancer therapy, whichever occurs first, and SAEs and AESIs will continue to be reported until 90 days after the last dose of study treatment or until initiation of new anti-cancer therapy.

All SAEs will be recorded in the AE CRF and reported to the sponsor within 24 hours of first awareness, or immediately upon awareness, if the SAE is fatal or life-threatening (i.e., causes an immediate risk of death) – regardless of the extend of available information. The above time frames also apply to any additional information (i.e., follow-up information) concerning previously submitted reports of a SAE.

Investigators are not obligated to actively seek AEs or SAEs in former study participants. However, if the investigator learns of any SAE and AESI, including a death, at any time after a participant has been discharged from the study, and he/she considers the event to be reasonably related to the study treatment or study participation, the investigator must promptly notify the sponsor.

The method of recording, evaluating, and assessing causality of AEs, SAEs and AESIs and the procedures for completing and transmitting SAE reports are provided in Appendix 8.

9.9.2. Method of Detecting AEs and SAEs

The investigator has to report all directly observed AEs and all spontaneously reported AEs by the study patient. In addition, each study patient will be questioned about AEs.

Care will be taken not to introduce bias when detecting AEs and/or SAEs. Open-ended and non-leading verbal questioning of the participant is the preferred method to inquire about AE occurrences.
At the last scheduled visit, the investigator should instruct each patient to report to the investigator any subsequent AEs that the patient’s personal physician believes to be possibly related to prior study treatment.

9.9.3. Follow-up of AEs and SAEs

After the initial AE/SAE report, the investigator is required to proactively follow each participant at subsequent visits/contacts. All SAEs will be followed until resolution, stabilization, the event is otherwise explained, or the participant is lost to follow-up. Further information on follow-up procedures is given in Appendix 8.

9.9.4. Regulatory Reporting Requirements for SAEs

The sponsor will promptly evaluate all SAEs and AESIs against cumulative product experience to identify and expeditiously communicate possible new safety findings to regulatory authority, Independent Ethics Committees (IEC), Marketing Authorization Holders, and investigators according to German regulatory requirements.

To determine reporting requirements for single AE cases, the sponsor will assess the expectedness of these events using the following reference documents:

- Atezolizumab IB
- Local prescribing information ("Fachinformation") for Paclitaxel, Carboplatin, Epirubicin, Cyclophosphamide

Investigator safety reports must be prepared for suspected unexpected serious adverse reactions (SUSAR) according to local regulatory requirements and sponsor policy and forwarded to investigators as necessary (fatal or life-threatening events within 7 calendar days, all other events within 15 calendar days).

An investigator who receives an investigator safety report describing a SAE or other specific safety information (e.g., summary or listing of SAEs) from the sponsor will review and then file it along with the IB or prescribing information/"Fachinformation" and will notify the IEC, if appropriate according to local requirements.

9.9.5. Reporting of SAEs

All SAEs, whether or not deemed drug-related or expected, must be reported by the investigator. Reporting has to be performed within 24 hours of first becoming aware of the event or immediately upon awareness if the SAE is fatal or life-threatening by means of EDC (RAVE).

In case the EDC is not available for the Investigator, the study-specific paper-based SAE Reporting Form has to be sent to the following address by fax or email:

palleos healthcare GmbH
Taunusstraße 5a
65183 Wiesbaden
Tel: +49 (0)611 950190 19
Fax: +49 (0)611 950190 29
Email: SAE@palleos.com

The sponsor will forward each SAE Report to the Drug Safety Department of Roche Pharma AG, Germany.

It is important that information regarding an SAE be reported within the established timelines. If a report is delayed, the reason for the delay should be clearly explained.

Examples of reasons for delay:

- Information missed due to clerical issues at the site
- Correction of previously transmitted information

There are no non-reportable protocol-specified SAEs in this study. All SAEs will be reported by the investigator as described above and will be managed accordingly in the safety database.

9.9.6. Reporting of AESIs

Certain types of adverse events (Adverse Events of Special Interest), as identified below, are reportable to the sponsor under the reporting processes and requirements for SAEs, even if they are not classified as serious. Like a SAE, an AESI has to be reported within 24 hours of awareness electronically in the EDC or, if the EDC is not available, on a SAE Reporting Form and followed up to determine outcome.

- Cases of potential drug-induced liver injury (DILI) that include an elevated ALT or AST in combination with either an elevated bilirubin or clinical jaundice, as defined by Hy's Law and based on the following observations:
  - Treatment-emergent ALT or AST > 3 x baseline value in combination with total bilirubin > 2 x ULN (of which ≥ 35% is direct bilirubin)
  - Treatment-emergent ALT or AST > 3 x baseline value in combination with clinical jaundice
- Suspected Transmission of Infectious Agents *via* a Medicinal Product, as defined below:
  - Any organism, virus, or infectious particle (e.g., prion protein-transmitting transmissible spongiform encephalopathy), pathogenic or non-pathogenic, is considered an infectious agent. A transmission of an infectious agent may be suspected from clinical symptoms or laboratory findings that indicate an infection in a patient exposed to a medicinal product. This term applies only when a contamination of study treatment is suspected.

- AESIs suggestive of potential immune-related aetiology:
  - Immune-related Pneumonitis
  - Immune-related Colitis
  - Immune-related Hepatitis, including AST or ALT > 10 x ULN
  - Immune-related Endocrinopathies: hypothyroidism, hyperthyroidism, adrenal insufficiency, hypophysistis, pancreatitis, type-1-diabetes mellitus)
  - Systemic lupus erythematosus
  - Immune-related Neurological disorders: Guillain-Barré syndrome, myasthenic syndrome/myasthenia gravis, meningoencephalitis
  - Events suggestive of hypersensitivity, infusion-related reaction, cytokine release syndrome, influenza-like illness, systemic inflammatory response syndrome, systemic immune activation, macrophage activating syndrome, hemophagocytic lymphohistiocytosis
  - Immune-related Nephritis
  - Immune-related Occular toxicities (e.g., uveitis, retinitis)
  - Immune-related Myositis
  - Myopathies, including rhabdomyolysis
  - Grade ≥ 2 cardiac disorders (e.g., atrial fibrillation, myocarditis, pericarditis)
  - Immune-related Myocarditis
  - Immune-related Vasculitis
  - Autoimmune hemolytic anemia
  - Severe cutaneous reactions (e.g., Stevens-Johnson syndrome, dermatitis bullous, toxic epidermal necrolysis)

Atezolizumab is associated with immune-related adverse reactions and there should always be a high level of suspicion that new symptoms are immune-mediated and related to treatment with Atezolizumab.

If an AE is suspected to be immune-related, thorough examination is necessary to confirm aetiology or to exclude other causes. The events listed above represent the currently identified

risks during treatment with Atezolizumab. Other AEs suggestive of potential immune-related aetiology grade ≥ 2 should be managed and reported in the same way.

9.9.7. Reporting of Special Situations

Certain events, as identified below, are reportable to the sponsor under the reporting processes and requirements for SAEs, even if they are not directly related to an AE. Like a SAE, such a *special situation* is to be reported within 24 hours of awareness electronically in the EDC or, if the EDC is not available, on a SAE Reporting Form and followed up to determine outcome.

- Overdose: This refers to the accidental or intentional administration of a quantity of an investigational medicinal product given per administration or cumulatively, which is above the maximum recommended dose according to the dose being studied per protocol.

- Misuse: This refers to situations where the investigational medicinal product is intentionally and inappropriately used by the patient in a manner that is not in accordance to the protocol.

- Abuse: This corresponds to the persistent or sporadic, intentional excessive use of the self-administered studied investigational medicinal product, which is accompanied by harmful physical or psychological effects.

- Medication Error: A medication error is an unintended deviation from the protocol, in the drug treatment process that leads to, or has the potential to lead to, harm to the patient. This would include medication errors (potential or confirmed) that were intercepted prior to the administration of the investigational medicinal product to the patient.

- Occupational exposure: This refers to an event that occurs when during performance of job duties, a person (whether a healthcare professional or otherwise) gets in unplanned direct contact with the product.

- Breastfeeding: This refers to a situation in which infants following exposure to a medicinal product from breast milk.

Reports of special situations may or may not include information about clinical consequences (i.e., AEs) that have to be recorded in the AE CRF

9.9.8. Reporting of Death Events

Deaths that occur during the protocol-specified AE reporting period will be recorded on a Death Report Form (DRF) in the EDC and in parallel expeditiously reported to the sponsor as a SAE Report. An independent DSMB will monitor the frequency of deaths from all causes.
When recording a death in the EDC, the event or condition that caused or contributed to the fatal outcome should be recorded as the single medical concept in the EDC. If the cause of death is unknown and cannot be ascertained at the time of reporting, record “Unexplained Death” on the DRF in the EDC.

9.9.9. Pregnancy

Pregnancy testing will be performed in premenopausal patients monthly during Atezolizumab treatment and until 5 months following the last dose of Atezolizumab.

Pregnancies and suspected pregnancies (including a positive pregnancy test regardless of age or disease state) of a female subject or the female partner of a male patient occurring while the subject is treated with study drug, or within 5 months of the subject's last dose of study drug, are considered reportable events. The pregnancy, suspected pregnancy, or positive pregnancy test shall be reported by the investigator to the sponsor on the paper-based Pregnancy Reporting Form to the following address within 24 hours:

palleos healthcare GmbH
Taunusstraße 5a
65183 Wiesbaden
Germany
Tel: +49 (0)611 950190 19
Fax: +49 (0)611 950190 29
Email: SAE@palleos.com

The sponsor will forward each Pregnancy Reporting Form to the Drug Safety Department of Roche Pharma AG, Germany.

The female subject may be referred to an obstetrician-gynecologist (not necessarily one with reproductive toxicity experience) or another appropriate healthcare professional for further evaluation.

The investigator will follow the female subject until completion of the pregnancy and must notify the sponsor immediately about the outcome of the pregnancy (either normal or abnormal outcome).

Any abortion should be classified as SAE (as the sponsor considers abortions to be medically significant), recorded on the AE CRF and reported to the sponsor immediately on SAE Reporting Form (according to section 9.9.5).

Any congenital anomaly/birth defect in a child born to a female patient or a female partner of a male study patient exposed to study treatment should be classified as SAE, recorded on the AE CRF and reported to the sponsor immediately on SAE Reporting Form (according to section 9.9.5).

Attempts should be made to collect and report infant health information. If the Authorization for the Use and Disclosure of Infant Health Information had been signed by both parents who have custody, the infant's health status at birth should be recorded on the Clinical Trial Pregnancy Reporting Form. In addition, the Sponsor may collect follow-up information on the infant's health status at 6 and 12 months after birth.

9.9.10. Annual Safety Report

The sponsor will prepare an annual safety report in form of a Development Safety Update Report (DSUR) and submit the report to the Competent Authorities and Ethics Committee. A copy of the DSUR is shared with Roche after completion.

9.9.11. Safety monitoring

9.9.11.1. DSMB

This study will use a DSMB whose members are therapeutic area experts and a statistician who are not employed by the sponsor of the study and have no material conflict of interest.
Overall safety will be assessed on an ongoing basis during the conduct of the study. The DSMB will also review the findings from the protocol defined interim analyses. The DSMB will monitor cumulative safety data at least once every 6 months during the course of the study. At the interim analyses, both efficacy and safety will be reviewed and recommendations will be based on the totality of data.

In particular, the DSMB will convene as soon as results for an interim efficacy analysis are available. Interim analysis reports prepared by the sponsor statistician will communicate data, decisions and consequences pertaining to the continuation of the ongoing trial according to the adaptive design with its decision rules for early stopping (see section 5.4), so that the DSMB may provide an independent review of trial design execution by the sponsor.

Safety monitoring will include protocol-defined AEs, SAEs and AESIs.

The DSMB can recommend changes to the study including study termination, if concern arises over the benefit risk profile of Atezolizumab and its combination therapy.

The DSMB related tasks and responsibilities will be defined in the DSMB charter.

9.9.11.2. Cardiac safety monitoring

Atezolizumab, Paclitaxel, Epirubicin and Cyclophosphamide and Carboplatin have rare reports of cardiovascular incidents (see SmPCs and current version of Atezolizumab IB).

Cardiac safety evaluation will include evaluation of cardiac AEs, measurement of LVEF by echocardiography and ECG as noted in the SOA table (section 2 table 1). Cardiac assessments should be performed according to the current clinical guidelines. It is the responsibility of the investigator to ensure that adequate resources and technical equipment for performance of echocardiography and ECG are available.
These examinations should be performed according to current clinical guidelines. A consultation by a cardiologist should be considered in case of a clinically relevant pathologic finding (as assessed by the investigator). The decision whether to continue or hold the treatment with TN-targeted agents has to be made as a result of the values of LVEF following the algorithm below.

Table 9: Cardiovascular safety monitoring

Cardiovascular Toxicity	Occurrence	Actions
Cardiovascular Toxicity (e.g. arrhythmias, CHF or LVEF grade ≥ 3) grade ≥ 3	First	Discontinue study treatment; Manage the cardiac condition; Patients under E/C/Atezolizumab regime may continue with Carboplatin/Paclitaxel/Atezolizumab when recovered to grade ≤ 1
	Second	Permanently discontinue all study treatment

9.10. Safety assessments

Planned time points for all safety assessments are provided in the SOA.

9.10.1. Physical examination

- A complete physical examination will include at a minimum, assessments of the cardiovascular, respiratory, gastrointestinal, skin, breast, lymph nodes and neurological systems. Height and weight will also be measured and recorded. Investigators should pay special attention to clinical signs related to previous serious illnesses.
- Baseline cancer related signs and symptoms will be recorded at the cycle 1 cay 1 visit and then reported as AEs during the trial if they worsen in severity or increase in frequency.

9.10.2. Vital signs and ECOG performance test

- Vital signs (to be taken before blood collection for laboratory tests) will consist of pulse and blood pressure.
- Performance Status: The ECOG performance status scale or KI will be used (see Appendix 6).

9.10.3. Electrocardiogram

- 12-lead ECG will be obtained as outlined in the SOA (see table 1) using an ECG machine with a 10-second rhythm strip. ECG measurements will include PQ interval, QT interval, RR interval, and QRS complex. It is preferable that the machine used has a capacity to calculate the standard intervals automatically.
- At each time point at which triplicate ECG are required, 3 individual ECG tracings should be obtained as closely as possible in succession, but no more than 2 min apart to determine the mean QTc interval.
- ECG interval readings by the ECG recorder's algorithm will be read and interpreted at the investigational site for eligibility determination and patient safety monitoring and documentation stored in the source documents.
- Additional ECGs may be performed as clinically indicated at any time.

9.10.4. Clinical safety laboratory assessments

- See Appendix 2 for the list of clinical laboratory tests to be performed and to the SOA for the timing and frequency.
- The investigator must review the laboratory report, document this review, and record any clinically relevant changes occurring during the study in the AE section of the CRF. The laboratory reports must be filed with the source documents. Clinically significant abnormal laboratory findings are those which are not associated with the underlying disease, unless judged by the investigator to be more severe than expected for the participant's condition.
- All laboratory tests with values considered clinically significantly abnormal during participation in the study or within 30 days after the last dose of study treatment should be repeated until the values return to normal or baseline or are no longer considered clinically significant by the investigator or Medical Monitor.
- If such values do not return to normal/baseline within a period of time judged reasonable by the investigator, the etiology should be identified, and the sponsor notified.
- All protocol-required laboratory assessments, as defined in Appendix 2, must be conducted in accordance with the SOA.

If laboratory values from non-protocol specified laboratory assessments performed at the institution's local laboratory require a change in participant management or are considered clinically significant by the investigator (e.g., SAE or AE or dose modification), then the results must be recorded in the CRF.

9.11. Markers for translational research

Translational analyses are planned especially regarding biomarkers of early prediction of resistance and response to immune therapies. It is expected that the landscape regarding biomarkers identifying resistance and response to immune therapies will change dramatically in the next two years. The definition of translational analyses of interest will take these future findings into account and cannot be presented at the time of submission of the protocol.

A detailed description of blood sampling is given in Appendix 3.

9.12. Protocol violations

Protocol Violations (PVs) can be detected in several different ways, e.g.:

- Detection of PVs by Data Management by applying edit checks to the data base
- Investigator proactively notifies sponsor personnel (clinical monitors, project manager) of PVs which have occurred at his investigational site
- PVs are detected during remote or on-site monitoring visits by clinical monitors
- PVs are detected during audits or regulatory inspections.

All PVs will be categorized as follows: source data; ICF, inclusion and exclusion criteria, randomization, primary endpoints, therapeutic scheme, SAE reporting, and others as planned by the study team. PVs are then analyzed by the Medical Monitor (MM).

Subsequently, the Medical Monitor will grade the PVs either in serious or non-serious breaches of the trial protocol and decides whether the patient can remain in the trial despite the PV or not.

The PVs will be acknowledged at each study site by the investigator. Sites will be trained again on the study procedures as required to avoid recurrence of PVs.

If applicable, PVs assessed as serious breaches of the trial protocol have to be notified to the ethic committees (ECs) (leading EC and concerned local EC) and the regulatory authorities. All PVs will be regularly analyzed and require appropriate corrective and preventive action (CAPA) which will be overseen by the palleos healthcare GmbH study team.

10. Statistical considerations

Detailed methodology for summary and statistical analyses of the data collected in this study will be documented in a Statistical Analysis Plan (SAP), which will be dated and maintained by the sponsor. This document may modify the plans outlined in the protocol; however, any major modifications will also be reflected in a protocol amendment.

10.1. Rationale and sample size determination

The neoMono statistical design adapts the idea of a proof-of-concept trial and uses Bayesian posterior and predictive probabilities for inference about the primary hypothesis. Up to 4 planned efficacy interim analyses provide decision points for early stopping for success or futility. A detailed explanation of statistical methods and the design with literature sources is provided in Appendix 9.

The primary objective is to show superiority of experimental Arm A vs. control Arm B in terms of pCR to neoadjuvant treatment.

The primary analysis is based on non-informative uniform (beta) priors for the pCR rates $p_A$and p_B in Arms A (experimental) and B (control) respectively. As in a proof-of-concept trial, a **dual criterion** is used to *simultaneously test for significant and relevant superiority* at different levels of certainty by requiring posterior probabilities, conditional on observed response counts $x_A,\ x_B \in N_0$ respectively in the two arms, to exceed the following thresholds:

$$P(p_A > p_B \mid x_A,\ x_B) \geq 0.975 \quad \wedge \quad P(p_A - p_B > \delta \mid x_A,\ x_B) \geq 0.85,$$

significance *relevance*

with a clinically meaningful difference of $\delta = 0.05$ (see Appendix 9 for a rationale).

The trial is planned to have a **maximal sample size of** $N_{max} = 370$ evaluable patients, with up to 4 planned interim analyses in order to assess early futility or success of the trial based on posterior predictive probabilities P for trial success. That is, the probability of claiming superiority in terms of the dual criterion if the trial were to continue to the maximal sample size N_{max}, conditional on the responses observed in the trial so far (see statistical Appendix 9 for mathematical details).

During the trial, up to 4 **interim analyses are to be performed after 100, 140, 180 and 220 patients evaluable for the primary endpoint in an ITT collective**. Interim analysis results will be presented to the DSMB for independent review. The following decision rules will be implemented at each interim analysis point:

- If $PP < 0.025$ the trial is stopped early for futility
- If $PP > 0.975$ the trial is stopped early for success

Interim analyses will be carried out and reported by the sponsor statistician**.** Based on these reports, the sponsor will carry out decisions regarding the continuation of recruitment according to the decision rules above. At each interim analysis timepoint, the DSMB will provide an independent review of interim efficacy results and trial design execution.

The maximal sample size N_{max} was determined by Monte Carlo simulation of the full adaptive trial with the parameters above using 10^8 repetitions and calculating the **global** operating characteristics (power and type I error) in different scenarios of interest. Multiple testing is implicitly accounted for by the simulation.

$N_{max} = 370$ is the smallest maximal sample size for the trial to reach at least **80% power** with the given interim analysis time points to rightly claim superiority in the scenario $p_A = 60\%,\ p_B = 45\%$ and at most a **2.5% type I error rate** to wrongly claim superiority in the scenario $p_A = 45\%,\ p_B = 45\%$. (see tables below; see statistical appendix for further details and a justification of pCR assumptions).

We assume an *analysis dropout* rate of 10% for the primary objective, where an *analysis dropout* is defined as any patient for whom a critical analysis-enabling covariate or the primary endpoint is not measurable for any reason, thus requiring 412 patients to be randomized (206 per arm with

1:1 randomization). In addition, we account for a 10% screening failure rate. **As a result, the expected number of patients to be recruited is set to 458**.

Scenario	**Operating Characteristics**	**P**(correct early stop)	$\mathbb{E}$[sample size]
H_0 ***significance***: $p_A = 45\%,\ p_B = 45\%$	type 1 error: 2.4%	68.5 %	296
H_0 ***relevance***: $p_A = 49\%,\ p_B = 45\%$	type 1 error: 10.9%	46.3%	321
H_1: $p_A = 60\%, p_B = 45\%$	power: 80.1%	34.9%	332

(Expected sample sizes refer to the number of recruited patients at the time of analysis and are based on assumptions of average recruitment rates explained in Appendix 9.)

See Appendix 9 for additional visualization of early stopping probabilities in different effect scenarios.

10.2. Analysis populations

Population	**Description**
ITT (intent-to-treat)	Full population containing all participants allocated to one of the two treatment arms.
PP (per protocol)	Population subset including only participants that are compliant with the protocol in terms of eligibility, interventions, and treatment plan as well as outcome assessment. This population will be used for sensitivity analyses of the primary endpoint.
AT (as treated, safety)	All participants randomly assigned to study treatment and who received at least one dose of study treatment. Participants will be analyzed according to the treatment they actually received.

10.3. Statistical analyses

The statistical analysis plan will be developed and finalized before first-patient-in. It will describe the participant populations and provide the mathematical details of all statistical methods and analyses, as well as additional literature references. This section provides a brief summary of the planned statistical analyses of primary and secondary endpoints. For details on the objectives and endpoints, see sections 4 and 9.8.

All efficacy analyses will be based on the intention-to-treat (ITT population. A sensitivity analysis on the per-protocol population will be carried out for the primary endpoint.

10.3.1. Analysis of primary endpoint

The primary endpoint is pCR. The associated estimators are pCR proportions in both arms and their risk difference.

The primary analysis is based on the joint posterior distribution of pCR prevalence in experimental and control arm, relative to an uninformative uniform prior distribution (see appendix 9 for details). Based on the posterior distribution, the two events of relevant and significant superiority of the experimental arm (risk difference) are simultaneously evaluated to yield a hypothesis test in terms of the dual criterion described in section 10.1.

If both probability thresholds specified by the dual criterion are exceeded simultaneously, superiority of the experimental arm is accepted. During interim analyses, superiority may be accepted based on thresholds specified for the predictive probability distribution of the dual criterion event (see section 10.1). In any case, marginal posterior distributions of pCR rates in both arms, as well as the posterior distribution of the risk difference will be reported, including corresponding expected value and 95% high posterior density interval (see also Appendix 9).

10.3.2. Analysis of secondary endpoints

Secondary endpoints include additional pCR definitions, biomarkers as continuous endpoints, biomarkers as thresholded binary response parameters, and survival outcomes.

Hypothesis testing for comparing efficacy endpoints across treatment arms is omitted in favor of reporting comparative estimators such as risk difference, odds ratios or hazard ratios with associated interval estimates. No further multiplicity adjustments are planned for secondary analyses, except in situations where joint prior distributions may be specified to that effect in Bayesian models.

In the following, we state fully Bayesian analyses whenever a non-informative prior distribution can be defined unambiguously for the estimand of interest (in which case the analyses should agree with maximum-likelihood based inference, see e.g. [6]). Otherwise, the primary reported inference will be based on maximum-likelihood methods for a common probability model. Additional supportive analyses using Bayesian inference with vague prior distributions for the same probability model may be specified in the SAP.

10.3.2.1. Biomarkers and response endpoints

Binary efficacy endpoints and thresholded biomarker endpoints (**secondary objectives 2 – 5**) will be analyzed in terms of their proportions in the respective treatment arms, analogous to section 10.3.2. As described in Appendix 9, we report posterior distributions for the risk difference (difference between proportions of responders), from which expected values are derived as point estimators, together with 95% high posterior density intervals (see e.g. (Gelman et al., 2013)). In addition, marginal posterior distributions for individual proportions will be reported.

Prognostic and predictive quality of biomarkers (**secondary objective 6**) will be evaluated by logistic regression on pCR. We report inference for univariate models (with and without treatment interaction terms) and multivariate models, including a selection of additional baseline parameters to be specified in the SAP, as well as subset selection procedures (e.g. LASSO, see (Tibshirani, 1996)) together with measures of robustness such as bootstrap inclusion frequencies of selected predictors (see (Royston & Sauerbrei, 2008)). A supportive Bayesian analysis may be carried out by employing t-family joint prior distributions for regression coefficients (see (Gelman et al., 2013), chp. 14).

10.3.2.2. Survival analysis

For the analysis of survival endpoints (**secondary objectives 7 - 9**) a semi-parametric constant hazard model is employed (see (Ibrahim et al., 2014) and (Klein & Moeschberger, 2010) respectively). The model is extended to account for proportional hazards between the two treatment arms in a Cox regression with treatment arm and additional predictors (baseline characteristics and biomarkers) as covariates. Time-varying regression coefficients may be employed to investigate deviations from the proportional hazard assumption.

Maximum likelihood point estimates for the survival functions (Kaplan-Meier method), the hazard ratios (Cox regression model), as well as median and n-year survival probabilities will be reported with (point-wise) 95% confidence intervals (see (Klein & Moeschberger, 2010)). Further details and additional supportive analyses will be specified in the SAP.

10.3.3. Safety analyses

The AT population will be the primary population for safety evaluation, comprising all patients who received at least one dose of study medication. Summaries of AEs and other safety parameters will be provided as appropriate.

AEs will be classified using the MedDRA classification system. The severity of the AEs will be graded according to the NCI CTCAE v5.0 whenever possible. AEs will be summarized by treatment and by the frequency of patients experiencing treatment emergent AEs corresponding to MedDRA system organ class preferred terms.

AEs will be summarized by cycle and by relatedness to trial treatment. Detailed information collected for each AE will include a description of the event, duration, whether the AE was serious, intensity, relationship to study drug, action taken, and clinical outcome. Emphasis in the analysis will be placed on AEs classified as treatment emergent.

AEs leading to death or discontinuation of trial treatment, events classified as NCI CTCAE v5.0 Grade 3 or higher, trial drug-related events, and SAEs will be considered with special attention.

10.3.4. Other analyses

Analyses of translational objectives will be described in the revised statistical analysis plan before final database lock and will be presented separately from the main clinical study report.

10.3.5. Interim analyses

During the trial, up to 4 interim analyses are to be performed after 100, 140, 180 and 220 patients evaluable for the primary endpoint in an ITT collective. Interim analysis results and any decisions for early stopping will be presented to the DSMB for independent review. Analyses of pCR rates are based on posterior predictive probabilities PP for trial success. That is, the probability of claiming superiority in terms of the dual criterion if the trial were to continue to the maximal sample size N_{max}, conditional on the responses observed in the trial so far (see statistical Appendix 9 for full mathematical details).

The following decision rules will be implemented at each interim analysis point:

- If $PP < 0.025$ the trial is stopped early for futility
- If $PP > 0.975$ the trial is stopped early for success

See Appendix 9 for additional visualization of early stopping probabilities in different effect scenarios. Note that recruitment will be ongoing continuously without interruption. Interim analyses are to be performed only as long as recruitment is not stopped or completed at N_{max}. Performing less than 4 interim analyses will not negatively impact the operating characteristics, see SAP and statistical Appendix 9 for further details.

10.4. Missing values

The sponsor will make every effort to prevent the occurrence of missing values, in particular for primary and secondary efficacy endpoints or analysis-enabling covariates, as well as treatment documentation. Reasons for missingness will be documented and queried whenever feasible. All efforts will be made to continue collection of *auxiliary variables*, in particular those that may relate to reasons of missingness, as well as *efficacy outcomes* for patients with missing primary parameters or in cases of treatment discontinuation, unless informed consent is withdrawn.

If missing values occur, robust statistical inference will be guided by the following sources:

- CHMP. "EMA Guideline on Missing Data in Confirmatory Clinical Trials (EMA/CPMP/EWP/1776/99)." (2010).
- National Research Council. (2010). The Prevention and Treatment of Missing Data in Clinical Trials. Panel on Handling Missing Data in Clinical Trials. Committee on National Statistics, Division of Behavioral and Social Sciences and Education. Washington, DC: The National Academies Press.
- Molenberghs, G., Fitzmaurice, G., Kenward, M. G., Tsiatis, A., & Verbeke, G. (Eds.). (2014). Handbook of missing data methodology. CRC Press.

A descriptive analysis of the number and proportion, timing, pattern, and reasons for missing values will be provided. An emphasis will be placed on displaying differences between treatment groups. Reasons for missingness will be investigated to check for potential bias and for validity of assumptions regarding the putative missing data mechanism.

Inference for the primary objective will address missing values by employing a full data model, including a missing data mechanism (see Molenberghs et al. (2014), chp. 5.4). The missing data distribution part of the model will feature a sensitivity parameter that indexes the missing data mechanism (see also Molenberghs et al. (2014), chp. 18). For the primary analysis, the sensitivity parameter will be centered at Missing At Random (MAR) assuming (Bayesian) ignorability of missing data, which will result in Bayesian proper imputation for inference.

Further sensitivity analyses may be carried out by centering the sensitivity parameter at different plausible Not Missing At Random scenarios (NMAR), in addition to performing a responder analysis. Further details will be provided in the SAP.

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Appendix A: Genes based on QIAseq Targeted DNA Panels

AKT1, AKT serine/threonine kinase 1; AR, Androgen receptor; BRCA1, DNA repair associated; BRCA2, BRCA2, DNA repair associated; EGFR, Epidermal growth factor receptor; ERCC4, ERCC excision repair 4, endonuclease non-catalytic subunit; ERBB2, Erb-b2 receptor tyrosine kinase 2, encoding for HER2; ERBB3, Erb-b2 receptor tyrosine kinase 3; ESR1, Estrogen receptor gene; FGFR1, fi3broblast growth factor receptor 1; KRAS, KRAS proto-oncogene, GTPase; MUC16, mucin 16, cell surface associated; PIK3CA, Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; PIK3R1, phosphoinositide-3-kinase regulatory subunit 1; PTEN, phosphatase and tensin homolog; PTGFR, prostaglandin F receptor; TGFB1, transforming growth factor beta 1

Appendix B: Genes based on nCounter® PanCancer Immune Profiling Panel

Please see more details on page 3 of
http://www.biosystems.com.ar/archivos/folletos/228/pdf.pdf

Appendix C: Genes based on nCounter® PanCancer Immune Profiling Panel and PAM50

Please see more details: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GPL17071

Please see more details on page 3 of
http://www.biosystems.com.ar/archivos/folletos/228/pdf.pdf

Genes from Yang et al.(2018):

Immunity Genes: *APOBEC3G, CCL5, CCR2, CD2, CD27, CD3D, CD52, CORO1A, CXCL9, GZMA, GZMK, HLA-DMA, IL2RG, LCK, PRKCB, PTPRC*, and *SH2D1A*.

Proliferation genes: *AURKA, BIRC5, CCNB1, CCNE1, CDC20, CDC6, CENPF, CEP55, EXO1, MKI-67, KIF2C, MELK, MYBL2, NDC80, ORC6, PTTG1, RRM2, TYMS, and UBE2C*

Appendix D: Genome-wide gene expression analysis for RNA-based biomarker signature related to response/resistance to Atezolizumab using ArrayXS microarrays

For RNA-based biomarker signature, TMA of all core biopsies (FFPE blocks) will be used.

Please see more details: https://www.oak-labs.com/gene-expression-ffpe-human/index.phtml

12. Appendices

12.1. Appendix 1: Breast Cancer Stages

UICC classification

The neoMono study will include patients with T1c-T4c TNBC. This will include:

- T1c: the tumor is larger than 10 mm but 20 mm or smaller
- T2: the tumor is larger than 20 mm but not larger than 50 mm
- T3: the tumor is larger than 50 mm
- T4:
    - T4a means the tumor has grown into the chest wall
    - T4b means the tumor has grown into the skin
    - T4c means the tumor has grown into the chest wall and skin
    - T4d means inflammatory breast cancer

The neoMono study will include patients with stage N0-N3.

Regional lymph nodes include:

- Lymph nodes located under the arm, called the axillary lymph nodes
- Above and below the collarbone
- Under the breastbone, called the internal mammary lymph nodes

N stages include:

N0: Either of the following:
- No cancer was found in the lymph nodes.
- Only areas of cancer smaller than 0.2 mm are in the lymph nodes.

N1: The cancer has spread to 1 to 3 axillary lymph nodes and/or the internal mammary lymph nodes. If the cancer in the lymph node is larger than 0.2 mm but 2.0 mm or smaller, is it called "micro metastatic" (N1mi).

N2: The cancer has spread to 4 to 9 axillary lymph nodes. Or it has spread to the internal mammary lymph nodes, but not the axillary lymph nodes.

N3: The cancer has spread to 10 or more axillary lymph nodes. Or it has spread to the lymph nodes located under the clavicle, or collarbone. It may have also spread to the internal mammary lymph nodes. Cancer that has spread to the lymph nodes above the clavicle, called the supraclavicular lymph nodes, is also described as N3.

Only patients with cM0 stage will be included. cM0 refers to patients who have no clinical indication of distant metastases.

For definition of tumor staging according to AJJC, please refer to:

Eight edition/editor-in-chief, Mahul B. Amin, MD, FCAP; editors, Stephen B. Edge, MD, FACS [and 16 others]; Donna M. Gress, RHIT, CTR - Technical editor; Laura R. Meyer, CAPM - Managing editor"AJCC cancer staging manual", American Joint Committee on Cancer, Springer (2017)

12.2. Appendix 2: Clinical laboratory tests

- The tests detailed in Table 10 will be performed by the investigator`s local laboratory.
- Protocol-specific requirements for inclusion or exclusion of participants are detailed in Section 6.1 and Section 6.2 of the protocol.
- Additional tests may be performed at any time during the study as determined necessary by the investigator or required by local regulations.

Table 10: Hematology and biochemistry assessments

Laboratory Assessments	**Parameters**		
Hematology	WBC	WBC Count with Differential*: Neutrophils Lymphocytes Monocytes Eosinophils Basophils	
	Platelet Count		
	Hemoglobin		
Clinical Chemistry[1]	Aspartate Aminotransferase (AST)	Alanine Aminotransferase (ALT)	Total (and direct**) bilirubin
	Creatinine		
	Sodium	Potassium	
	TSH; FT4		

Investigators must document their review of each laboratory safety report.
* Differential WBC Count is mandatory before start of CTX. During the ongoing therapy, it is sufficient to analyze WBC/leucocytes, neutrophils, platelets and hemoglobin.
** Direct bilirubin has only to be assessed if total bilirubin shows values beyond normal range.

12.3. Appendix 3: Tests for translational research

Blood samples

Blood for translational research is drawn at the time points described in section 2, SOA.

For patients in Arm A, 5 blood samples will be taken over the whole period of the study: one at baseline and 4 during the neoadjuvant treatment.

For patients in Arm B, 4 blood samples will be taken over the whole period of the study: one at baseline and 3 during the neoadjuvant treatment.

At each time point, 4 collection tubes à 8.5 mL blood (total amounts 34 mL) will be withdrawn from the patient. Collection tubes will be supplied together with corresponding adapters and butterflies. Each tube contains 1.5 mL fixate that stabilizes the samples for up to 7 days. After withdrawing the blood, each collection tube should be inverted gently.

Blood should be withdrawn and shipped **only from Monday to Thursday** to avoid any delay over a weekend. Holidays should also be considered when sampling and shipment of blood samples are planned.

Shipment of the blood samples should take place on the same day using overnight shipment to the laboratory at the University Hospital Essen using the supplied shipment containers to:

Klinik für Frauenheilkunde und Geburtshilfe
Universitätsklinikum Essen
z.Hd. Frau Prof. Dr. Sabine Kasimir-Bauer
Forschungslabor, Ebene -1, Raum-1.06
Hufelandstraße 55
45147 Essen

The laboratory processes the samples (Separation of plasma and solid blood component by centrifugation) and subsequently stores all blood samples at -80 °C.

From blood samples the multiplexed gene expression panels specified in Appendix A - C will be analyzed.

12.4. Appendix 4: Cardiac safety monitoring

Cardiac safety evaluation

Cardiac safety monitoring for toxicity of CTX combination and toxicity other than CTX combination has been reported. Hence, cardiac AEs should be followed closely.
Refer to section 9.9.11.2 of the protocol for cardiac safety evaluation.

Definitions of cardiac toxicity

Cardiac toxicity will be classified as follows:

- **Cardiac death**

Cardiac death will be defined as death due to one of the following:
- Confirmed CHF
- Myocardial infarction
- Documented primary arrhythmia
- Probable cardiac death i.e. sudden death without documented aetiology.

An autopsy is preferred in cases where cause of death has a cardiac aetiology.

➢ **Congestive Heart Failure (CHF)**

Clinical signs and symptoms suggesting CHF (dyspnoea, tachycardia, cough, neck vein distension, cardiomegaly, hepatomegaly, paroxysmal nocturnal dyspnoea, orthopnoea, peripheral edema, etc.) must be investigated.
The suspicion of CHF, based on the signs and symptoms mentioned above, must be confirmed by a LVEF decrease in echocardiography, with a chest X-ray. LVEF assessment should be repeated 4 to 7 days afterwards to confirm a diagnosis of CHF.

➢ **Cardiac arrhythmias, grade 3 or grade 4**

The NCI Common Toxicity Criteria, version 5.0 will be used to classify an arrhythmia as grade 3, which is symptomatic and requiring treatment, or grade 4 which is an arrhythmia considered to be life-threatening e.g. an arrhythmia associated with CHF, hypotension, syncope, shock.

➢ **Cardiac ischemia/Infarction, grade 3 or grade 4**

The NCI Common Toxicity Criteria, version 5.0 will be used to classify the severity of cardiac ischemia/infarction. Grade 3 ischemia is defined as angina without evidence of infarction. Grade 4 is defined as an acute myocardial infarction.
Patients showing one of those symptoms will consult a cardiologist and will be followed as defined by the institution's routine.

Cardiac safety analysis

The incidence of cardiac AEs (cardiac deaths, CHF, grade 3 or grade 4 ischemia/infarction, grade 3 or grade 4 arrhythmias) will be calculated for each treatment arm.

Reporting of cardiac toxicities

Cardiac toxicities will be documented in the CRF at each visit during treatment and follow-up.

12.5. Appendix 5: Fluid retention severity grading

Edema	Severity grading	Effusion
• Asymptomatic *and/or* • Very well tolerated *and/or* • Dependent in evening only	MILD 1	• Asymptomatic • No intervention required
• Moderate functional impairment *and/or* • Pronounced and well tolerated *and/or* • Dependent throughout day	MODERATE 2	•Symptomatic: - exertional dyspnoea *and/or* - chest pain *and/or* • ECG changes *and/or* • Abdominal distension • Drainage may be required
• Significant impairment of function *and/or* • Pronounced and not well tolerated *and/or* • Generalized anasarca	SEVERE 3	• Symptomatic effusion - dyspnoea at rest *and/or* - tamponade *and/or* - pronounced abdominal distension • Drainage urgently required

12.6. Appendix 6: ECOG Performance Status

Eastern Cooperative Oncology Group (ECOG) Performance Status Description Grade

Description	***Grade***
Fully active, able to carry on all pre-disease performance without restriction.	0
Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature, i.e., light housework, office work.	1
Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours.	2
Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	3
Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	4

12.7. Appendix 7: Karnofsky Index

100 – Normal; no complaints; no evidence of disease.
90 – Able to carry on normal activity; minor signs or symptoms of disease.
80 – Normal activity with effort; some signs or symptoms of disease.
70 – Cares for self; unable to carry on normal activity or to do active work.
60 – Requires occasional assistance, but is able to care for most of their personal needs.
50 – Requires considerable assistance and frequent medical care.
40 – Disabled; requires special care and assistance.
30 – Severely disabled; hospital admission is indicated although death not imminent.
20 – Very sick; hospital admission necessary; active supportive treatment necessary.
10 – Moribund; fatal processes progressing rapidly.
0 – Dead

12.8. Appendix 8: Adverse Events: definitions and procedures for recording, evaluating, follow-up, and reporting

Reporting of SAEs

SAE Reporting to Palleos
Electronic reporting is the preferred method to transmit SAE information to the Safety Manager of palleos healthcare GmbH. Facsimile or email transmission of the SAE paper CRF is an alternative method to transmit this information to the Safety Manager of palleos healthcare GmbH. Contacts for paper-based SAE reporting can be found in Section 9.9.5.

Definition of AE

AE Definition
An AE is any untoward medical occurrence in a patient or clinical study participant, temporally associated with the use of study treatment, whether or not considered related to the study treatment. NOTE: An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of study treatment.

Events Meeting the AE Definition
• Any abnormal laboratory test results (hematology, clinical chemistry, or urinalysis) or other safety assessments (e.g., ECG, radiological scans, vital signs measurements), including those that worsen from baseline, considered clinically significant in the medical and scientific judgment of the investigator (i.e., not related to progression of underlying disease). • Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition. • New conditions detected or diagnosed after study treatment administration even though it may have been present before the start of the study. • Signs, symptoms, or the clinical sequelae of a suspected drug-drug interaction. • Signs, symptoms, or the clinical sequelae of a suspected overdose of either study treatment or a concomitant medication. Overdose per se will not be reported as an AE/SAE unless it is an intentional overdose taken with possible suicidal/self-harming intent. Such overdoses should be reported regardless of sequelae. • Signs, symptoms, or the clinical sequelae of a drug abuse. • Signs, symptoms, or the clinical sequelae of a drug withdrawal. • Hypersensitivity • Events that are related to a protocol-mandated intervention, including those that occur prior to assignment of study treatment (e.g. screening invasive procedures such as biopsies.

Events NOT Meeting the AE Definition
• Any clinically significant abnormal laboratory findings or other abnormal safety assessments which are associated with the underlying disease, unless judged by the investigator to be more severe than expected for the participant's condition. • The disease/disorder being studied or expected progression, signs, or symptoms of the disease/disorder being studied, unless more severe than expected for the participant's condition. • Medical or surgical procedure (e.g. endoscopy, appendectomy): the condition that leads to the procedure is the AE. • Situations in which an untoward medical occurrence did not occur (Social and/or convenience admission to a hospital). • Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the study that do not worsen.

Definition of SAE

If an event is not an AE per definition above, then it cannot be an SAE even if serious conditions are met (e.g., hospitalization for signs/symptoms of the disease under study, death due to progression of disease).

An SAE is defined as any untoward medical occurrence that, at any dose:
Results in death
Is life-threatening The term 'life-threatening' in the definition of 'serious' refers to an event in which the participant was at risk of death at the time of the event. It does not refer to an event, which hypothetically might have caused death, if it were more severe.
Requires inpatient hospitalization or prolongation of existing hospitalization In general, hospitalization signifies that the participant has been detained at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or outpatient setting. Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization or fulfills any other serious criteria, the event is serious. When in doubt as to whether "hospitalization" occurred or was necessary, the AE should be considered serious. Any AE leading to hospitalization or prolongation of hospitalization will be considered as serious, UNLESS at least one of the following exceptions is met: - The admission is pre-planned (i.e., elective or scheduled surgery arranged prior to the start of the study) or - The admission is not associated with an adverse event (e.g., social hospitalization for purposes of respite care). - The admission is designated to perform an efficacy measurement for the study

- The admission is designated to receive scheduled therapy for the target disease of the study However, it should be noted that invasive treatment during any hospitalization may fulfil the criteria of 'medically important' and as such may be reportable as a SAE dependent on clinical judgment. In addition, where local regulatory authorities specifically require a more stringent definition, the local regulation takes precedent.
Results in persistent disability/incapacity The term disability means a substantial disruption of a person's ability to conduct normal life functions. This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza, and accidental trauma (e.g. sprained ankle) which may interfere with or prevent everyday life functions but do not constitute a substantial disruption.
Is a congenital anomaly/birth defect
Other situations Medical or scientific judgment should be exercised in deciding whether SAE reporting is appropriate in other situations such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the participant or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These events should usually be considered serious. Examples of such events include invasive or malignant cancers, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse. As guidance for determination of important medical events refer to the "WHO Adverse Reaction Terminology – Critical Terms List". These terms either refer to or might be indicative of a serious disease state. Such reported events warrant special attention, because of their possible association with a serious disease state and may lead to more decisive action than reports on other terms.

The terms "severe" and "serious" are not synonymous. Severity refers to the intensity of an AE (rated according to NCI CTCAE v5.0; see below); the event itself may be of relatively minor medical significance (such as severe headache without any further findings).

Recording an AE and/or SAE

AE and SAE Recording
When an AE/SAE occurs, it is the responsibility of the investigator to review all documentation (e.g., hospital progress notes, laboratory reports, and diagnostics reports) related to the event. The investigator will record all relevant AE/SAE information in the CRF. SAEs have to be reported to the sponsor within the protocol-specified timeframes either electronically or paper-based as specified above. It is not acceptable for the investigator to send photocopies of the participant's medical records to palleos healthcare GmbH in lieu of completion of the AE/SAE CRF page. There may be instances when copies of medical records for certain cases are requested by palleos healthcare GmbH. In this case, all participant identifiers, with the exception of the participant number, will be redacted on the copies of the medical records before submission to palleos healthcare GmbH. The investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. Whenever possible the diagnosis (not the individual signs/symptoms) will be documented as the AE/SAE.

Assessment of Severity
The investigator will make an assessment of severity for each AE and SAE reported during the study and assign it according to NCI CTCAE v5.0 to one of the following grades: Grade 1/Mild: An event that is easily tolerated by the participant, causing minimal discomfort and not interfering with everyday activities. Grade 2/Moderate: An event that causes sufficiently discomfort and interferes with normal everyday activities. Grade 3/Severe: An event that prevents normal everyday activities. An AE that is assessed as severe should not be confused with an SAE. Severe is a category utilized for rating the intensity of an event; and both AEs and SAEs can be assessed as severe. An event is defined as 'serious' when it meets at least 1 of the predefined outcomes as described in the definition of an SAE, NOT when it is rated as severe. Grade 4/Life-threatening: An event that places the patient or subject at immediate risk of death and indicates urgent interventions. Grade 5/Fatal: Death related to AE.

Assessment of Causality

The investigator is obligated to assess the relationship between study treatment and each occurrence of each AE/SAE.

A "reasonable possibility" of a relationship conveys that there are facts, evidence, and/or arguments to suggest a causal relationship, rather than a relationship cannot be ruled out.

The investigator will use clinical judgment to determine the relationship.

Alternative causes, such as underlying disease(s), concomitant therapy, and other risk factors, as well as the temporal relationship of the event to study treatment administration will be considered and investigated.

The investigator will also consult the IB and/or SmPC, for marketed products, in his/her assessment.

For each AE/SAE, the investigator must document in the medical notes that he/she has reviewed the AE/SAE and has provided an assessment of causality.

There may be situations in which an SAE has occurred, and the investigator has minimal information to include in the initial report to palleos healthcare GmbH. However, it is very important that the investigator always makes an assessment of causality for every event before the initial transmission of the SAE data to palleos healthcare GmbH.

The investigator may change his/her opinion of causality in light of follow-up information and create a SAE follow-up report with the updated causality assessment.

The causality assessment is one of the criteria used when determining regulatory reporting requirements.

Causal relationship between the study treatment and a certain AE will be graded according to the following criteria (WHO-UMC system):

Certain
- Event or laboratory test abnormality, with plausible time relationship to drug intake
- Cannot be explained by disease or other drugs
- Response to withdrawal plausible (pharmacologically, pathologically)
- Event definitive pharmacologically or phenomenologically (i.e. an objective and specific medical disorder or a recognized pharmacological phenomenon)
- Rechallenge satisfactory, if necessary

Probable/Likely
- Event or laboratory test abnormality, with reasonable time relationship to drug intake
- Unlikely to be attributed to disease or other drugs
- Response to withdrawal clinically reasonable
- Rechallenge not required

Possible
- Event or laboratory test abnormality, with reasonable time relationship to drug intake
- Could also be explained by disease or other drugs
- Information on drug withdrawal may be lacking or unclear

Unlikely

- Event or laboratory test abnormality, with a time to drug intake that makes a relationship improbable (but not impossible)
- Disease or other drugs provide plausible explanations
- Conditional/Unclassified
- Event or laboratory test abnormality
- More data for proper assessment needed, or
- Additional data under examination

Unassessable/Unclassifiable

- Report suggesting an adverse reaction
- Cannot be judged because information is insufficient or contradictory
- Data cannot be supplemented or verified

For patients receiving combination therapy, causality will be assessed individually for each protocol-mandated therapy.

Sponsor`s Assessment of Expectedness

The specificity or severity of an unexpected AE or SUSAR is not consistent with the current IB or SmPC for the study treatment.

Also, reports which add significant information on specificity or severity of a known, already documented AE constitute unexpected AEs.

For example, an event more specific or more severe than described in the SmPC (prescribing information/"Fachinformation") would be considered “unexpected”.

Specific examples would be; (a) acute renal failure as a labelled AE with a subsequent new report of interstitial nephritis and (b) hepatitis with a first report of fulminant hepatitis.

An expected AE with fatal outcome must be regarded as unexpected, if the IB or SmPC does not explicitly state the option of fatal outcome for this event.

Follow-up of AEs and SAEs

The investigator is obligated to perform or arrange for the conduct of supplemental measurements and/or evaluations as medically indicated or as requested by palleos healthcare GmbH to elucidate the nature and/or causality of the AE or SAE as fully as possible. This may include additional laboratory tests or investigations, histopathological examinations, or consultation with other health care professionals.

If a participant dies during participation in the study or during a recognized follow-up period, the investigator will provide palleos healthcare GmbH with a copy of any post-mortem findings including histopathology.

New or updated information concerning AEs will be recorded in the originally completed CRF.

The investigator will submit any updated SAE data to palleos healthcare GmbH within 24 hours of receipt of the information.

New, updated, or corrected information about a previously reported SAE should be submitted on a new SAE Report Form that includes the data that are new or revised from the previous report. Follow-up information should never be added to a previously submitted report form.

12.9. Appendix 9: Statistical design and methods

In the neoMono trial we employ Bayesian inference calculus to report posterior probability distributions in the inference step (details are provided in subsection 1.2 below, for a general reference see [7]). In particular, inference for the primary objective is focused on comparing efficacy between an experimental Arm A versus a control Arm B. Efficacy is measured in terms of the endpoint pCR *rate*. In the following, the effect θ denotes a difference in pCR rates of experimental Arm A relative to Arm B.

Primary objective

For the inference step of the primary objective, we adapt the idea of a *proof-of-concept trial* ([1], see also [2] – [5] for additional examples) to show superiority of an experimental arm versus a control arm in terms of a simultaneous *dual criterion*:

1. **Significance**: *high* confidence that the effect θ of experimental arm relative to control is bigger than zero

$$\mathbf{Pr}(\theta > 0 \mid \text{data}) \geq 1 - \alpha$$

2. **Relevance**: *moderate* confidence that the experimental effect, relative to control, is larger than a clinically meaningful difference δ

$$\mathbf{Pr}(\theta > \delta \mid \text{data}) \geq \gamma$$

We choose a clinically meaningful difference of $\delta = 0.05$. Given that both burden and expense for the addition of 14 d Atezolizumab mono-window in Arm A is low for the patients, a δ of 5% is sufficient to consider a result clinically relevant in this context. As a reference for this choice, consider also the Taxane meta-analysis by Peto et al. in [10]. As probability threshold for accepting significant superiority, $1 - \alpha = 0.975$ is chosen, and $\gamma = 0.85$ for relevant superiority. Both thresholds must be exceeded simultaneously to accept superiority in terms of the *dual criterion*. A more formal statement is provided in the next section after introducing additional notation. The use of the dual criterion is motivated by requiring more certainty during interim decision-making based on lower sample sizes. Inclusion of a relevance criterion decreases the chance of acting based on spurious sampling results at an interim analysis and also notably reduces the type I error rate in simulations of the trial.

Bayesian inference calculus

For a complete summary and derivation of all probability terms involved in a proof-of-concept trial with binary endpoints, see [2]. Below we give a short description for illustrative purposes.

Bayesian inference calculus formalizes all uncertainty in a given problem context in terms of probability distributions. Before observing data, prior distributions are assigned to all random variables in the problem context. After observing data, an inference step is carried out by updating prior probabilities to conditional posterior probabilities *via* Bayes theorem.

We assign a joint uniform prior to pCR rates (p_A, p_B) in experimental (A) and control arm (B) respectively to obtain a joint posterior distribution as *product of beta distributions*. In the notation of a Bayesian hierarchical model where $x_A, x_B \in \{0, 1, \ldots, n\}$ denote observed responses in the two arms with n patients evaluable in each arm,

$$\begin{aligned} p_A &\sim U(0,1) \\ p_B &\sim U(0,1) \\ p_A|x_A &\sim Beta(\alpha_A, \beta_A) \\ p_B|x_B &\sim Beta(\alpha_B, \beta_B) \end{aligned}$$

Assuming a uniform prior distribution entails the knowledge that pCR can be obtained in both arms but expresses complete uncertainty with respect to its actual rate. The main inference step is realized by reporting the joint posterior probability distribution

$$\mathbf{Pr}(p_A, p_B \mid x_A, x_B) = \mathbf{P}(p_A \mid x_A)\ \mathbf{P}(p_B \mid x_B)$$

This leads to the following parametrization for the joint posterior distribution above which realizes the update step *via* Bayes theorem from uniform prior to informed posterior:

$$\begin{aligned} \alpha_A &= 1 + x_A, \quad \beta_A = 1 + n - x_A \\ \alpha_B &= 1 + x_B, \quad \beta_B = 1 + n - x_B \end{aligned}$$

The joint distribution and its marginals are the basis for all further inference and can be used to

- directly derive $\mathbf{Pr}(\theta \geq \delta \mid x_A, x_B)$ for $\theta = p_A - p_B$ (see [2] for details),
- compute point estimators (expected value) for θ, p_A and p_B,
- compute confidence intervals (high posterior density interval)

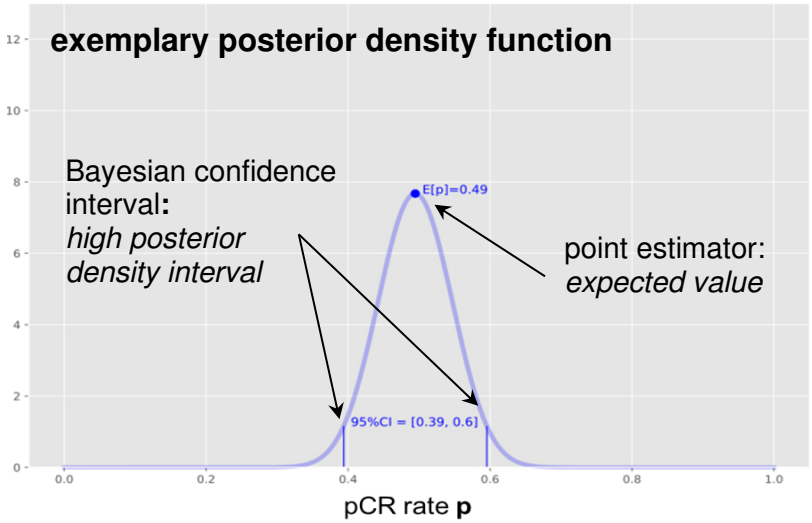

Fig. 1: Exemplary posterior density function for a hypothetical pCR rate p.

We report full (marginal) posterior distributions for the inference step. The primary objective can now be stated as follows.

Primary objective: to show superiority in terms of the dual criterion, i.e. accept superiority of experimental arm over control arm at the end of the trial if

$$P(p_A > p_B \mid x_A, x_B) \geq 0.975 \quad \wedge \quad P(p_A - p_B > \delta \mid x_A, x_B) \geq 0.85$$

with a clinically meaningful difference of $\delta = 0.05$.

Planned interim analyses

Prior estimates for pCR rates in the two therapy arms are subject to a high degree of uncertainty. To make an attempt at accounting for ethical and financial risks of the trial in the statistical design we make use of the flexibility provided by Bayesian inference calculus to plan a maximal number of interim analyses, so as to be able to conclude the trial for futility or success at an early time, depending on the true unknown effect size.

We employ *predictive probabilities* of trial success to specify stopping rules for futility and success and use Monte Carlo simulations of the full trial, including a grid of interim analyses to be determined, to evaluate corresponding frequentist operating characteristics (see recommendations in [6], and further theory in [7], chp. 4).

Assuming a balanced randomization between arms, at the i'th interim analysis m_i additional patients are yet to be observed per arm. A predictive probability *PP*(i) is then defined informally as

PP(i) := **P**{trial success | current pCR rates in arms A and B}.

More formally (see also [2] and [7] for reference), at an interim analysis where in both arms $n < n_{max}$ patients have been observed, with $n_{\max}$ the maximum sample size per arm,

- let *Y* be the *number of responses* in the potential $m = n_{max} - n$ future patients of one particular arm after x responses have been observed in n patients so far,

$$Y \sim BetaBinomial(m,\ 1 + x,\ 1 + n - x)$$

- let B(i, j, δ) = **Pr**($\theta \geq \delta$ | $X_A = x_A + Y_A$, $X_B = x_B + Y_B$, $Y_A = i$, $Y_B = j$), where $\theta = p_A - p_B$,
- let $I_{i,j} = I$[B(i, j, 0) ≥ 0.975 ∧ B(i, j, 0.05) ≥ 0.85] be the indicator function of the dual criterion, then

$$PP := E[I_{i,j}|x_A, x_B, n] = \sum_{i=0}^{m} \sum_{j=0}^{m} \Pr(Y_A = i \mid x_A, n, m) \times \Pr(Y_B = j \mid x_B, n, m) \times I_{i,j}.$$

At any interim analysis the trial is stopped early for *futility* if $PP \leq 0.025$, or stopped early for *success* if $PP \geq 0.975$, i.e. predictive probabilities for trial success in terms of the dual criterion are exceedingly low or high, respectively.

The statistical design is evaluated by simulation (further details follow below) to determine an optimal grid of interim analyses, assuming that recruitment is ongoing during the analyses and will not be stopped. The analysis grid ends once the actual recruitment state affords no further decision making in terms of early termination. The analysis grid is chosen to provide a positive cost-benefit ratio with regard to the possibility of early termination. That is, we require a high probability of early termination if a true effect is absent, and generally an expected sample size that is significantly lower than a planned maximum $N_{max} = 2n_{max}$ across different pCR scenarios that might obtain during the trial.

The chosen grid starts with the first interim analysis after a burn-in period of 100 patients evaluable (50 per arm) and continues in blocks of 40 (20 per arm) unless recruitment is stopped early or has reached N_{max}. Assuming a moderate to high recruitment rate of 15 patients per month (e.g. 30 sites each recruiting 0.5 patients per month), we estimate a period of 32 weeks between randomization of the last patient in a block of 20 to monitored data of baseline and surgery results ready for analysis in each block. This creates an assumed minimum offset of 120 patients that have been recruited during the preparation of each interim analysis and defines the last feasible interim analysis time point in terms of evaluable patients before the trial has reached maximum recruitment numbers. At that point, the recruitment state affords no further decision making. The planned number of interim analyses thus depends on the maximal sample size that is estimated in simulations, as described in the following section.

Sample size calculation and operating characteristics

As recommended in [6], chp. VI.A, we calibrate the maximal sample size N_{max} according to *frequentist operating characteristics* (OC) by numerical simulation. That is, we perform 10 million repetitions in a comprehensive Monte Carlo simulation of the full adaptive trial with all interim analyses. Based on the simulation results we count decisions for accepting or rejecting the dual criterion superiority hypothesis stated in section 1.2. By evaluating corresponding relative counts in dedicated scenarios for a *nullhypothesis* ($p_A = p_B$) or *specific alternative* (e.g. $p_A - p_B \geq 0.15$) *type I error rate* and *power* are determined respectively for a given maximal sample size and interim analysis grid.

Note that *multiple testing issues* are automatically addressed here by controlling the *global* type I and II error rates in the full trial simulation.

Due to the nature of the dual criterion, the nullhypothesis scenarios for the true effect size θ entail $0 \leq \theta \leq 0.05$. We require for the nullhypothesis with respect to *significant superiority* (scenario $p_A = p_B$) that the type I error rate $\alpha \leq 0.025$. In the context of a proof-of-concept trial, a larger α around 0.1 is acceptable for the nullhypothesis with respect to *relevant superiority* (scenario $0 < \theta \leq 0.05$). With regard to a specific alternative hypothesis, the trial must yield a power ≥ 0.8 to detect a difference in pCR rates of $\theta = 0.15$, which is suggested based on preliminary results published in [8]. A visual representation of the different θ scenarios and required operating characteristics is given in Fig. 2 below.

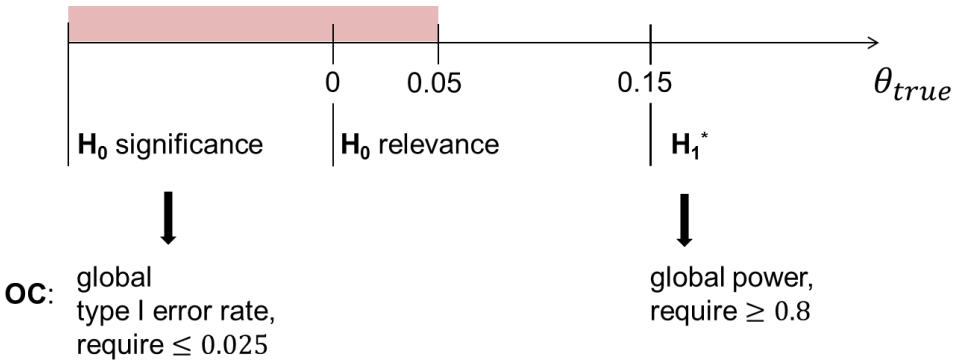

Fig. 2: Scenarios for true effect size θ and required operating characteristics.

Based on extensive simulation results, $N_{max} = 370$ (185 per arm) evaluable was determined, with a planned grid of **4 interim analyses** to be performed at the following counts for *patients evaluable*, assuming balanced randomization across both arms:

[100, 140, 180, 220]

Following results in the Keynote-522 trial (see [9]), investigating 602 patients treated with Pembrolizumab in combination with 4 cycles of Paclitaxel + Carboplatin followed by 4 cycles of Doxorubicin or Epirubicin + Cyclophosphamide, we chose a conservative lower bound for pCR rates at 45% to anchor the following scenarios for the true effect θ:

Scenario	**OC**	**P(*correct early stop*)**	**expected sample size**
H_0 *significance*: $p_A = 45\%,\ p_B = 45\%$	type I error: 2.4%	68.5%	296
H_0 *relevance*: $p_A = 49\%,\ p_B = 45\%$	type I error: 10.9%	46.3%	321
H_1: $p_A = 60\%, p_B = 45\%$	power: 80.1%	34.9%	332

Note that our primary measure of confidence is given in terms of the Bayesian posterior probability thresholds (see section 1.2, primary objective dual criterion), which we fix a priori and then proceed to select a sample size such that the common standards for frequentist operating characteristics in case of one-sided superiority (i.e. $\alpha = 0.025, 1 - \beta = 0.8$) are met as upper or lower bounds respectively. We accept the slightly conservative type I error rate that obtains as a result in conjunction with the discrete event space.

We assume an *analysis dropout* rate of 10% for the primary objective, where an *analysis dropout* is defined as any patient for whom a critical analysis-enabling covariate or the primary endpoint is not measurable for any reason, thus requiring 412 patients to be randomized, i.e. 206 per arm in a 1:1 randomization. In addition, we account for a 10% screening failure rate. As a result, **the maximum number of patients to be recruited is set to 458.**

In Fig. 3 below we show how the probability of early stopping changes as a function of effect size. The graph shows a trough in overall stopping probability (black) in between the H_1 scenario and the H_0-relevance scenario, where early discriminatory power based on the predictive probabilities is lowest. While in the H_0 scenario the futility stopping criterion is most active (red graph), the roles change and early stopping for success (blue) increases beyond the trough area. Note that in the H_0 scenario the blue success curve is practically zero, whereas the red futility curve is still of substantial magnitude in the H_1 scenario, which reflects the different utility weighting of type I error (only 2.5%) and type II error (20% acceptable) at the level of probabilities for early termination causes.

In addition, we show in Figs. 4 and 5 cumulative stopping probabilities (for all causes) across the interim and final analysis time points for the dedicated H_0 and H_1 scenarios respectively, as a function of expected recruited patient numbers using the same recruitment assumptions as before.

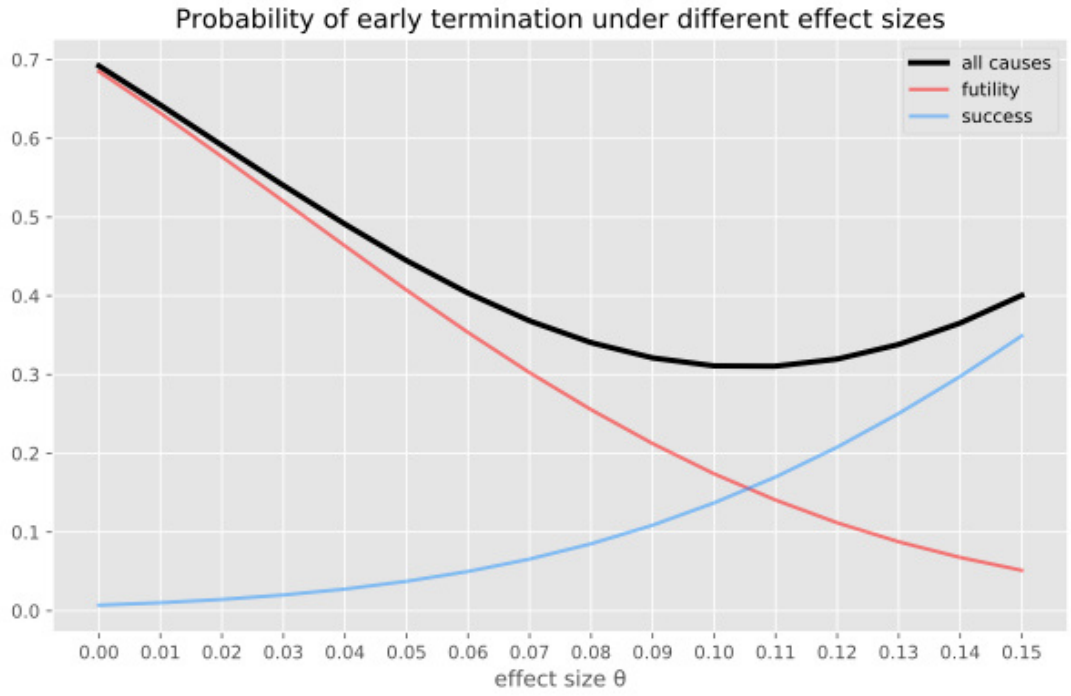

Fig. 3: Probability of early stop as a function of effect size. Stopping for any reason is counted (black graph), including erroneous stopping decisions (type I or II error, depending on the scenario, cmp. Fig. 2). Directional stopping is indicated by the two additional graphs, red for futility and blue for success.

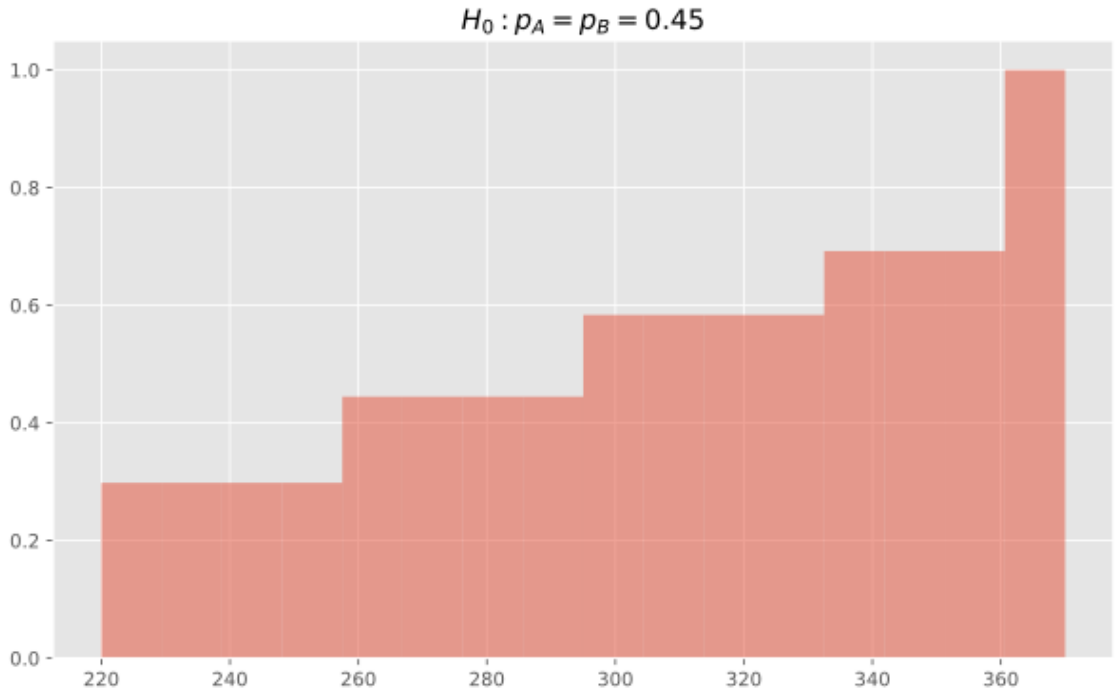

Fig. 4: Cumulative stopping probabilities (for all causes) across interim analyses in H_0 scenario.

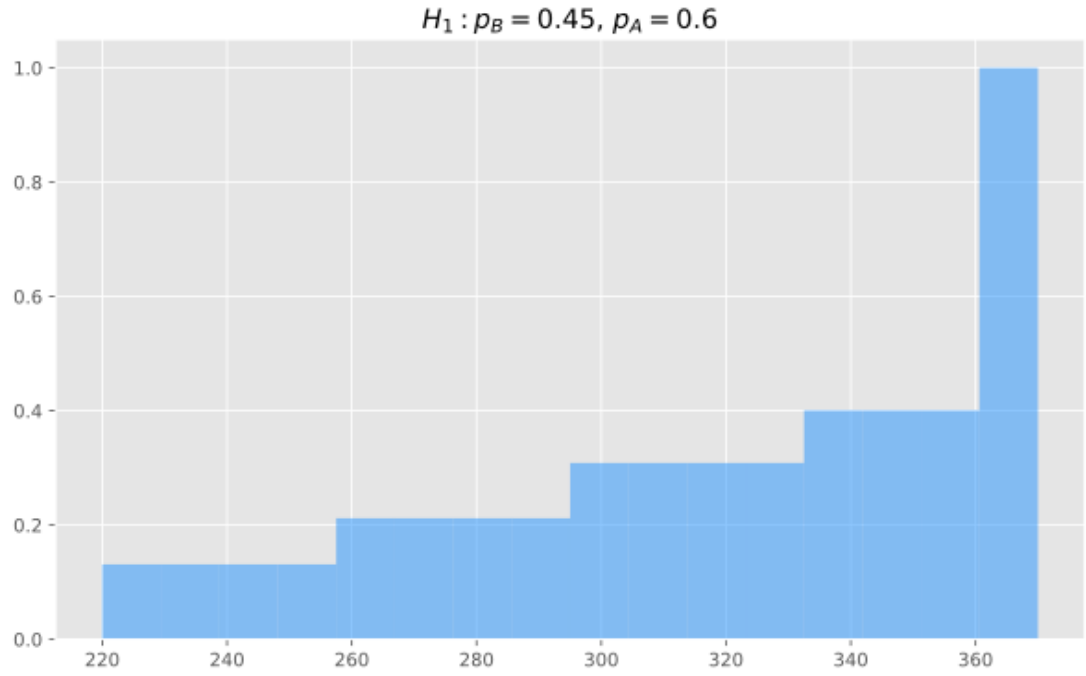

Fig. 5: Cumulative stopping probabilities (stopping for all causes) across interim analyses in H_1 scenario.

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12.10. Appendix 10: Study governance considerations

12.10.1. Regulatory and ethical considerations

This study will be conducted in accordance with the protocol and with the following:

- Consensus ethical principles derived from international guidelines including the Declaration of Helsinki and Council for International Organizations of Medical Sciences (CIOMS) International Ethical Guidelines

- Applicable ICH Good Clinical Practice (GCP) Guidelines

- Applicable laws and regulations. For this protocol, the European regulation 536/2014 for clinical studies (if applicable) and the German drug law (AMG) as well as the German GCP Ordinance apply.
  - ✓ The protocol, protocol amendments, ICF, SmPCs, and other relevant documents must be submitted to the relevant Competent Authority and an IEC by the sponsor and reviewed and approved by the CA and IEC before the study is initiated.
  - ✓ Any amendments to the protocol will require CA and IEC approval before implementation of changes made to the study design, except for changes necessary to eliminate an immediate hazard to study participants.

- The sponsor will be responsible for the following:
  - ✓ Providing written summaries of the status of the study to the CA and IEC annually
  - ✓ Notifying the CA and IEC of SAEs or other significant safety findings as required by the German drug law.

- The investigator will be responsible for the following:
  - ✓ Providing oversight of the conduct of the study at the site and adherence to ICH guidelines, the IEC, European regulation 536/2014 for clinical studies (if applicable, when the portal will be available), and all other applicable local regulations.
  - ✓ Maintaining all study and site-specific approvals and correspondence with the CA and IEC within the investigator site file.
  - ✓ Patient Alert Card: all prescribers of Tecentriq® (Atezolizumab) must be familiar with the safety section of the Tecentriq® (Atezolizumab) IB and with the SmPCs of the chemotherapeutic agents used as IMP in the neoadjuvant phase. The prescriber must discuss the risks of Tecentriq® (Atezolizumab) therapy and of the CTX administration with the patient. The patient will be provided with the Patient Alert Card and instructed to carry the card at all times.

12.10.2. Financial disclosure

Investigators and deputy will provide the sponsor with sufficient, accurate financial information as requested to allow the sponsor to submit complete and accurate financial disclosure statements to the IECs. Investigators are responsible for providing information on financial interests during the course of the study and for 1 year after completion of the study.

12.10.3. Safety issues and serious breaches of the protocol or ICH GCP

A “serious breach” is a breach which is likely to effect to a significant degree:

- The safety of physical or mental integrity of the subjects of the trial; or
- The scientific value of the trial.

The PI at each participating site is responsible for notifying the sponsor within 24 hours of becoming aware of a serious breach.

The sponsor is responsible for notifying the regulatory authorities (leading EC, local EC, competent authority (CA) and national CA) in writing of any serious breach (refer to section 9.12 Protocol Violations).

12.10.4. Informed consent process

- The informed consent document(s) used during the informed consent process must be reviewed and approved by the sponsor, approved by the IEC before use, and available for inspection.
- The investigator or his/her representative will explain the nature and objectives of the study and the possible risks associated with participation to the study patient or her legally authorized representative and answer all questions regarding the study.
- Participants must be informed that their participation is voluntary. Participants or their legally authorized representative will be required to sign a statement of informed consent that meets the requirements of regulatory and legal regulations and the ICH GCP guidelines.
- The investigator will retain the original of each patient’s signed consent document. The patient or its legally authorized representative will be provided with a copy of the signed ICF(s).
- The medical record must include a statement that written informed consent was obtained before the participant was enrolled in the study and the date the written consent was obtained. The authorized person obtaining the informed consent must also sign and date the ICF.
- Participants must be re-consented to the most current version of the ICF(s) during their participation in the study.

12.10.5. Data protection

- Study patients will be assigned a unique identifier by the sponsor. Any study patient records or datasets that are transferred to the sponsor will contain the identifier only; study patients names or any information which would make the participant identifiable will not be transferred. The European General Data protection Regulation as well as the AMG will apply for this study.
- The study patients must be informed that their personal study-related data will be used by the sponsor in accordance with local data protection law. The level of disclosure must also be explained to the study patient.
- The participant must be informed that her medical records may be examined by Clinical Quality Assurance auditors or other authorized personnel appointed by the sponsor, by appropriate IEC members, and by inspectors from regulatory authorities.
- By signing the ICF the patient gives this consent to the above-mentioned handling of his study-related data.

12.10.6. Publication policy

- The results of this study may be published or presented at scientific meetings. If this is foreseen, the investigators agree to submit all manuscripts or abstracts to ROCHE before submission. This allows ROCHE to protect proprietary information and to provide comments.
- Any publication will comply with the requirements for publication of study results and refer to the recommendations of the International Committee of Medical Journal Editors for the Conduct, Reporting, Editing, and Publication of Scholarly Work in Medical Journals (http://www.icmje.org/icmje-recommendations.pdf).
- ROCHE will be mentioned as financier within publications of the results of this trial.
- ROCHE will support the first publication of trial results in their entirety and not as individual site data.
- ROCHE will have an opportunity to review the first publication 60 days (14 days for abstracts) before it will be submitted for publication or otherwise disclosed.
- In case of Congress presentations ROCHE will have an opportunity for review at least 15 days before submission.
- For any publication/abstract review, the ROCHE's proposed changes will be taken into consideration.
- After the first publication of the trial results the participating investigators shall be at liberty to publish trial results in accordance with the sponsor.
- Authorship will be determined by mutual agreement and in line with International Committee of Medical Journal Editors authorship requirements.

12.10.7. Data quality assurance

- All participant data relating to the study will be recorded on electronic CRF. The investigator is responsible for verifying that data entries are accurate and correct by electronically signing the CRF.
- The investigator must maintain accurate documentation (source data) that supports the information entered in the CRF.
- The investigator must permit study-related monitoring, audits, IEC review, and regulatory agency inspections and provide direct access to source data documents.
- The sponsor is responsible for the data management of this study including quality checking of the data.
- The clinical monitors will perform an ongoing off-site combined with a risk-assessed on-site monitoring to confirm that data entered into the CRF by authorized site personnel are accurate, complete, and selected critical data are verifiable from source documents, that the safety and rights of participants are being protected; and that the study is being conducted in accordance with the currently approved protocol and any other study agreements, ICH GCP, and all applicable regulatory requirements (refer to Risk based monitoring (RBM) below).
- During onsite monitoring visits investigator(s) and their relevant staff must be available.
- Records and documents, including signed ICFs, pertaining to the conduct of this study must be retained by the investigator for 10 years after study completion or discontinuation unless local regulations or institutional policies require a longer retention period. No records may be destroyed during the retention period without the written approval of the sponsor. No records may be transferred to another location or party without written notification to the sponsor.

12.10.8. Risk Based Monitoring (RBM) strategy

Monitoring clinical data is a quality control (QC) activity which involves a system of ongoing checks to detect failures, to correct them, and prevent the failure from recurring. The overall goal of monitoring is to produce clinical data consistently.

In this protocol, the RBM strategy will be applied as followed: RBM is an adaptive approach that directs monitoring focus and activities to the areas which have the most potential to impact patient safety and data quality. RBM provides an ability to evaluate and plan for risks before a study starts and continuously adapt monitoring activities to areas that have the most potential to impact patient safety and data quality. Study oversight is accomplished through an appropriate mix of central, off-site and on-site monitoring activities.

On-site monitoring visit: In person evaluation carried out by sponsor/CRO personnel at the investigational site. On-site monitoring visits will focus on reviewing completeness and accuracy of ICFs, drug supply reconciliation, source document verification (SDV) and Source Data Review (SDR) of original records, and other issues that may occur during the course of the clinical trial. The activities are conducted regardless of the type of study, safety risks, phase of the study, stage of the study, or experience of the site personnel conducting the study.

Off-site monitoring or remote monitoring: Evaluation carried out by sponsor/CRO personnel outside the investigative site. It is a centralized review of individual site data. Data check is accomplished through regular phone monitoring with the study site personnel using monitoring questionnaires.

Central monitoring: Review of centralized data focusing on risk indicator data between investigative sites within a clinical trial or across studies. This review may be performed by medical monitors, data managers, project managers, statisticians.

The risks will be evaluated through a Key Risk Indicator (KRI) analysis which determines risks that could affect patient safety, data integrity, and/or regulatory compliance and:

- Identifies how and by which functions risks will be managed
- Categorizes risks which will be managed by and affect the monitoring plan (MP)
- Determines overall risk level (green, yellow, red) for monitoring activities
- Ensures that monitoring strategies (mitigation actions) are tailored to risks that are focused on critical data and processes

The detailed risk analysis is described in the risk management plan (RMP). The monitoring processes are described in the study MP.

12.10.9. Source documents

- Source documents provide evidence for the existence of the participant and substantiate the integrity of the data collected. Source documents are filed at the investigator's site.
- Data reported on the eCRF that are transcribed from source documents must be consistent with the source documents or the discrepancies must be explained. The investigator may need to request previous medical records or transfer records, depending on the study. Also, current medical records must be available.
- Any source document that is not directly integrated in the patient's notes and created separately for the study or documented electronically may be used as "source document" only if this is specifically documented in the protocol. If a copy is made from an original document, the copy must be certified. In that case it will be made available at every study visit and will be filed and archived as study relevant source documents.

12.10.10. Site closures

The sponsor reserves the right to close the study site or terminate the study at any time for any reason at the sole discretion of the sponsor. In any other case, study sites will be closed upon study completion. An active study site is considered closed when all required documents and study supplies have been collected and a study-site closure visit has been performed. For sites which didn't recruit patients (inactive site) no closure visit is necessary.

The investigator may initiate study-site closure at any time, provided there is reasonable cause and sufficient notice is given in advance of the intended termination.

Reasons for the early closure of a study site by the sponsor or investigator may include but are not limited to:

- Failure of the investigator to comply with the protocol, the requirements of the IEC or local health authorities, the sponsor's procedures, or GCP guidelines
- No recruitment of study patients by the investigator in a defined period as defined by the sponsor study team.

12.10.11. Study design and management: initiation, managing, funding of the clinical trial

The study is funded by ROCHE. palleos healthcare GmbH is conducting this trial as a sponsor (organizing, managing and running the clinical trial)

12.10.12. Patient expenses/payments

There are no participants study payments designated.

12.10.13. Insurance

The sponsor will provide patient insurance for the clinical trial for all study participants in accordance with the AMG.

12.10.14. Steering Committee (SC)

The SC members are listed on page 3. The role of the SC will be to provide overall supervision of the trial and to ensure that it is being conducted in accordance with the principles of GCP and the relevant regulations. The SC will provide advice to the investigators on all aspects of the trial.

12.11. Appendix 11: Abbreviations and trademarks

AE	Adverse event
AESI	Adverse event of special interest
AGO	Arbeitsgemeinschaft Gynäkologische Onkologie E.V
ALK	Alkaline phosphatase
ALT	Alanine aminotransferase
AMG	“Arzneimittelgesetz” (German drug law)
ANC	Absolute neutrophile count
Anti-TNF	Anti-tumor necrosis factor
AST	Aspartate aminotransferase
AT	As treated
AUC	Area under the curve
BRCA	Breast cancer
CA	Competent authority
CAPA	Corrective and preventive action
CCCA	Complete Cell Cycle Arrest
CelTIL	Combined score based on tumor cellularity and TILs
CHF	Congestive heart failure
CIOMS	Council for International Organizations of Medical Sciences
CT	Computerized tomography
CTC	Common Terminology Criteria
CTCAE	Common Terminology Criteria for Adverse Events
ctDNA	circulating tumor Deoxyribonucleic acid
CTX	Chemotherapy
DC	Dendritic cell
DCIS	Ductal carcinoma *in-situ*
DFS	Disease Free Survival
DRF	Death report form
DSMB	Data Safety Monitoring Board

DSUR	Development Safety Update Report
EC	Ethic committee
ECG	Electrocardiogram
ECOG	Eastern Cooperative Oncology Group
EDC	Electronic Data Capture
EFS	Event Free Survival
EGFR	Epidermal growth factor receptor
EOS	End of study
EOT	End of treatment
(e)CRF	(Electronic) case report form
ER	Estrogen receptor
FPI	First patient in
GCP	Good clinical practice
G-CSF	Granulocyte Colony Stimulating Factor
hCG	Human Choriongonadotropin
HER	Human epidermal growth factor receptor
IB	Investigator's brochure
ICF	Informed consent form
IEC	Independent Ethics Committee
IHC	ImmunoHistoChemistry
IMP	Investigational Medicinal Product
irAE	Immune-related adverse event
ISH	*In-Situ*-Hybridization
ITT	Intention-to-treat
IUD	Intrauterine device
IV	Intravenous
KI	Karnofsky index
KRI	Key Risk Indicator
LPLV	Last patient last visit

LVEF	Left ventricular ejection fraction
MAR	Missing at random
MedDRA	Medical Dictionary for Regulatory Activities
MM	Medical monitor
MP	Monitoring plan
MRI	Magnetic Resonance Imaging
mTNBC	Metastatic triple negative breast cancer
NCI	National Cancer Institute
NMAR	Not-missing at random
NYHA	New York Heart Association
OS	Overall survival
pCR	Pathological complete response
PD	Progressive disease
PD-L1	Programmed cell Death 1 Ligand 1
PET	Positron emission tomography
PI	Principal investigator
PP	Per Protocol
PP (*italic*)	Predictive probability
PR	Progesterone Receptor
QC	Quality control
QTc	QT corrected interval
RBM	Risk based monitoring
RMP	Risk management plan
SAE	Serious adverse event
SC	Steering Committee
SDR	Source data review
SmPC	Summary of Product Characteristics
SOA	Schedule of Activity
SOC	Standard of Care

SUSAR	Suspected unexpected serious adverse reaction
TILs	Tumor-Infiltrating Lymphocytes
TMA	Tissue Microarrays
TNBC	Triple Negative Breast Cancer
UICC	The Union for International Cancer Control
ULN	Upper Limit of Normal