PHOENIX

PHOENIX DDR/Anti-PD-L1 Trial:

A pre-surgical window of opportunity and post-surgical adjuvant biomarker study of DNA damage response inhibition and/or anti-PD-L1 immunotherapy in patients with neoadjuvant chemotherapy resistant residual triple negative breast cancer

TRIAL SUMMARY

PROTOCOL TITLE	**PHOENIX DDR/Anti-PD-L1 Trial:** A pre-surgical window of opportunity and post-surgical adjuvant biomarker study of DNA damage response inhibition and/or anti-PD-L1 immunotherapy in patients with neoadjuvant chemotherapy resistant residual triple negative breast cancer
PROTOCOL CONTEXT	This trial is part of the PHOENIX Platform: A post-neoadjuvant chemotherapy resistant residual triple negative breast cancer, phase IIa novel therapy window of opportunity platform aiming to identify biomarker response signals of biological activity to inform phase II and/or efficacy endpoint trials
TARGET DISEASE	Neoadjuvant chemotherapy (NACT) resistant residual early triple negative breast cancer (TNBC) with high risk of metastatic relapse
TRIAL HYPOTHESIS	In patients with TNBC who have moderate to significant residual disease following NACT, short exposure to a DNA damage response (DDR) inhibitor and/or anti-programmed death ligand 1 (PD-L1) immunotherapy in the post-NACT, preoperative window of opportunity (WOP) will show a signal of biological activity in residual disease tissue and for those patients who have post-operative evidence of presence of micro-metastatic relapse exposure to the same therapy will show a signal of anti-tumour activity.
PRIMARY OBJECTIVE	To assess whether short exposure to a DDR inhibitor and/or anti-PD-L1 immunotherapy in a preoperative WOP in patients with post-NACT high risk residual disease, generates a signal of anti-tumour biological activity within residual disease tissue.

SECONDARY OBJECTIVES	1. To characterise the safety of designated IMPs in a WOP trial context. 2. To examine biomarkers of cancer or stroma pathway reprogramming/ signalling following trial treatment.
EXPLORATORY OBJECTIVES	1. To examine the overall effect on the growth index (calculated as Ki67/ apoptosis %) of the residual tumour. 2. Explore within patient associations between responses seen in PART 1 and changes in circulating tumour DNA (ctDNA) in PART 2. 3. Explore associations between ctDNA detection and disease related outcomes in PART 2.
TRIAL DESIGN	WOP, open-label, multi-centre, phase IIa trial comprising multiple non-comparative treatment cohorts with patient allocation via minimisation. The trial consists of two parts: a post-NACT preoperative WOP component (PART 1); and a post-operative component (PART 2).
TRIAL POPULATION	Patients with early TNBC who are poor responders to NACT with significant viable residual disease, as defined by imaging, and for whom definitive complete surgical excision of disease is planned.
PART 1 – WINDOW OF OPPORTUNITY COMPONENT	In PART 1 patients identified as poor responders to NACT during mid-assessment scan as per standard imaging guideline (i.e. predicted to have ≥1cm residual disease at the end of NACT) will be approached to consent for Trial Registration. Registered patients with confirmed residual disease ≥1cm on the trial-specific imaging performed at least 1 week following day 1 of the final cycle of NACT will be approached to consent for Trial Entry. The trial will comprise initially of 4 cohorts allocated via minimisation: a standard care reference cohort (Cohort A); 2 DDR inhibitor treatment cohorts (Cohorts B and C); and an anti-PD-L1 immunotherapy treatment cohort (Cohort D). Eligible patients will be allocated a cohort via minimisation (to ensure unbiased selection of patient characteristics within each cohort) and commence trial treatment, as applicable as indicated below, within the WOP defined as the 2-week time period starting at least 3 weeks after the first day of the final cycle of NACT and 2 weeks prior to the patient's scheduled surgical intervention. The WOP is referred to as Day 1 – Day 14. **Cohort A**: Standard care reference cohort (no trial treatment during Part 1) **Cohort B**: pre-operative exposure to AZD6738

- 160mg AZD6738 to be administered orally twice daily on **Days 5-14** of the WOP.

Cohort C: pre-operative exposure to olaparib

- 300mg of olaparib to be administered orally twice daily on **Days 1-14** of the WOP.

Cohort D: pre-operative exposure to durvalumab

- 1,500mg durvalumab to be administered via intravenous (IV) infusion on **Day 1 only** of the WOP*

** If there is an absence of a response signal in Cohort D co-primary endpoints, consideration will be given to protocol amendment to add a new durvalumab cohort starting durvalumab 6 weeks prior to surgery with or immediately following the final cycle of NACT supported by associated safety data given the possible latency of T-cell response after anti-PDL1 therapy [2].*

Potential future cohorts:

Once sufficient safety data is available the following combination treatment cohorts may be added to the protocol via future amendment:

- AZD6738 in combination with olaparib
- AZD6738 in combination with durvalumab
- Olaparib in combination with durvalumab

Research blood and tissue samples will be collected from all patients at the beginning and end of the WOP to allow pre- and post-treatment comparison.

All patients will be followed up with a visit at 30 days post-surgery (including collection of research blood samples), at 3 months post-surgery (this will incorporate the PART 2 Pre-Treatment Assessments visit for patients screened ctDNA positive at 30 days post-surgery) and every 3 months for 24 months following the 3-month post-surgery/30-day post-surgery visit (for those screened ctDNA positive at 30 days post-surgery).

At Trial Registration patients will be required to provide consent for access to their primary archival diagnostic tissue block, except if unavailable in exceptional circumstances upon discussion with the trial team. For those patients who go on to consent to randomisation, this tissue block will be analysed at the central laboratory for the presence of trackable mutations for which a ctDNA assay can be developed to facilitate continuation to PART 2. In cases where archival diagnostic tissue block is unavailable, trackable mutations identified in the residual disease from the patient's surgical resection formalin-fixed paraffin-embedded (FFPE) block may be used to develop a ctDNA assay. The ctDNA assays will be personalised and developed for the trackable mutations found in each individual patient's cancer and will facilitate continuation to PART 2.

If no trackable mutations can be detected in the patient's tumour tissue, or if it is not possible to develop a ctDNA assay, this will be considered a mutation analysis screen failure. Patients with a mutation analysis screen failure will not be approached for continuation to PART 2. These patients will be followed up for a total of 24 months from the 3 month post-surgery visit with data collected on survival and further treatment received.

PART 2 – POST-OPERATIVE COMPONENT

All patients will be provided with further information about continuation to PART 2 during Part 1. Once trackable mutation status has been confirmed, eligible patients will be asked to consent for continuation to PART 2. Consenting patients will be asked to provide blood samples for ctDNA screening to detect minimal residual disease (MRD) at the 30-day post-surgery visit, or at the 3-month post-surgery visit if the results of the trackable mutation status are not available at the time of the 30 day post-surgery visit.

DNA extracted from the plasma will be analysed for the presence of tumour ctDNA, and the patient will be identified as ctDNA positive (ctDNA detected) or ctDNA negative (ctDNA not detected). Patients who test ctDNA negative at the 30 day post-surgery visit will be asked to undergo repeat ctDNA screening at the 3-month post-surgery visit.

Patients allocated to Cohorts B-D with a ctDNA positive result, at either the 30 day or 3 month post-surgery visit, may resume treatment with the same trial treatment administered in PART 1 as indicated below. Patients allocated to Cohort A with a ctDNA positive result may commence durvalumab treatment as for Cohort D. In all cases treatment can only commence provided that the pre-treatment assessments confirm suitability to receive trial treatment in PART 2 and that the treatment cohort is not closed due to safety concerns. Patients whose clinical management includes the use of adjuvant capecitabine will not be invited to participate in PART 2 of the trial, but will be followed up every 3 months for a total of 24 months from the 3 month post-surgery visit as per Part 1 follow-up schedule.

Cohort A: durvalumab monotherapy

- 1,500mg durvalumab to be administered via intravenous (IV) infusion on Day 1 only of each 28 day cycle.

Cohort B: AZD6738 monotherapy

- 160mg AZD6738 to be administered orally twice daily on Days 1 – 14 of each 28 day cycle.

Cohort C: olaparib monotherapy

- 300mg olaparib (2 x 150mg tablets) to be administered orally twice daily on a continuous schedule Day 1-28 of each 28 day cycle.

Cohort D: durvalumab monotherapy

- 1,500mg durvalumab to be administered via intravenous (IV) infusion on Day 1 only of each 28 day cycle.

For each treatment cohort a cycle consists of 28 days and the planned trial treatment duration is 12 months (13 cycles).

Patients will continue on trial treatment for up to a maximum of 12 months with serial ctDNA blood samples collected every 4 weeks.

Follow up

All patients who provide consent for continuation to PART 2 (including those patients identified as ctDNA negative or those who did not resume trial treatment in PART 2 for any reason) should be followed up every 3 months for a total of 24 months from the 3 month post-surgery/30 day post-surgery visit (for those screened ctDNA positive at 30 days post-surgery) with follow up data collected on survival and further treatment received.

Those patients who were identified as ctDNA negative or those identified as ctDNA positive who **did not** have evidence of macroscopic disease on imaging **but** were deemed unsuitable to resume trial treatment in PART 2 for another reason should also have ctDNA blood samples collected at each follow up visit (i.e. every 3 months (+/-2 weeks) for a period of 24 months from the 3 month post-surgery/30 day post-surgery visit (for those screened ctDNA positive at 30 days post-surgery).

RECRUITMENT TARGET

A maximum of 81 evaluable patients will be recruited into the trial as outlined below:

PART 1:

Cohort A – 9 patients
Cohort B – max. 24 patients
Cohort C – max. 24 patients
Cohort D – max. 24 patients

PART 2:

Any patients who are identified as ctDNA positive at 30 days or 3 months post-surgery will be given the opportunity to receive trial treatment in PART 2 provided they are confirmed suitable to commence treatment. Therefore a maximum of 81 patients may resume trial treatment within PART 2, however it is anticipated that the proportion of patients identified as ctDNA positive at this time point – and thus receiving trial treatment in PART 2 – will be substantially lower (approximately 20%).

PRIMARY ENDPOINTS

The primary endpoint is specific to each treatment cohort based on the nature of the target and the biological effect being targeted.

The co-primary endpoints for Cohorts B, C and D are as outlined below:

Cohort B and C:

1. **Change in mean proliferation index** (as measured by tumour cell Ki67 staining) post-WOP intervention within post-treatment biopsy compared to pre-treatment baseline biopsy.
   A patient will be defined as being a Ki67 responder if they experience a relative decrease in Ki67 positive cells of ≥33% in the post-treatment biopsy sample

AND/OR

2. **Changes in the proliferation gene expression signature** post WOP intervention within the post-treatment biopsy compared to pre-treatment baseline biopsy.
   A patient will be defined as being a responder if there is a ≥1.5-fold decrease in the proliferation gene expression score in the post-treatment biopsy sample

Cohort D:

1. **Change in $CD8^+$ stromal tumour infiltrating lymphocytes (sTILs)** post anti-PD-L1 immunotherapy within the post-treatment biopsy compared to pre-treatment baseline biopsy.
   A patient will be defined as being a responder if they experience an absolute increase of ≥ 10% in the percentage CD8+ sTILs within the post-treatment biopsy sample

AND/OR

2. **Changes in the interferon gamma-positive ($IFN\gamma^+$) signature** post WOP intervention within the post-treatment biopsy compared to pre-treatment baseline biopsy.
   A patient will be defined as being a responder if there is a ≥2-fold increase in the IFNγ+ gene expression in the post-treatment biopsy sample

Cohort A (standard care reference cohort) will allow some characterization of any (artefactual) biopsy effects on the co-primary endpoints assessed in treatment Cohorts B, C and D.

SECONDARY ENDPOINTS

1. Incidence of adverse events (AEs) during trial treatment (including surgical complications) by treatment cohort at 1 month post-surgery.
2. Changes in phosphorylation of ataxia telangiectasia and Rad3-related protein (ATR) and its downstream effectors (Chk1, γH2AX, TAO upon drug exposure: including but not limited to levels of phosphorylation of p53, p38, p21/p27, cyclin dependent kinases (CDC25)).
3. Changes in biomarkers of DDR and adaptive and innate response, including but not limited to 53BP1, RAD51, RPA, RPA32, pRPA, BRCA1/2, PARP expression and immune checkpoint ligands and receptors and adaptive and innate immune response markers (IFNγ, cGAS-STING pathway, NKG2D receptors, ligands and

cell markers) in the post-treatment biopsy compared to pre-treatment baseline biopsy using gene expression profiling.

4. Assessment of associated expression of co-inhibitory immune checkpoint receptors and ligands and frequency and function of TILs and myeloid cells subsets using immune cell markers and high content image de-convolution.
5. Changes in the levels of Th1/IFNγ response as measured by transcriptional and proteomic profiling.
6. Immune cell population sub-set characterisation using appropriate and T and B cell receptor DNA sequencing methodologies.
7. Assess change in the Ki67:CD8+ ratio within the post-treatment biopsy compared to pre-treatment baseline biopsy.

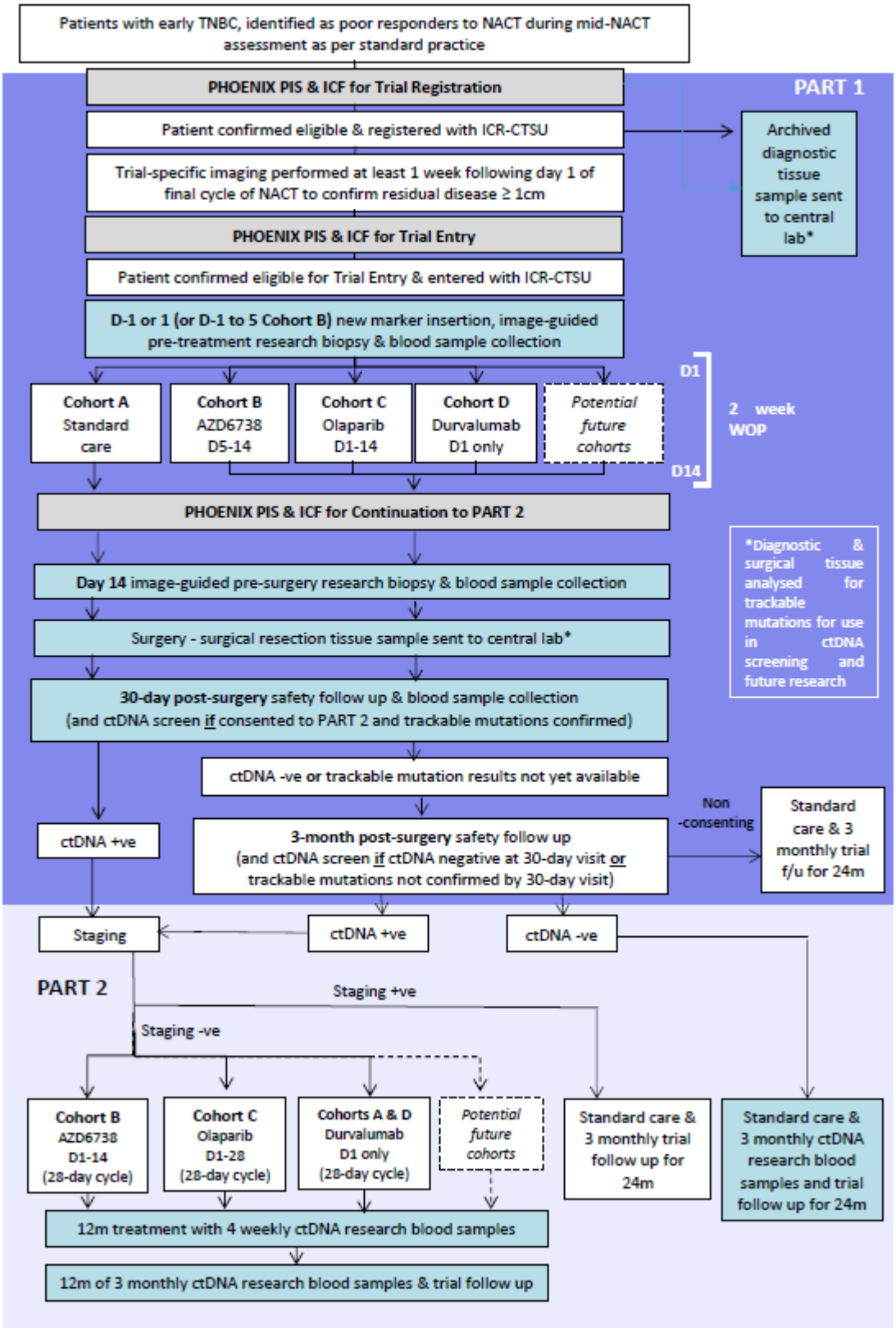
EXPLORATORY ENDPOINTS

1. Assess the ratio change in apoptosis and tumour cell proliferation in the post-treatment biopsy compared with pre-treatment baseline biopsy.
2. Relationship between the primary and secondary endpoints with the mutational landscape of the treated tumour as assessed by deep sequencing of the primary and residual disease and any subsequent metastatic relapse tumour genome and of tumour ctDNA in plasma.
3. Descriptive relationship between changes in ctDNA compared to biomarkers response to PART 1 in all patients from cohorts B, C and D.
4. Descriptive relationship between response status in PART 1 and changes in ctDNA in PART 2 within patients from cohorts B, C and D identified as ctDNA positive 30 days/3 months post-surgery who receive trial treatment in PART 2.
5. Descriptive relationship between ctDNA mutational profile in mutated genes in ctDNA pre-treatment and post-treatment ctDNA profiles in patients in both PART 1 and PART 2.
6. Descriptive differences in time between ctDNA detection and time to recurrence by 2 years in both the treated groups and observation group in PART 2.
7. Changes in biomarkers of DDR and adaptive and innate response, including but not limited to 53BP1, RAD51, RPA, RPA32, pRPA, BRCA1/2, PARP expression and immune checkpoint ligands and receptors and adaptive and innate immune response markers (IFNγ, cGAS-STING pathway, NKG2D receptors, ligands and cell markers) in the post treatment biopsy compared to pre-treatment baseline biopsy using reverse phase protein array (RPPA) and other proteome profiling.

TRIAL DURATION AND FOLLOW UP

It is anticipated that recruitment will take approximately 36 months to complete.

All patients will be followed up for a total of 24 months from the 3 month post-surgery/30 day post-surgery visit (for those screened ctDNA positive at 30 days post-surgery).


Patients with early TNBC, identified as poor responders to NACT during mid-NACT assessment as per standard practice
PART 1
PHOENIX PIS & ICF for Trial Registration
Patient confirmed eligible & registered with ICR-CTSU
Archived diagnostic tissue sample sent to central lab*
Trial-specific imaging performed at least 1 week following day 1 of final cycle of NACT to confirm residual disease ≥ 1cm
PHOENIX PIS & ICF for Trial Entry
Patient confirmed eligible for Trial Entry & entered with ICR-CTSU
D-1 or 1 (or D-1 to 5 Cohort B) new marker insertion, image-guided pre-treatment research biopsy & blood sample collection
D1
D14
2 week WOP
Cohort A Standard care
Cohort B AZD6738 D5-14
Cohort C Olaparib D1-14
Cohort D Durvalumab D1 only
Potential future cohorts
PHOENIX PIS & ICF for Continuation to PART 2
*Diagnostic & surgical tissue analysed for trackable mutations for use in ctDNA screening and future research
Day 14 image-guided pre-surgery research biopsy & blood sample collection
Surgery - surgical resection tissue sample sent to central lab*
30-day post-surgery safety follow up & blood sample collection (and ctDNA screen if consented to PART 2 and trackable mutations confirmed)
ctDNA -ve or trackable mutation results not yet available
Non -consenting
Standard care & 3 monthly trial f/u for 24m
ctDNA +ve
3-month post-surgery safety follow up (and ctDNA screen if ctDNA negative at 30-day visit or trackable mutations not confirmed by 30-day visit)
Staging
ctDNA +ve
ctDNA -ve
PART 2
Staging +ve
Staging -ve
Cohort B AZD6738 D1-14 (28-day cycle)
Cohort C Olaparib D1-28 (28-day cycle)
Cohorts A & D Durvalumab D1 only (28-day cycle)
Potential future cohorts
Standard care & 3 monthly trial follow up for 24m
Standard care & 3 monthly ctDNA research blood samples and trial follow up for 24m
12m treatment with 4 weekly ctDNA research blood samples
12m of 3 monthly ctDNA research blood samples & trial follow up