Recipient Information	
Family name: Achutti Duso	First name: Bruno
ESMO Membership ID: 360469	
Phone (at current workplace/mobile): +39 0294372728 +39 3485513138	Email: bruno.achuttiduso@ieo.it

Grant Information	
Start date: 21/08/2017	End date: 20/08/2018

Host Institute Information	
Institution: European Institute of Oncology	City, Country: Milan, Italy
Mentor / Contact: Prof. Dr. Giuseppe Curigliano	Email: giuseppe.curigliano@ieo.it

Clinical Research Fellowship – Final Report

Title of the project
Combination of pembrolizumab with oral metronomic cyclophosphamide in patients with chest wall breast cancer (PERICLES): A phase II study. *EudraCT NUMBER: 2017-003343-37*

1. Background and rationale

In spite of the current efforts for diagnostic standardization[1–3] and recommended trimodality approach - with neoadjuvant chemotherapy followed by modified radical mastectomy, radiation and targeted therapy when appropriate -, local recurrence rates for inflammatory breast cancer (IBC) are still high. A majority of patients progress to a lymphangitic spread to the chest wall ("chest wall disease"[CWD]) and/or become metastatic, with a median overall survival (OS) of only 26 months[4]. Retrospective data analyses regarding previously known breast cancer molecular subtypes have limited predictive and prognostic power in IBC. Unlike non-IBC (nIBC), IBC with hormone receptor (HR)-positive status are not associated with a favorable prognosis. Similarly, receiving trastuzumab for HER2-positive disease does not prolong OS or disease free survival (DFS)[5]. These striking differences in clinical presentation and outcomes of IBC compared to nIBC suggest that its' pathological and molecular biology may also significantly diverge. IBC appears to be a disease of the whole breast, presenting as a combination of tumor cell-intrinsic oncogenic pathways and breast tissue abnormalities that create the characteristic IBC phenotype. The anti-programmed cell death protein-1 (PD-1) is a critical checkpoint molecule expressed on the cell surface of T-cells upon activation. It acts primarily in the periphery by dampening ongoing immune responses and preventing damage to self-tissues. This inhibitory action is thought to be a key in the immune evasion process as PD-1, PD-L1 and PD-L2 have been found to be abnormally expressed by both malignant cells and lymphocytes in the tumor microenvironment (TME)[6]. Also, PD-L1 messenger ribonucleic acid (mRNA) is expressed in nearly 60% of breast tumors, independently of HR status, and is positively correlated with PD-L1 protein expression and increased tumor infiltrating lymphocytes (TILs)[7]. Pembrolizumab is a selective, humanized IgG4/kappa isotype, anti-PD-1 monoclonal antibody (mAb) that exhibits dual ligand blockade of the PD-1 pathway. By blocking interactions between PD-L1/PD-L2 and PD-1, pembrolizumab may reactivate immune surveillance, leading to improved

anti-tumor activity. Dying cancer cells that release endogenous molecules - damage-associated molecular patterns (DAMPs) - after exposure to certain cytotoxic agents can render these tumor cells recognizable by antigen presenting cells (APCs), such as dendritic cells (DCs), and prompt the T-cell-mediated adaptive immunity[8,9]. Generation of these tumor-reactive CD8 T cells requires a complex path, starting with successful processing and presentation of tumor-associated peptide by APCs and recognition of these antigenic peptides by MHC I/II. A unique T-cell receptor recognizes MHC-bound tumor antigen, providing the first signal for T-cell activation. Full T-cell activation follows the engagement of the co-stimulatory CD28 receptor on T cells by B7 on the APC. Tumor-specific CD8 T cells subsequently differentiate into Teff, undergo clonal expansion, traffic to the TME, and ultimately kill tumor cells, displaying those tumor-associated antigens on HLA, via release of cytolytic effector molecules[10–12]. Some cytotoxic agents, in turn, may lead to immunogenic cell death resulting in activation of dendritic cells (DCs) and priming of anti-tumor immune responses. This promotion of DC maturation might also explain the capacity of some chemotherapy regimens to reduce regulator T cells (Treg), as a higher frequency of proliferating cells is observed in Treg compared to the non-Treg compartment, tilting the balance from Treg towards effector T cells (Teff)[13]. Cyclophosphamide (CTX) is one of the main products of this therapeutic class and some studies in humans have shown a selective reduction in Treg numbers after low dose/metronomic CTX administration[14,15]. According to Ghiringhelli et al.[16], after 1 month of metronomic CTX regimen, initially designed to reduce tumor angiogenesis through its effect on endothelial cells found in growing tumor-associated blood vessel capillaries as well as up-regulation of the endogenous angiogenesis inhibitor thrombospondin-1, - the number of circulating Treg was decreased and T cell proliferation as well as NK cell effector function were restored in patients with end-stage tumors. Thus, we hypothesize that the specific depletion of these CD4+CD25+FOXP3+ Treg cells along with the upregulation of DCs by metronomic CTX in combination with pembrolizumab may have an impact on inducing a significant clinical response in the IBC/CWD population[17].

A previous study already investigated this combination in patients with soft tissue sarcoma (STS). Toulmonde et al. ran an open-label, multicenter, phase 2 trial, on 4 cohorts of patients with advanced leiomyosarcoma (LMS), undifferentiated pleomorphic sarcoma (UPS), other sarcomas (others) and gastrointestinal stromal tumor (GIST)[18]. All patients received CTX 50 mg twice daily 1 week on and 1 week off, and 200 mg of IV pembrolizumab every 3 weeks. Between June 2015 and July 2016, 57 patients were included (median age 59.5 years; 24 women [42%]) and 50 patients were assessable for the efficacy end point. Only three patients experienced tumor shrinkage, resulting in PR in a single solitary fibrous tumor. The 6-month non-progression rates were 0%, 0%, 14.3% (95% CI, 1.8%-42.8%) for LMS, UPS, and others, respectively, and 11.1% (95% CI, 2.8%-48.3%) for GIST. The most frequent adverse events were grade 1-2 fatigue, diarrhea, and anemia. The only patient who experienced partial response (PR) was the only one with strong PD-L1-positive staining in immune cell. Strong infiltration by macrophage expressing the inhibitory enzyme indoleamine 2,3-dioxygenase 1 (IDO1) was also observed in the majority of cases. Authors concluded that PD-1 inhibition has limited activity in selected STS and GIST. This may be explained by an immunosuppressive TME resulting from M2 macrophage infiltration and IDO1 pathway activation. STS are known to be immunologically “cold tumors”, with limited lymphocytic infiltration and low expression of PD-L1. We certainly cannot extrapolate these data for IBC/CWD, which presents as a clinical spectrum ranging from inflammatory to lymphangitic breast cancer. Inflammation and the immune response have long been viewed as a delicate balance that have the ability to promote a durable tumor regression or promote tumor progression. Preclinical models and biomarker studies suggest that IBC counts on an important role of the TME, including immune cell infiltration and vasculogenesis, especially lympho-angiogenesis[19]. The activation of mature DCs through toll like receptors (TLRs) or by inflammatory cytokines converts immature DCs into mature DCs that present specific antigen to T cells, thereby activating them. Maturation of DCs is accompanied by co-stimulatory molecules and secretion of inflammatory cytokines polarizing lymphocytic, macrophages and fibroblast infiltration. It is unknown until which level those immune cells associated to the IBC microenvironment transiently promote epithelial to mesenchymal transition (EMT) in this scenario. Immune and TME factors can induce phenotypic, morphological, and functional changes in breast cancer cells. We can hypothesize that similar inflammatory conditions in vivo may support both the rapid metastasis and tight tumor emboli

that are characteristic of CWD and that targeted anti-inflammatory therapy may be key in this specific patient population. The activity of pembrolizumab was also investigated among patients with advanced triple negative breast cancer (TNBC) in the Keynote 012 trial[20]. Among 111 subjects whose tumor samples were screened for PD-L1 expression, 58.6% had PD-L1 positivity. 32 women (median age 50.5 years; range 29 to 72 years) were enrolled and assessed for safety and antitumor activity. The median number of doses administered was five (range 1 to 36 doses). Common toxicities were mild and similar to those observed in other tumor cohorts (e.g., arthralgia, fatigue, myalgia, and nausea), and included five (15.6%) patients with toxicity grade ≥3 and one treatment-related death. Among the 27 patients who were evaluable for antitumor activity, the overall response rate (ORR) was 18.5%, the median time to response was 17.9 weeks (range 7.3 to 32.4 weeks), and the median duration of response (DoR) was not yet reached (range 15.0 to ≥ 47.3 weeks). Another trial addressing the efficacy and safety of pembrolizumab as a single-agent was the KEYNOTE-086. On cohort A[21], consisting of previously treated metastatic TNBC, regardless of PD-L1 expression, patients had pembrolizumab 200 mg every 3 weeks for up to 24 months. Of the total 170 women enrolled (median age 54 years), 62% had positive PD-L1 status at screening (combined positive score [CPS] ≥1%); 44% had ≥3 prior lines of therapy, 51% had elevated LDH and 74% had visceral disease. After a median follow-up of 10.9 months, 5% patents remained on trial. Treatment-related AEs (TRAEs) of any grade and grade 3-4 occurred in 60% and 12% of patients, respectively; 4% discontinued due to TRAEs. There were no deaths due to AE. ORR was 5%, regardless of PD-L1 expression. Best overall response was 0.6% complete response (CR), 4% PR, 21% stable disease (SD); 3% not evaluable. Disease control rate (DCR [CR + PR + SD ≥24 weeks]) was 8% (95% CI, 4-13). Median DoR was 6.3 months. Median PFS and OS were 2.0 (95% CI, 1.9-2.0) and 8.9 months (95% CI, 7.2-11.2), respectively. ORR was numerically lower in patients with poor prognostic factors (e.g., high LDH, visceral disease). On cohort B[22], corresponding to first line metastatic TNBC patients with tumor PD-L1 CPS ≥1, the same schedule was given to 84 women (median age 52.5 years) of which 48% had elevated LDH, 65% had visceral and/or non-visceral metastases, and 87% received prior (neo)adjuvant therapy. After 10.6 months of median follow-up, 21% remained on pembrolizumab. TRAEs occurred in 63% of patients and were of grade 3-4 in 8%; no patients died or discontinued pembrolizumab because of TRAEs. The most common TRAEs were fatigue (26%), nausea (13%), and diarrhea (12%). The most common immune-mediated AE was hypothyroidism (10%). Three patients had CR and 16 had PR for an ORR of 23% (95% CI, 15-33). Of the 11 patients with a best response of SD, 1 had SD for ≥24 weeks, leading to a DCR of 24% (95% CI, 16-34). 12 of 19 (63%) responses were ongoing at data cutoff, and median DoR was 8.4 months (range 2.1 to 13.9). Median PFS was 2.1 months (95% CI, 2.0-2.2), with an estimated 6 months PFS rate of 26%. Median OS was 16.1 months (95% CI 11.3-NR), with an estimated 6 months OS rate of 83%.

The present study is a proof-of-concept clinical trial that will evaluate the hypothesis that the combination of an immune reactivation strategy with an anti-PD-1 mAb and oral metronomic CTX in the setting of advanced IBC/CWD can induce objective response and improve clinical outcomes in this special population of patients where there is a massive lymphocytic infiltration and where mechanisms of inflammation and tolerance are upregulated. To support our translational analysis, data gathered in recent years show that defining only one or two immune markers as predictive of therapeutic success is unlikely to be sufficient in terms of prognosis and prediction[23], encouraging a more comprehensive and multiparametric approach regarding the potential elements responsible for segregating the history of this particular disease[24]. A series of gene signatures have been identified in IBC patients so far, but none of them with enough value to be applied on current clinical practice as a therapeutic guiding tool[25–29]. As we better appreciate the immune context and the unprecedented impact of hitting it on specific populations among phase II and phase III trials - associated or not with cytotoxic agents[30–32] -, we may rethink multimodality and how the environment that affects progression can also affect response to treatment. Additionally, accumulating evidence supports a strict contribution of the gut and possibly breast microbiomes and their metabolic activities at enhancing host antitumor immune response[33–35].

2. Aims

2.1. Primary endpoints: assessment of the objective response rate (ORR) - confirmed complete response (CR) or PR as best overall responses - to pembrolizumab plus metronomic CTX as therapy for IBC/CWD patients with PD-L1 status positive (IHC will be performed to test PD-L1 CPS) and/or with positive TILs. ORR will be evaluated according to Immune-related RECIST (iRECIST)[36] criteria and clinical response. The rationale for the use of iRECIST criteria is that immunotherapeutic agents may produce antitumor effects by potentiating endogenous cancer-specific immune responses, which may be functionally anergic prior to treatment. The response patterns seen with such an approach may extend beyond the typical time course of responses seen with cytotoxic agents, and can manifest as a clinical response after an initial increase in tumor burden or even the appearance of new lesions (transient tumor flare). Thus, standard RECIST criteria may not provide a complete response assessment of immunotherapeutic agents such as pembrolizumab.

2.2. Secondary endpoints: duration of response (DoR); time to progression (TTP); progression-free survival (PFS) and OS.

2.3. Exploratory endpoints: assess the composite results of six biological parameters from all the enrolled subjects in correspondence to the aforementioned outcomes, those parameters being:

2.3.1. Immunologic constant of rejection (ICR)

Gene expression profiling investigations in the context of cancer immunotherapy have elucidated the main overlapping molecular pathways activated in lesions more likely to have a better prognosis. The same observations have been done in terms of response to immune modulation, such as checkpoint inhibition[37,38]. These pathways include Th-1 signaling (IFNG, TXB21, CD8B, CD8A, IL12B, STAT1, and IRF1), CXCR3/CCR5 chemokine ligands (CXCL9, CXCL10, and CCL5) and effector immune functions (GNLY, PRF1, GZMA, GZMB, and GZMH). Since these modules are coordinately activated in other forms of immune-mediated tissue destruction such as autoimmunity, graft-versus-host disease or allograft rejection[39], they were referred to as the ICR. A pivotal study by Hendrickx et al.[40] using RNA-sequencing data from 1,004 breast cancer samples collected by The Cancer Genome Atlas (TCGA) consortium managed to segregate tumors though a Calinski index into four groups (ICR 1 to ICR4) according to the range of overall expression magnitude of these specific genes. ICR4 tumors, marked by the highest levels of immune gene expression, equated with a strong Th-1 immune activation, whereas ICR1 tumors, characterized by the lowest immune gene expression, lacked an activated immune polarization.

2.3.2. Alterations in specific driver genes

A genetic profiling previously published by Ross et al.[41] identified the most frequently relevant altered genes in 53 specimens of IBC, those being: TP53 (62%), MYC (32%), PIK3CA (28%), ERBB2 (26%), FGFR1 (17%), BRCA2 (15%), and PTEN (15%). Although there are no available therapies that can directly target all of these alterations, disruption on particular drivers that favor immune-exclusion can candidate for alternative combinations with either standard or investigational treatments. Recent preclinical evidence suggests that cells with overexpression of MYC protein may be sensitive to CDK aurora kinase inhibitors[42,43]. Also, tumors with FGFR1 amplification or activating mutations may be sensitive to FGFR family inhibitors and clinical trials of these agents in a range of solid tumor are awaited[44]. Interestingly, aberrant expression of these oncogenic pathways have recently been described to significantly impact the expression of PD-L1 in tumor cells and are currently being exploited as to improve the stratification of patients for better targeting the PD-1/PD-L1 axis[45].

2.3.3. Tumor mutational load

Identifying immune responses to antigens unique to tumors and not expressed on normal tissue

can be a drawback. Tumor mutational burden (TMB) is defined as the total number of somatic, coding, base substitution, and indel mutations per megabase of genome examined. It has been used as a proxy for the presence of T cell epitopes derived from these neoantigens has recently been correlated with a clinical benefit from anti-PD-1 and anti-CTLA-4 therapy in various tumor types[46], including melanoma[47,48] and non-small cell lung cancer (NSCLC)[49,50]. Although this statement is most likely true for some populations, other factors including specific features of the immune microenvironment, germline single-nucleotide polymorphisms, and epigenetic signatures may also influence response. In a recent trial[51], a robust mutational burden threshold associated with evidence of immune checkpoint activation could not be identified in TNBC, a subtype particularly expected to achieve response rates under immune checkpoint blockade[20]. Here, features other than the tumor mutational load are probably responsible for immune checkpoint activation. In TNBC, the genomic landscape is dominated by gene rearrangements, which are not always captured by exome sequencing[52]. In a parallel with the ICR in breast cancer[40], the TMB progressively increased from ICR1 to ICR4 groups. Nevertheless, a considerable proportion of samples belonging to ICR4 group had a relatively low mutational burden while a fraction of ICR1 tumors had a relatively high number of mutations, also suggesting that mutational load cannot fully explain the observed differences in terms of immune activation, pushing our efforts towards a more integrative approach when evaluating such a stressed correlation.

2.3.4. Microsatellite instability (MSI)/mismatch repair deficiency (dMMR)

MSI is a hypermutator phenotype seen in tumors with a variability in the length of base pair repeated sequences (<5 bp) caused by replication slippage that is usually kept stable by the DNA mismatch repair (MMR) system. In sporadic cases, when the inactivation of MMR genes (e.g., MLH1, MSH2, MSH3, MSH6 and PMS2) occurs through somatic mutations, it leads to an increased number of neoepitopes, tumor-infiltrating cytotoxic lymphocytes and responsiveness to anti-PD-1 therapy[53–56]. A recent prediction of MSI status from exome-sequencing of 7.919 tumors and matched normal pairs from TCGA across 23 cancer types found a frequency of 1,7% of MSI-High (MSI-H) for breast cancer samples[57]. In another trial, tumor genomes from patients specifically with IBC showed more frequent complex rearrangement patterns as well as a higher percentage of genes with copy number alterations per sample, suggesting that IBC may harbor a higher degree of genomic instability[41].

2.3.5. Quality and spatial distribution of the tumor infiltrate

The local infiltrate undergoes dynamic changes according to the pre-existing immune status, the continuous tumor interactions and in response to therapy. As a result, this immune contexture can yield information that is relevant to prognosis, prediction of therapeutic success and many other parameters regarding the equipoise between tumor suppression and tolerance[58]. Tumor-infiltrating lymphocytes (TILs) can be identified in breast cancer by the stromal tissue adjacent to the tumor (sTILs) or actively infiltrating intratumoral areas (iTILs). Current evidence indicate that the higher the number of sTILs, the higher is the probability of cure, especially on early stage TNBC and HER2-positive breast cancer[59]. Nonetheless, the evaluation of TILs in hematoxylin and eosin (H&E)-stained sections is not able to evaluate specific subsets of immune cells and may not correspond to the levels of active antitumor TILs. This functional blindfolding may explain, at least in part, why some patients with high levels of TILs do not show improved prognosis[60]. Lymphocytes with opposite functions, such as CD4+ T cells with Th1 orientation versus Th2 orientation versus immune cells with regulatory functions, Tregs, or NK cells, B cells or cytotoxic CD8+ T cells, are indistinguishable without proper marker evaluation and require antibody labelling by immunohistochemistry (IHC). Also, TILs may be exhausted or rendered inactive through immune checkpoint pathways such as PD-1:PD-L1 signaling, or lack of immune

stimulatory pathways such as OX-40:OX-40L signaling. Preliminary data of one trial quantifying TILs in 36 IBC patients found a 72% of sTILs in the invasive tumor pretreatment biopsies. Mean TILs infiltrate did not significantly vary among classic immune subtypes (10.0-11.5%), except for HER2-/HR+ tumors (3.6%). CD8 and PD-L1 IHC staining were performed on samples with >1% TILs. An average of 42% was positive for CD8, with no significant correlation to pCR, stage or receptor status. No patient presented with PD-L1 positivity. We plan to stratify TILs using IHC staining for CD3, CD8, CD45RO, FOXP3[61] and observe their distribution across tumor center, margins and around microemboli[61–63], aiming to broader the classification of the IBC microenvironment and its effect in response to therapy.

2.3.6. Breast and gut dysbiosis

To date, there is substantial evidence on the role for bacteria in hindering cancer at sites that are distant from the gut, mainly through fostering of host antitumor immune responses[33]. This relationship is most likely bidirectional and malignancy-driven changes in the microbiota occur as a result of the disease but can also contribute to its progression[64]. Patients proposed to have a favorable gut microbiome (e.g., high diversity and abundance of Ruminococcaceae and *Faecalibacterium*) have an enhanced antitumor immune response mediated by increased antigen presentation and improved Teff cell function in the periphery and the tumor microenvironment. By contrast, patients with a proposed unfavorable gut microbiome (e.g., low diversity and high relative abundance of Bacteroidales) have impaired immune responses mediated by limited intratumoral lymphoid and myeloid infiltration and weakened antigen presentation capacity[34]. It has also been insinuated that local breast microbiota - which differs both quantitatively and qualitatively in patients with and without breast cancer[65] - and their specific components' ability to degrade carcinogens all contribute to the maintenance of healthy breast tissue by stimulating resident immune cells. In the study by Xuan, using next-generation sequencing (NGS) on breast tumors paired with normal adjacent tissue from the same patient, differences in breast microbiome composition were found to be largely driven by the increase of *Methylobacterium radiotolerans* population in tumor tissue and by increase of the bacterium *Sphingomonas yanoikuyae* in normal tissue. In another trial, an unknown genus of family Alcaligenaceae was increased in cancer compared to non-cancer samples. Furthermore, both PD-1 blockade[34,66] and metronomic cyclophosphamide[67] suffer from a direct influence on their activity regarding the gut microbiota.

3. Study design

This is a phase II, single center, open-label, non-randomized trial for patients with locally recurrent, inoperable, and/or metastatic IBC/CWD who had received at least one cytotoxic treatment. The definition of IBC will follow the international consensus diagnostic criteria: rapid onset (less than 6 months) of breast erythema, edema, and/or *peau d'orange*, and/or warm breast, with or without an underlying palpable mass. Patients will be treated with pembrolizumab administered as an intravenous infusion at 200 mg in 21-day treatment cycles and oral CTX 50 mg per day in metronomic administration as a 21 days cycle (figure 1). Forty-six subjects are planned to be enrolled. Key exclusion criteria consists prior anti-PD-1, anti-CTLA-4 or other immune pathway-targeted therapy. Patients with autoimmune diseases and/or receiving drugs who interfere with the immune system will not be eligible. Patients will be monitored carefully for the development of adverse events (AEs) as well as for clinical and/or radiographic evidence of disease progression according to usual standards of clinical practice. AEs will be evaluated according to criteria outlined in the NCI Common Terminology Criteria for Adverse Events (CTCAE), version 4.0. For individual patients that experience dose-limiting toxicities (DLT), criteria for dose modification of pembrolizumab and CTX are outlined in details at section 5.1. Treatment with pembrolizumab and metronomic CTX will continue until documented PD, unacceptable AEs, intercurrent illness that prevents further administration of

treatment, investigator's decision to withdraw the subject, subject withdraws consent, pregnancy of the subject, noncompliance with trial treatment or procedure requirements, completion of 24 months of treatment with pembrolizumab, or administrative reasons requiring the cessation of treatment. Subjects who attain CR confirmed by central radiology review may consider stopping therapy after receiving at least 24 weeks of treatment. Subjects who discontinue treatment after 24 months of therapy for reasons other than disease progression or intolerability or who discontinue treatment after attaining a CR may be eligible for up to one year of retreatment after they have experienced radiographic disease progression. The decision to retreat will be at the discretion of the investigator, only if no cancer treatment was administered since the last dose of pembrolizumab, the subject still meets the safety parameters listed in the inclusion/exclusion criteria and the trial remains open. A Simon's two-stage design will be used and the null hypothesis that the true objective response rate is 7% will be tested against a one-sided alternative of 22%. In the first stage, 17 patients will be enrolled. If there are 1 or fewer responses in these 17 patients, enrollment will be stopped. Otherwise, 29 additional patients will be accrued for a total of 46. The null hypothesis will be rejected if a total of 6 or more objective responses are observed on those 46 patients. This design yields a type I error rate of 0.05 and power of 85% when the true objective response rate is 22%. PFS and OS will be evaluated using the Kaplan-Meier estimates of the survival curves, and median TTP will be calculated accordingly, with 95% confidence intervals (95% CI).

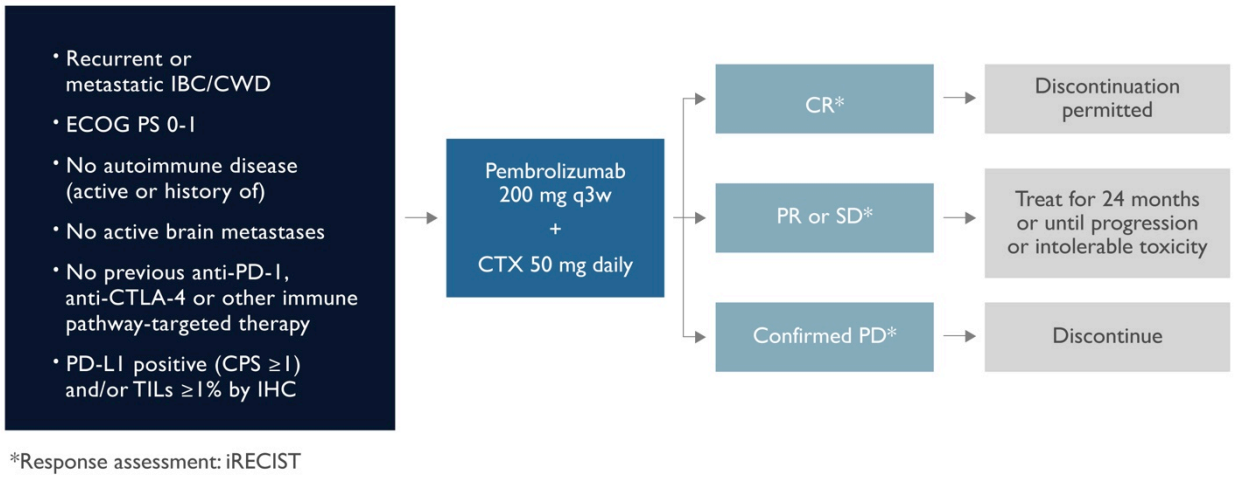

Figure 1. Trial diagram

4. Inclusion/exclusion criteria

4.1. Subject inclusion criteria

In order to be eligible for participation in this trial, the subject must:

4.1.1. Histologically proven, PD-L1 (≥1%) positive and/or tumor infiltrating lymphocyte positive (≥1%) locally advanced "chest wall" breast cancer (with or without distant metastases), who have been treated with chemotherapy or radiation therapy may be eligible for this study. Patients with cutaneous metastases only (with or without evidence of primary tumor) are eligible for the study;

4.1.2. Patients must have tissue accessible for serial biopsies;

4.1.3. Expected survival of >3 months;

4.1.4. Be willing and able to provide written informed consent for the trial. The subject may also provide consent for future biomedical research. However, the subject may participate in the main trial without participating in future biomedical research;

4.1.5. Be 18 years of age on day of signing informed consent;

4.1.6. Be a female or male subject with IBC with lymphangitic spread to the chest wall. ER, PgR and

HER2 status determination is required for enrolment;

4.1.7. Have provided tissue for PD-L1 biomarker analysis and TILs evaluation from a newly obtained core or excisional biopsy of a tumor lesion (mandatory) and received permission for enrollment from the core lab based on the adequacy of the biopsy specimen. Repeat samples may be required if adequate tissue is not provided. *Note: newly obtained is defined as a specimen obtained up to 6 weeks (42 days) prior to initiation of treatment on Day 1. Subjects for whom newly obtained samples cannot be provided (e.g., inaccessible or subject safety concern) may submit an archived specimen only upon agreement from the Sponsor*;

4.1.8. Have measurable metastatic disease based on iRECIST criteria as determined by central radiology review. Tumor lesions situated in a previously irradiated area are considered measurable, if progression has been demonstrated in such lesions. *Note: the exact same image acquisition and processing parameters should be used throughout the study*;

4.1.9. Have a performance status of 0 or 1 on the ECOG Performance Scale. Assessment should be performed within 10 days of treatment initiation;

4.1.10. Subjects of childbearing potential must be willing to use an adequate method of contraception for the course of the study through 120 days after the last dose of study medication. *Note: Abstinence is acceptable if this is the usual lifestyle and preferred contraception for the subject*;

4.1.11. Female subjects of childbearing potential should have a negative urine or serum pregnancy test within 72 hours prior to receiving the first dose of study medication. If the urine test is positive or cannot be confirmed as negative, a serum pregnancy test will be required;

4.1.12. Patients with HR-positive and/or HER2-positive breast cancer would be eligible for the study only if their disease is considered refractory to hormonal or anti-HER2 agents, respectively, and no further hormonal or anti-HER2 treatment is indicated;

4.1.13. Demonstrate adequate organ function as defined in Table 1. All screening labs should be performed within 10 days of treatment initiation.

System	Laboratory Value
Hematological	
Absolute neutrophil count (ANC)	≥1,500 /mcL
Platelets	≥100,000 / mcL
Hemoglobin	≥9 g/dL or ≥5.6 mmol/L without transfusion or EPO dependency (within 7 days of assessment)
Renal	
Serum creatinine **or** Measured or calculated[a] creatinine clearance (GFR can also be used in place of creatinine or CrCl)	≤1.5 X upper limit of normal (ULN) **or** ≥60 mL/min for subject with creatinine levels > 1.5 X institutional ULN
Hepatic	
Serum total bilirubin	≤1.5 X ULN **or** Direct bilirubin ≤ ULN for subjects with total bilirubin levels > 1.5 ULN
AST and ALT	≤2.5 X ULN **or** ≤5 X ULN for subjects with liver metastases
Albumin	≥2.5 mg/dL
Coagulation	

International Normalized Ratio (INR) or Prothrombin Time (PT)	≤1.5 X ULN unless subject is receiving anticoagulant therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulants
Activated Partial Thromboplastin Time (aPTT)	≤1.5 X ULN unless subject is receiving anticoagulant therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulants
[a]Creatinine clearance should be calculated using CKD-EPI Creatinine Equation (2009) available at *https://www.kidney.org/professionals/kdoqi/gfr_calculator*.	

Table 1. Adequate organ function laboratory values.

5. Trial treatments

The treatment to be used in this trial is outlined in Table.

Drug	Dose	Dose Frequency	Route of Administration	Regimen/Treatment Period
Pembrolizumab	200 mg	Every 3 weeks	IV infusion	Day 1 of each 3 week cycle
Cyclophosphamide	50 mg	Daily	Oral	Continuously

Table 2. Trial treatment.

Trial treatment should begin on the day of randomization or as close as possible to the date on which treatment is allocated/assigned.

5.1. Dose selection/modification

5.1.1. Dose selection for pembrolizumab: an open-label, phase I trial was conducted to evaluate the safety and clinical activity of single agent pembrolizumab. The dose escalation portion of this trial evaluated three dose levels, 1 mg/kg, 3 mg/kg, and 10 mg/kg, administered every 2 weeks (q2w) in subjects with advanced solid tumors. All three dose levels were well tolerated and no dose-limiting toxicities (DLT) were observed. The study showed evidence of target engagement and objective evidence of tumor size reduction at all dose levels (1 mg/kg, 3 mg/kg and 10 mg/kg q2w and q3w). No maximum tolerated dose (MTD) has been identified. 10 mg/kg q3w was sufficient for target engagement and clinical activity. Pharmacokinetic (PK) data analysis of pembrolizumab administered q2w and q3w showed slow systemic clearance, limited volume of distribution, and a long half-life. Pharmacodynamic (PD) data (IL-2 release assay) suggested that peripheral target engagement is durable (>21 days). A population PK analysis has been performed using serum concentration time data from 476 patients. Within the resulting population PK model, clearance and volume parameters of pembrolizumab were found to be dependent on body weight. The relationship between clearance and body weight, with an allometric exponent of 0.59, is within the range observed for other antibodies and would support both body weight normalized dosing or a fixed dose across all body weights. Pembrolizumab has been found to have a wide therapeutic range based on the melanoma indication. The differences in exposure for a 200 mg fixed dose regimen relative to a 2 mg/kg q3w body weight based regimen are anticipated to remain well within the established exposure margins of 0.5-5.0

for pembrolizumab in the melanoma indication. The exposure margins are based on the notion of similar efficacy and safety in melanoma at 10 mg/kg q3w vs. the proposed dose regimen of 2 mg/kg q3w (i.e., 5-fold higher dose and exposure). The population PK evaluation revealed that there was no significant impact of tumor burden on exposure. In addition, exposure was similar between the NSCLC and melanoma indications. The choice of the 200 mg q3w as an appropriate dose for the switch to fixed dosing is based on simulations performed using the population PK model of pembrolizumab showing that the fixed dose of 200 mg every 3 weeks will provide exposures that are optimally consistent with those obtained with the 2 mg/kg dose every 3 week; will maintain individual patient exposures in the range established in melanoma as associated with maximal efficacy response; will maintain individual patients exposure in the range established in melanoma that are well tolerated and safe. A fixed dose regimen will also simplify the dosing regimen to be more convenient for physicians and to reduce potential for dosing errors. A fixed dosing scheme will also reduce complexity in the logistical chain at treatment facilities and reduce wastage.

5.1.2. **Dose selection for CTX:** Cyclophosphamide (INN, trade names Endoxan, Cytoxan, Neosar, Procytox, Revimmune, Cycloblastin), also known as cytophosphane, is a nitrogen mustard alkylating agent from the oxazaphosphorine group. An alkylating agent adds an alkyl group to DNA. It attaches the alkyl group to the guanine base of DNA, at the number 7 nitrogen atom of the imidazole ring. This interferes with DNA replication by forming intrastrand and interstrand DNA crosslinks. As a prodrug, it is converted by liver cytochrome P450 (CYP) enzymes to form the metabolite 4-hydroxy cyclophosphamide that has chemotherapeutic activity. The main effect of cyclophosphamide is due to its metabolite phosphoramide mustard. This metabolite is only formed in cells that have low levels of aldehyde dehydrogenase (ALDH). Phosphoramide mustard forms DNA crosslinks both between and within DNA strands at guanine N-7 positions (known as interstrand and intrastrand crosslinkages, respectively). This is irreversible and leads to cell apoptosis. Cyclophosphamide has relatively little typical chemotherapy toxicity as ALDHs are present in relatively large concentrations in bone marrow stem cells, liver and intestinal epithelium. ALDHs protect these actively proliferating tissues against toxic effects of phosphoramide mustard and acrolein by converting aldophosphamide to carboxycyclophosphamide that does not give rise to the toxic metabolites phosphoramide mustard and acrolein. This is because carboxycyclophosphamide cannot undergo β-elimination (the carboxylate acts as an electron-donating group, forbidding the transformation), preventing nitrogen mustard activation and subsequent alkylation. Using low, continuous dosage of CTX (50 mg daily) is a determining factor in the selective effect of this treatment on Treg number and function. Indeed, in a series of patients receiving a similar metronomic regimen but with a higher dosage of CTX (i.e., 200 mg per day), a profound decrease in all circulating lymphocytes was observed, with no specificity on Treg subpopulation[16,68,69]. These results stablished low doses of CTX as relevant for obtaining a selective Treg depletion. Moreover, this regimen decreased the NK cell-dependent cytotoxicity and T cell proliferation capacity[15].

5.2. **Timing of dose administration:** trial treatment should be administered on Day 1 of each cycle after all procedures/assessments have been completed as detailed on the PERICLES Flow Chart (Supplementary 1) . Trial treatment may be administered up to 3 days before or after the scheduled Day 1 of each cycle due to administrative reasons. All trial treatments will be administered on an outpatient basis. Pembrolizumab 200 mg will be administered as a 30 minute IV infusion every 3 weeks. All efforts should be made to target infusion timing to be as close to 30 minutes as possible. However, given the variability of infusion pumps, a window of -5 minutes and +10 minutes is permitted (i.e., infusion time is 30 minutes: -5 min/+10 min). The specific instructions for the

preparation of the pembrolizumab infusion fluid and administration of infusion solution is on a separate document.

6. Trial blinding/masking

This is an open-label trial; therefore, the Sponsor, investigator and subject will know the treatment administered.

7. Enrollment/baseline and treatment

All patients are given a fully comprehensive informed consent (IC) and can be screened from there. All inclusion/exclusion criteria will be checked during the registration procedure. As soon as the eligibility is verified the patient is registered. All patients will be assigned in a progressive number fashion.

8. Subject withdrawal/discontinuation

Subjects may withdraw consent at any time for any reason or be dropped from the trial at the discretion of the investigator should any untoward effect occur. In addition, a subject may be withdrawn by the investigator or the Sponsor if enrollment into the trial is inappropriate, the trial plan is violated, or for administrative and/or other safety reasons. However, a subject must be discontinued from the trial for any of the following reasons:

- The subject or legal representative (such as a parent or legal guardian) withdraws consent;
- Confirmed radiographic PD. *Note: a subject may be granted an exception to continue on treatment with confirmed radiographic PD if clinically stable or clinically improved after staff discussion and approval from the Sponsor global team*;
- Unacceptable AEs;
- Intercurrent illness that prevents further administration of treatment;
- Investigator's decision to withdraw the subject;
- The subject has a confirmed positive serum pregnancy test;
- Noncompliance with trial treatment or procedure requirements;
- The subject is lost to follow-up;
- Completed 24 months of uninterrupted treatment with pembrolizumab and CTX or 35 administrations of study therapy, whichever is later. *Note: 24 months of study therapy is calculated from the date of first dose. Subjects who stop pembrolizumab and CTX after 24 months may be eligible for up to one year of additional study treatment if they progress after stopping study treatment provided they meet the requirements detailed in section 4*;
- Administrative reasons.

The end of treatment (EoT) and follow-up visit procedures are listed in the PERICLES Flow Chart. After EoT, each subject will be followed for 30 days for adverse event monitoring (SAEs will be collected for 90 days after EoT).

8.1. Discontinuation of study therapy after CR: discontinuation of treatment may be considered for subjects who have attained a confirmed CR and have been treated for at least 24 weeks with pembrolizumab plus CTX and had received at least two doses beyond the date when the initial CR was declared. Subjects who then experience radiographic PD may be eligible for up to one year of additional treatment with pembrolizumab at the discretion of the investigator if no cancer treatment was administered since the last dose of pembrolizumab and CTX, the subject meets the safety parameters listed in the inclusion/exclusion criteria (section 4), and the trial is open. Subjects will resume therapy at the same dose and schedule administered by the time of initial

discontinuation.

8.2. Clinical criteria for early trial termination: early trial termination will be the result of the criteria specified below:

8.2.1. Quality or quantity of data recording is inaccurate or incomplete;

8.2.2. Poor adherence to protocol and regulatory requirements;

8.2.3. Incidence or severity of adverse drug reaction in this or other studies indicates a potential health hazard to subjects.

9. Translational analyses

9.1. ICR, target gene alterations, TMB and dMMR

Archival or newly obtained (≤6 weeks) tissue will be collected at screening visit for all 46 planned patients. Blood will also be draw at baseline and stored at -20 °C in microtubes containing RNA stabilizer. From each sample, total RNA and DNA will be extracted according to previously validated workflow[40]. Samples will be shipped to Sidra Medical Research Center for processing and analysis of transcriptome. Gene expression analysis will be conducted *ad hoc* on the NanoString nCounter gene expression platform (NanoString Technologies). A custom code set consisting of a 680-gene panel related to T cell biology, immune regulation pathways and cellular markers of tumor-infiltrating lymphocytes/tumor-associated macrophages will be used. Mutational analysis, calculation of TMB and MSI status will be conducted using the Illumina TruSight Tumor 170© panel. All patients with an evidence of MSI will be sequenced for germline mutations of MSH2, MSH6, PMS2, and MLH1 genes to determine whether the dMMR is associated with an inherited change in one of these genes.

9.2. TILs: density, distribution and organization into tertiary lymphoid structures

Tumor-infiltrating lymphocytes (TILs) will be evaluated in all the full-face hematoxylin and eosin (H&E) sections originally sampled from each patient included in the trial, carefully following the criteria proposed by the International TILs Working Group[60] and blinded of clinical information. Briefly, all mononuclear cells (including lymphocytes and plasma cells) in the stromal compartment within the borders of the invasive tumor will be evaluated and reported as a percentage values. TILs outside of the tumor border, around DCIS and normal breast tissue, as well as in areas of necrosis, if any, will not be included in the analysis. TILs will be evaluated by one expert pathologist in all cases. IHC staining for CD3, CD8, CD45RO and FOXP3 will be performed in tumors with >1%TIL.

9.3. Microbiome

Comparison of abundances of different potential groupings of bacteria will be based on 16S ribosomal RNA (rRNA) gene sequencing[33,34,72].

10. Expected results and impact on cancer

There is a potential role of immune-checkpoint inhibitors in treating patients with IBC/CWD, especially when combined with agents that heighten the balance towards inflammation cascade abrogation, and we're exploiting it with a strong rationale with the current protocol. In addition, by arranging each patient's characteristics in a radar plot fashion (figure 2), we may comprehensively assemble different profiles and observe their correlated outcomes during the study period. A positive clustering among similar plot areas from the tumor-host metaorganism and its correlation to both primary and secondary endpoints (e.g., SD, PR, CR) may prompt further investigation in a larger setting. The current trial may elucidate the way we approach IBC and may bring out new potential therapeutic strategies for this population.

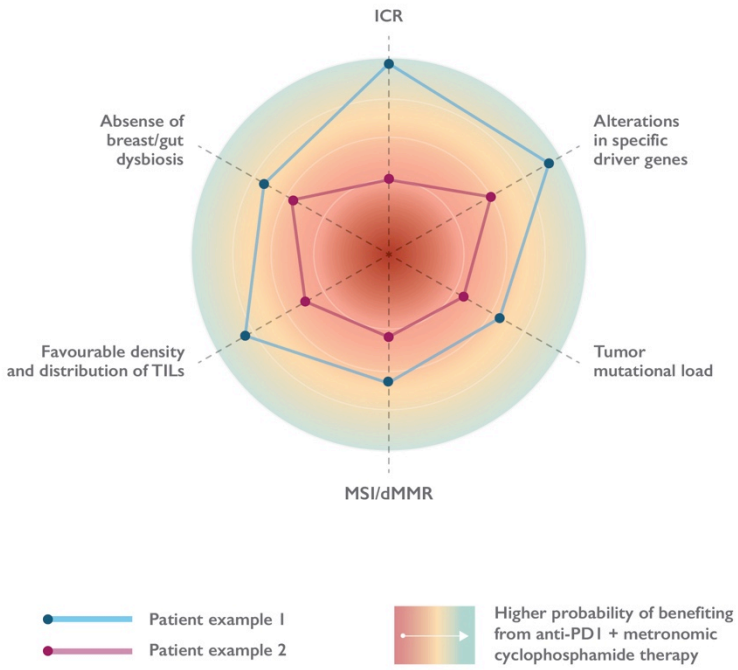

Figure 2. Radar plot with six axes containing the most prominent variants under investigation in breast cancer as well as two example of their composite quantified analysis.

References

1. Dawood S, Merajver SD, Viens P, et al. International expert panel on inflammatory breast cancer: Consensus statement for standardized diagnosis and treatment. *Ann Oncol*. 2011;22(3):515-523. doi:10.1093/annonc/mdq345.
2. Ueno NT, Espinosa Fernandez JR, Cristofanilli M, et al. International Consensus on the Clinical Management of Inflammatory Breast Cancer from the Morgan Welch Inflammatory Breast Cancer Research Program 10th Anniversary Conference. *J Cancer*. 2018;9(8):1437-1447. doi:10.7150/jca.23969.
3. Rea D, Francis A, Hanby AM, et al. Inflammatory breast cancer: Time to standardise diagnosis assessment and management, and for the joining of forces to facilitate effective research. *Br J Cancer*. 2015;112(9):1613-1615. doi:10.1038/bjc.2015.115.
4. Matro JM, Li T, Cristofanilli M, et al. Inflammatory breast cancer management in the National Comprehensive Cancer Network: The disease, recurrence pattern, and outcome. *Clin Breast Cancer*. 2015;15(1):1-7. doi:10.1016/j.clbc.2014.05.005.
5. Masuda H, Brewer TM, Liu DD, et al. Long-term treatment efficacy in primary inflammatory breast cancer by hormonal receptor-and HER2-defined subtypes. *Ann Oncol*. 2014;25(2):384-391. doi:10.1093/annonc/mdt525.
6. Binnewies M, Roberts EW, Kersten K, et al. Understanding the tumor immune microenvironment (TIME) for effective therapy. *Nat Med*. 2018. doi:10.1038/s41591-018-0014-x.
7. Dawood S, Lei X, Dent R, et al. Survival of women with inflammatory breast cancer: a large population-based study†. *Ann Oncol*. 2014;25(6):1143-1151. doi:10.1093/annonc/mdu121.
8. Inoue H, Tani K. Multimodal immunogenic cancer cell death as a consequence of anticancer cytotoxic treatments. *Cell Death Differ*. 2014;21(1):39-49. doi:10.1038/cdd.2013.84.
9. Munzone E, Colleoni M. Clinical overview of metronomic chemotherapy in breast cancer. *Nat Rev Clin Oncol*. 2015;12(11):631-644. doi:10.1038/nrclinonc.2015.131.

10. Schumacher TN, Schreiber RD. Realising the Promise: Neoantigens in cancer immunotherapy. *Sci Mag*. 2015;348(6230).
11. Blank c, Haanen JB, Ribas A, Schumacher TN. The "cancer immunogram" Visualizing the state of cancer–immune system interactions may spur personalized therapy. *Science (80-)*. 2016;352(6286):658-660. doi:10.1126/science.aaf2834.
12. Jenkins RW, Barbie DA, Flaherty KT. Mechanisms of resistance to immune checkpoint inhibitors. *Br J Cancer*. 2018;118(1):9-16. doi:10.1038/bjc.2017.434.
13. Loeffler M, Krüger JA, Reisfeld RA, Kru A. Immunostimulatory Effects of Low-Dose Cyclophosphamide Are Controlled by Inducible Nitric Oxide Synthase Immunostimulatory Effects of Low-Dose Cyclophosphamide Are Controlled by Inducible Nitric Oxide Synthase. *Cancer Res*. 2005;65(12):5027-5030. doi:10.1158/0008-5472.CAN-05-0646.
14. Zitvogel L, Galluzzi L, Smyth MJ, Kroemer G. Mechanism of Action of Conventional and Targeted Anticancer Therapies: Reinstating Immunosurveillance. *Immunity*. 2013;39(1):74-88. doi:10.1016/j.immuni.2013.06.014.
15. Ghiringhelli F, Larmonier N, Schmitt E, et al. CD4+CD25+ regulatory T cells suppress tumor immunity but are sensitive to cyclophosphamide which allows immunotherapy of established tumors to be curative. *Eur J Immunol*. 2004;34(2):336-344. doi:10.1002/eji.200324181.
16. Ghiringhelli F, Menard C, Puig PE, et al. Metronomic cyclophosphamide regimen selectively depletes CD4 +CD25+ regulatory T cells and restores T and NK effector functions in end stage cancer patients. *Cancer Immunol Immunother*. 2007;56(5):641-648. doi:10.1007/s00262-006-0225-8.
17. Nars MS, Kaneno R. Immunomodulatory effects of low dose chemotherapy and perspectives of its combination with immunotherapy. *Int J Cancer*. 2013;132(11):2471-2478. doi:10.1002/ijc.27801.
18. Toulmonde M, Penel N, Adam J, et al. Use of PD-1 targeting, macrophage infiltration, and IDO pathway activation in sarcomas a phase 2 clinical trial. *JAMA Oncol*. 2018;4(1):93-97. doi:10.1001/jamaoncol.2017.1617.
19. Cristofanilli M, Valero V, Buzdar AU, et al. Inflammatory breast cancer (IBC) and patterns of recurrence: Understanding the biology of a unique disease. *Cancer*. 2007;110(7):1436-1444. doi:10.1002/cncr.22927.
20. Nanda R, Chow LQM, Dees EC, et al. Pembrolizumab in patients with advanced triple-negative breast cancer: Phase Ib keynote-012 study. *J Clin Oncol*. 2016;34(21):2460-2467. doi:10.1200/JCO.2015.64.8931.
21. Adams S, Schmid P, Rugo HS, et al. Phase 2 study of pembrolizumab (pembro) monotherapy for previously treated metastatic triple-negative breast cancer (mTNBC): KEYNOTE-086 cohort A. *J Clin Oncol*. 2017;35(15_suppl):1008. doi:10.1200/JCO.2017.35.15_suppl.1008.
22. Adams S, Loi S, Toppmeyer D, et al. Abstract PD6-10: KEYNOTE-086 cohort B: Pembrolizumab monotherapy for PD-L1–positive, previously untreated, metastatic triple-negative breast cancer (mTNBC). *Cancer Res*. 2018;78(4 Supplement):PD6-10-PD6-10. doi:10.1158/1538-7445.SABCS17-PD6-10.
23. Woodward WA. Inflammatory breast cancer: Unique biological and therapeutic considerations. *Lancet Oncol*. 2015;16(15):e568-e576. doi:10.1016/S1470-2045(15)00146-1.
24. Church SE, Galon J. Tumor Microenvironment and Immunotherapy: The Whole Picture Is Better Than a Glimpse. *Immunity*. 2015;43(4):631-633. doi:10.1016/j.immuni.2015.10.004.
25. Bertucci F, Finetti P, Vermeulen P, et al. Genomic profiling of inflammatory breast cancer: A review. *The Breast*. 2014;23(5):538-545. doi:10.1016/j.breast.2014.06.008.
26. Van Laere SJ, Ueno NT, Finetti P, et al. Uncovering the molecular secrets of inflammatory breast cancer biology: An integrated analysis of three distinct affymetrix gene expression datasets. *Clin Cancer Res*. 2013;19(17):4685-4696. doi:10.1158/1078-0432.CCR-12-2549.
27. Bekhouche I, Finetti P, Adelaïde J, et al. High-resolution comparative genomic hybridization of Inflammatory breast cancer and identification of candidate genes. *PLoS One*. 2011;6(2). doi:10.1371/journal.pone.0016950.

28. Nguyen DM, Sam K, Tsimelzon A, et al. Molecular heterogeneity of inflammatory breast cancer: A hyperproliferative phenotype. *Clin Cancer Res*. 2006;12(17):5047-5054. doi:10.1158/1078-0432.CCR-05-2248.
29. Marquart J, Chen EY, Prasad V. Estimation of The Percentage of US Patients With Cancer Who Benefit From Genome-Driven Oncology. *JAMA Oncol*. 2018;97239:1-7. doi:10.1001/JAMAONCOL.2018.1660.
30. Gandhi L, Rodríguez-Abreu D, Gadgeel S, et al. Pembrolizumab plus Chemotherapy in Metastatic Non–Small-Cell Lung Cancer. *N Engl J Med*. 2018:NEJMoa1801005. doi:10.1056/NEJMoa1801005.
31. Reck M, Rodríguez-Abreu D, Robinson AG, et al. Pembrolizumab versus Chemotherapy for PD-L1–Positive Non–Small-Cell Lung Cancer. *N Engl J Med*. 2016;375(19):1823-1833. doi:10.1056/NEJMoa1606774.
32. Langer CJ, Gadgeel SM, Borghaei H, et al. Carboplatin and pemetrexed with or without pembrolizumab for advanced, non-squamous non-small-cell lung cancer: a randomised, phase 2 cohort of the open-label KEYNOTE-021 study. *Lancet Oncol*. 2016;17(11):1497-1508. doi:10.1016/S1470-2045(16)30498-3.
33. Zitvogel L, Daillère R, Roberti MP, Routy B, Kroemer G. Anticancer effects of the microbiome and its products. *Nat Rev Microbiol*. 2017;15(8):465-478. doi:10.1038/nrmicro.2017.44.
34. Gopalakrishnan V, Spencer CN, Nezi L, et al. Gut microbiome modulates response to anti – PD-1 immunotherapy in melanoma patients. *Science (80-)*. 2018;359(6371):97-103. doi:10.1126/science.aan4236.
35. Xuan C, Shamonki JM, Chung A, et al. Microbial dysbiosis is associated with human breast cancer. *PLoS One*. 2014;9(1):1-7. doi:10.1371/journal.pone.0083744.
36. Seymour L, Bogaerts J, Perrone A, et al. iRECIST: guidelines for response criteria for use in trials testing immunotherapeutics. *Lancet Oncol*. 2017;18(3):e143-e152. doi:10.1016/S1470-2045(17)30074-8.
37. Galon J, Angell HK, Bedognetti D, Marincola FM. The Continuum of Cancer Immunosurveillance: Prognostic, Predictive, and Mechanistic Signatures. *Immunity*. 2013;39(1):11-26. doi:10.1016/j.immuni.2013.07.008.
38. Ayers M, Lunceford J, Nebozhyn M, et al. IFN-gamma-related mRNA profile predicts clinical response to PD-1 blockade. *J Clin Invest*. 2017;127(8):2930-2940. doi:10.1172/JCI91190.
39. Wang E, Worschech A, Marincola FM. The immunologic constant of rejection. *Trends Immunol*. 2008;29(6):256-262. doi:10.1016/j.it.2008.03.002.
40. Hendrickx W, Simeone I, Anjum S, et al. Identification of genetic determinants of breast cancer immune phenotypes by integrative genome-scale analysis. *Oncoimmunology*. 2017;6(2):e1253654. doi:10.1080/2162402X.2016.1253654.
41. Ross JS, Ali SM, Wang K, et al. Comprehensive genomic profiling of inflammatory breast cancer cases reveals a high frequency of clinically relevant genomic alterations. *Breast Cancer Res Treat*. 2015;154(1):155-162. doi:10.1007/s10549-015-3592-z.
42. Horiuchi D, Kusdra L, Huskey NE, et al. MYC pathway activation in triple-negative breast cancer is synthetic lethal with CDK inhibition. *J Exp Med*. 2012;209(4):679-696. doi:10.1084/jem.20111512.
43. Hook KE, Garza SJ, Lira ME, et al. An Integrated Genomic Approach to Identify Predictive Biomarkers of Response to the Aurora Kinase Inhibitor PF-03814735. *Mol Cancer Ther*. 2012;11(3):710-719. doi:10.1158/1535-7163.MCT-11-0184.
44. Dieci MV, Arnedos M, Andre F, Soria JC. Fibroblast Growth Factor Receptor Inhibitors as a Cancer Treatment: From a Biologic Rationale to Medical Perspectives. *Cancer Discov*. 2013;3(3):264-279. doi:10.1158/2159-8290.CD-12-0362.
45. Wellenstein MD, de Visser KE. Cancer-Cell-Intrinsic Mechanisms Shaping the Tumor Immune Landscape. *Immunity*. 2018;48(3):399-416. doi:10.1016/j.immuni.2018.03.004.
46. Goodman AM, Kato S, Bazhenova L, et al. Tumor Mutational Burden as an Independent Predictor of Response to Immunotherapy in Diverse Cancers. *Mol Cancer Ther*. 2017;16(November):molcanther.0386.2017. doi:10.1158/1535-7163.MCT-17-0386.
47. Snyder A, Makarov V, Merghoub T, et al. Genetic Basis for Clinical Response to CTLA-4 Blockade in

Melanoma. *N Engl J Med*. 2014;371(23):2189-2199. doi:10.1056/NEJMoa1406498.

48. Van Allen EM, Miao D, Schilling B, et al. Genomic correlates of response to CTLA-4 blockade in metastatic melanoma. *Science (80-)*. 2016;352(6283):207-212. doi:10.1126/science.aaf8264.
49. Hellmann MD, Ciuleanu T-E, Pluzanski A, et al. Nivolumab plus Ipilimumab in Lung Cancer with a High Tumor Mutational Burden. *N Engl J Med*. 2018:NEJMoa1801946. doi:10.1056/NEJMoa1801946.
50. Rizvi NA, Hellmann MD, Snyder A, et al. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. *Science (80-)*. 2015;348(6230):124-128. doi:10.1126/science.aaa1348.
51. Panda A, Betigeri A, Subramanian K, et al. Identifying a Clinically Applicable Mutational Burden Threshold as a Potential Biomarker of Response to Immune Checkpoint Therapy in Solid Tumors. *JCO Precis Oncol*. 2017;(1):1-13. doi:10.1200/PO.17.00146.
52. Nik-Zainal S, Davies H, Staaf J, et al. Landscape of somatic mutations in 560 breast cancer whole-genome sequences. *Nature*. 2016;534(7605):47-54. doi:10.1038/nature17676.
53. Gnjatic S, Bronte V, Brunet LR, et al. Identifying baseline immune-related biomarkers to predict clinical outcome of immunotherapy. *J Immunother Cancer*. 2017;5(1):1-18. doi:10.1186/s40425-017-0243-4.
54. Le DT, Durham JN, Smith KN, et al. Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade. *Science*. 2017;357(6349):409-413. doi:10.1126/science.aan6733.
55. Dudley JC, Lin M-T, Le DT, Eshleman JR. Microsatellite Instability as a Biomarker for PD-1 Blockade. *Clin Cancer Res*. 2016;22(4):813-820. doi:10.1158/1078-0432.CCR-15-1678.
56. Le DT, Uram JN, Wang H, et al. PD-1 Blockade in Tumors with Mismatch-Repair Deficiency. *N Engl J Med*. 2015;372(26):2509-2520. doi:10.1056/NEJMoa1500596.
57. Cortes-Ciriano I, Lee S, Park WY, Kim TM, Park PJ. A molecular portrait of microsatellite instability across multiple cancers. *Nat Commun*. 2017;8:1-12. doi:10.1038/ncomms15180.
58. Fridman WH, Zitvogel L, Sautès–Fridman C, Kroemer G. The immune contexture in cancer prognosis and treatment. *Nat Rev Clin Oncol*. 2017;14(12):717-734. doi:10.1038/nrclinonc.2017.101.
59. Savas P, Salgado R, Denkert C, et al. Clinical relevance of host immunity in breast cancer: From TILs to the clinic. *Nat Rev Clin Oncol*. 2016;13(4):228-241. doi:10.1038/nrclinonc.2015.215.
60. Hendry S, Salgado R, Gevaert T, et al. Assessing Tumor-infiltrating Lymphocytes in Solid Tumors: A Practical Review for Pathologists and Proposal for a Standardized Method from the International Immunooncology Biomarkers Working Group: Part 1: Assessing the Host Immune Response, TILs in Invasi. *Adv Anat Pathol*. 2017;24(5):235-251. doi:10.1097/PAP.0000000000000162.
61. Galon J, Mlecnik B, Bindea G, et al. Towards the introduction of the “Immunoscore” in the classification of malignant tumours. *J Pathol*. 2014;232(2):199-209. doi:10.1002/path.4287.
62. Mlecnik B, Bindea G, Kirilovsky A, et al. The tumor microenvironment and Immunoscore are critical determinants of dissemination to distant metastasis. *Sci Transl Med*. 2016;8(327):327ra26-327ra26. doi:10.1126/scitranslmed.aad6352.
63. Xiao Y, Ye Y, Zou X, et al. The lymphovascular embolus of inflammatory breast cancer exhibits a Notch 3 addiction. *Oncogene*. 2011;30(3):287-300. doi:10.1038/onc.2010.405.
64. Zitvogel L, Ma Y, Raoult D, Kroemer G, Gajewski TF. The microbiome in cancer immunotherapy: Diagnostic tools and therapeutic strategies. *Science (80-)*. 2018;359(6382):1366-1370. doi:10.1126/science.aar6918.
65. Wang H, Altemus J, Niazi F, et al. Breast tissue, oral and urinary microbiomes in breast cancer. *Oncotarget*. 2017;8(50):88122-88138. doi:10.18632/oncotarget.21490.
66. Routy B, Le Chatelier E, Derosa L, et al. Gut microbiome influences efficacy of PD-1-based immunotherapy against epithelial tumors. *Science (80-)*. 2018;359(6371):91-97. doi:10.1126/science.aan3706.
67. Viaud S, Viaud S, Saccheri F, et al. The Intestinal Microbiota Modulates Cyclophosphamide. 2013;971(November):971-977. doi:10.1126/science.1240537.
68. Cerullo V, Diaconu I, Kangasniemi L, et al. Immunological effects of low-dose cyclophosphamide in cancer patients treated with oncolytic adenovirus. *Mol Ther*. 2011;19(9):1737-1746.

doi:10.1038/mt.2011.113.

69. Lutsiak MEC, Semnani RT, Pascalis R De, et al. Inhibition of CD4+ 25+ T regulatory cell function implicated in enhanced immune response by low-dose cyclophosphamide. *Blood*. 2005;105(7):2862-2868. doi:10.1182/blood-2004-06-2410.
70. Bottini A, Generali D, Brizzi MP, et al. Randomized phase II trial of letrozole and letrozole plus low-dose metronomic oral cyclophosphamide as primary systemic treatment in elderly breast cancer patients. *J Clin Oncol*. 2006;24(22):3623-3628. doi:10.1200/JCO.2005.04.5773.
71. Wildiers H, Tryfonidis K, Dal Lago L, et al. Pertuzumab and trastuzumab with or without metronomic chemotherapy for older patients with HER2-positive metastatic breast cancer (EORTC 75111-10114): an open-label, randomised, phase 2 trial from the Elderly Task Force/Breast Cancer Group. *Lancet Oncol*. 2018;19(3):323-336. doi:10.1016/S1470-2045(18)30083-4.
72. Guerrero-Preston R, Godoy-Vitorino A, Jedlicka A, Rodriguez H, Gonzalez H, Sidransky D. 16S rRNA amplicon sequencing identifies microbiota associated with oral cancer, Human Papilloma Virus infection and surgical treatment. *Oncotarget*. 2016;7(32):1-15. doi:10.18632/oncotarget.9710.

Acknowledgements

Writing this protocol and working closely with the staff of the Division of Early Drug Development for Innovative Therapies at the European Institute of Oncology was a game changer. From day 1 they had me onboard, everything was about looking for chances and opportunities to patients with uncertain fate. I saw great talent and commitment fine-tuned in a year of massive learning about clinical trial design rationales, how to take care of patients under a first-in-human setting, as well as all the logistics behind it and it was no less then overwhelming. The PERICLES trial needed a lot of effort to be put together and I could certainly not have done it without the unconditional support from Prof. Giuseppe Curigliano, who stood up for me with patience and wisdom through all phases of this process. For bureaucratic matters, enrollment start took longer than we expected and the detailed timetable is reported above:

Date	Event
08/08/2017	First version of the protocol is finished; EudraCT number is requested.
From August 2017 to November 2017	Internal administrative evaluation, along with Merck, regarding the established contract (profit/no profit).
November 2017	Clinical Trials Office (CTO) requests support from the Ethical Committee (EC).
13/12/2017	Minor changes on the protocol regarding recent released toxicity updates from pembrolizumab; New version sent to CTO.
From December 2017 to February 2018	CTO decides to ask the Clinical Pharmacy and Merck for support in fulfilling the Clinical Trial Authorization (CTA).
01/03/2018	The documents were sent to the EC for registration upon the National Observatory on Clinical Trials' website.
April 2018	A translational scope consisting of the comprehensive analysis of 6 molecular variables has being built to address the landscape of prediction to IO response in the study population.
07/05/2018	EC confirms trial input at the National Observatory on Clinical Trials' website.
08/05/2018	Awaiting on Italian Pharma Agency (AIFA) position.
22/06/2018	AIFA suggests amendments in the main protocol.
10/07/2018	Study becomes a pure phase II trial in the base of dose/safety data of the trial therapeutic combination in sarcomas; Inclusion criteria are modified based on recent published data regarding PD-L1 expression and TILs on breast cancer.
31/08/2018	Official study title changes to "Combination of pembrolizumab with oral metronomic cyclophosphamide in patients with chest wall breast cancer (PERICLES): A phase II study".
01/09/2018	Awaiting final OK from AIFA.

AIFA asks 60 days for final analysis. We are ready to start enrolling as soon as the approval comes in (deadline 01/11/2018).

During this period, I've also followed all the clinical and research activities within the Division, namely: day hospital of patients under early phase clinical trials, daily clinics with potential patients for enrollment on phase I/II trials conducted in house, medical oncology ward supporting any foreseen inpatient condition and/or serious adverse events management regarding targeted therapy and immunotherapy under early development.

The trials which I had participated, directly or indirectly, on accrual and patient management were the following:

- A phase I/Ib, open label study of LSZ102 single agent and LSZ102 in combination with either LEE011 (LSZ102 + LEE011) or BYL719 (LSZ102 + BYL719) in patients with advanced or metastatic ER+ breast cancer who have progressed after endocrine therapy - NCT02734615;
- A phase 2, multicenter, open-label study of DS-8201a, an anti-HER2-antibody drug conjugate (ADC) for HER2-positive, unresectable and/or metastatic breast cancer subjects who are resistant or refractory to TDM-1 - NCT03248492;
- A Phase Ib/II, open label, multicenter study of MCS110 in combination with PDR001 in patients with advanced malignancies - NCT02807844;
- A phase 1 study of durvalumab and IPH2201 in adult subjects with select advanced solid tumors - NCT02671435;
- A Phase 1 Study of the Highly-selective RET Inhibitor, BLU-667, in Patients with Thyroid Cancer, Non-Small Cell Lung Cancer (NSCLC) and Other Advanced Solid Tumors - NCT03037385;
- Multicenter, international, non-controlled, phase II trial to identify the molecular mechanisms of resistance and sensitivity to palbociclib re-challenge upon progression to a palbociclib combination in ER-positive metastatic breast cancer patients (BioPER) - NCT03184090;
- An open -label, multicenter, dose-escalation, phase Ia/Ib study to evaluate safety, pharmacokinetics, and therapeutic activity of RO6874281, an immunocytokin 2 variant (IL-2v) targeting fibroblast activation protein-α (FAP), as a single agent (part A) or in combination with trastuzumab or cetuximab (part B or C) - NCT02627274;
- A Phase I/Ib, open-label, multi-center dose-escalation and dose-expansion study of the safety and tolerability of intratumorally administered LHC165 single agent and in combination with PDR001 in patients with advanced malignancies - NCT03301896;
- A Phase 1/2a Dose Escalation and Cohort Expansion Study of the Safety, Tolerability, and Efficacy of Anti-LAG-3 Monoclonal Antibody (BMS-986016) Administered Alone and in Combination with Anti-PD-1 Monoclonal Antibody (Nivolumab, BMS-936558) in Advanced Solid Tumors - NCT01968109;
- A phase I-Ib/II, open-label, multi-center study of the safety and efficacy of MBG453 as single agent and in combination with PDR001 in adult patients with advanced malignancies - NCT02608268;
- Phase 1/2a Study of BMS-986205 Administered in Combination with Nivolumab (anti-PD-1 Monoclonal Antibody) and in Combination with Both Nivolumab and Ipilimumab (anti-CTLA-4 Monoclonal Antibody) in Advanced Malignant Tumors - NCT02658890;
- A first-in-human, open-label, phase 1/2 study to evaluate the safety, pharmacokinetics, pharmacodynamics, and clinical activity of JNJ-63723283, an anti-PD-1 monoclonal antibody, in subjects with advanced cancers - NCT02908906;
- A phase 2, fast real-time assessment of combination therapies in immuno-oncology study in subjects with advanced non-small cell lung cancer (FRACTION-Lung) - NCT02750514;
- A pase Ia/Ib study evaluating TAS-116 in patients with advanced solid tumors - NCT02965885;

- Safety and tolerability of single and repeated doses of odm-203: an open-label, non-randomised, uncontrolled, dose escalation, multicentre, first-in-human study in subjects with advanced solid tumours - NCT02264418;
- A phase 1/2 study on the safety of rovalpituzumab tesirine administered in combination with nivolumab or nivolumab and ipilimumab for adults with extensive-stage small cell lung cancer - NCT03026166;
- Phase 1 dose escalation and cohort expansion study of TSR-042, an anti-PD-1 monoclonal antibody, in patients with advanced solid tumors - NCT02715284;
- A phase II, randomized, multicenter study to assess the efficacy of nab-paclitaxel-based doublet as first line therapy in patients with cancer of unknown primary (CUP): the AGNOSTOS trial - NCT02607202;
- Multicenter, translational study to investigate the genomic landscape of cancer of unknown primary (CUP): AGNOSTOS profiling - NCT02607202.

My peer-reviewed publications derived from this period are listed above and correlates with our expertise within the Division:

Taylor & Francis

Expert Opinion on Pharmacotherapy

ISSN: 1465-6566 (Print) 1744-7666 (Online) Journal homepage: http://www.tandfonline.com/loi/ieop20

Clinical efficacy of ribociclib as a first-line therapy for HR-positive, advanced breast cancer

Bruno Achutti Duso, Dario Trapani, Giulia Viale, Carmen Criscitiello, Paolo D'Amico, Carmen Belli, Luca Mazzarella, Marzia Locatelli, Ida Minchella & Giuseppe Curigliano

To cite this article: Bruno Achutti Duso, Dario Trapani, Giulia Viale, Carmen Criscitiello, Paolo D'Amico, Carmen Belli, Luca Mazzarella, Marzia Locatelli, Ida Minchella & Giuseppe Curigliano (2018): Clinical efficacy of ribociclib as a first-line therapy for HR-positive, advanced breast cancer, Expert Opinion on Pharmacotherapy, DOI: 10.1080/14656566.2018.1429407

To link to this article: https://doi.org/10.1080/14656566.2018.1429407

Cancer Treatment Reviews 65 (2018) 22–32

Contents lists available at ScienceDirect

Cancer Treatment Reviews

journal homepage: www.elsevierhealth.com/journals/ctrv

Tumour Review

Targeting the microenvironment in solid tumors ☆

Carmen Belli [a,*], Dario Trapani [a], Giulia Viale [a], Paolo D'Amico [a], Bruno Achutti Duso [a], Paolo Della Vigna [b], Franco Orsi [b], Giuseppe Curigliano [a]

[a] Division of Early Drug Development for Innovative Therapies, European Institute of Oncology, via Ripamonti 435, 20141 Milan, Italy
[b] Interventional Radiology Division, European Institute of Oncology, via Ripamonti 435, 20141 Milan, Italy

Co-authored manuscripts recently accepted and/or under submission:

- The evolving landscape of "Next Generation" Immune Modulators. A review;
- Homologous recombination deficiency (HRD) in triple negative breast cancer (TNBC);
- Complexity of Genome Sequencing and Reporting: Next generation sequencing (NGS) technologies and implementation of Precision Medicine in Real Life;
- Liver Toxicity in the Era of Immune Checkpoint Inhibitors: A Practical Approach.

I would also like to deeply thank the ESMO Fellowship and Awards Committee for giving me this opportunity. I believe IBC/CWD is a much relevant entity to be focused at and in clear need of better enlightening on new therapeutic approaches. The PERICLES trial was tailored to make a point in a number of questions regarding this population and promote direct benefit for its participants and for the scientific community after all data have being collected and processed. As a young oncologist, going through this path with the endorsement of ESMO was extremely flattering and stimulating. Last but not least, I would like to show my honest appreciation for the attention, kindness and technical support that all of the following colleagues showed towards me during the last year: Dario Trapani, Luca Mazzarella, Giulia Viale, Emanuela Ferraro, Paolo D'Amico, Paolo Tarantino, Stefania Morganti, Carmen Belli, Carmen Criscitiello, Marzia Locatelli, Ida Minchella, Giulia Zampino, Andrea Vingiani, Luigi Nezi and Davide Bedognetti.

Division of Early Drug Development for Innovative Therapies at the European Institute of Oncology, Milan.