Protocol for a Pilot Trial to Implement Diagnostics for Clonal Hematopoiesis of Indeterminate Potential into Routine Clinical Care of Older Patients with Breast Cancer

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Abstract: Clonal hematopoiesis of indeterminate potential (CHIP) refers to the presence of a hematopoietic clone with a common leukemia driver mutation without diagnosis of an underlying hematopoietic disease. The prevalence of CHIP is increasing with age and is associated with pro-inflammatory states, higher risk of cardiovascular diseases (CVD) and therapy-induced leukemia. However, these CHIP-associated risks overlap with treatment-related toxicities of breast cancer therapy, which potentially supports the integration of CHIP into treatment- and survivorship plans. However, so far no data on the feasibility and acceptance of a CHIP-based aftercare are available.

In this pilot trial, the feasibility to integrate pre-treatment CHIP diagnostics into the routine care of breast cancer patients is evaluated. Early-stage breast cancer is common among older women and has an excellent long-term outcome. Thus, 80-100 patients with limited stage breast cancer aged ≥ 60 years without known hematological disease will be included. CHIP is assessed by targeted next generation sequencing from peripheral blood samples. The primary outcome measures the estimation of willingness to participate. Secondary outcome measures include evaluation of patient acceptance of the study process, potential fears in relation to CHIP-positivity, and cardiovascular risk profile of CHIP-positive versus CHIP-negative patients.
In case this study meets its primary endpoint, the results are used to design a larger cohort study that integrates an intensified CHIP-tailored survivorship program, in order to minimize late treatment-related toxicities and improve long-term outcomes of older breast-cancer patients.

**Key words:** Clonal haematopoiesis of indeterminate potential (CHIP); Breast cancer; Older adults; Cardiovascular risk factors; Survivorship

1. Introduction

1.1 Background

With 46,000 new cases, breast cancer is the most common solid cancer in women ≥ 65 years in Germany [1]. Especially when diagnosed at early stages, the long-term outcomes are excellent [2]. Thus, the balance between long-term control of breast cancer and treatment-related toxicities is of utmost importance. Clonal hematopoiesis of indeterminate potential (CHIP) confers to the presence of a hematopoietic clone carrying a hematological malignancy-associated mutation without an underlying hematological malignancy [3]. The incidence of CHIP is rising with age [4, 5]. CHIP is associated with an increased risk of (therapy-related) myeloid neoplasms [6–9], and cardiovascular events [4, 10]. As several of CHIP-associated risks overlap with known long-term toxicities of breast cancer therapy, it seems indispensable to understand its interplay and to integrate CHIP into treatment and survivorship concepts of older adults with breast cancer. However, so far no data on the implementation of a CHIP-based strategy into survivorship care are available. Thus, incorporation of CHIP into real-world medicine needs to be established and evaluated. These requirements led to the concept of Crunchy-CHIPs (Cardiovascular and hematological survivorship in breast cancer patients at high risk caused by clonal hematopoiesis of indeterminate potential - study).

1.1.1 CHIP: Definition and general aspects

The most abundant definition of CHIP, that was recently included in the 2022 World Health Organization (WHO) classification of myeloid malignancies [11] is the presence of a hematopoietic clone carrying a hematological malignancy-associated mutation with a variant allele frequency (VAF) of ≥ 2% without otherwise unexplained cytopenias [3]. Despite that, much smaller VAFs were described depending on the depth of sequencing and the number of sequenced genes [5, 12]. At least in some circumstances (e.g., risk of acute myeloid leukemia (AML) related to Tumor Protein 53 (TP53) and isocitrate dehydrogenase (IDH)-mutations [13], or mutations in DNA damage repair (DDR) genes [14]), these smaller VAFs seem clinically relevant, as they provide a fitness advantage and can expand easily [13].

CHIP occurs most commonly in genes of the epigenetic modifiers DNA methyltransferase 3 alpha (DNMT3A), Tet methylcytosine dioxygenase 2 (TET2), and Additional Sex Combs-Like 1 (ASXL1) (‘DTA mutations’) [4, 5]. Besides, common mutations affect IDH1/2, spliceosome genes, and DDR genes, such as TP53, and Protein Phosphatase Mg2+/Mn2+ Dependent ID (PPM1D) [4, 5, 13, 15]. Of note, the spectrum of CHIP mutations that were studied in different trials was not standardized; thereby, the variance in CHIP incidence between different data sets is easily explained.

Although CHIP was recently described to appear as early as in utero at very small VAFs [16], its incidence strongly increases with age, affecting at least 10% of individuals ≥ 60 years [4]. The CHIP incidence in breast cancer patients might differ depending on age and prior exposure to genotoxic agents which includes a smoking history. Albeit, the CHIP incidence in women with breast cancer ≥ 70 years was described as high as 44% [17]. CHIP is associated with an increased overall mortality in adults ≥ 70 years [4].

Most of the mutations confer to a pro-inflammatory phenotype, which is based on pre-clinical [18–20] and clinical data [16, 21]. As a clinical surrogate marker, high-sensitive C-reactive protein (hsCRP) was increased in CHIP-positive individuals [22]. Furthermore, other cytokines, such as interleukin-6 (IL-6) [23, 24] or interleukin-8 (IL-8) [10] were also shown to be elevated. As a consequence, it is not surprising that the presence of CHIP fuels diseases like chronic obstructive pulmonary disease [25, 26], liver cirrhosis [27], autoimmune diseases [28], and infections, such as Clostridium difficile enterocolitis and severe COVID-19 infection [29] (Figure 1), most likely via a pro-inflammatory crosstalk.

1.1.2 The interplay between CHIP and solid cancers

CHIP is frequently found incidentally when peripheral
blood is used as comparator for mutational profiling of solid cancers \cite{30, 31} and acts as a confounder in liquid biopsies \cite{32, 33}. Recently, infiltration of the tumor microenvironment by CHIP-positive tumor-infiltrating leukocytes (TILs) was described \cite{34}. This prompts the question whether the pro-inflammatory properties of CHIP-positive leukocytes modify the TIL-tumor interactions and promote the tumor growth. This is not sufficiently studied yet but first results in an experimental model of colon cancer with the experimental equivalent for \textit{DNMT3A}-CHIP showed increased infiltration of colon cancer by immune cells and, in preliminary data, a decreased phagocytic capacity \cite{35}, suggesting a permissive microenvironment. Given the known tumor-promoting effect of chronic inflammation, it could be expected that CHIP acts as a tumor-promoting factor. First data that support such a negative impact showed an increased mortality due to progressive solid cancers in CHIP-positive patients \cite{6}. This needs to be confirmed in larger data sets and tumor-entity specific analyses. With regard to breast cancer, CHIP could further influence tumorigenesis and tumor promotion via the cancer-metabolic interplay. Metabolic syndrome and its components (diabetes, dyslipidemia, and obesity) are well-known risk factors, especially for hormone-receptor positive breast cancer in postmenopausal woman \cite{36}. Although the pathophysiology behind this risk is complex and multifaceted, it is largely determined by the chronic inflammatory and endocrine properties of the adipose tissue \cite{37}. Thus, CHIP comes again into play with its pro-inflammatory nature and its interacting role within the metabolic axis. Specifically, \textit{TET2}-Chip has been shown to potentiate age- and obesity-related insulin resistance in a diabetic mouse model that can be modified via inhibition of the inflammasome \cite{38}. Whether a reduction of CHIP-related inflammation and/or a consequent control of metabolic disturbances can abrogate the tumor promotion remains elusive and will be a vibrant research question for the next years as it provides a promising target for cancer prevention together with cardiovascular risk reduction.

Besides the possible CHIP-cancer interplay, CHIP could have a very practical impact on therapy-related toxicities during cancer therapies which is largely understudied so far. With regard to therapy-induced cytopenia during chemotherapy, data are lacking although CHIP has been shown to be associated with a delayed time to neutrophil reconstitution after autologous stem-cell transplantation \cite{39}.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{chip-associated-risks.png}
\caption{CHIP-associated risks in healthy older individuals, breast cancer treatment-related toxicities, and their suspected interplay with CHIP.}
\end{figure}
Abbreviations: CAD, coronary artery disease; CHIP, clonal hematopoiesis of indeterminate potential; COPD, chronic obstructive pulmonary disease; TIL, tumor-infiltrating leukocytes; TMN, therapy-related myeloid neoplasia.

1.1.3 Treatment-related toxicities after breast cancer therapy: Therapy-related myeloid neoplasms

Therapy-related myeloid neoplasms (TMN) after breast cancer therapy are rare, as they occur in ~0.5% of patients within 10 years of chemotherapy-including treatments \[40\] but their outcomes are grim with a 3-year overall survival of ~30% \[41\], even after allogeneic stem-cell transplantation. CHIP is related to the exposure with genotoxic agents and smoking \[9\] and has been identified as a predisposing factor for TMN \[6-9\]. On a mechanistical basis, pre-existing CHIP clones might own a growth advantage upon genotoxic stress and can acquire secondary mutations that lead to leukemic transformation of these clones. In addition, genotoxic therapy can induce mutations that lead to the development of CHIP; later, secondary acquisition of further mutations initiates a leukemic transformation. These two potential mechanisms leading to TMN are depicted in Figure 2. These hypotheses are underlined by data from the MSK-IMPACT study \[9\]. In this, CHIP clones that harboured a mutation in the DNA damage genes TP53, Checkpoint kinase 2 (CHEK2), or PPM1D showed an increase in allelic burden during chemo- or radiation therapy \[9\] as a sign for a fitness advantage upon these respective therapies. In 59% of patients who developed a TMN, at least one leukemia driver mutation that was present in TMN could be detected as CHIP prior to initial therapy \[9\]. This is underlined by the findings from the Woman Health Study, in which 100% of participants with CHIP in TP53 or IDH1/2 developed spontaneously a myeloid neoplasia over time \[13\]. However, it should be noted that even analyses of large cohorts include only small numbers of individuals with a respective mutation, thus, a detailed risk assessment to predict the development of (T)MN remains an unmet clinical need. In the context of early breast cancer care, this could become a highly relevant topic in future if the risk of TMN is weighed against the risk reduction of cancer recurrence by adjuvant chemotherapy. Thus, depending on the individual risk, adapting the (neo-)adjuvant therapy might provide the necessary balance of risks, but needs thorough evaluation before such an approach is integrated into clinical routine.

Figure 2. Possible pathways to therapy-related myeloid neoplasms in relation to CHIP.
By mechanistical view, CHIP can pave two pathways to the development of therapy-related myeloid neoplasms: Firstly, in an individual with pre-existing CHIP, the application of cytotoxic chemotherapy can lead to a selection of CHIP clones with fitness advantage. Further acquisition of mutations by genotoxic therapies can finally cause transformation into a myeloid neoplasm. Secondly, in an individual without pre-existing CHIP, genotoxic therapy can induce CHIP clones which can advance into myeloid neoplasms after further acquisition of mutations.

Abbreviations: T-AML, therapy-related acute myeloid leukemia; T-MDS, therapy-related myelodysplastic syndrome.

1.1.4 CHIP and the risk for cardiovascular diseases
CHIP was shown to increase the overall mortality in the general population, which was not caused by an increased leukemia-induced death-rate but increased cardiovascular (CV) events \[4\]. Further, CHIP was demonstrated to be associated with an increased risk for coronary artery disease (CAD)\[4\], peripheral artery disease (PAD) \[42\], and stroke \[4, 45, 46\] (Figure 1).

Recent work has elucidated the pathophysiological mechanism of the promotion of artherosclerosis by CHIP: Based on the atherosclerosis-prone, low-density lipoprotein receptor–deficient (Ldlr-/-) mouse model combined with competitive Tet2-deficient hematopoietic stem-cell (HSC) transplantation that resembles TET2-CHIP, Tet2-deficient macrophages were shown to have an increased NOD-, LRR- and pyrin domain-containing protein 3 (NLRP3) inflammasome–mediated secretion of interleukin-1ß (IL-1ß) after stimulation with Lipopolysaccharide and interferon-gamma. In addition, other pro-inflammatory cytokines were also upregulated \[18\]. This IL-1ß secretion is potentially the most potent causative link to the accelerated atherosclerosis as it orchestrates low-grade inflammation of endothelial cells and enhances proliferation of smooth muscle cells \[45\]. Thus, it is not surprising that inhibition of NLRP3 inflammasome was demonstrated to reverse the pro-atherosclerotic effect of Tet2-deficiency in the same experimental model \[18\]. In concordance, an exploratory analysis from the Canakinumab Antiinflammatory Thrombosis Outcome Study (CANTOS) trial demonstrated a reduction in major adverse cardiac event (MACE) for patients receiving the neutralizing anti-IL-1ß antibody canakinumab (hazard ratio (HR), 0.38 [95% confidence interval (CI), 0.15-0.96]) in comparison to placebo-treated patients \[46\]. This is further underlined by a recent analysis of a cohort of ST segment elevation myocardial infarction (STEMI) patients. Among these, patients with DNMT3A/TET2 – CHIP experienced an increased rate of death (30.9% vs 15.5%, P = 0.001) and MACE (44.5% vs 21.8%, P < 0.001) during a medium follow up of 3 years \[24\].

Of interest, the relationship between CHIP and atherosclerosis is most likely not only unidirectional as an enhanced proliferation of HSCs in the presence of atherosclerosis was recently suggested, presumably due to increased inflammation and hyperlipidemia \[67\]. Although CHIP was shown to act as CV risk factor independent of established risk factors, such as smoking, or hyperlipidaemia \[4\], it remains elusive whether CHIP confers any substantial CV risk in individuals without additional risk factors, such as dyslipidemia, smoking, or hypertension, as most of the studied cohorts included many metabolic high-risk individuals (e.g., diabetes) \[4\]. In line, the seminal work in the atherosclerosis-prone mouse model resembling TET2-CHIP only demonstrated an accelerated plaque formation upon high fat/cholesterol diet, whereas mice fed with normal diet did not show this acceleration \[18\]. This suggests the interplay of traditional CV risk factors with CHIP/pro-inflammatory state as necessary requisite for atherosclerosis acceleration rather than a causative role of CHIP as single risk factor. As a consequence, reduction of CHIP-induced CV risk might be achieved by consequent control of established risk factor or require additional anti-inflammatory treatment. As this question is crucial for future management and reduction of CHIP-related CV risk, this needs to be determined in different patient cohorts, most likely with an additional focus on early endothelial dysfunctions.

Besides the increased risk for CAD, CHIP was demonstrated to increase the risk for heart failure \[48, 49\]. This finding is further supported in experimental mouse models resembling TET2 \[50\] and Janus kinase 2 (JAK2) \[51\] CHIP. Furthermore, CHIP with DNMT3A- or TET2-mutation was shown to be independently associated with an accelerated heart failure progression in terms of death and/or heart failure hospitalization (DNMT3A: HR, 4.50; 95% CI, 2.07-9.74; p < 0.001, TET2: HR, 3.18; 95%
CL, 1.52-6.66; p = 0.002) irrespective of the underlying ischemic/non-ischemic etiology\(^\text{[49]}\).

### 1.1.5 Treatment-related toxicities after breast cancer therapy: Cardiovascular Sequelae and cardio-metabolic interplay

Many components of breast cancer therapy carry a risk for cardiovascular side-effects and sequelae, such as anthracycline-induced left ventricular dysfunction (AILVD)\(^\text{[52, 53]}\), radiation-induced atherosclerosis\(^\text{[54]}\), taxane-related early endothelial dysfunctions\(^\text{[55]}\), or the prothrombotic properties of tamoxifen\(^\text{[56]}\) (Figure 1). Given the role of cardio-metabolic risk factors for the development of breast cancer, it is not surprising that breast cancer patients show an increased prevalence of cardio-metabolic risk factors, even at baseline\(^\text{[56, 57]}\) that further predispose to such treatment-related toxicities\(^\text{[53, 54]}\).

As CHIP is in between this cardio-metabolic interplay and confers an increased CV risk, as described above, it might have a further impact on these toxicities during treatment and survivorship (Figure 1). First data support this view: In lymphoma patients receiving anthracyclines, TET2-CHIP was associated with an increased rate of AILVD\(^\text{[58]}\). Furthermore, in AML patients treated with intensive anthracycline-containing regimens, the presence of CHIP-mutations was associated with an increased risk for CV events (HR, 1.74; 95% CI, 1.03-2.93; p = 0.037)\(^\text{[59]}\). The same accounted specifically for TP53 (HR, 4.18; 95% CI, 2.07-8.47; p < 0.001) and ASXL1 (HR, 2.37; 95% CI, 1.21-4.63 p = 0.012) mutations\(^\text{[59]}\). These data are supported in a mouse model of AILVD with underlying TP53 – CHIP\(^\text{[60]}\). Should this be further confirmed, intensification of primary preventive measures to mitigate toxicities, or even adjusting the treatment according to the individualized risk might be advantageous although this is still a long way off.

During breast cancer survivorship, the most common causes of death are CV events, including CAD and heart failure\(^\text{[61-67]}\). Data from childhood cancer survivors suggest that traditional CV risk calculation in this population underestimates the risk\(^\text{[68]}\), which was also demonstrated for the use of Framingham risk calculation in adult breast cancer patients\(^\text{[69]}\). In part, that can be related to the increase in cardio-metabolic risk factors after breast cancer therapy\(^\text{[56, 70]}\). Whether CHIP-positive patients carry an even higher cardio-metabolic risk during survivorship that further impacts these events, is unknown, but appears likely given the multifaceted interplay between these risks. Another open question is, whether CHIP-positive patients require an intensified survivorship program, including stringent reduction of traditional CV risk factors or even an anti-inflammatory approach.

### 1.1.6 Genetic counselling in older adults

Although CHIP diagnostics do not refer to germline but somatic mutations, it is a kind of acquired predisposition for leukemia and CV conditions; thus, it represents a form of predictive genetic testing. This is especially important as no explicit recommendations for individualized prevention exist in case of positivity. Predictive genetic testing can potentially result in psychological adverse reactions, such as anxiety, or distress, which might be dependent on the perception of controllability of the respective condition\(^\text{[71]}\). Despite, a recent systematic review revealed mostly no serious negative impact of genetic testing for CV diseases and cancer on quality of life (QoL), anxiety, or distress\(^\text{[72]}\). As several hereditary cancer syndromes or cardiovascular diseases tend to have an early onset in life, studies are focussed on younger adults, although older adults were shown to be willing to participate in genetic testing\(^\text{[72]}\). Despite, acceptance and psychological distress of predictive genetic testing is understudied in older adults.

A recent survey among survivors of breast cancer at young age on preferences and attitudes towards CHIP testing revealed an interest in CHIP testing in >75% of participants after receiving some education on this condition. 70.8% expressed that they would be willing to participate in genetic testing\(^\text{[72]}\). Despite that, 22.7% stated hypothetically that learning that they would be CHIP positive would be “more than [they] could handle emotionally”\(^\text{[73]}\). Notably, 92% of participants had an educational level of college or above and the median age was 46 years (range: 41-54 years)\(^\text{[73]}\). Although these results are very informative about patient preferences with regard to CHIP testing, the older age group which is mainly affected by CHIP, was not included into the survey. As a consequence, we see a particular need to assess patient acceptance of CHIP evaluation during our pilot trial.
1.1.7 Study rationale
Given the pro-inflammatory properties of CHIP and its suspected role within the (hemato-) cardio-metabolic crosstalk, CHIP will likely gain an important role within geriatric oncology, especially in a patient-centred individualized survivorship care that takes into account the individual risks for cardiometabolic diseases and TMN. Hitherto, effects of CHIP are almost exclusively studied in retrospective data sets. Integration in routine clinical care is not systematically performed and evaluated, and the acceptance by patients of such an approach is unknown. In this pilot trial, we determine the feasibility of integrating CHIP diagnostics into the routine work-up during breast cancer diagnostics and treatment initiation. A main goal of this piloting is to assess the willingness to participate, the prevalence of CHIP and cardiovascular risk factors, and overall patient satisfaction in our cohort in order to determine sample sizes and potential measurements for a large CHIP-tailored survivorship trial in future.

2. Methods
2.1 Aims, Study Design, Setting, and Recruitment
2.1.1 Aims
Our study aims are as follows: 1) The primary aim is to estimate the willingness of participation with an accuracy of +/- 7.5%. 2) Secondary aims are to assess the patient acceptance of the study procedures and addition of CHIP diagnostics to the routine work-up, to evaluate the overall prevalence of pre-therapeutic CHIP in this cohort of breast cancer patients, to assess the feasibility to recruit 80 – 100 patients within 12 months, to assess the frequency of guideline-adherent cardiovascular preventive medications at baseline and after 12 months, and to characterize the prevalence of cardiovascular risk factors in CHIP-positive versus CHIP-negative patients.

2.1.2 Trial design and setting
80-100 patients will be recruited from the Essen University Hospital, Breast Cancer Center, Department for Obstetrics and Gynecology. The protocol was approved by the Institutional Ethics Committee, University Duisburg-Essen on May 18, 2022. The study is funded by a grant from the Medical Faculty of the University Duisburg-Essen. The trial is registered at German Clinical Trials Register (Deutsches Register für klinische Studien, DRKS), DRKS00031021.

2.1.3 Recruitment and Consensus
A trial participation is offered to all patients ≥ 60 years presenting at our breast cancer center who are firstly diagnosed with breast cancer at a curative stage and further fulfil the eligibility criteria (Table 1). These eligibility criteria are very broad to avoid any selection bias. Written informed consent is obtained by all participants. The number of patients who reject participation is recorded without documentation of personal data.

<table>
<thead>
<tr>
<th>Table 1. Eligibility criteria</th>
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<td><strong>Inclusion criteria</strong></td>
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<td>Age ≥ 60 years</td>
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<td>Female gender</td>
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<td>First diagnosis of non-metastasized breast cancer (stage I-III)</td>
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<td>Must be able to understand written and spoken German</td>
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<td>Willing to comply with study procedures for the entire lengths of the study.</td>
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<td><strong>Exclusion criteria</strong></td>
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<td>Male gender</td>
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<td>Metastasized breast cancer</td>
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<td>Former history of acute myeloid leukemia, myeloproliferative diseases, or myelodysplastic syndrome</td>
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<td>Prior therapies with cytotoxic agents (excluding methotrexate for autoimmune diseases)</td>
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<td>Inability to give informed consent</td>
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2.2 Study procedures
At baseline, pre-treatment blood samples will be collected. Patients undergo their planned breast cancer treatment (surgery, radiation therapy, hormone-/chemotherapy) without any modification (Figure 3). Study personnel will document cancer and treatment...
specific details. Approx. three months after diagnosis, results of CHIP diagnostics are discussed with patients at the hematological outpatient clinic. At this point in time, no additional laboratory values are taken. In case, the baseline assessment was incomplete (e.g., cardiac risk assessment), measures are completed. Patients who are CHIP-positive receive a written summary on associated risks, their general practitioner also obtain a summary of current evidence about risks for TMN and CV events together with the information that current preventive measures remain unchanged (Figure 3). Treating physicians are not blinded for CHIP results as treatment decisions are already determined at this point in time. Patients in whom one or more CHIP-mutations with high risk for TMN which we define currently as CHIP with mutations in the DNA damage pathway (including TP53, PPM1D, and CHEK2) are detected are offered a differential blood count and a hematology consultation every 3-6 months. The definition of high-risk CHIP can be adapted during the study if new data are published that suggest a modification. In case, CHIP occurs together with cytopenia, a standard work-up for cytopenia is offered, independent of the study procedures. If myelodysplastic neoplasia is excluded, this condition is termed clonal cytopenia of undetermined significance (CCUS) and further hematological surveillance according to the local standard-of-care is offered.

After the informative hematological consultation, patients are asked to answer the European Organisation for Research and Treatment of Cancer Quality of Life Questionnaire C30 (EORTC-QLQ-C30) questionnaire\(^{[75]}\) together with a questionnaire on patient satisfaction and possibly associated fears with...
regard to CHIP diagnosis (Supplementary Table S3). Another consultation is scheduled after 12 months; during that visit, another blood sample is collected and regular medication is recorded. An additional, posttreatment CHIP assessment is facultative at this point in time. Patients with high-risk CHIP mutations are offered to continue hematology consultations, for the other participants the trial ends after 12 months.

### 2.3 Measures

Measures will be obtained at baseline (T1), approximately three months after diagnosis (T2), and after 12 months (T3). Study measures are listed in Table 2.

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<tr>
<th>Measure</th>
<th>Description</th>
<th>Time point</th>
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<tr>
<td>Blood draw</td>
<td>CHIP analysis by NGS</td>
<td>Baseline, (+12 months, facultative)</td>
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<td></td>
<td>Cytokine panel (IL-1ß, IL-6, IL-18, c-peptide, leptin, adiponectin, IGF-1, iridin, soluble Klotho)</td>
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<td>Differential blood count, RDW, MCV</td>
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<td>Breast cancer-related data</td>
<td>Stage, grading, histological subtype</td>
<td>Baseline</td>
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<tr>
<td>Treatment-related data</td>
<td>Intended treatment, accomplished treatment; treatment-related toxicities [acc. to CTCAE]: duration and severity of During treatment cytopenia, therapy-related infections, treatment modifications</td>
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<td>CV risk assessment (lab)</td>
<td>hsTropI</td>
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<td>CV risk assessment (clin)</td>
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<td>h/o arterial hypertension, as documented by antihypertensive medication or former diagnosis</td>
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<td>h/o + dx heart failure (HFREF, HFMREF, HFpEF)</td>
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<td>h/o thromboembolic events (stroke, myocardial infarction, thrombosis, pulmonary embolism)</td>
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<td>echocardiography (LVEF, E/A, E/E')</td>
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<td>h/o migraine with aura</td>
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<td>Family h/o MACE</td>
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<td>Medication history</td>
<td>ACE inhibitors</td>
<td>Baseline, + 12 months</td>
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<td>ARBs</td>
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<td>β-blockers</td>
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<td></td>
<td>Anti-inflammatory drugs: NSAIDs, MTX, cholchicine, TNFα-blocker</td>
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<td>SGLT2 inhibitors</td>
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<td>GLP-1 receptor antagonents</td>
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Table 2. Trial measures
2.3.1 Blood Sample Collection and CHIP assessment

Blood will be drawn at baseline (pre-treatment), and after 12 months during a routine laboratory test. Approximately 4 ml of serum will be stored after centrifugation at -80°C, peripheral blood mononuclear cells (PMBCs) will be collected and processed by BD Biosciences CPT vacutainer system according to the manufacturer’s instructions and further stored at -80°C.

Genomic DNA is extracted from PBMCs at baseline with QIAamp DNA Blood Mini Kit (Qiagen) and quantified by Qubit™ 1X dsDNA HS Assay Kit (ThermoFisher Scientific). 200 ng of genomic DNA will be subjected to next generation sequencing using Illumina NextSeq2000 at 2x 150 bp. A customized panel will be used (SOPHiA Genetics), covering 112 genes (Supplementary Table 2). Data are processed with SophiaDDM Software (SOPHiA Genetics).

We will call genomic variants from the obtained sequence reads using the Snakemake[^77] workflow dna-seq-varlociraptor (https://github.com/snakemake-workflows/dna-seq-varlociraptor), consisting of the following steps. Sequence reads will be mapped with BWA-mem[^78], followed by conversion of polymerase-chain reaction (PCR) duplicates into consensus reads with rust-bio-tools (https://github.com/rust-bio/rust-bio-tools) and base recalibration with GATK[^79]. Next, the known CHIP-associated variants are called and the posterior allele frequency distribution of each variant is obtained with Varlociraptor. Finally, an interactive, portable visual report is generated using Datavzrd (https://github.com/datavzrd/datavzrd) and Snakemake.

In addition, a differential blood count, high-sensitive troponin I, N-terminal prohormone of brain natriuretic peptide (NT-proBNP), total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), and high-sensitive C-reactive protein (CRP) will be determined at baseline.

2.3.2 Clinical Measures

Standard information on breast cancer stage, histology, treatment details, and measures of treatment tolerability are documented (Table 2). A detailed CV history and risk assessment is performed during outpatient visit at hematology department as listed in Table 2. QoL is assessed by EORTC-QLQ-C30 questionnaire together with patient acceptance evaluation (Supplementary Table S3) after patient education about their CHIP status at hematology outpatient department.

2.3.3 Feasibility Metrics

In order to assess the feasibility of integration CHIP diagnostics into routine care, we will collect several feasibility metrics. The enrolment rate (corresponding to the percentage of patients who are eligible and consented) will be documented, as well as the completeness of documentation (e.g., details of provided therapy, duration and severity of treatment-associated cytopenias during chemotherapy) as some patients receive parts of their therapy outside our center (e.g., radiation therapy), the retention rate (percentage...
of patients who complete also the 12-months follow-up), and the rate of sufficient sequencing results in \( \geq 80\% \) of samples.

In addition, patient satisfaction with information about the study and CHIP positivity itself and acceptance of procedures will be evaluated after the patients are informed about their CHIP status at our hematology outpatient service via a questionnaire (Supplementary Table S3). As fluctuations in QoL could potentially influence patient’s satisfaction, completion of EORTC-QLQ-C30 \(^{[83]}\) and EORTC-QLQ-BR23 \(^{[84]}\) questionnaires will be requested in parallel. This allows a comparison with other cohorts of breast cancer patients (e.g., \(^{[85]}\)) in case of unexpected results.

### 2.3.4 Cytokine profiling

Cytokines that are of interest in cardiometabolics are measured by custom-plex cytokine arrays (Eve Technologies) pre-treatment (Table 2). Measures will be performed in duplicates. These measurements will only be performed if \( \geq 15\% \) of participants are CHIP-positive as a lower percentage would not allow for comparison between the CHIP-positive and -negative patients.

### 2.3.5 Quality Control and Missing Data

Anonymized data will be entered in a database that complies with good clinical practice guidelines (Maganamed, https://maganamed.com/de). As patients might receive their therapy and routine blood work during therapy outside our center, missing data are expected. Thus, the rate of complete documentation is a part of the feasibility measures.

### 2.4 Sample Size

The primary endpoint, defined as the estimation of willingness to participate with an accuracy of \( \pm 7.5\% \), is dependent on the real participation rate (p) and the number of approached patients (N). Tight confidence intervals (CIs) are associated with a superior accuracy of estimation and the width of the CI broadens with increased number of approached patients. In case of a real participation rate (p) of \( \sim 50\% \) (p = 0.5), this estimation is the lowest and 200 patients would be required to achieve an accuracy of estimation of \( \pm 7.5\% \). The lower the real participation rate, the higher is the probability of a selection bias to characterize our cohort. That could potentially hamper the design of a future trial which aims at CHIP-tailored survivorship care in a real-world patient cohort. As an example: If the real participation rate is \( \geq 80\% \) which is expected, 80-100 patients would be in the range to estimate with the aimed accuracy. Supplementary Table 1 summarizes the size of CIs in dependency of p and N. Based on case numbers at our center and the probability to include \( \sim 10-20\% \) of patients who are CHIP-positive, we aim at a sample size of 80-100 patients to achieve in addition a sufficient power for assessment of feasibility metrics.

### 2.5 Statistical Analysis

The primary analysis will assess the estimation of willingness to participate with an accuracy of \( \pm 7.5\% \). This estimation of willingness will be calculated with the Clopper-Pearson interval for the parameter p of the binominal distribution. For that, the limits of the confidence interval (95% CI(p)) represent the largest and the smallest, respectively, value of p that allows the assumption to achieve the estimated number of participants.

Secondary aims are to assess the patient acceptance of the study procedures and addition of CHIP diagnostics to the routine work-up, to evaluate the overall prevalence of pre-therapeutic CHIP in this cohort of breast cancer patients, to assess the feasibility to recruit 80 – 100 patients within 12 months, to assess the frequency of guideline-adherent cardiovascular preventive medications at baseline and after 12 months, and to characterize the prevalence of cardiovascular risk factors in CHIP-positive versus CHIP-negative patients.

### 3. Discussion

A growing body of evidence suggests that CHIP plays an important role for the development of TMN and cardio-metabolic toxicities besides its well-known impact on overall CV mortality. As early breast cancer has an excellent long-term survival, improvement of survivorship care is an unmet need. CHIP likely represents an important key factor for individualized, risk-tailored survivorship care. To the author’s knowledge, this is the first study that prospectively implements CHIP diagnostics into routine clinical care of older adults with breast cancer. One major reason why the integration into routine clinical paths was neglected so far might be the lack of evidence
how CHIP-positivity should be managed during survivorship care. Our pilot study will not answer this question but prepare further trials that focus on such topics. Thus, feasibility of recruitment and patient acceptance are our major outcomes to legitimate future trials or modify their design. Most survivorship programs are focused on younger adults. As CHIP is related to aging, it provided the unique perspective to develop a tailored survivorship concept for older adults and their specific needs.

Most cancer patients have regularly scheduled visits with their cancer doctors (gynecologists, oncologists, hematologists). Thus, consultations with primary care specialists for purpose of prevention are often neglected. In line, cancer patients less often receive indicated preventive care assessments [86], cardiovascular medication [87], and counselling [88]. In concordance, a recent Canadian survey among hematologists revealed that 20% of them do not regularly consider the indication before they stop cardiovascular medication in acute myeloid leukemia patients and up to 28% did not recommence this medication after interruption [87]. Another survey described a discordance in perceived responsibilities for general preventive health between oncologists and general care physicians involved in survivorship care [89]. Similar data for a German cohort of cancer patients are lacking. To further elucidate this topic in our patient cohort, we will assess whether indicated preventive cardiovascular medication is prescribed at baseline and after 12 months. Although our cohort has a limited size, we expect an excellent quality of data in comparison to retrospective registry data as patients are scheduled for an outpatient visit that included a detailed history. If our assumption would be confirmed that breast cancer patients receive fewer preventive measures than indicated, this would provide a rationale to implement a close interaction between gynecologists, oncologists, and primary care physicians in our area.

4. Strength and possible limitations

Several limitations should be addressed. First, trial results can be limited by the small sample size. If fewer than 15-20% of participants are CHIP-positive, this can result in a less informative result on patient satisfaction. In addition, serum cytokine analysis will not be performed in such a case. This would limit the significance of a sample size calculation, in order to prepare for the subsequent trial. As cardiovascular prevention is mainly initiated and supervised by primary care physicians, a further limitation is that their involvement is not intended so far. This could be compensated by a separate subsequent evaluation of general care physician’s attitudes towards cardiovascular prevention in breast cancer patients based on the results of our study to address potential barriers.

We believe that the evaluation of patient satisfaction is a key strength of this study as the assessment and integration of patient’s needs are a key requirement for future trial considerations. Another strength of this study are the broad inclusion criteria to avoid any relevant selection bias. This is especially important as prevalence of CV risk factors in breast cancer survivors was shown to be dependent on age [88] and socioeconomic factors [90]. Although the recruiting center is a tertiary care hospital and academic institution, it serves as the main provider for breast cancer care in our region. Thus, no selection bias against older or unfit patients is expected. In addition, we expect an excellent retention rate at 12 months, as these visits are coupled with routine appointments.

5. Conclusions

The results of this study will inform us about the feasibility to integrate CHIP diagnostics into routine care of older breast cancer patients. If the primary endpoint is met and patient satisfaction is sufficient, patient characteristics of this cohort will allow us to design a large real-world study to assess a CHIP-tailored survivorship concept in order to improve treatment-related outcomes in this vulnerable patient group.

Consent for publication

Not applicable.

Availability of data and material

Not applicable.

Author contributions

Nina Rosa Neuendorff: Conception and Design, Data Collection, Manuscript Writing, Approval of Final Article.

Ann-Kathrin Bittner: Conception and Design,
Approval of Final Article.

Tessy Mauer: Manuscript Writing, Approval of Final Article.

Florian Schmitz: Conception and Design of the patient satisfaction questionnaire, Approval of Final Article.

Oliver Hoffmann: Conception and Design, Approval of Final Article.

Bastian von Tresckow: Conception and Design, Approval of Final Article.

Hans Christian Reinhardt: Conception and Design, Approval of Final Article.

Sara Hirt: Statistical conception, Manuscript Writing, Approval of Final Article.

Nils von Neuhoff: Conception and Design, Approval of Final Article.

Markus Schneider: Conception and Design, Approval of Final Article.

Johannes Köster: Conception and Design, Manuscript Writing, Approval of Final Article.

Felix Mölder: Conception and Design, Manuscript Writing, Approval of Final Article.

Amin T. Turki: Clinical expertise, correction of manuscript, Approval of Final Article.

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ATT: Consultancy for CSL Behring, Maat Pharma, Biomarin and Onkowissen.

All other authors do not report COI related to this article.

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Appendices

Supplementary Table 1. Size of confidence intervals in relation to number of approached versus participating patients.

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<th>p</th>
<th>N</th>
<th>50</th>
<th>100</th>
<th>150</th>
<th>200</th>
<th>250</th>
<th>300</th>
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<td>0.289</td>
<td>0.203</td>
<td>0.165</td>
<td>0.143</td>
<td>0.127</td>
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<td>0.125</td>
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<td>0.187</td>
<td>0.152</td>
<td>0.131</td>
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<td>0.237</td>
<td>0.165</td>
<td>0.134</td>
<td>0.115</td>
<td>0.103</td>
<td>0.094</td>
</tr>
<tr>
<td>0.9</td>
<td></td>
<td>0.185</td>
<td>0.127</td>
<td>0.103</td>
<td>0.088</td>
<td>0.078</td>
<td>0.071</td>
</tr>
</tbody>
</table>

Abbreviations: N, number of approached patients; p, real participation rate

Supplementary Table 2. Genes included in targeted sequencing (myeloid panel)

<table>
<thead>
<tr>
<th>Gene</th>
<th>Gene</th>
<th>Gene</th>
<th>Gene</th>
<th>Gene</th>
<th>Gene</th>
<th>Gene</th>
</tr>
</thead>
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<td>ABL1</td>
<td>CSNK1A1</td>
<td>IDH2</td>
<td>PAX5</td>
<td>SF3B1</td>
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<td></td>
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<tr>
<td>ANKRD26</td>
<td>CTCF</td>
<td>IKZF1</td>
<td>PDGFRA</td>
<td>SH2B3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ARID1A</td>
<td>CUX1</td>
<td>JAK1</td>
<td>PHF6</td>
<td>SMC1A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ASXL1</td>
<td>CXCR4</td>
<td>JAK2</td>
<td>PIGA</td>
<td>SMC3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ASXL2</td>
<td>DDX41</td>
<td>KAK3</td>
<td>PML</td>
<td>SOS1</td>
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<tr>
<td>ATM</td>
<td>DHX15</td>
<td>KDM6A</td>
<td>PPM1D</td>
<td>SRP72</td>
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<tr>
<td>ATRX</td>
<td>DNMT3A</td>
<td>KIT</td>
<td>PRPF40B</td>
<td>SRSF2</td>
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<tr>
<td>BCR</td>
<td>EED</td>
<td>KMT2A</td>
<td>PTEN</td>
<td>STAG1</td>
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<tr>
<td>BCORL1</td>
<td>ELANE</td>
<td>KMT2D</td>
<td>RAD21</td>
<td>STAG2</td>
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<td>ETV6</td>
<td>LUC7L2</td>
<td>RB1</td>
<td>STAT5B</td>
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<td>BRCC3</td>
<td>EZH2</td>
<td>MECOM</td>
<td>RBBP6</td>
<td>SUZ12</td>
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<td>FANCA</td>
<td>MET</td>
<td>RPS19</td>
<td>TERC</td>
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<tr>
<td>CBL</td>
<td>FANCL</td>
<td>MPL</td>
<td>RTEL1</td>
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<td></td>
</tr>
<tr>
<td>CBLC</td>
<td>FLT</td>
<td>MYC</td>
<td>RUNX1</td>
<td>TET2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CCND2</td>
<td>GATA1</td>
<td>NF1</td>
<td>SAMD9</td>
<td>THPO</td>
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</tr>
<tr>
<td>CDKN2A</td>
<td>GATA2</td>
<td>NOTCH1</td>
<td>SAMD9L</td>
<td>TP53</td>
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<td></td>
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<tr>
<td>CEBPA</td>
<td>GNAS</td>
<td>NOTCH2</td>
<td>SBDS</td>
<td>U2AF1</td>
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<tr>
<td>CHEK2</td>
<td>GNB1</td>
<td>NPM1</td>
<td>SETBP1</td>
<td>U2AF2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CREBBP</td>
<td>HNRNPK</td>
<td>NRAS</td>
<td>SETD2</td>
<td>WT1</td>
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<td>SF3A1</td>
<td>ZRSR2</td>
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</tbody>
</table>

Genes in bold are included as required in WHO classification of myeloid malignancies.

Supplementary Table 3. Questionnaire on patient satisfaction and potential fears.

<table>
<thead>
<tr>
<th>I totally agree</th>
<th>I agree</th>
<th>I neither agree nor disagree</th>
<th>I don’t agree</th>
<th>I strongly disagree</th>
</tr>
</thead>
</table>

Questions on satisfaction with the study

1) I was happy to take part in the study.
   It gives me a very good feeling personally,
2) that I was able to contribute to the research
   about my illness.
3) I think that the participation in this study
   has an additional value on my health.
<table>
<thead>
<tr>
<th></th>
<th></th>
<th>I totally agree</th>
<th>I agree</th>
<th>I neither agree nor disagree</th>
<th>I don’t agree</th>
<th>I strongly disagree</th>
</tr>
</thead>
<tbody>
<tr>
<td>4)</td>
<td>Dealing with my illness was an emotionally draining task.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5)</td>
<td>The process and the explanation of the study was clear and comprehensible.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6)</td>
<td>I basically understood why individual study parts were realized.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7)</td>
<td>I was satisfied with the information about the CHIP-diagnostic.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8)*</td>
<td>The CHIP-Positivity was intelligibly explained to me.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9)*</td>
<td>The information letter concerning CHIP helped me to have the most important information at hand again after the medical consultation.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10)*</td>
<td>I think it’s reasonable that my primary care physician received information about my CHIP-Positivity.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11)*</td>
<td>The presence of CHIP worries me a lot.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12)*</td>
<td>Knowing I’m CHIP-positive stresses me out.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13)*</td>
<td>I wish I didn’t know that I’m CHIP-positive.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Questions 8-13 are only provided to patients who are tested as CHIP positive