HIGHLIGHT
DIVISION BLOOD

FEMKE DE VRIES

Cracking the Code of Ageing: Multimodal Imaging in Multimorbidity

In recent decades, medicine and health care have made rapid progress, with evolving patient needs and transformative technologies. This is reflected in our average lifespan, which rose from 32 to 76 years over the past 200 years. This rise, however, also resulted in an increase in age- and lifestyle-related diseases, such as cardiovascular disease (CVD), cancer, type 2 diabetes, and neurological cognitive decline. A common denominator of these chronic diseases consists of changes in vascular remodelling, resulting in reduced elasticity of vessel walls and an impaired ability to control blood flow and pressure.

To comprehensively investigate the relationship between cancer and CVD, and their impact on mental health, our group focuses on *in vitro* and *in vivo* research and clinical studies, within a partnership involving the research institutes CARIM, GROW and MHeNs. We have initiated *in vitro* research to investigate the direct effect of breast cancer cell conditioned media on vascular smooth muscle cell (VSMC) function. Simultaneously, we are developing a multi-morbidity experimental animal model that allows us to study interactions between cancer and vascular diseases, and their influence on mental health. Additionally, the risk of thrombosis and vascular calcification in breast cancer patients will be assessed. Below we present the highlights of our research lines.

IN VITRO MODELS TO STUDY THE RELATIONSHIP BETWEEN BREAST CANCER AND VASCULAR REMODELLING

Breast cancer is the most common cancer in the world, and 12.5% of all new annual cancer cases concern breast cancer. Survival rate is greatly influenced by the stage and type of the tumour (e.g., luminal A, luminal B, and HER2 positive). Breast cancer survivors are at elevated risk of dying from CVD, compared to women without breast cancer. Additionally, the risk of breast cancer and CVD increases with age. Elucidating the relationship between breast cancer and CVD will provide molecular and cellular cues to improve current patient and preventive care.

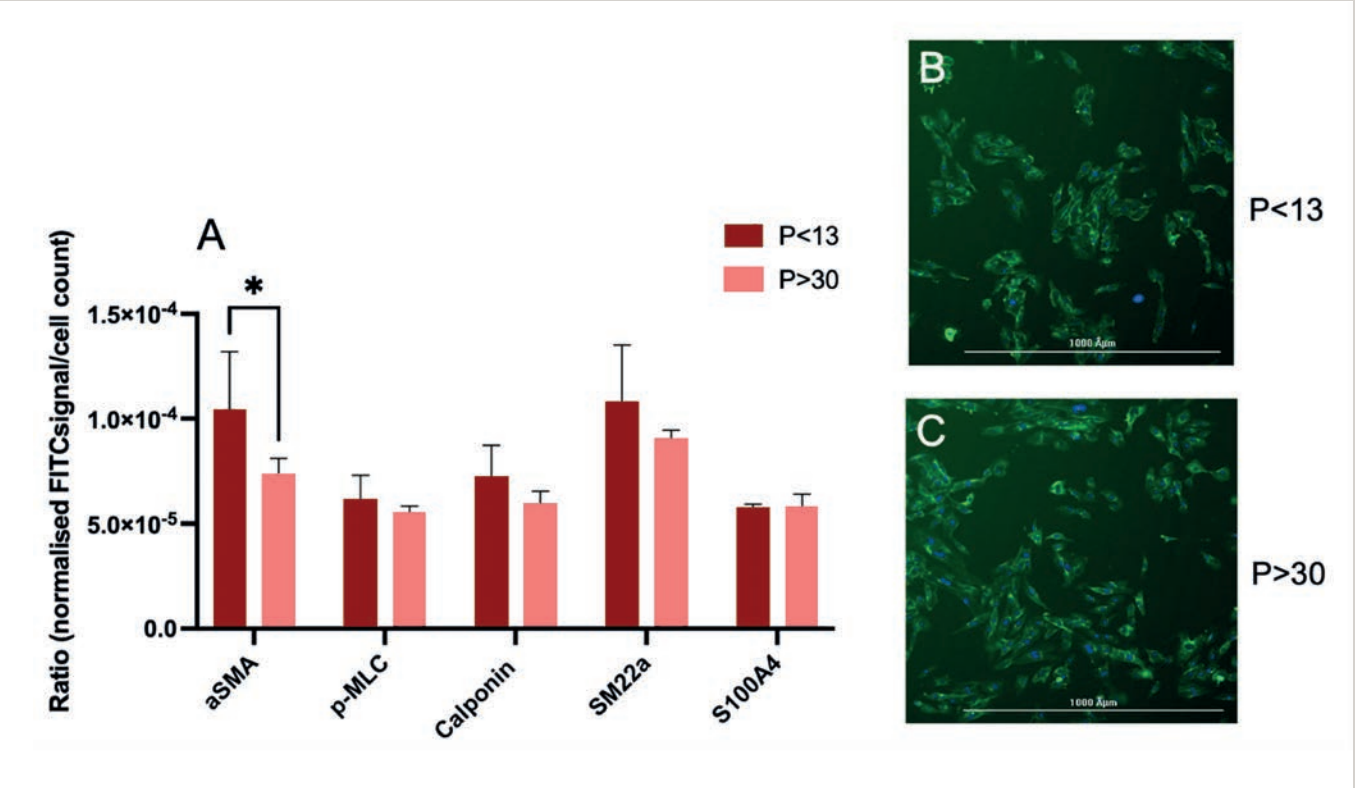

FIGURE 1 iVSMC phenotype was assessed using five different characterisation markers, namely α-SMA, p-MLC, calponin, SM22α, and S100A4. Panel A shows the different levels measured in young and aged iVSMCs. B shows the α-SMA levels measured using fluorescence in young iVSMCs. C shows the α-SMA levels measured using fluorescence in aged iVSMCs. α-SMA: α-smooth muscle actin; p-MLC: phospho-myosin light chain; SM22α: smooth muscle 22α.

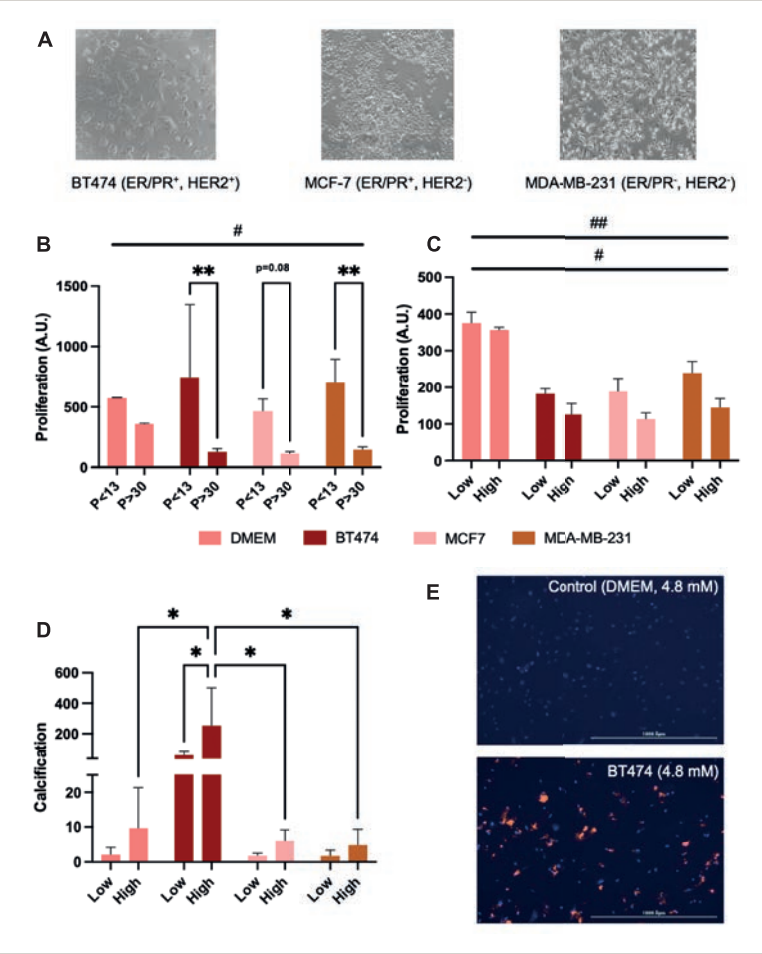

FIGURE 2 The effect of young and aged iVSMCs exposed to human breast cancer conditioned media on proliferation and vascular calcification.

Panel A: Microscopic images of BT474, MCF-7 and MDA-MB-231 in culture conditions.

B: Proliferation measured by xCELLigence of young and aged iVSMCs exposed to control (DMEM), BT474, MCF-7 or MDA-MB-231 conditioned media.

C: Proliferation measured by xCELLigence of aged iVSMCs exposed to control (Dulbecco's Modified Eagle Medium (DMEM)), BT474, MCF-7 or MDA-MB-231 conditioned media with normal or high levels of Ca^{2+}.

D: Calcification measured by Fetuin-A of aged iVSMCs exposed to control (DMEM), BT474, MCF-7 or MDA-MB-231 conditioned media with normal or high levels of Ca^{2+}.

E: * p<0.05 ** p<0.01 # significant age difference between conditions (p<0.001) ## significant calcium difference (p<0.0001)

Our research aims to investigate the influence of human breast cancer on vascular ageing, using induced-pluripotent stem cells (iPSC) VSMCs (iVSMCs, Stem Cell Research University Maastricht; SCRUM). For this research, we selected three breast cancer cell lines, each with its own phenotype, namely triple hormone positive BT474 (ER+/PR+ and HER2+), triple hormone negative MDA-MB-231 (ER-/PR- and HER2-), and HER2-negative MCF-7 (ER+/PR+ and HER2-). iVSMCs were cultured as either young (passage<13) or aged (passage>30) cells. To confirm iVSMC phenotype, we characterised expression levels of the smooth muscle markers a-SMA, p-myosin light chain (p-MLC), calponin, smooth muscle 22a and S100A4. As expected, aged iVSMCs showed a significant decrease in a-SMA levels (p=0.016) compared to young iVSMCs (**Figure 1**), in line with the literature.

To investigate the effect of breast cancer cell conditioned media on iVSMC proliferation and calcification, cells were exposed for 72h to ensure that transcriptional and translational changes took place. Aged iVSMCs conditioned with BT474 (p=0.004) and MDA-MB-231 (p=0.008) media showed a significant decrease in proliferation compared to

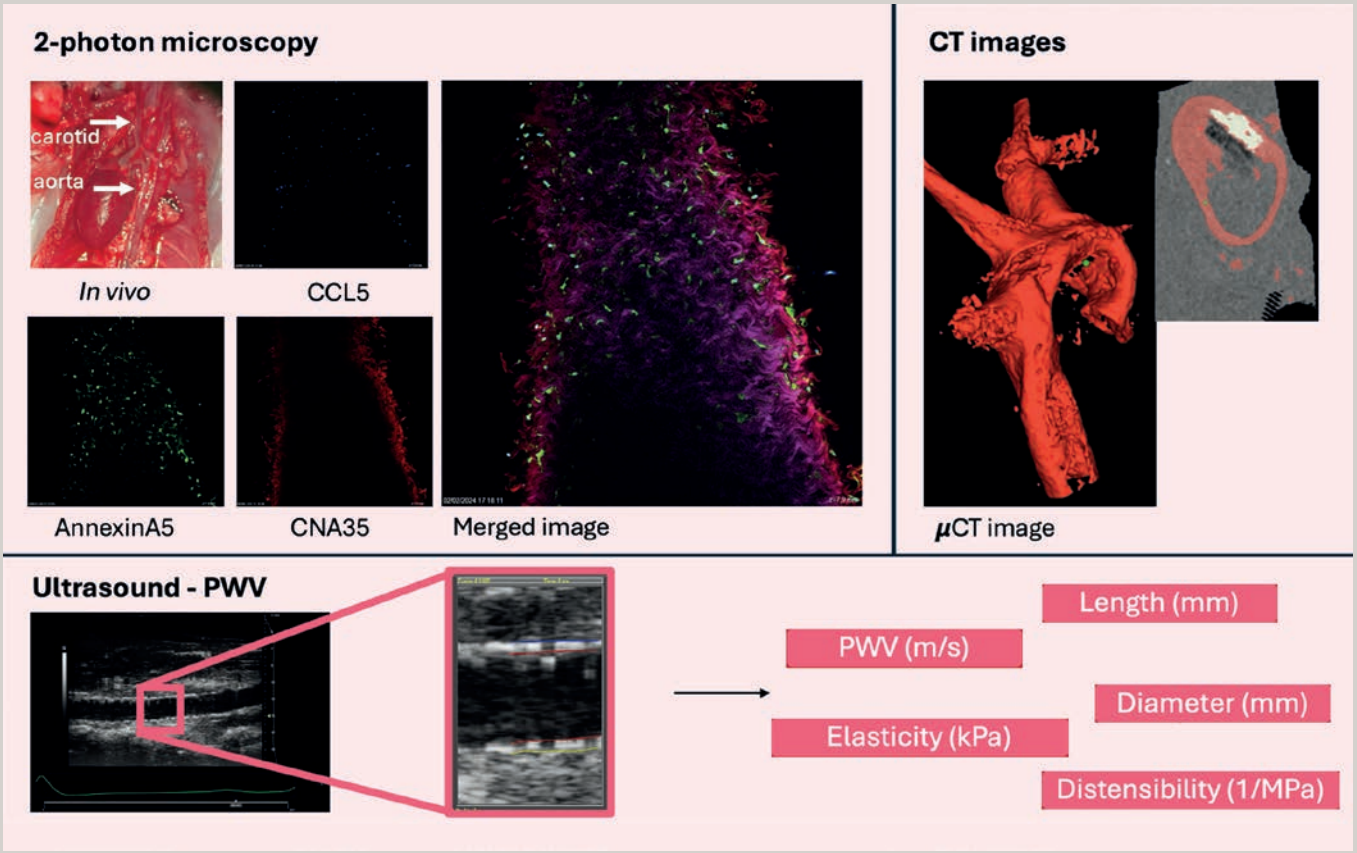

FIGURE 3 Examples of the different imaging modalities used for the *in vivo* animal model. PWV: Pulse Wave Velocity.

young iVSMCs, while iVSMCs conditioned with MCF-7 ($p=0.08$) only showed a trend (**Figure 2B**). When calcium conditions were raised, aged iVSMCs showed a significant decrease in proliferation ($p<0.0001$; **Figure 2C**). Aged iVSMCs exposed to BT474-conditioned media with high calcium levels showed a significant increase in calcification compared to control, MCF-7 and MDA-MB-231 conditions, with high calcium levels ($p<0.05$; **Figure 2D/E**).

MULTIMORBIDITY ANIMAL MODEL TO STUDY THE RELATIONSHIP BETWEEN CVD AND CANCER, AND THEIR INFLUENCE ON MENTAL HEALTH

Over the past years, increasing life expectancy has resulted in clinical care shifting from treatment to prevention and early detection. Interactions between morbidities and the consequences of these interactions for treatment are currently insufficiently understood. Additionally, studying the causal interaction between morbidities in the clinical setting is far from ideal, due to the complexity of the diseases and the specific expertise required. Therefore, developing and using a multi-modal animal model is of crucial importance to answer complex future research questions. As such, examining how comorbidities drive disease could reveal as yet unknown fundamental pathways and provide new therapeutic angles for future translation to the clinic. Several animal models for multi-morbidity have been developed in recent years, including models combining CVD and renal failure, CVD and chronic obstructive pulmonary disease (COPD), hypertension and stroke and CVD/COPD and smoking. However, these models do not combine CVD, cancer, and mental health.

In our approach, we will develop an atherosclerotic and vascular calcification model using ApoE knockout rats, in combination with rhabdomyosarcoma R1 cancer. Our multi-modal model will be evaluated using advanced imaging (**Figure 3**). The carotid artery will be assessed using 2-photon microscopy, staining for collagen (CNA-35-AF564), calcification (Fetuin-A-AF546), inflammation (CCL5-Pacific Blue), and apoptosis (Annexin-A5-AF488). Furthermore, the aorta will be assessed using μCT/PET; immunohistochemistry staining for collagen (CNA-35), calcification (Alizarin red), inflammation (CCL5/CD68/Mac3), apoptosis (Annexin-A5) and hypoxia (Pimonidazole); and tissue stiffness using the Pavone. Vessel properties (e.g., pulse wave velocity, vessel diameter, and vessel elasticity) are evaluated by ultrasound measurements at baseline and endpoint. Additionally, to assess the influence of CVD on mental health, five different behavioural and stress tests will be performed (e.g., Y-maze, sucrose preference test, object recognition test, and open field).

CLINICAL STUDY: THE INFLUENCE OF BREAST CANCER ON VASCULAR CALCIFICATION AND THROMBOSIS

To validate our research results obtained from *in vitro* models, we have initiated a partnership with the Department of Oncology at Maastricht UMC+. As mentioned above, the relationship between cancer (including breast cancer) and CVD has as yet been insufficiently studied. Our clinical study will focus on the relationship between breast cancer and CVD, specifically vascular calcification and thrombosis. This translational partnership will make it possible to initiate patient sample collection, including early-stage breast cancer patients, before treatment. In this line of research, patients will be analysed for the risk of vascular calcification and thrombosis. By elucidating which breast cancer patients are more prone to vascular calcification and thrombosis, and which factors cause an increased risk, we ultimately aim to provide preventive care for these patients.