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Insights into drug-induced heart damage from cancer therapy

Submission date 27/08/2020	**Recruitment status** No longer recruiting	[_] Prospectively registered [X] Protocol [_] Statistical analysis plan [_] Results [_] Individual participant data [_] Record updated in last year
Registration date 20/11/2020	**Overall study status** Completed	
Last Edited 21/11/2023	**Condition category** Circulatory System	

Plain English summary of protocol

Background and study aims
During cancer treatment, patients can be given drugs as part of their therapy regime. Different drugs work in different ways to destroy cancer cells and, whilst they can work effectively against the cancer cells, some drugs can also unfortunately cause damage to the heart cells during the process. This is known as drug-induced cardiotoxicity and can occur both during treatment and sometime after treatment has been completed. This damage can be irreversible in some cases and its occurrence during treatment can often lead to that treatment being changed or withdrawn. It is not yet possible to know which patients will develop these complications or when, nor is it possible to say how or why they do. In order to improve treatment and care for these patients it is necessary to find out how and why these drugs cause damage to the heart. In this study, the researchers aim to reprogram blood cells into stem cells which they can then make heart cells from. They want to use these heart cells to explore the possible differences between those who do develop heart-related complications from their treatment and those who don't, and also the possible reasons why some people do and some people don't.

Who can participate?
Adults (aged 16+) who have received doxorubicin chemotherapy treatment can be considered for this study by their oncologist and/or cardiologist at one of the participating hospital sites who will base their suitability on a range of inclusion criteria.

What does the study involve?
Participants will be asked to attend an appointment at a participating hospital site where they will sign a consent form and a health care professional will take a blood sample. If someone agrees to take part in this study, the researchers are asking for their informed consent to store this blood for use in research projects. The blood sample stored will usually be very small and may be processed to remove the cells, which will be stored separately. The researchers would also seek consent for authorised members of research staff to access participant health records. These will be reviewed by staff in order to update information on their research database relevant to their findings. All information will be treated with the strictest confidence and held securely within the University of Liverpool for this study only. In addition, the researchers may seek to access information held by other sources such as NHS Trusts, Disease Registries (such as the North West Cancer Intelligence Service, NWCIS) and the UK Statistics Authority. This

information will help researchers to further understand the nature and process of the samples taken and relate what is found in the laboratory to what happens to patients. Details such as names, addresses and any other personal data will be removed before any information is given to research groups and no participant will be identifiable to research staff.

What are the possible benefits and risks of participating?
The samples taken for the research study will only be taken once the necessary diagnostic tests have been performed. The results of research carried out using blood from this study may help in the future discovery of new drugs and treatments for patients with drug-induced cardiotoxicity. There will be no direct benefit to participants other than the knowledge of taking part in clinical research. There will be no additional risks from choosing to participate. The risks associated with any planned clinical procedure will be explained to participants separately by the medical team as part of their treatment. When the blood sample is taken there is the small chance that some bruising may occur at the site.

Where is the study run from?
University of Liverpool (UK)

When is the study starting and how long is it expected to run?
January 2020 to May 2025

Who is funding the study?
University of Liverpool (UK)

Who is the main contact?
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Contact information

Type(s)
Scientific

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Additional identifiers

EudraCT/CTIS number

Nil known

IRAS number
285910

ClinicalTrials.gov number
Nil known

Secondary identifying numbers
IRAS 285910

Study information

Scientific Title
Translational insights into the underlying pathogenesis of anthracycline-induced cardiotoxicity

Study objectives
According to the World Health Organisation, cardiovascular diseases (CVD) are the number one cause of death worldwide, with almost 23.6 million people estimated to die from CVD by 2030. The second leading cause of death globally is cancer, with 9.6 million people estimated to have died from the disease in 2018. The greatest single non-cancer cause of death in cancer survivors is CVD.
Cancer Research UK states that the cancer survival rate in the UK has doubled in the last 40 years from 24% to 50% at 10 years in many of the most commonly diagnosed cancers. This can be attributed to improvements in the understanding of the cause and management of the disease as well as the availability of new and evolving treatment options.
One of the most prominent and effective forms of cancer treatment to date has been the use of anthracyclines, which are known to cause cardiotoxicity in patients both during and several years following treatment. This is also true in teenagers and young adults who receive anthracycline therapy during childhood. The dose-dependent cardiotoxic effects of chemotherapeutic anthracyclines can limit patient exposure and therapeutic efficacy. Even at relative low cumulative doses, an 8% increase in adverse cardiac events has been observed which increases to 26% with cumulative doses and adjuvant therapy with targeted monoclonal antibodies. Whilst improvements have been made into understanding the cause and management of cancer over recent years, the same level of understanding has not yet been reached into the cause and management of cancer-related comorbidities like anthracyclines-induced CVD.
With cancer survival statistics on the rise, recent consultation with oncologists and cardiologists from the Liverpool area highlights the fact that attention is now switching to the need for more effective monitoring and management of cancer patients for CVD following treatment. The clinical management of such complications however currently lacks scientific support. To effectively aid this, a better understanding is warranted to explain why some cancer patients are at a higher risk of developing and dying from CVD compared to others. CVD risk in patients receiving anthracycline therapy has been shown to correlate with several clinical and lifestyle factors, such as age, sex and smoking, but being able to stratify baseline risk in patient populations on additional histological and genetic factors would greatly advance and improve supportive therapeutic approaches. This study aims to achieve an insight into what the histological and genetic factors might be and whether they have any predictive or prognostic value.
The primary aim of this study is to generate cardiomyocytes from patient-derived induced pluripotent stem cells (iPSC) and characterise the mechanisms of anthracycline-induced cardiotoxicity by comparing the electrical, structural and functional differences between cells

derived from patients exhibiting cardiotoxicity as a result of chemotherapy treatment compared with those on a matched treatment regime with no evidence of cardiotoxicity. Studies have shown that patient-derived iPSC cardiomyocytes recapitulate the characteristics seen in the patients from whom they are derived, and since the heart is a non-regenerative organ the availability of biopsies is rare, particularly from healthy volunteers, therefore limiting the analysis and comparisons from the primary source.
The incidence of anthracycline-induced cardiotoxicity in the adult population is difficult to determine as follow-up time and monitoring policies are often currently inadequate. Due to the limited underpinning of scientific judgement on clinical management in these patients, current recommendations are based on expert consensus and local multidisciplinary protocols.
This study should help to identify candidate risk factors for anthracycline-induced cardiotoxicity on a physiological level that will provide a more specific prediction model than that which currently exists, thus aiding in improving both life expectancy and quality of life following cancer treatment.

Ethics approval required
Old ethics approval format

Ethics approval(s)
Approved 17/11/2022, London - Harrow Research Ethics Committee (Level 3, Block B, Whitefriars, Lewins Mead, Bristol, BS1 2NT, UK; +44 (0)207 104 8154, +44 (0)207 104 8357; harrow.rec@hra.nhs.uk), ref: 20/PR/0880

Study design
Multi-centre case-control study

Primary study design
Observational

Secondary study design
Case-control study

Study setting(s)
Hospital

Study type(s)
Other

Participant information sheet
Not available in a web format, please use contact details to request a participant information sheet

Health condition(s) or problem(s) studied
Cardiotoxicity in doxorubicin-treated patients

Interventions
Participants will be recruited to the study by the clinical team by means of a verbal explanation and the provision of a Patient Information Sheet (PIS). Potential participants may provide their contact details, or they can make contact themselves at a later stage. They will then be contacted by the clinical team and given further information over the telephone. Those wishing to proceed will have arrangements made to attend their nearest participating hospital site to

sign the consent form and have a blood sample taken. A record of consent will be stored by the clinical team and a copy given to both the patient and the research team prior to sample processing taking place at the University of Liverpool research site. Patient demographics and clinical test results will be recorded by the clinical team for the purposes of the study and will be accessible to the clinical team only. Access to patient records to identify potential participants and check whether they meet the relevant inclusion criteria will be restricted to the patient's existing clinical care team only.

All experimental methods carried out during this study will be conducted at the University of Liverpool by the study's designated research assistant. All cell lines used in this study will be generated from somatic patient cells. The cells used for reprogramming will be PBMCs isolated and cultured from whole blood or, potentially, dermal fibroblasts isolated and cultured from skin biopsies. Cells will be reprogrammed using either Sendai-virus transduction or by electroporation of episomal plasmid vectors. Cell lines generated will be allocated a pseudoanonymised form of identification by the research staff at the University of Liverpool. Surplus primary material (PBMC/fibroblasts) will be cryopreserved in cryopreservation medium. Sendai lines will be transduced with the CytoTune iPSC Sendai Reprogramming Kit (Invitrogen), which contains F-gene deficient Sendai-virus expressing the four Yamanaka transcription factors (SOX2, OCT3/4, c-MYC and KLF4). The reprogramming will be conducted according to the manufacturer's instructions. After transduction, the Sendai-lines will be re-plated onto cell-free matrix-coated 6-well plates and cultured until iPSC colonies are ready to differentiate. Brightfield microscopy will be used to analyse cellular reprogramming and differentiation efficiency as well as to assess the regular progress and overall health of the cells whilst in culture. Reprogramming efficiency will be assessed by microscopy in two ways – firstly, following transduction/transfection, all newly generated iPSC colonies will be counted and divided by the number of input cells used for reprogramming. Secondly, the number of iPSC colonies that survive following picking will be divided by the number of colonies that are selected for picking. The cardiomyocyte differentiation efficiency will be determined by dividing the number of observed beating areas by the total number of iPSC colonies that are used for differentiation. The researchers will use PCR to characterise the gene expression of iPSC and cardiomyocyte cell lines. PCR data will be generated using several established techniques. Standard PCR and RT-PCR will be used to confirm the absence of exogenic genetic material from electroporated and Sendai-virus iPSC lines, respectively. The expression of EBNA-1, which will be present in the transfection plasmids, will be assessed for electroporated lines. For Sendai-virus lines, the four viral transgenes (KLF-4, SOX-2, c-MYC and OCT-3/4) will be assessed. After verifying the absence of exogenic genetic material, the expression of endogenic pluripotency genes will be studied at the mRNA level by RT-PCR. GAPDH will be used as an endogenic control. Nucleic acid samples will be collected using commercially available reagents and collected samples will be stored at -80°C until extraction. Nucleic acid extraction will be performed using a commercially available extraction kit according to the manufacturer's instructions. Sample concentration will be measured spectrophotometrically and extracted samples will be stored at -80°C. RNA samples will be transcribed into cDNA using a commercially available kit according to manufacturer's instructions and synthesised cDNA will be stored at -80oC until ready for use. PCR products will be separated and analysed using agarose gel electrophoresis and the results viewed using a UV gel documentation system. The data images will be further interpreted using an appropriate imaging software programme.
To obtain quantitative PCR data which will allow us to compare the expression of pluripotency genes both within the same subject and between individual subjects, the researchers will use a fluorescent reporter probe-based qPCR method. cDNA samples will be synthesised as described above from cell lines at two different passages (early and late) and studied to detect the expression levels of endogenous pluripotency genes with GAPDH levels used as an endogenic control. Samples will be processed in triplicate and analysed using the double-delta ct method to

calculate relative expression. Statistical analyses will be performed to study (1) the differences in relative gene expression at early and late passages in the same subject (Mann-Whitney U test) and, (2) significant differences between subjects at the same passage number (Kruskal-Wallis test). Statistical analysis will be conducted using the R computational software environment. To determine the chromosome complement of the cells the researchers will carry out chromosomal karyotyping using colcemid solution. Cells will be incubated in media containing 0.5 µg/ml colcemid solution (Gibco) and staining carried out according to manufactures instructions. Cells will be fixed in ice-cold methanol and mounted onto microscope slides which will be stained using Giemsa stain and Gurrs 6.8 buffer (Gibco). Chromosomes will be analysed using a brightfield microscope. The researchers will use indirect immunocytochemistry (ICC) to characterise iPSC and cardiomyocyte cell lines at the protein level. This method will allow us to generate data that will confirm the simultaneous expression and cellular location of key pluripotent stem cell markers and cardiac-specific markers such as Troponin-T. The stained cells will be viewed using a fluorescence microscope and the images will be captured using a digital camera. Captured images will be interpreted and analysed with the aid of appropriate imaging software, such as Adobe Photoshop, and data may be manipulated to include the addition of scale bars, contrast adjustment and image overlays. Data manipulation will not affect the result of the experiments in any way. Our interpretations of the data will be validated with the use of positive and negative staining controls and imaging parameters for the controls will be the same as those applied to the test dataset. In addition to obtaining data on protein expression levels, the cardiomyocyte differentiation efficiency will also be assessed by ICC. A select number of images (<5) will be captured at random and the number of cardiac TroponinT- positive cells will be divided by the total cell count. Cells will be counterstained with a nucleic acid stain (DAPI) to achieve this. To generate quantitative data on the whole proteome of doxorubicin-treated cardiomyocytes, cardiomyocytes from all experimental groups will be subjected to iTRAQ-based MS. To determine whether protein expression is similar between cardiotoxicity and non-cardiotoxicity samples, the data will be subjected to principal component analysis to analyse the variance across the sample sets and identify whether there is a distinction between them based on protein expression. A two-tailed t-test will be carried out using the R computational environment to determine the statistical significance of differences in protein expression between cardiotoxicity and non-cardiotoxicity samples

To validate the iTRAQ-MS data, the researchers will use Western blot analysis to verify the differential protein expression of a select number of identified proteins. The identity of the target proteins will be confirmed by comparison to a molecular weight marker (for size) and a positive control if possible (for size and signal). A loading control will be used to allow us to normalise the data and compare the expression levels between the target proteins. The data produced will be interpreted using imaging software, such as ImageJ, and a semi-quantitative comparison will be made of the signals generated between protein bands.
Following validation, proteins which are found to have a statistically significant higher or lower level of expression by MS in the control samples will be explored further using computational enrichment analysis to identify functional pathways involved in cardiotoxicity.

The researchers will use standard electrophysiological and fluorescence imaging techniques to characterise the electrical activity and calcium homeostasis in iPSC and cardiomyocyte cell lines. Patch-clamp recordings will be made from single cells using microelectrodes formed from thick-walled borosilicate glass filled with an electrolyte solution connected to an industry-standard amplifier (Axopatch 200B), digitised (Digidata 1440) and recorded using specialised electrophysiological recording software (pCLAMP10.7). It is anticipated that the researchers will measure ionic currents, in particular K+ and Ca2+ along with the cardiac action potential to investigate morphological and pathophysiological changes in the whole-cell electrical signalling that may correlate with disease states. In particular, current amplitude will be measured using

voltage-protocol appropriate to the current under investigation. Action potential amplitude and duration will be measured as these are common markers of electrical dysregulation.

In order to study the responses to electrical signalling, the researchers will use state-of-the-art fluorescence measurements to measure intracellular Ca2+ changes correlating to each contractile cycle (using fluo-3, fluo-4 or Fura-2), along with intracellular ATP (using MgGreen), mitochondrial function (using TMRE/TMRM) and membrane potential (using Di-4-ANNEPS or Di-8-ANNPES) across the syncytium of cells using cell-permeant fluorescent markers. Fluorescence will be excited using a PTI monochromator attached to a Nikon TiU microscope with fluorescence signals detected using an Andor Zyla camera controlled by Winfluor4.5 software. In all cases, daily control data sets will be gathered along with any test data sets. Calcium fluorescence signals from either spontaneous action potentials or from electric field stimulation for pacing of cells, will be analysed for peak amplitude, transient duration and measurement of the area under the curve to assess changes. ATP and mitochondrial membrane potential will be measured for responsiveness to simulated ischaemia, where cells exposed to toxic conditions generally show an increased rate of mitochondrial depolarisation and rapid ATP depletion. Finally, membrane potential indicators will be used to measure the spread of excitation through the syncytium. Measurements of the rate of depolarisation and the delay across the syncytium will be assessed. These protocols are well established in the group, and standard data analysis protocols and statistical analysis are established and are used in peer-reviewed published manuscripts.

Intervention Type
Other

Primary outcome measure
1. Reprogramming efficiency of PBMC into iPSC determined by PCR and ICC after colony formation
2. Chromosomal stability determined by karyotype analysis at 2-6 weeks after colony formation
3. Differentiation efficiency of iPSC into cardiomyocytes determined by ICC at 10-14 days after initiation
4. Amenability of iPSC and cardiomyocytes to experimental analysis determined by PCR, ICC, electrophysiology, and Western blotting at 1-2 weeks after formation

Secondary outcome measures
1. A doxorubicin-specific signature in cardiomyocytes derived from cardiotoxicity subjects, identified using proteomics profiling at 3 weeks
2. Candidate biomarkers that are of functional relevance to doxorubicin-induced cardiotoxicity, identified by proteomics profiling at 3 weeks

Overall study start date
05/01/2020

Completion date
03/05/2025

Eligibility

Key inclusion criteria
Cardiotoxicity cohort:
1. Over 16 years of age at the time of consent

2. Capable of providing informed consent as determined by the consenting clinician
3. Receiving doxorubicin chemotherapy treatment at the time of consent or previously received doxorubicin chemotherapy treatment prior to consent
4. Clinical presentation of left ventricular systolic dysfunction at the time of consent secondary to receiving doxorubicin chemotherapy treatment as determined by the clinical team

Non-cardiotoxicity cohort:
1. Over 16 years of age at the time of consent
2. Capable of providing informed consent as determined by the consenting clinician
3. Receiving doxorubicin chemotherapy treatment at the time of consent or previously received doxorubicin chemotherapy treatment prior to consent
4. Normal left ventricular systolic function at the time of consent secondary to receiving doxorubicin chemotherapy treatment as determined by the clinical team

Participant type(s)
Patient

Age group
Adult

Lower age limit
16 Years

Sex
Both

Target number of participants
12

Key exclusion criteria
1. Under 16 years of age at the time of consent
2. Lacking ability to provide informed consent as determined by the consenting clinician
3. Judged to have been coerced to consent as determined by the consenting clinician
4. Pre-existing left ventricular systolic dysfunction to be reviewed by the clinical team on a case by case basis
5. Recent surgery (<3 months)
6. Excessive alcohol consumption (>30 units a week) and/or recreational drug use
7. Active immunological disease as determined by the clinical team
8. On current steroid therapy with the exception of corticosteroid inhaler <2 mg/kg
9. Active or recent (<6 weeks) serious infection at the discretion of the clinical team
10. Inability to comply with study procedures

Date of first enrolment
01/10/2020

Date of final enrolment
01/03/2024

Locations

Countries of recruitment
England

United Kingdom

Study participating centre
Liverpool Heart and Chest Hospital
Thomas Drive
Liverpool
United Kingdom
L14 3PE

Study participating centre
Clatterbridge Cancer Centre
65 Pembroke Place
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L7 8YA

Sponsor information

Organisation
University of Liverpool

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Sponsor type
University/education

Website
http://www.liv.ac.uk/

ROR
https://ror.org/04xs57h96

Funder(s)

Funder type
University/education

Funder Name
University of Liverpool

Alternative Name(s)
The University of Liverpool, , Universidad de Liverpool, UoL

Funding Body Type
Government organisation

Funding Body Subtype
Universities (academic only)

Location
United Kingdom

Results and Publications

Publication and dissemination plan
Planned publication in a high-impact peer-reviewed journal. The relevant documents for this study are still undergoing sponsorship committee approval prior to submission for ethics approval. As a result they are still subject to change and are therefore not available at this time. It is unknown which documents, if any, will be made available.

Intention to publish date
01/12/2025

Individual participant data (IPD) sharing plan
The datasets generated and/or analysed during the current study will be published as a supplement to the results publication

IPD sharing plan summary
Published as a supplement to the results publication

Study outputs

Output type	Details	Date created	Date added	Peer reviewed?	Patient-facing?
Protocol file	version 2.2	20/09/2022	21/11/2023	No	No