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13. SUPPLEMENTARY NOTES

14. ABSTRACT

Epidemiologic evidence shows alteration of myocardial properties with age resulting in progressive decline in diastolic function. The presence of a population of senescent cardiomyocytes and the soluble factors they secrete (senescence-associated secretory phenotype) could be a major player in the progressive deterioration of myocardial function observed during aging. To test this hypothesis, we propose 1) to generate a new model of cardiac-specific inducible senescence and evaluate cardiac function in association with cellular senescence and 2) to develop a method for *in vivo* imaging of cellular senescence. The animal model of cardiac-specific inducible senescence was generated by crossing mice with cardiac specific inducible Cre recombinase (αMHC-MerCreMer) with enhancer of zeste homolog 2-floxed mice ($Ezh2^{fl/fl}$), enabling tissue-specific conditional deletion of Ezh2, a key component of a protein complex involved in the repression of $p16^{INK4a}$, a master regulator of cellular senescence. The validation of genetic recombination, resulting cellular senescence and its association with diastolic dysfunction remains to be evaluated. The generation of malondialdehyde-vimentin, a marker expressed at the membrane of senescent cells, was unsuccessful. An alternative maker of cellular senescent, NOTCH3, was shown to be upregulated in senescent murine cardiomyocytes. This marker will be validated in the generated mouse model and single-domain antibodies targeting NOTCH3 will be generated as used to produce technetium-99m labeled tracers. Radiotracers will be characterized and used for single-photon emission computed tomography imaging to determine the ability to specifically detect cardiac cellular senescence *in vivo* in the generated animal model.

15. SUBJECT TERMS
Cellular senescence, diastolic dysfunction, molecular imaging

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1. INTRODUCTION:

Indirect evidence suggest that cellular senescence could play a major role in age-related myocardial alteration leading to diastolic dysfunction. This work proposes to generate a murine model of cardiac specific inducible senescence, *via* tissue specific conditional knockout of enhanced of zeste homolog 2 (Ezh2), and evaluate the impact of cardiac specific cellular senescence on the development of diastolic dysfunction (Aim 1). Currently, there is no non-invasive imaging method to directly assess senescent cell burden in vivo. A second aspect of this work is to generate a tracer for molecular imaging of cellular senescence *in vivo* and validate its functionality and utility in the generated mouse model (Aim 2).

2. KEYWORDS:

Cellular senescence, diastolic dysfunction, molecular imaging

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Aim 1: Development of a preclinical model of cardiac-specific inducible senescence and evaluation of the relationship between cardiac cellular senescence and myocardial dysfunction.

Four key tasks were identified for Aim 1, covering the majors tasks 1 and 2 presented in the statement of work:

- Generation of homozygote double transgenic animals.

The generation of the mouse model of cardiac-specific and inducible senescence was expected to be achieved by crossing the cardiac-specific inducible Cre mouse (αMHC-MerCreMer), with whole body Ezh2-floxed mouse ($Ezh2^{fl/fl}$). As stated in the statement of work, the generation of the double transgenic line ($Ezh2^{fl/fl}$:αMHC-MerCreMer) was expected to be achieved within 11 months of the beginning of the award. This task was achieved in January 2020, with the set-up of the first homozygous double transgenic breeders (completion: 100 %, expected: 100%).

- Test and optimization of genomic recombination

After obtaining the double transgenic animals, initial tests of the efficiency of genetic recombination were expected to be carried out and optimized to achieve a burden of cellular senescence that, according to the proposed working hypothesis would lead to diastolic dysfunction over time, in absence of acute alteration of cardiac function and tissue damage. Test and optimization of genomic recombination condition was expected to be verified within 1 months after obtaining adult double transgenic animals. This task was started with an initial evaluation of adult double transgenic animals in early March 2020 but has been interrupted due to the institution wide lockdown caused by COVID-19 pandemic (estimated completion: 25%, expected: 100 %).

- Cross-sectional study

Once the proper condition for genetic recombination were identified, the cross-sectional study assessing the time frame for the development of diastolic dysfunction in presence of senescent cells was expected to begin and last for the last 6 months of the award. This task was not scheduled to start until the second year of the award, and is delayed (completion: 0 %, expected: 0%).

- Validation study

Relying on the identified time point in the cross-sectional study was expected to validate the relationship between cellular senescence and the development of diastolic dysfunction in a properly powered group matched with control animals. This task was expected to be run in parallel, in the later phase of the cross-sectional study in the last 3 months of the award (completion: 0 %, expected: 0%).

Aim 2: Development and validation of novel tracer for non-invasive imaging of cellular senescence.

Four key tasks were identified for Aim 2, covering the major tasks 3 and 4 presented in the statement of work:

- Antigen preparation

The first task of Aim 2 was to generate the antigen to be used for the immunization and selection of single-domain antibodies, subsequently used to generate the tracer. The proposed antigen was identified on bibliographical search, based on a study by Frescas *et al.* (Proc Natl Acad Sci U S A. 2017, 114:E1668) showing the presence of vimentin, modified by the oxidative adduct malondialdehyde (MDA) on the cysteine 328, at the cell membrane of senescent cells. The original approach was to induce the chemical modification on the recombinant protein and was expected to be performed in the first 2 months of the award. Considerable difficulties were encountered at this stage. Alternative approaches were explored, but the generation of the antigen was not successful. The detailed account of work undertaken in this task and the current approach are presented below.

- Immunization, phage-display and nanobodies production
- Radiolabeling and screening of tracer candidates
- Evaluation of the selected tracer

Key tasks 2, 3 and 4 rely on the results of task 1. Therefore, no progress is reported in either task. For time frame reference, task 2 was expected to be completed at 11 months of the award, and by the beginning of year 2, task 3 was expected to be completed at 25%.

What was accomplished under these goals?

Aim 1: Development of a preclinical model of cardiac-specific inducible senescence and evaluation of the relationship between cardiac cellular senescence and myocardial dysfunction.

The specific objectives of the tasks to be realized within the first year of the project in in Aim 1 were to generate homozygote double transgenic $Ezh2^{fl/fl}$:αMHC-MerCreMer animals, and test and optimized the conditions of genomic recombination in those animals.

Mice with loxP sites flanking exons 14-15 of enhancer of zeste homolog 2 gene ($Ezh2^{fl/fl}$) and mice with tamoxifen-inducible Cre recombinase under the promoter of cardiac-specific alpha-myosin

heavy (αMHC-MerCreMer) were obtained from the Jackson Laboratories (JAX). Methods to identify the presence of transgene by PCR were developed based on the protocols described by JAX and validated in animals with known genotype. Single transgenic animals were crossed to obtain a first generation (F1) of double heterozygote animals ($Ezh2^{fl/+}$:αMHC-MerCreMer/wt). The heterozygotes $Ezh2^{fl/+}$;αMHC-MerCreMer/wt mice were crossed to obtained F2 animals, which were genotyped to assess the presence of transgenes. Assuming Mendelian distribution 6.25 % of F2 animals were expected to be double transgenic. Overall, in the initial breeding a Mendelian distribution was observed and a sufficient number of double transgenic animals (7/75) was obtained to set up breeding of those animals. Additional breading cages were set up using heterozygote animals to increase the number of generated double transgenic animals and maintain animals to control for the effect of tamoxifen ($Ezh2^{fl/fl}$;wt) and the expression of Cre in cardiomyocytes ($Ezh2^{+/+}$;αMHC-MerCreMer).

A first group of double transgenic animals at 8 weeks of age (n = 4) was treated with 2 doses of tamoxifen (3 × 30 mg/kg, n = 1, or 3 × 100 mg/kg, n = 2) or vehicle solution (n = 1). The highest dose was included because of concerns about the dose preparation. Animals were imaged by echocardiography to assess cardiac function at baseline and at 1 week after treatment. The evaluation of cardiac function at 1 week was used to assess overt cardiac toxicity anticipated at a high rate of genetic recombination and associated cardiomyocytes apoptosis/senescence, not the dysfunction associated with the presence of a smaller population of senescence cells and the associated secretory phenotype, anticipated to develop over a longer period, which this model aims to recapitulate. Only the animal treated with the highest dose showed an impact on cardiac function while the treatment at 3 × 30 mg/kg of tamoxifen and the control animal did not show any impact of the ejection fraction on this preliminary evaluation (Fig. 1). At 1 week of treatment, all animals were euthanized and samples of tissues (cardiac and none-cardiac) were harvested to evaluate the efficiency of genetic recombination and the presence of senescent cells. However, these evaluations could not be carried out before the institutional lock down.

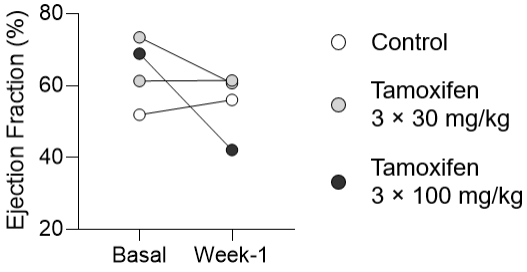

Fig. 1: Acute impact of tamoxifen on cardiac function in $Ezh2^{fl/fl}$;αMHC-MerCreMer/wt mice. Ejection fraction of $Ezh2^{fl/fl}$;αMHC-MerCreMer/wt mice evaluated before and at 1 week after the treatment with tamoxifen at 3 × 30 mg/kg, 3 × 100 mg/kg or vehicle solution.

The double transgenic line ($Ezh2^{fl/fl}$:αMHC-MerCreMer) was successfully generated within the anticipated time frame; the original animals lines were maintained as control ($Ezh2^{fl/fl}$;wt and $Ezh2^{+/+}$;αMHC-MerCreMer) by crossing selected F1 animals. The test of genetic recombination was started in early March and could not be concluded before the institutional lock down.

Succinct methods:

Genotyping. Genomic DNA was extracted form ear tissue (Direct PCR, ear) and used as DNA template for standard PCR (34 cycles, annealing temperature 60 °C) with 3 sets of primers to detect αMHC-MerCreMer wild-type, αMHC-MerCreMer mutant, and Ezh2 flox and wild-type alleles; PCR products were run on an 1% agarose gel containing Sybr Safe.

Genetic recombination. Animals at 8 weeks of age were injected i.p. with tamoxifen in corn oil solution or vehicle solution for 3 consecutive days at 30 mg/kg or 100 mg/kg. At 1 week after the end of the treatment, animals were euthanized and samples of heart tissue and control, non-cardiac tissue were harvested. Efficiency of genomic recombination was set to be analyzed using the ratio of recombined (1-lox) to non-recombined (2-lox) alleles.

Echocardiography. Animals underwent echocardiography (Vevo2100, VisualSonics) prior and at one week after tamoxifen (or control) treatment. Mice under 2% isoflurane anesthesia were imaged in short axis, parasternal long axis and by power and tissue doppler to assess various cardiac function parameters including left ventricular ejection fraction and mitral flow and motion during early diastole and atrial contraction.

Aim 2: Development and validation of novel tracer for non-invasive imaging of cellular senescence.

The specific objectives of the tasks to be achieved within the first year of the project in Aim 2 were to prepare the antigen specific to senescent cells: MDA-vimentin and use this antigen to generate single domain antibodies.

The original approach, as presented in the statement of work, was to order recombinant vimentin protein and chemically modify the protein to obtain the targeted antigen: MDA adduct on the cysteine 328, which was observed *in vivo* and reported as specific of cellular senescence.

To that end, an initial approach was to set in place an analytic method capable of identifying the occurrence of the specific modification and absence of undesired modifications, which could create undesired, potentially immunogenic antigen. A method based on LC-MS analysis of peptides obtained from the cleavage of the protein by trypsin, in comparison with in silico predicted peptides, was set in place. The implementation of this analytical method, described by Frescas et al. (Proc Natl Acad Sci U S A. 2017, 114(9):E1668), required multiple iteration of testing to find proper conditions for this experimental setting.

In parallel, a series of experiments on amino acid was carried out with the objective of finding optimal condition for specific modification of cysteine residues with MDA. Preparation of MDA was carried out following described methods (J. Am. Chem. Soc. 1981, 103:3030; J. Sci. Food Agric 2009, 89:1416) by acid hydrolysis of 1,1,3,3-tetramethoxypropane. The formation of MDA was confirmed by liquid chromatography–mass spectrometry (LC-MS) analysis (Fig. 2a). The reaction between MDA, either crystallized or freshly prepared, and amino acids (N-acetylated methyl esters of cysteine and lysine) was tested using various concentration ratios, buffer conditions, pH, temperature and oxidative conditions (sodium azide). The progress of the reaction was monitored by LC-MS to identify modified amino acids (Fig. 2b, c). While some conditions showed complete reaction of N-acetyl-L-lysine methyl ester, no condition was found where the modification of N-acetyl-L-cysteine methyl ester was observed. After a failure to obtain the expected modification, it was hypothesized that the reaction on the cysteine would be more favorable in conditions closer related to the *in vivo* situation, with the cysteine engaged in peptide bonds with adjacent residues. A peptide corresponding to the residues 316 to 333 of vimentin

protein was synthetized and similar experiments of reaction with MDA were performed. However, no condition was found where the modification of the cysteine by MDA was observed.

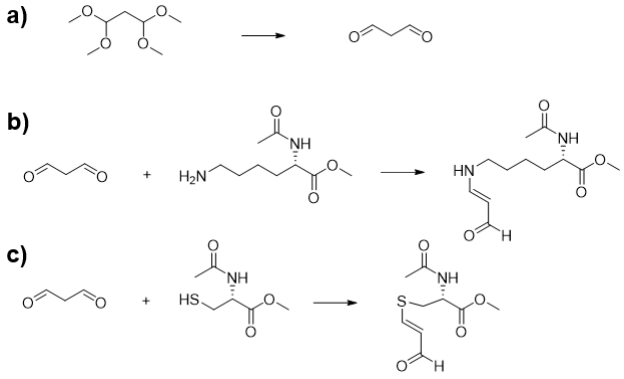

Fig. 2: Model reactions to test MDA modification on amino acids.
a) Preparation of MDA by acid hydrolysis of 1,1,3,3-tetramethoxypropane; **b)** model reaction of MDA with N-acetyl-L-lysine methyl ester, expected to produce MDA modified lysine (m/z: 256.14); **c)** model reaction of MDA with N-acetyl-L-cysteine methyl ester, expected to produce MDA modified cysteine (m/z: 231.06).

The inability to modify cysteine thiol group with the oxidation adduct MDA lead to renounce to the generation of MDA-vimentin as a target. Instead, effort was put to identify an alternative target specific of cellular senescence in the heart. Of note, this change in the molecular target, for imaging the same biological process was not considered as a significant change in the project or its direction, since the all anticipated studies are expected to be perform without alteration to the initial plan with an alternative target.

A first step was an extensive review of the literature to identify potential targets. A total of 24 potential targets: protein expressed at the cell membrane, upregulated in senescent cells, were identified. However, those results were obtained primary in established models of cellular senescence, mainly in fibroblast cell lines. A set of experiments was designed to test the validity of those results in cardiomyocytes, to ensure the relevance for this project. First, cardiomyocyte cell line HL-1 were obtained and amplified. HL-1 cells were treated with doxorubicin to induce cellular senescence. After 1 weeks, cells were harvested, and gene expression analysis was performed. Successful induction of p21-mediated cellular senescence was observed along with the upregulation of several alternative targets identified in the literature review. The highest upregulation in gene expression was observed for *Notch3* (Fig.3, log2 fold change: 4.35).

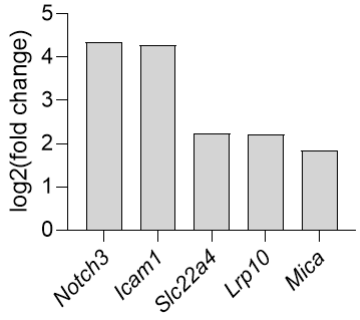

Fig. 3: Gene expression analysis of targets overexpressed in senescent murine cardiomyocytes.
Top 5 overexpressed gene, among the tested 24 target genes, in murine HL-1 cardiomyocytes where cellular senescence was induced by doxorubicin compared to control HL-1. Gene expression was normalized to *Gapdh*.

By the time NOTCH3 was identified as a potential alternative target, double transgenic animals have been generated (Aim 1). Given the failure to obtain the initially planned antigen and the related delay in the realization of the project, the initiation of a no-cost extension to the expiration date of the award for a period of up to 12 months was deemed necessary. Therefore, it was decided to wait for the validation of upregulation of NOTCH3 (in association with cellular senescence) in

the generated animal model before committing to the production of single domain antibodies against this target.

Succinct methods:

Chemistry. MDA by acid hydrolysis (1:5 HCl mM) of 1,1,3,3-tetramethoxypropane 8.4 mM (40 °C, 30 min) neutralized to pH 7.5 with NaOH. Amino acid reaction were carried out for different ratio of MDA (up to 0.2 M) to N-acetylated lysine or cysteine methyl esters (or vimentin peptide) in 50 mM PBS or 100 mM acetate buffer, with pH range from 2 to 12, at temperature range from room temperature to 80 °C, in presence or absence of sodium azide 2.5 mM, with reaction time range from 1 h to 18 h (overnight). LC-MS analysis was perform using Agilent LC-MS 6120B Quadrupole, 6490 Triple Quad, 6550A iFunnel Q-TOF.

Cell culture. Murine cardiomyocytes HL-1 cells were cultured on gelatin/fibronectin coated cell culture dishes in Claycomb culture media supplemented with HL-1 qualified FBS, norepinephrine, L-glutamine and penicillin/streptomycin. Cellular senescence was induced by 24 h exposure to doxorubicin 10 μM; senescent cells were harvested at 1 week after the exposure.

Gene expression analysis. Senescent and control cells were lysed and total RNA was extracted and reverse-transcribed to cDNA (Qiagen kits). Quantitative PCR analysis was performed using Sybr Green master mix and appropriate primer sets (7500 Real-Time PCR System, Applied Biosystems). Amplification results were normalized to *Gapdh* ($2^{\Delta CT}$) and analyzed as fold change (senescent to control cells).

What opportunities for training and professional development has the project provided?

Nothing to Report

How were the results disseminated to communities of interest?

Nothing to Report.

What do you plan to do during the next reporting period to accomplish the goals?

Aim 1 studies will be continued following the plan described in the statement of work. As presented above, the generation of the double transgenic line ($Ezh2^{fl/fl}$:αMHC-MerCreMer) was achieved within the expected time frame. The test and optimization of the condition for genomic recombination was interrupted by the institutional lockdown. Only the initial (high dose) of tamoxifen was administrated to animals. The next step is to ascertain the genomic recombination occurred in those animals. Following this, lower doses will be tested to find a minimal dose for genomic recombination occurs at a lower level, detectable by PCR on genomic DNA extracted from heart tissue. This should set stage for the cross-sectional study. Induction of cardiac specific senescence will be performed in 8 weeks-old double transgenic mice. Animals will be followed by echocardiography to monitor the development of diastolic dysfunction prior to animals' euthanasia at various time point after treatment (up to 28 weeks). The presence of cellular senescence, tissue inflammation and fibrosis will be evaluated *ex vivo* in cardiac and control tissues. This evaluation is expected to provide the time frame of the development of diastolic dysfunction in association with cardiomyocytes cellular senescence. Based on this time point, a validation study will be performed

including higher number of animals and appropriate controls (e.g. double transgenic treated with vehicle solution, as described in the statement of work, but also single transgenic animals, to control for the effect of tamoxifen, in $Ezh2^{fl/fl}$;wt, and the expression of Cre in cardiomyocytes, in $Ezh2^{+/+}$;αMHC-MerCreMer).

Aim 2 studies will continue with the validation of NOTCH3 in the model established (Aim 1), as an alternative target of cellular senescence. After this validation, the studies in Aim 2 will be carried out as described in the statement of work. Briefly, polyhistidine-tagged single domain antibodies (sdAb) targeting NOTCH3 will be generated by an expert external contractor. The sdAb will be radiolabeled with technetium-99m (^{99m}Tc). Stability and affinity to NOTCH3 will be evaluated in vitro. The biodistribution of ^{99m}Tc-sdAb will be evaluated in control animals. Those evaluation will lead to the selection of lead compound among the tested sdAb. The efficiency of *in vivo* imaging cellular senescence via NOTCH3 targeting will be tested *in vivo* in animals from the validation group (Aim 1) by single photon emission computed tomography imaging.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

Identification of makers specific of cellular senescence and the validation of their relevance in various tissues is of interest for various application; this is particularly true for makers expressed at the cell membrane and thus potentially of use for targeting senescent cells (Cell 2019, 179:813). Overexpression of NOTCH3 was previously reported in human fibroblast and cancer cell lines (Cancer Res. 2013, 73:3451; Cell Death Dis. 2014, 5:e1528). Here NOTCH3 was validated a marker of cellular senescence induced by doxorubicin in murine cardiomyocytes.

What was the impact on other disciplines?

Malondialdehyde (MDA) oxidative adduct on the side chain of cysteine has been reported to occur *in vivo* (Proc Natl Acad Sci U S A. 2017, 114:E1668). However, only sparse literature on MDA chemistry with thiol groups have been reported so far (Free Radic Biol Med. 1991, 11:81). Here a systematic study aiming at the chemically modification of cysteine with MDA was performed and no conditions were found where the chemical modification was observed. This negative data is planned to be reported as a short communication, providing that no additional experiments are requested for the publication.

What was the impact on technology transfer?

Nothing to Report.

What was the impact on society beyond science and technology?

Nothing to Report.

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change

In the tasks related to Aim 1, no significant deviation from the statement of work are report. The obtention of double transgenic animals occurred within the expected time frame. The second task of Aim 1 has been interrupted by the institution wide lockdown. Therefore, delay in the realization of the subsequent task are to be expected. A minor modification to the project was the maintenance of single transgenic animal lines, as additional controls for subsequent studies in Aim 1 (see part 3).

In Aim 2, different conditions were tested to modify the side chain of cysteine with the oxidation adduct MDA but this approach remained unsuccessful. Therefore, MDA-vimentin, issue from the modification of the recombinant protein (original approach), or MDA-vimentin peptide (considered alternative) could not be generated and used as antigen to generated single domain antibodies. It was decided to pursue with an alternative target. This change is not considered as significant in objective and scope, since the purpose and strategy (targeting cellular senescence using single domain antibodies) remain the same, with an alternative molecular target. Based on cardiomyocyte cell assay, NOTCH3 was identified as the most relevant of the alternative targets identified by bibliographical search. However, by the time NOTCH3 was validated as the most promising alternative target, the project has seen considerable delay, and it was anticipated that the no-cost extension to the expiration date of the award will be requested. With the anticipated extra time, it was decided that NOTCH3 upregulation should be validated in the generated animal model before committing to the production of single domain antibodies against this target.

Actual or anticipated problems or delays and actions or plans to resolve them

The first task for the Aim 2 of the project was to generate MDA-modified vimentin as an antigen specific of cellular senescence and use this antigen for the generation of single domain antibodies. The planed approach was to chemically modify recombinant vimentin with MDA. To this end, an analytical method to verify the chemical modification of the protein had to be set in place and conditions for the chemical reaction had to be determined. The implementation of the methods to analyze peptides obtained with trypsin digestion required more testing than anticipated. To determine reaction conditions, initial tests with amino acids (lysine and cysteine) were performed. A wide range of conditions was tested, but no formation of MDA-cysteine was observed in any conditions (while condition for the reaction with the side chain of lysine were determined). It was hypothesized that conditions for the modification of the side chain of the cysteine might be more favorable using the peptide sequence where this modification was observed in vivo. Therefore, an 18-mer peptide corresponding to the sequence of vimentin including the cysteine 328 was synthetized. Of note, peptides of this size can be used as antigen for the generation of single domain antibodies, usually conjugated with keyhole limpet haemocyanin (KLH) for increased immunogenicity. The use of the peptide, which does not contain lysine residues, was considered as an alternative strategy providing that conditions for cysteine modification were found, regardless if in those condition lysine residues are also modified, which, using the recombinant protein would lead to the creation of undesired, potentially immunogenic epitopes. However, similarly to the study with amino acids, no conditions was found were the modification of the cysteine with MDA was

found. Therefore, the alternative approach that was pursued was to identify an alternative target: a cell membrane protein overexpressed in senescent cardiomyocytes. NOTCH3 was identified and once validated in the animal model, single domain antibodies against NOTCH3 will be generated to continue the studies planned in Aim 2 of the project.

Changes that had a significant impact on expenditure,

Due to the delay in the preparation of the antigen, the main expenditures related to Aim 2 (all steps from immunization to the production of single-domain antibodies, to be performed by an expert external contract) has not yet been processed.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Significant changes in use or care of human subjects

Nothing to Report.

Significant changes in use or care of vertebrate animals

Nothing to Report.

Significant changes in use of biohazards and/or select agents

Nothing to Report.

6. PRODUCTS:

- **Publications, conference papers, and presentations**

  Journal publications.

  Nothing to Report.

  Books or other non-periodical, one-time publications.

  Nothing to Report.

  Other publications, conference papers and presentations.

  Nothing to Report.

- **Website(s) or other Internet site(s)**

  Nothing to Report.

- **Technologies or techniques**

  Nothing to Report.

- **Inventions, patent applications, and/or licenses**

  Nothing to Report.

- **Other Products**

Generation of a murine model of cardiac-specific cellular senescence: $Ezh2^{fl/fl}$;αMHC-MerCreMer mice (remains to be validated).

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	Jakub Toczek
Project Role:	PI
Researcher Identifier (ORCID ID):	0000-0003-0639-8075
Nearest person month worked:	2 (2.4)
Contribution to Project:	Dr. Toczek designed the experiments, provided supervision to the postgraduate associate recruited on the project, participated in animals breeding, experimentation and data interpretation
Funding Support:	20 % from this award, 80 % from American Heart Association postdoctoral fellowship (AHA, 19POST34450142)

Name:	Nowshin Sanzida
Project Role:	Postgraduate Associate
Researcher Identifier (ORCID ID):	0000-0002-2432-5489
Nearest person month worked:	10 (9.6)
Contribution to Project:	Ms. Sanzida, participated in animals breeding, experimentation and data interpretation

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to Report.

What other organizations were involved as partners?

The laboratory INSERM UMR_S 1039 is an academic institution, part of the French national institute for biomedical research (INSERM). This group possess considerable expertise in the development of antibody-based radiotracers.

Organization Name: INSERM UMR_S 1039
Location of Organization: France
Partner's contribution to the project: Consulting on study and experiments design.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

Not applicable

QUAD CHARTS:

Not applicable

9. APPENDICES:

None.