

Systematic review protocol	
Title:	Ferroptosis inhibitors in organ ischemia reperfusion injury and transplantation: a systematic review
Version:	V1.0 – Initial submission

## 1. Review team members and affiliations

Team member	Contribution	Department
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## 2. Conflicts of interest/funding resources

### 2.1. Conflicts of interest

Not applicable, as there are no conflicts of interest.

### 2.2. Funding resources

FWO SBO (S001522N): “Blocking ischemia reperfusion injury during dynamic preservation of organ grafts using lead ferroptosis inhibitors”

- Role: Funding of the general research project, under which this systematic review will be performed.

## 3. Introduction

### 3.1. Rationale

Ferroptosis is a novel type of cell death that is characterized by an iron-dependent accumulation of lipid reactive oxygen species. Especially, the oxidation of membrane lipids is essential in the induction of ferroptosis, as these oxidized lipids contribute to the disruption of the structure and dynamics of the cell membrane. Glutathione peroxidase 4 (GPX4) is an important regulator in this process, among other regulators such as system  $X_c^-$  and NPR. (1-3) Despite the current knowledge of the mechanism behind ferroptosis, there is still a lot to discover about its contribution in disease and the potential clinical relevance of regulating this process.

In ischemia reperfusion injury (IRI), a form of oxidative damage that occurs in re-oxygenated tissue after a period of lack of oxygen (e.g.: transplantation, stroke, etc.), ferroptosis has already shown to have a role in the induction of cellular injury. (4) Moreover, targeting ferroptosis in models of IRI has shown to alleviate cellular injury in multiple organs, suggesting clinical relevance of ferroptosis inhibition in IRI-induced conditions. (5-7) In this systematic review, the efficacy of ferroptosis inhibition on the read-outs and clinical endpoints in the IRI setting will be investigated.

### 3.2. Review question

What is the efficacy of ferroptosis inhibition on read-outs in ischemic reperfusion injury or transplantation models (rodent or higher) in lung, liver, pancreas, heart, kidney and intestines?

### 3.3. Objectives

- Ischemia reperfusion injury (IRI) and/or transplantation setup:
  - Which animal model was used? Specific strain? Age?
  - How was the IRI induced?
  - Where was the organ transplanted (*in vivo*, *ex vivo*)? (or e.g.: in the neck for heart transplantation)
  - Which conditions were used (temperature, duration, incision, pre-conditioning etc.)?
  - Which conditions were used for the sham / control animals?
- Ferroptosis inhibitor:
  - Which inhibitor(s) was(were) used?
  - What pathway does this(these) inhibitor(s) affect?
  - Specific vehicle used as control?
- Read-out:
  - Which samples were collected (tissue, blood, urine, perfusate etc.)?
  - Which parameters/markers were analyzed?
  - Which techniques were used (histology, ELISA, Western blot, isolations, lipid extraction, FACS (Fluorescence-Activated Cell Sorting))?

## 4. Methods

### 4.1. Information sources

The following information sources will be used in order to have sufficient coverage for our research question:

- Pubmed
- Embase (Elsevier)
- Web of Science (Core collection)
- Cochrane library (Wiley)
- Gray literature

### 4.2. Search strategy

Two concepts will be used as search terms for this systematic review and will be combined with the Boolean operator “AND”: “ferroptosis” AND “ischemia/reperfusion injury”. In the latter concept, the search term “transplantation” will also be included with Boolean operator “OR”. Note that the term “inhibition” was not included as a concept, because this term is not included in the Mesh or Emtree database and could therefore potentially lead to missing relevant articles.

Advanced search strategies will be used in each database. An overview of the different search strategies within each database can be found in the subsections below. For each concept, synonyms, abbreviations and/or related terms will be included as well, using the Boolean operator “OR”.

In contrast, the concept “ferroptosis” has only been termed in 2012 by Dixon et al. and was included in search databases at the earliest in 2017. For this reason, the Boolean operator “AND” will be used in the first concept in order to combine concepts of “cell death”, “iron”, and terms related to oxidative stress, lipid peroxidation, etc. By including these additional terms, we intend to avoid missing out on relevant articles that were published before 2012.

Lastly, although ferroptosis is a different type of cell death than apoptosis, it was decided to include apoptosis as a related term to cell death. This decision was based on the fact that “apoptosis” appeared in several relevant articles as a Mesh term during a preliminary literature search.

#### 4.2.1. Pubmed

(concept 1) AND (concept 2)

- Concept Ferroptosis:  
"Ferroptosis"[Mesh] OR "ferroptosis"[tiab] OR "ferroptotic"[tiab] OR "Oxytosis"[tiab] OR (("Regulated Cell Death"[Mesh:NoExp] OR "Cell Death"[Mesh:NoExp] OR "cell death"[tiab] OR "cell necrosis"[tiab] OR "Cell Survival"[Mesh] OR "cell survival"[tiab] OR "cellular survival"[tiab] OR "cell life span"[tiab] OR "cell lifespan"[tiab] OR "cell viabilit\*" [tiab] OR "cellular viabilit\*" [tiab] OR "non-apoptotic"[tiab] OR "necrotic"[tiab] OR "Apoptosis"[Mesh:NoExp] OR "apoptosis"[tiab]) AND ("Iron"[Mesh:NoExp] OR "iron"[tiab] OR "Iron Overload"[Mesh:NoExp] OR "Fe"[tiab] OR "Fe2+" [tiab] OR "Fe3+" [tiab] OR "fenton"[tiab]) AND ("Lipid Peroxidation"[Mesh] OR "Lipid Peroxid\*" [tiab] OR "Lipid Peroxides"[Mesh] OR "Fatty Acid Hydroperoxid\*" [tiab] OR "Lipoperoxid\*" [tiab] OR "Lipid Hydroperoxid\*" [tiab] OR "lipohydroperoxid\*" [tiab] OR "Lipid Metabolism"[Mesh:NoExp] OR "lipid metabol\*" [tiab] OR "fat metabol\*" [tiab] OR "lipometabol\*" [tiab] OR "Reactive Oxygen Species"[Mesh:NoExp] OR "Reactive Oxygen Species"[tiab] OR "ROS"[tiab] OR "reactive oxygen metabol\*" [tiab] OR "Oxidative Stress"[Mesh:NoExp] OR "Oxidative stress\*" [tiab] OR "oxidant stress\*" [tiab] OR "Oxidative Damage\*" [tiab] OR "Oxidative Injur\*" [tiab]))
- Concept Ischemia/reperfusion injury:  
"Reperfusion Injury"[Mesh] OR "reperfusion injur\*" [tiab] OR "reperfusion damage\*" [tiab] OR "IR injur\*" [tiab] OR "IRI" [tiab] OR "reperfusion cell injur\*" [tiab] OR "reperfusion organ injur\*" [tiab] OR "reperfusion tissue injur\*" [tiab] OR "reperfusion-associated injur\*" [tiab] OR "reperfusion-induced injur\*" [tiab] OR "reperfusion-induced organ injur\*" [tiab] OR "reperfusion-induced tissue injur\*" [tiab] OR "reperfusion-related injur\*" [tiab] OR "reperfusion hepatic injur\*" [tiab] OR "reperfusion hepatocyte injur\*" [tiab] OR "reperfusion liver injur\*" [tiab] OR "reperfusion-induced hepatic injur\*" [tiab] OR "reperfusion-induced hepatocyte injur\*" [tiab] OR "reperfusion-induced liver injur\*" [tiab] OR "reperfusion lung injur\*" [tiab] OR "reperfusion pulmonary injur\*" [tiab] OR "reperfusion-induced lung injur\*" [tiab] OR "reperfusion-induced pulmonary injur\*" [tiab] OR "reperfusion cardiac injur\*" [tiab] OR "reperfusion heart injur\*" [tiab] OR "reperfusion myocardial injur\*" [tiab] OR "reperfusion-induced

cardiac injur\*[tiab] OR "reperfusion-induced cardiomyocyte injur\*[tiab] OR "reperfusion-induced heart injur\*[tiab] OR "reperfusion-induced myocardial injur\*[tiab] OR "Post-Cardiac Arrest Syndrome\*[tiab] OR "Postresuscitation Disease\*[tiab] OR "post heart arrest syndrome"[tiab] OR "reperfusion kidney injur\*[tiab] OR "reperfusion renal injur\*[tiab] OR "reperfusion-induced kidney injur\*[tiab] OR "reperfusion-induced renal injur\*[tiab] OR "Transplantation"[Mesh] OR "transplant\*[tiab] OR "graft\*[tiab] OR "organ procurement\*[tiab] OR "allograft\*[tiab] OR "alloplastic implant\*[tiab] OR "allograft\*[tiab] OR "homograft\*[tiab] OR "homotransplant\*[tiab] OR "homoiotransplant\*[tiab] OR "autograft\*[tiab] OR "autotransplant\*[tiab] OR "engraft\*[tiab] OR "harvest\*[tiab] OR "isograft\*[tiab] OR "isotransplant\*[tiab] OR "retransplant\*[tiab] OR "xenograft\*[tiab] OR "heterograft\*[tiab] OR "heterotransplant\*[tiab] OR "xenotransplant\*[tiab] "Ischemia"[Mesh] OR "ischemi\*[tiab] OR "ischaemi\*[tiab] OR "circulation disorder\*[tiab] OR "blood flow disorder\*[tiab] OR "circulatory failure\*[tiab] OR "circulation failure\*[tiab] OR "circulatory disorder\*[tiab] OR "infarct\*[tiab] OR "bloodless"[tiab] OR "Thromboembolism"[Mesh] OR "Embolism"[Mesh] OR "embol\*[tiab] OR "thromboembol\*[tiab] OR "thrombo embol\*[tiab] OR "cardiovascular stroke\*[tiab] OR "heart attack\*[tiab] OR "no-reflow phenomenon"[tiab] OR "slow-flow phenomenon"[tiab] OR "Organ Preservation"[Mesh] OR "organ preservation\*[tiab] OR "organ conservation\*[tiab] OR "organ storage"[tiab] OR "heart preservation\*[tiab] OR "cardiac preservation\*[tiab] OR "cardiac storage"[tiab] OR "heart storage"[tiab] OR "kidney preservation\*[tiab] OR "kidney storage"[tiab] OR "renal preservation\*[tiab] OR "renal storage"[tiab] OR "liver preservation\*[tiab] OR "hepatic storage"[tiab] OR "liver conservation\*[tiab] OR "Tissue and Organ Procurement"[Mesh:NoExp] OR "tissue procurement\*[tiab] OR "Tissue and Organ Harvesting"[Mesh:NoExp] OR "organ retrieval\*[tiab]

#### 4.2.2. Embase (Elsevier) (concept 1) AND (concept 2)

- Concept Ferroptosis:  
'ferroptosis'/exp OR 'ferroptosis':ti,ab,kw OR 'ferroptotic':ti,ab,kw OR 'oxytosis':ti,ab,kw OR (('cell death'/de OR 'cell death':ti,ab,kw OR 'cell necrosis':ti,ab,kw OR 'regulated cell death'/de OR 'cell survival'/de OR 'cell\* survival':ti,ab,kw OR 'cell life span':ti,ab,kw OR 'cell lifespan':ti,ab,kw OR 'cell viability'/exp OR 'cell\* viabilit\*':ti,ab,kw OR 'non-apoptotic':ti,ab,kw OR 'necrotic':ti,ab,kw OR 'apoptosis'/de OR 'apoptosis':ti,ab,kw) AND ('iron'/exp OR 'iron':ti,ab,kw OR 'iron overload'/exp OR 'Fe':ti,ab,kw OR 'Fe2+':ti,ab,kw OR 'Fe3+':ti,ab,kw OR 'Fenton reaction'/exp OR 'fenton':ti,ab,kw) AND ('lipid peroxidation'/exp OR 'lipid peroxid\*':ti,ab,kw OR 'lipoperoxid\*':ti,ab,kw OR 'lipid peroxide'/de OR 'lipid hydroperoxide'/de OR 'lipid hydroperoxid\*':ti,ab,kw OR 'lipohydroperoxid\*':ti,ab,kw OR 'Fatty Acid Hydroperoxid\*':ti,ab,kw OR 'Lipoperoxid\*':ti,ab,kw OR 'lipid metabolism'/de OR 'lipid metabl\*':ti,ab,kw OR 'fat metabol\*':ti,ab,kw OR 'lipometabol\*':ti,ab,kw OR 'reactive oxygen metabolite'/exp OR 'reactive oxygen metabol\*':ti,ab,kw OR 'reactive oxygen

species':ti,ab,kw OR 'ROS':ti,ab,kw OR 'oxidative stress'/exp OR 'oxidative stress\*':ti,ab,kw OR 'oxidant stress\*':ti,ab,kw OR 'oxidative damage'/exp OR 'Oxidative Damage\*':ti,ab,kw OR 'Oxidative Injur\*':ti,ab,kw))

- Concept Ischemia/reperfusion injury:

'Reperfusion injury'/exp OR 'reperfusion injur\*':ti,ab,kw OR 'reperfusion damage\*':ti,ab,kw OR 'IR injur\*':ti,ab,kw OR 'IRI':ti,ab,kw OR 'reperfusion cell injur\*':ti,ab,kw OR 'reperfusion organ injur\*':ti,ab,kw OR 'reperfusion tissue injur\*':ti,ab,kw OR 'reperfusion-associated injur\*':ti,ab,kw OR 'reperfusion-induced injur\*':ti,ab,kw OR 'reperfusion-induced organ injur\*':ti,ab,kw OR 'reperfusion-induced tissue injur\*':ti,ab,kw OR 'reperfusion-related injur\*':ti,ab,kw OR 'reperfusion hepatic injur\*':ti,ab,kw OR 'reperfusion hepatocyte injur\*':ti,ab,kw OR 'reperfusion liver injur\*':ti,ab,kw OR 'reperfusion-induced hepatic injur\*':ti,ab,kw OR 'reperfusion-induced hepatocyte injur\*':ti,ab,kw OR 'reperfusion-induced liver injur\*':ti,ab,kw OR 'reperfusion lung injur\*':ti,ab,kw OR 'reperfusion pulmonary injur\*':ti,ab,kw OR 'reperfusion-induced lung injur\*':ti,ab,kw OR 'reperfusion-induced pulmonary injur\*':ti,ab,kw OR 'reperfusion cardiac injur\*':ti,ab,kw OR 'reperfusion heart injur\*':ti,ab,kw OR 'reperfusion myocardial injur\*':ti,ab,kw OR 'reperfusion-induced cardiac injur\*':ti,ab,kw OR 'reperfusion-induced cardiomyocyte injur\*':ti,ab,kw OR 'reperfusion-induced heart injur\*':ti,ab,kw OR 'reperfusion-induced myocardial injur\*':ti,ab,kw OR 'post-cardiac arrest syndrome':ti,ab,kw OR 'Postresuscitation Disease\*':ti,ab,kw OR 'post heart arrest syndrome':ti,ab,kw OR 'reperfusion kidney injur\*':ti,ab,kw OR 'reperfusion renal injur\*':ti,ab,kw OR 'reperfusion-induced kidney injur\*':ti,ab,kw OR 'reperfusion-induced renal injur\*':ti,ab,kw OR 'transplantation'/exp OR 'transplant\*' OR 'graft\*':ti,ab,kw OR 'organ procurement\*':ti,ab,kw OR 'allograft\*':ti,ab,kw OR 'alloplastic implant\*':ti,ab,kw OR 'allograft\*':ti,ab,kw OR 'homograft\*':ti,ab,kw OR 'homotransplant\*':ti,ab,kw OR 'homoiotransplant\*':ti,ab,kw OR 'autograft\*':ti,ab,kw OR 'autotransplant\*':ti,ab,kw OR 'engraft\*':ti,ab,kw OR 'harvest\*':ti,ab,kw OR 'isograft\*':ti,ab,kw OR 'isotransplant\*':ti,ab,kw OR 'retransplant\*':ti,ab,kw OR 'xenograft\*':ti,ab,kw OR 'heterograft\*':ti,ab,kw OR 'heterotransplant\*':ti,ab,kw OR 'xenotransplant\*':ti,ab,kw OR 'cold ischemia'/exp OR 'ischemia'/exp OR 'isch\*mi\*':ti,ab,kw OR 'circulat\* disorder\*':ti,ab,kw OR 'blood flow disorder\*':ti,ab,kw OR 'circulat\* failure\*':ti,ab,kw OR 'infarct\*':ti,ab,kw OR 'bloodless':ti,ab,kw OR 'thromboembolism'/exp OR 'embolism'/exp OR 'embol\*':ti,ab,kw OR 'thromboembol\*':ti,ab,kw OR 'thromboembol\*':ti,ab,kw OR 'cardiovascular stroke\*':ti,ab,kw OR 'heart attack\*':ti,ab,kw OR 'no reflow phenomenon'/exp OR 'no-reflow phenomenon':ti,ab,kw OR 'slow-flow phenomenon':ti,ab,kw OR 'organ preservation'/exp OR 'organ preservation\*':ti,ab,kw OR 'organ conservation\*':ti,ab,kw OR 'organ storage':ti,ab,kw OR 'heart preservation\*':ti,ab,kw OR 'cardiac preservation\*':ti,ab,kw OR 'cardiac storage':ti,ab,kw OR 'heart storage':ti,ab,kw OR 'kidney preservation\*':ti,ab,kw OR 'kidney storage':ti,ab,kw OR 'renal preservation\*':ti,ab,kw OR 'renal storage':ti,ab,kw OR 'liver preservation\*':ti,ab,kw OR 'hepatic storage':ti,ab,kw OR 'liver

conservation\*':ti,ab,kw OR 'tissue and organ procurement\*':ti,ab,kw OR 'tissue procurement\*':ti,ab,kw OR 'graft harvesting'/exp OR 'organ retrieval\*':ti,ab,kw

#### 4.2.3. Web of Science (Core collection)

(concept 1) AND (concept 2)

- Concept Ferroptosis:

TS=(("ferroptosis" OR "ferroptotic" OR "Oxytosis" OR (("cell death" OR "cell necrosis" OR "cell survival" OR "cellular survival" OR "cell life span" OR "cell lifespan" OR "cell viabilit\*" OR "cellular viabilit\*" OR "non-apoptotic" OR "necrotic" OR "apoptosis")) AND ("iron" OR "Fe" OR "Fe2+" OR "Fe3+" OR "fenton") AND ("Lipid Peroxid\*" OR "Fatty Acid Hydroperoxid\*" OR "Lipoperoxid\*" OR "Lipid Hydroperoxide\*" OR "lipohydroperoxid\*" OR "lipid metabolism" OR "lipid metabol\*" OR "fat metabol\*" OR "lipometabol\*" OR "Reactive Oxygen Species" OR "ROS" OR "reactive oxygen metabol\*" OR "Oxidative stress\*" OR "oxidant stress\*" OR "Oxidative Damage\*" OR "Oxidative Injur\*"))))

- Concept Ischemia/reperfusion injury:

TS=("reperfusion injur\*" OR "reperfusion damage\*" OR "IR injur\*" OR "IRI" OR "reperfusion cell injur\*" OR "reperfusion organ injur\*" OR "reperfusion tissue injur\*" OR "reperfusion-associated injur\*" OR "reperfusion-induced injur\*" OR "reperfusion-induced organ injur\*" OR "reperfusion-induced tissue injur\*" OR "reperfusion-related injur\*" OR "reperfusion hepatic injur\*" OR "reperfusion hepatocyte injur\*" OR "reperfusion liver injur\*" OR "reperfusion-induced hepatic injur\*" OR "reperfusion-induced hepatocyte injur\*" OR "reperfusion-induced liver injur\*" OR "reperfusion lung injur\*" OR "reperfusion pulmonary injur\*" OR "reperfusion-induced lung injur\*" OR "reperfusion-induced pulmonary injur\*" OR "reperfusion cardiac injur\*" OR "reperfusion heart injur\*" OR "reperfusion myocardial injur\*" OR "reperfusion-induced cardiac injur\*" OR "reperfusion-induced cardiomyocyte injur\*" OR "reperfusion-induced heart injur\*" OR "reperfusion-induced myocardial injur\*" OR "Post-Cardiac Arrest Syndrome\*" OR "Postresuscitation Disease\*" OR "post heart arrest syndrome" OR "reperfusion kidney injur\*" OR "reperfusion renal injur\*" OR "reperfusion-induced kidney injur\*" OR "reperfusion-induced renal injur\*" OR "transplant\*" OR "graft\*" OR "organ procurement\*" OR "allograft\*" OR "alloplastic implant\*" OR "allograft\*" OR "homograft\*" OR "homotransplant\*" OR "homoiotransplant\*" OR "autograft\*" OR "autotransplant\*" OR "engraft\*" OR "harvest\*" OR "isograft\*" OR "isotransplant\*" OR "retransplant\*" OR "xenograft\*" OR "heterograft\*" OR "heterotransplant\*" OR "xenotransplant\*" OR "ischemi\*" OR "ischaemi\*" OR "circulation disorder\*" OR "blood flow disorder\*" OR "circulatory failure\*" OR "circulation failure\*" OR "circulatory disorder\*" OR "infarct\*" OR "bloodless" OR "embol\*" OR "thromboembol\*" OR "thrombo embol\*" OR "cardiovascular stroke\*" OR "heart attack\*" OR "no-reflow phenomenon" OR "slow-flow phenomenon" OR "organ preservation\*" OR "organ conservation\*" OR "organ storage" OR "heart preservation\*" OR "cardiac preservation\*" OR "cardiac storage" OR "heart storage" OR "kidney preservation\*" OR "kidney storage" OR "renal preservation\*" OR "renal storage" OR "liver

preservation\*" OR "hepatic storage" OR "liver conservation\*" OR "tissue procurement\*" OR "organ retrieval\*")

#### 4.2.4. Cochrane library (Wiley)

(concept 1) AND (concept 2)

- Concept Ferroptosis:
  - #1: [mh "ferroptosis"]
  - #2: ("ferroptosis" OR "ferroptotic" OR "oxytosis"):ti,ab,kw
  - #3: #1 OR #2
  - #4: [mh ^"regulated cell death"] OR [mh ^"cell death"] OR [mh "cell survival"] OR [mh ^"apoptosis"]
  - #5: ("cell death" OR "cell necrosis" OR "cell survival" OR "cellular survival" OR "cell life span" OR "cell lifespan" OR "cell viabilit\*" OR (cellular NEXT viabilit\*) OR "non-apoptotic" OR "necrotic" OR "apoptosis"):ti,ab,kw
  - #6: #4 OR #5
  - #7: [mh ^"iron"] OR [mh ^"iron overload"]
  - #8: ("iron" OR "Fe" OR "Fe2+" OR "Fe3+" OR "fenton"):ti,ab,kw
  - #9: #7 OR #8
  - #10: [mh "lipid peroxidation"] OR [mh "lipid peroxides"] OR [mh ^"lipid metabolism"] OR [mh ^"reactive oxygen species"] OR [mh ^"oxidative stress"]
  - #11: ((lipid NEXT peroxid\*) OR ("fatty acid" NEXT hydroperoxid\*) OR "lipoperoxid\*" OR (lipid NEXT hydroperoxid\*) OR "lipohydroperoxid\*" OR (lipid NEXT metabol\*) OR (fat NEXT metabol\*) OR "lipometabol\*" OR "reactive oxygen species" OR "ROS" OR ("reactive oxygen" NEXT metabol\*) OR (oxidative NEXT stress\*) OR (oxidant NEXT stress\*) OR (oxidative NEXT damage\*) OR (oxidative NEXT injur\*)):ti,ab,kw
  - #12: #10 OR #11
  - #13: #6 AND #9 AND #12
  - #14: #3 OR #13
  
- Concept Ischemia/reperfusion injury:
  - #1: [mh "reperfusion injury"]
  - #2: ((reperfusion NEXT injur\*) OR (reperfusion NEXT damage\*) OR (IR NEXT injur\*) OR "IRI" OR ("reperfusion cell" NEXT injur\*) OR ("reperfusion organ" NEXT injur\*) OR ("reperfusion tissue" NEXT injur\*) OR ("reperfusion-associated" NEXT injur\*) OR ("reperfusion-induced" NEXT injur\*) OR ("reperfusion-induced organ" NEXT injur\*) OR ("reperfusion-induced tissue" NEXT injur\*) OR ("reperfusion-related" NEXT injur\*) OR ("reperfusion hepatic" NEXT injur\*) OR ("reperfusion hepatocyte" NEXT injur\*) OR ("reperfusion liver" NEXT injur\*) OR ("reperfusion-induced hepatic" NEXT injur\*) OR ("reperfusion-induced hepatocyte" NEXT injur\*) OR ("reperfusion-induced liver" NEXT injur\*) OR ("reperfusion lung" NEXT injur\*) OR ("reperfusion pulmonary" NEXT injur\*) OR ("reperfusion-induced lung" NEXT injur\*) OR ("reperfusion-induced pulmonary" NEXT injur\*) OR ("reperfusion cardiac" NEXT injur\*) OR ("reperfusion heart" NEXT injur\*) OR ("reperfusion myocardial" NEXT injur\*) OR ("reperfusion-induced cardiac" NEXT injur\*) OR

- ("reperfusion-induced cardiomyocyte" NEXT injur\*) OR ("reperfusion-induced heart" NEXT injur\*) OR ("reperfusion-induced myocardial" NEXT injur\*) OR ("Post-Cardiac Arrest" NEXT Syndrome\*) OR (Postresuscitation NEXT Disease\*) OR "post heart arrest syndrome" OR ("reperfusion kidney" NEXT injur\*) OR ("reperfusion renal" NEXT injur\*) OR ("reperfusion-induced kidney" NEXT injur\*) OR ("reperfusion-induced renal" NEXT injur\*):ti,ab,kw
- #3: #1 OR #2
  - #4: [mh "transplantation"]
  - #5: ("transplant\*" OR "graft\*" OR (organ NEXT procurement\*) OR "allograft\*" OR (alloplastic NEXT implant\*) OR "allotransplant\*" OR "homograft\*" OR "homotransplant\*" OR "homoiotransplant\*" OR "autograft\*" OR "autotransplant\*" OR "engraft\*" OR "harvest\*" OR "isograft\*" OR "isotransplant\*" OR "retransplant\*" OR "xenograft\*" OR "heterograft\*" OR "heterotransplant\*" OR "xenotransplant\*"):ti,ab,kw
  - #6: #4 OR #5
  - #7: [mh "ischemia"]
  - #8: ("ischemi\*" OR "ischaemi\*" OR (circulation NEXT disorder\*) OR ("blood flow" NEXT disorder\*) OR (circulatory NEXT failure\*) OR (circulation NEXT failure\*) OR (circulatory NEXT disorder\*) OR "infarct\*" OR "bloodless"):ti,ab,kw
  - #9: #7 OR #8
  - #10: [mh "thromboembolism"] OR [mh "embolism"]
  - #11: ("embol\*" OR "thromboembol\*" OR (thrombo NEXT embol\*) OR (cardiovascular NEXT stroke\*) OR (heart NEXT attack\*) OR "no-reflow phenomenon" OR "slow-flow phenomenon"):ti,ab,kw
  - #12: #10 OR #11
  - #13: [mh "organ preservation"]
  - #14: ((organ NEXT preservation\*) OR (organ NEXT conservation\*) OR "organ storage" OR (heart NEXT preservation\*) OR (cardiac NEXT preservation\*) OR "cardiac storage" OR "heart storage" OR (kidney NEXT preservation\*) OR "kidney storage" OR (renal NEXT preservation\*) OR "renal storage" OR (liver NEXT preservation\*) OR "hepatic storage" OR (liver NEXT conservation\*)):ti,ab,kw
  - #15: #13 OR #14
  - #16: [mh ^"tissue and organ procurement"] OR [mh ^"tissue and organ harvesting"]
  - #17: ((tissue NEXT procurement\*) OR (organ NEXT retrieval)):ti,ab,kw
  - #18: #16 OR #17

#### 4.2.5. Gray literature

In addition to the search strategies enlisted above, the reference lists of the articles that are included in this review will be screened for eligibility, with the same inclusion and exclusion criteria that were used for the title, abstract and full text screening. This technique is called "snowballing".



### 4.3. Eligibility criteria

After running these searches, duplicates will be identified and excluded by two independent reviewers, using EndNote. Afterwards, the same reviewers will screen the remaining records against inclusion and exclusion criteria enlisted below. First the reviewers will perform this screening method on the title and abstract, and finally, on the full text of the remaining records.

#### 4.3.1. Inclusion criteria

1. Language: English, Dutch, or French
2. Article type: original research articles, systematic reviews
3. Content:
  - Animal models using rodents or higher
  - IRI and/or transplantation models using liver, kidney, intestine, heart, lungs, or pancreas (or multiple).
  - The paper should describe experiments where ferroptosis is inhibited.
4. Full text available (freely online or via KU Leuven Association)

#### 4.3.2. Exclusion criteria

1. Language: other than English, Dutch, or French
2. Article type: non-original research articles (review articles, letter to editor, conference abstracts, editorials etc.)
3. Content:
  - Animal models lower than rodents
  - Studies focusing on *in vitro* experiments only
  - Studies focusing on cell transplantation or organs other than liver, kidney, intestine, heart, pancreas, or lungs.
  - Studies using ferroptosis models that are not related to IRI or transplantation.
  - Studies that do not inhibit ferroptosis.
  - Studies that do not specifically touch the concept of ferroptosis (including iron-dependent cell death, etc.)
4. No full text available

### 4.4. Data extraction

Specific data will be extracted from the final set of articles and will be enlisted in an extraction table by each reviewer. If inconsistencies exist a third reviewer will be consulted.

The extraction table will contain at least the following elements:

- Article information (author, publication date, journal, study type)
- Species
- Organ
- Ferroptosis inhibitor(s)
- Vehicle/placebo
- IRI/transplantation method
- Specific conditions (temperature, pre-conditioning etc.)
- Which samples were taken/analyzed at which time points?
- Which parameters/markers/read-outs were evaluated at which time points?

- Which analyzing techniques were used for these parameters/markers/read-outs?
- Cellular pathways by which the ferroptosis inhibitor exerts its effects.

#### **4.5. Risk of bias and quality assessment**

Mostly animal intervention studies are expected in this systematic review. Therefore, the Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE)'s risk of bias tool will be used to evaluate the methodologies used in the design, conduct and analysis of the studies. (8) The quality of these papers will be assessed by using the ARRIVE guidelines. (9, 10)

In case clinical studies are found, the NIH quality scoring tool will be used in order to assess the quality if these studies.

#### **5. References**

1. Ferroptosis in Health and Disease. Tang D, editor: Springer, Cham; 2019. 1-324 p.
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