**AWARD NUMBER: W81XWH-19-1-0714**

**TITLE: In Vivo and Ex Vivo Models to Study Ischemia/Reperfusion Injury, Endothelial Cell Protection, and Limb Preservation in a Prolonged Field Care Scenario**

**PRINCIPAL INVESTIGATOR: Robert Rieben**

**CONTRACTING ORGANIZATION: Universität Bern**

**REPORT DATE: SEPTEMBER 2022**

**TYPE OF REPORT: Annual**

**PREPARED FOR:** U.S. Army Medical Research and Development Command,
Fort Detrick, Maryland, 21702-5012

# REPORT DOCUMENTATION PAGE

*Form Approved*
*OMB No. 0704-0188*



| **1. REPORT DATE** | **2. REPORT TYPE** | **3. DATES COVERED** |
|---|---|---|
| SEPTEMBER 2022 | Annual | 1 Sep 2021 – 31 Aug 2022 |

**4. TITLE AND SUBTITLE**
In Vivo and Ex Vivo Models to Study Ischemia/ Reperfusion Injury, Endothelial Cell Protection, and Limb Preservation in a Prolonged Field Care Scenario

**5a. CONTRACT NUMBER**
W81XWH-19-1-0714

**5b. GRANT NUMBER**
OR180176

**5c. PROGRAM ELEMENT NUMBER**

**6. AUTHOR(S)**

**Robert Rieben,**

**E-Mail:** robert.rieben@dbmr.unibe.ch

**5d. PROJECT NUMBER**

**5e. TASK NUMBER**

**5f. WORK UNIT NUMBER**

**7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)**
Universität Bern
Hochschulstrasse 6
3012 Bern, Switzerland

**8. PERFORMING ORGANIZATION REPORT NUMBER**
3

**9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)**
U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

**10. SPONSOR/MONITOR'S ACRONYM(S)**
CDMRP

**11. SPONSOR/MONITOR'S REPORT NUMBER(S)**

**12. DISTRIBUTION / AVAILABILITY STATEMENT**
Approved for Public Release, Distribution Unlimited

**13. SUPPLEMENTARY NOTES**

**14. ABSTRACT**
In this project, endothelial cell protection and extracorporeal machine perfusion will be combined to achieve a massive prolongation of the time between traumatic amputation and replantation. In the third year, all animal experiments for Milestone 2: 'Validation of ex vivo – in vivo models for the effect of EC protection on extremity I/R injury in a PFC scenario' were performed, as well as most of the respective laboratory analyses. We found that the use of the endothelial cell protectant C1-INH led to reduced histological tissue damage in the reperfused limbs, both ex vivo and in vivo after replantation. However, this did not result in less weight increase or a lower wet/dry ratio of ex vivo reperfused limbs. In addition, we could show that replantation and in vivo reperfusion of a 9h ischemic limb led to remote organ damage of lungs, kidney and liver, which was clearly visible by classical HE-staining as well as by immunofluorescence analysis for complement deposition. This project was granted a cost-neutral 12-month extension, and all aims as set out in the original SOW will be reached until 08/23.

**15. SUBJECT TERMS**
Ischemia/reperfusion injury, complement, endothelial activation, muscle damage, perfusion time, glycocalyx

| **16. SECURITY CLASSIFICATION OF:** | | | **17. LIMITATION OF ABSTRACT** | **18. NUMBER OF PAGES** | **18. NUMBER OF RESPONSIBLE PERSON** |
|---|---|---|---|---|---|
| | | | | | USAMRDC |
| **a. REPORT** | **b. REPORT** | **c. THIS PAGE** | Unclassified | 17 | **19. TELEPHONE NUMBER** *(include area code)* |
| Unclassified | Unclassified | Unclassified | | | |

## TABLE OF CONTENTS

1. **INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

   The study consists in the development of *in vivo* and *ex vivo* models to assess strategies for the prevention of ischemia reperfusion (I/R) injury in a Prolonged Field Care (PFC) scenario. There is a limited intervention capacity at the point of injury that may force the use of battlefield tourniquet to prevent fatal blood loos. Injured Service members then need to be transported to a hospital where revascularization or replantation can be performed. As this may take several hours or even days, severe I/R injury will occur. Extensive muscle tissue damage will be the local consequence in the affected extremity, but severe I/R injury may also lead to systemic inflammatory response syndrome and even multiorgan failure. The models to be developed will therefore be used to provide guidance in the management of I/R injury and to test technical feasibility and clinical efficacy of promising therapeutic interventions in the RUCK-TRUCK-HOUSE-PLANE operational context. Our project aims at reducing I/R injury after surgical revascularization or replantation of traumatically devascularized or amputated extremities in a PFC scenario in order to reduce limb loss and prevent systemic consequences of I/R injury. The goal of the project is to prove that a combination of pharmacological endothelial protection via simple perfusion of the devascularized or amputated extremity at RUCK or TRUCK level, followed by machine perfusion of the extremity at HOUSE / PLANE level, will allow prevention of I/R injury and successful surgical revascularization or replantation even if performed 24 or more hours after the injury was incurred.

2. **KEYWORDS:** *Provide a brief list of keywords (limit to 20 words).*

   Ischemia/reperfusion injury, complement, endothelial activation, muscle damage, perfusion time, glycocalyx shedding

3. **ACCOMPLISHMENTS:** *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.*

   **What were the major goals of the project?**

   *List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.*

   **Our specific aims are:**

   **1. Develop an ex vivo – in vivo large-animal model to study the consequences of I/R injury in extremity vascular injury as occurring in a PFC scenario.**

   Milestone 1: Establishment of ex vivo – in vivo models for extremity I/R injury in a PFC scenario
   Target date: CY19/20-Models for extremity I/R injury in a PFC scenario.
   Completion: Both -*ex vivo* and -*in vivo* models have been established.

   **2. Validate these models for the screening of pharmacological interventions that can be applied at RUCK/TRUCK level to promote EC protection and reduce/control I/R injury.**

Milestone 2: Validation of ex vivo – in vivo models for the effect of EC protection on extremity I/R injury in a PFC scenario.
Target date: End CY20 and CY21.
Completion: All animal experiments and most of the laboratory analyses were completed by 08/22.

**3. Combine and test the full PFC scenario comprising pharmacological EC protection and prolonged machine perfusion in the surgical replantation setting.**
Milestone 3: Experimental assessment of the whole PFC scenario for massive prolongation of the time window to replantation / revascularization as compared to the situation today – 33 hours vs. 6 hours.
Target date: CY22 – the project has been prolonged to 8/23, which is the new target date for this milestone

## What was accomplished under these goals?

*For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.*

**Validation of the models for testing the efficacy of pharmacological interventions aimed at preventing endothelial cell (EC) activation at RUCK/TRUCK stage**

Major activities and specific objectives

1. Performed experiments with both ex vivo and in vivo reperfusion using C1-INH as EC protectant after 2h of ischemia, followed by another 7h of ischemia. Reperfusion was for 12h, either ex vivo at the heart-lung-machine (groups 5-6) or in vivo after replantation of the limbs (groups 7-8). Completed the groups 5 to 8, all blinded for C1-INH or vehicle in the vascular rinse solution.
2. Analyzed the clinical data (limb weight post/pre-reperfusion, wet/dry ratio) and muscle samples of reperfused limbs of groups 5-8. Standard histology as well as immunofluorescence analysis was performed for complement and antibody deposition as well as EC activation.
3. Analyzed the tissue samples of remote organs (lung, kidney, liver) of in vivo groups 7-8. Standard histology as well as immunofluorescence analysis was performed for complement and antibody deposition as well as EC activation.

Significant results and/or key outcomes, including major findings, developments and/or conclusions

1. No difference in limb weight of ex vivo perfused pig limbs after 9h ischemia limbs with or without vascular rinse by C1-INH

9h ischemic limbs treated with either C1-INH or vehicle buffer were weighed at baseline (immediately after amputation) and at endpoint after 12h of ex vivo reperfusion. There was no difference in the limb weight between two groups (Fig. 1A), nor in the wet/dry ratios of the limbs (Fig. 1B).

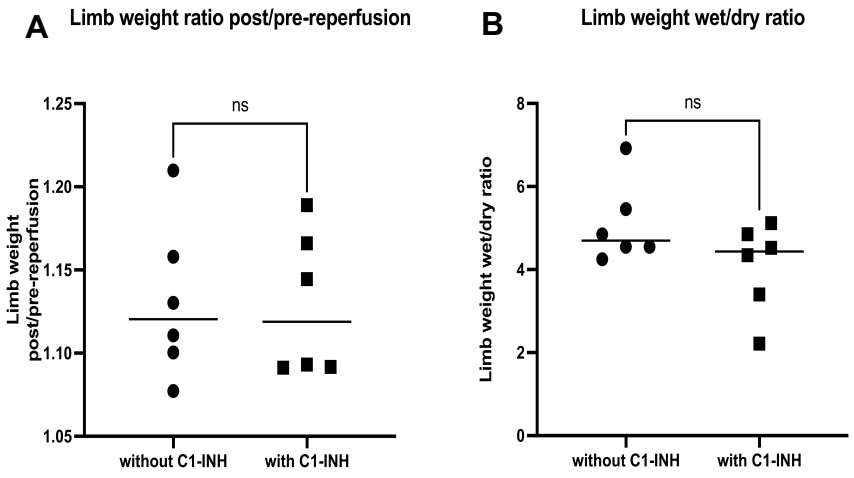


**Figure 1. (A) Limb weight ratio post- / pre-perfusion.** Amputated limbs were weighed before and after ex vivo reperfusion. Post- to pre-reperfusion weight ratios are shown for vascular rinse of the limbs after 2h of ischemia with vehicle buffer (without C1-INH) or with C1-INH. **(B) Limb wet/dry ratio after extracorporeal reperfusion.** Muscle tissue biopsies were weighed immediately after extracorporeal perfusion for 12h and after they were left to try dry for 40h at 80°C. Data are presented as dots for a single experiment with indication of the median values by a horizontal line. Inter-group differences were non-significant as tested by Mann Whitney U-test (n=6 per group).

2. Reduced histological tissue damage in ex vivo perfused limbs perfused with C1-INH solution to protect the endothelium

HE staining of muscle tissue samples from 9h ischemic limbs that underwent extracorporeal perfusion for 12h reveals extensive tissue damage. However, vascular rinse of the limbs by C1-INH solution after 2h of ischemia reduced the damage as revealed both by HE- and dystrophin-staining (Fig. 2).

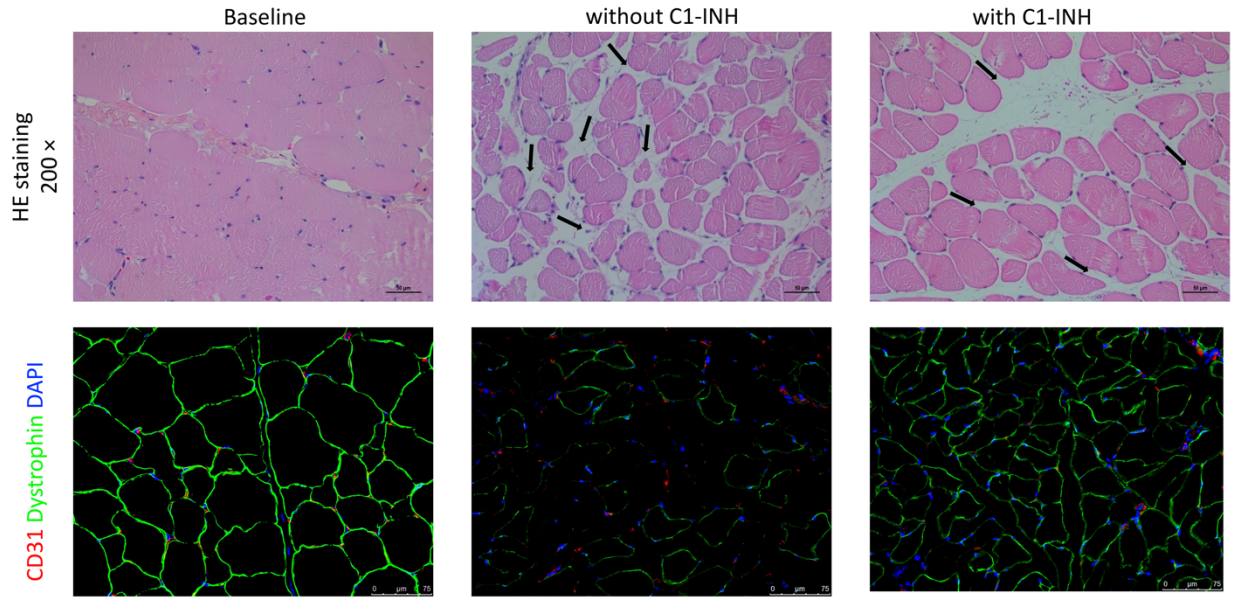


**Figure 2. HE staining (upper panel) and Dystrophin distribution (lower panel) in muscle of porcine limbs after extracorporeal reperfusion with or without C1-INH intervention.** Black arrows show the formation of edema. Dystrophin (in green) distribution in limb tissue collected at baseline (before perfusion) (left), from ischemic limbs (9h of ischemia) after extracorporeal perfusion without C1-INH intervention (middle), and with C1-INH intervention (right).

3. Lung injury after 9-hour ischemia, followed by 12h in vivo reperfusion

Lung injury is a feared systemic consequence of reperfusion of an extremity after prolonged ischemia. It can cause the acute respiratory distress syndrome (ARDS), which may further develop

into multiorgan failure. To investigate lung injury in our in vivo reperfusion model, we compared the histological changes between replanted limbs after 9h and 1h ischemia. HE staining showed that there was diffuse alveolar damage, characterized by edema, cellular debris, and early hyaline membrane formation, as well as immune cell infiltration, in pigs which had a limb replanted after 9h of ischemia. In contrast, much less histological lung damage was observed when limbs were replanted after only 1h of ischemia (Figure 3).

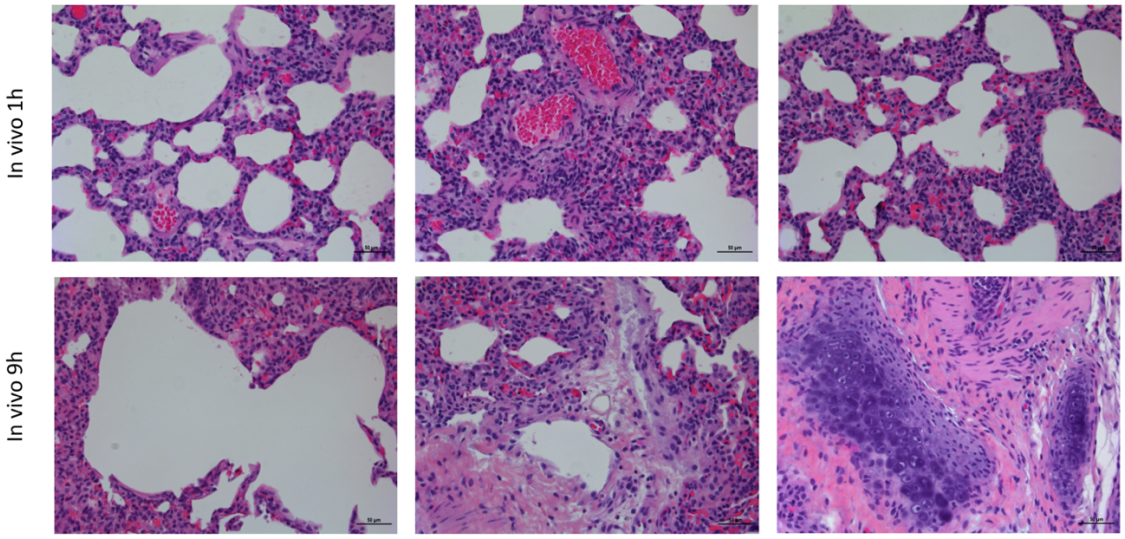


**Figure 3. Lung damage after 9h ischemia** (HE staining, x200).

4. Kidney injury after 9-hour ischemia, followed by 12h in vivo reperfusion

To investigate kidney damage as a systemic consequence of I/R injury, we compared the histological changes in kidneys of pigs whose limbs were replanted after 9h and 1h of ischemia, respectively. HE staining showed normal kidney histology when limbs were replanted after 1h ischemia. In contrast, replantation of limbs after 9h ischemia led to massive histological kidney injury with loss of cell integrity and intracellular vacuolization, injured tubules and debris (Figure 4).

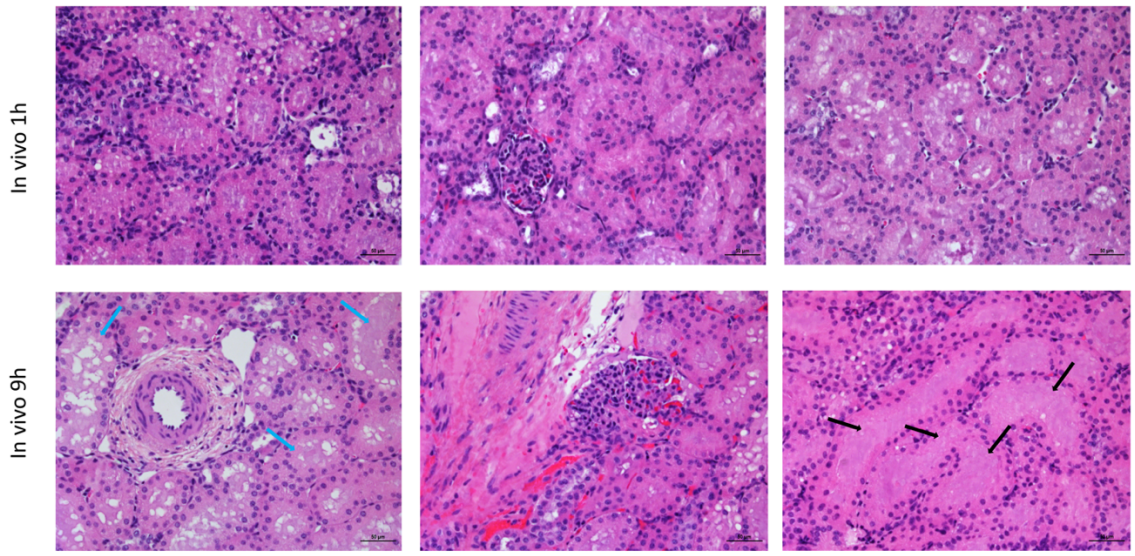


**Figure 4. Kidney damage after 9h ischemia** (HE staining, x200).

5. Liver injury after 9h ischemia upon in vivo reperfusion

Similar to lung and kidney injury, HE staining of liver samples showed that histological appearance was normal after replantation of a 1h-ischemic limb, while it was clearly pathological when limbs were replanted after 9h of ischemia. In the latter pigs, HE staining showed dilatation, congestion, hemorrhagic areas, and immune cell infiltration. In some areas of the liver after 9h ischemia, there were also increased fiber deposits and even necrosis and edema around the central vein and portal areas (Figure 5).

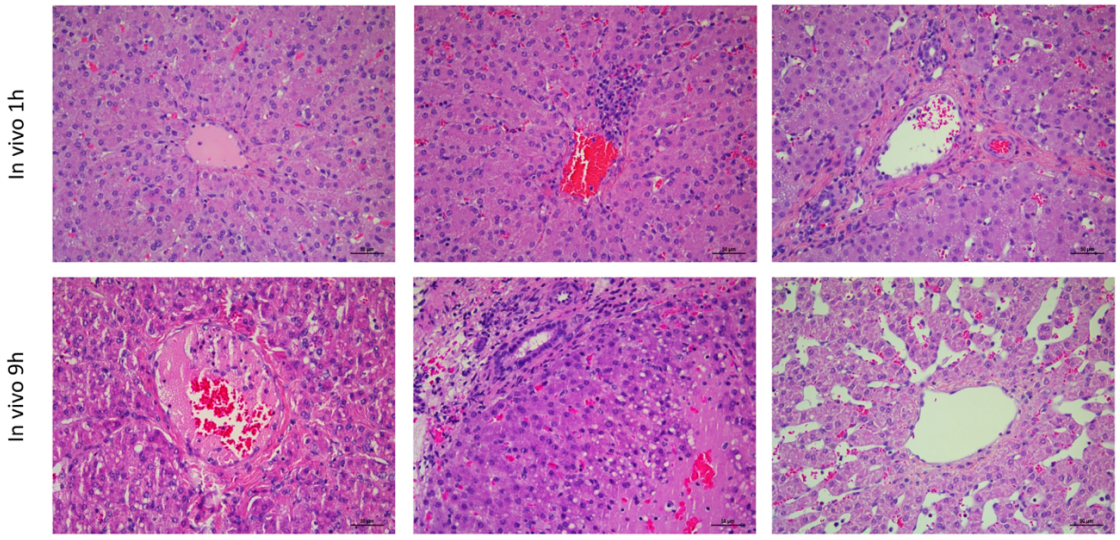


**Figure 5. Liver damage after 9h ischemia** (HE staining, x200).

6. Effect of C1-INH on remote lung damage after replantation of a 9h-ischemic limb

HE stainings of remote organs for groups 7-8, in which limbs were replanted after 9h of ischemia, with or without C1-INH treatment, are still being processed. However, results of immunofluorescence stainings for deposition of the complement components C5b-9 and C4b/c are available. Complement deposition in tissue is a classical hallmark of damage by the innate immune system / inflammation. C1-INH is an inhibitor of the classical complement cascade, and it was shown earlier, that it is able to reduce local I/R injury of the limbs when these are rinsed with a C1-INH containing solution 2h after the onset of ischemia. C1-INH was not used systemically in the replantation experiments and the hypothesis was therefore, that a reduction of local I/R injury of the limbs would also reduce the damage of remote organs like lung, kidney, and liver.

Immunofluorescence stainings showed that the terminal complement component C5b-9 was largely deposited in the porcine lungs after in vivo reperfusion of 9h ischemic limbs for 12h. A vascular rinse of the limbs with C1-INH after 2h of ischemia seems to reduce C5b-9 deposition in lungs (Figure 6). A similar picture was also seen for deposition the classical pathway component C4b/c (Figure 7). Together these still preliminary data suggest that the vascular rinse of the amputated limbs with a C1-INH containing solution not only has local effects in the replanted limbs – as shown above for ex vivo perfused limbs – but also leads to a lower remote organ injury after in vivo replantation.

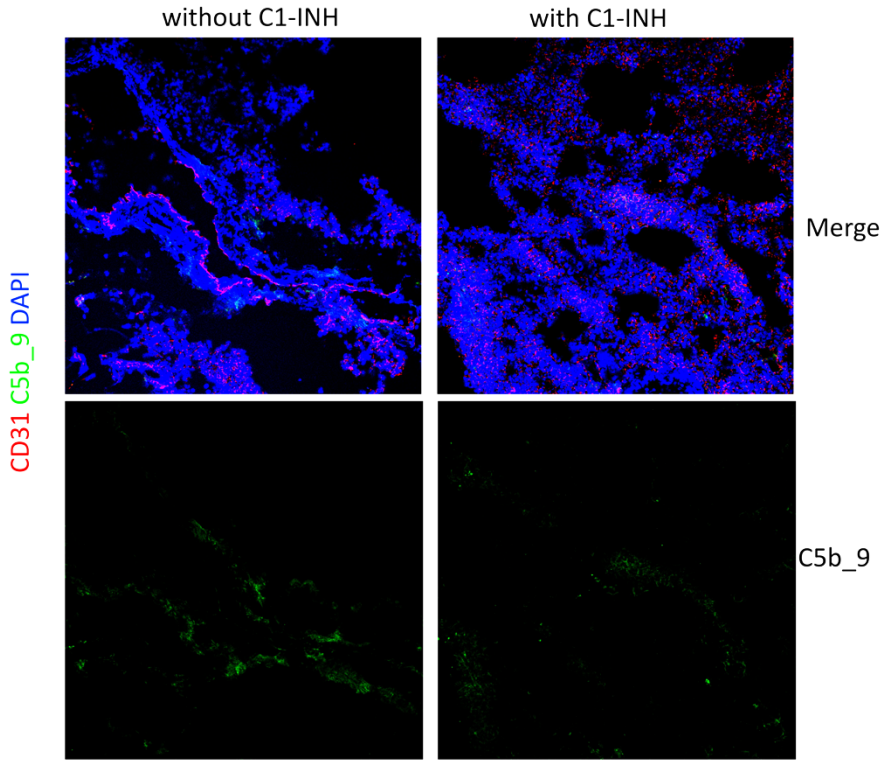


**Figure 6. C5b-9 deposition in lungs after 9h limb ischemia without or with C1-INH intervention upon in vivo reperfusion:** DAPI (in blue), CD31 (in red), C5b_9 (in green) distribution in lung tissue collected at endpoint (after 12h reperfusion), without C1-INH intervention (left), and with C1-INH intervention (right). The upper panel shows the merged image with all the colors, lower panel only shows C5b-9 in green.

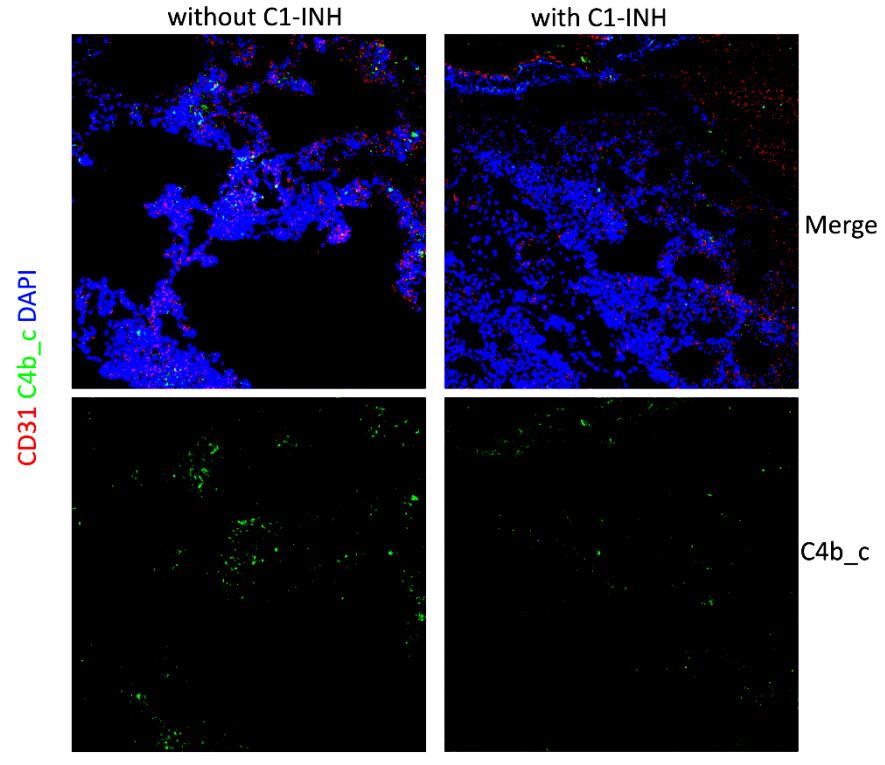


**Figure 7. C4b/c deposition in lungs after 9h limb ischemia without or with C1-INH intervention upon in vivo reperfusion:** DAPI (in blue), CD31 (in red), C4b_c (in green) distribution in lung tissue collected at endpoint (after reperfusion), without C1-INH intervention (left), and with C1-INH intervention (right). The upper panel shows the merged image with all the colors, the lower panel only shows C4b/c in green.

7. Effect of C1-INH on remote kidney damage after replantation of a 9h-ischemic limb
Immunofluorescent staining showed that, similar to lungs, C5b-9 was also deposited in the kidneys of pigs whose limbs were replanted after 9h of ischemia. Again, this was more pronounced when no C1-INH rinse was used before replantation (Figure 8).

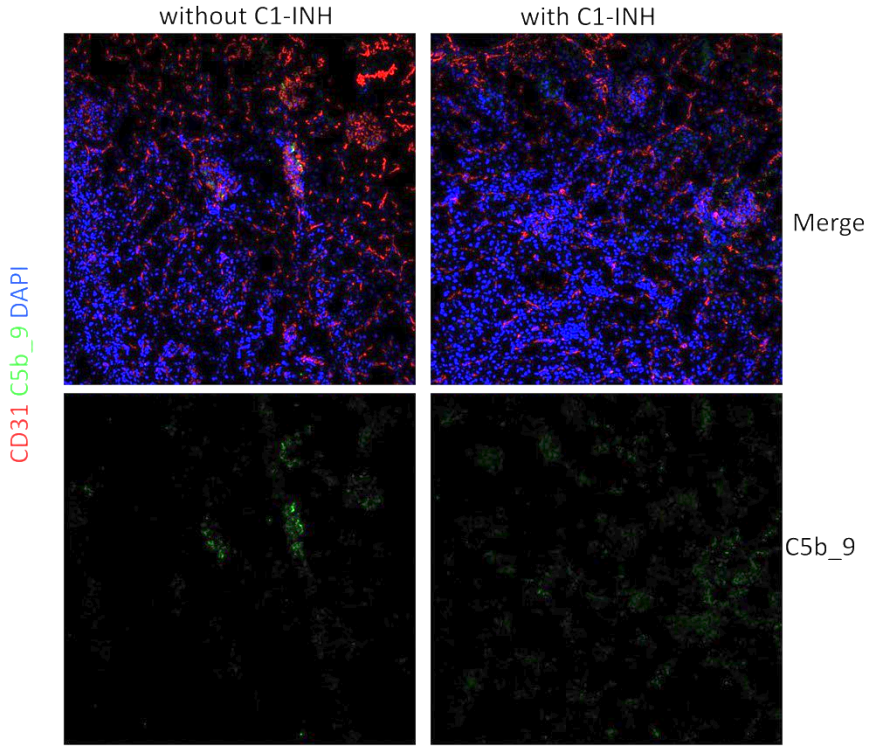


**Figure 8. C5b-9 deposition in the kidney after 9h limb ischemia without or with C1-INH intervention upon in vivo reperfusion:** DAPI (in blue), CD31 (in red), C5b_9 (in green) distribution in lung tissue collected at endpoint (after reperfusion), without C1-INH intervention (left), and with C1-INH intervention (right). The upper panel shows the merged image with all the colors, the lower panel only shows C5b_9 in green.

**What opportunities for training and professional development has the project provided?**
*If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."*

*Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.*

While not formally part of this project, training for medical students and professional development for assistant surgeons was provided for early career colleagues who attended the in vivo work in the Experimental Surgery Facility or analyses of tissue and blood samples in the wet lab. Medical students also prepared poster and oral presentation for internal meetings.

**How were the results disseminated to communities of interest?**
*If there is nothing significant to report during this reporting period, state "Nothing to Report."*

*Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.*

Nothing to report.

**What do you plan to do during the next reporting period to accomplish the goals?**
*If this is the final report, state "Nothing to Report."*

*Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.*

The project has been granted a cost-neutral extension for one year. This last year will be used to perform all animal experiments planned in WP3 'Combine and test the full PFC scenario comprising pharmacological EC protection and prolonged machine perfusion in the surgical replantation setting' as well as the related laboratory analyzes. The remaining analyzes of WP2 will also be performed and the two manuscripts, which are currently under preparation, finalized and submitted for publication. We should then be ready to report our final results at the 2023 MHSRS, expecting that this meeting will take place in autumn 2023, and submit the third manuscript, focusing on the data of WP3, towards the end of 2023. All work requiring financial support by the DoD will be finished by August 30, 2023.

***4.*** **IMPACT:** D*escribe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

**What was the impact on the development of the principal discipline(s) of the project?**
*If there is nothing significant to report during this reporting period, state "Nothing to Report."*

*Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).*

Nothing to report as yet, publications pending

**What was the impact on other disciplines?**
*If there is nothing significant to report during this reporting period, state "Nothing to Report."*

*Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.*

Nothing to report

**What was the impact on technology transfer?**
*If there is nothing significant to report during this reporting period, state "Nothing to Report."*

*Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:*

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to report

**What was the impact on society beyond science and technology?**
*If there is nothing significant to report during this reporting period, state "Nothing to Report."*

*Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:*

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to report

5. **CHANGES/PROBLEMS:** *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:*

An NCE was granted to this project until 08/23.

**Changes in approach and reasons for change**
*Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.*

There will be no changes to the project with the exception of the shifted timeline.

**Actual or anticipated problems or delays and actions or plans to resolve them**
*Describe problems or delays encountered during the reporting period and actions or plans to resolve them.*

As described in our NCE request, the reasons for the delay of our project are purely logistic. Our Experimental Surgery Facility was closed for a whole year due to the COVID pandemic and renovation. Many projects therefore incurred a delay and we have not been able to catch up with

our own project. The last series of experiments will now be performed in spring and early summer 2023. All milestones and deliverables remain the same and will be met / provided.

**Changes that had a significant impact on expenditures**
*Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.*

Nothing to report

**Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**
*Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.*

**Significant changes in use or care of human subjects**

Nothing to report

**Significant changes in use or care of vertebrate animals**

Our IACUC permit had to be renewed because the experiments will take longer than the maximum possible permit duration of 3 years. As formally the new IACUC permit was a rewrite, also the ACURO approval required a review. This has been done and we now have a new ACURO permit as well.

**Significant changes in use of biohazards and/or select agents**

Nothing to report

6. **PRODUCTS:** *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

- **Publications, conference papers, and presentations**
  *Report only the major publication(s) resulting from the work under this award.*

  **Journal publications.** *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

  Nothing to report

**Books or other non-periodical, one-time publications.** *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to report

**Other publications, conference papers and presentations**. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

**Poster presentation at the 18th Meeting on Complement in Human Disease, Bern, Switzerland**

Junhua Wang, Stefanie Hirsiger, Christine Lee, Lena Fuest, Bilal Ben Brahim, Valentina Zollet, Isabel Arenas Hoyos, Daniela Casoni, Hansjoerg Jenni, Alain Despont, Kay Nettelbeck, Luisana García, Yara Banz, Kirsten Irmler, Carole Gygax, Jane Shaw-Boden, Nicoletta Sorvillo, Esther Vögelin, Robert Rieben

**C1-inhibitor is protective against local and remote reperfusion injury of amputated pig limbs after prolonged ischemia**

*Molecular Immunology 2020, 150:186*

- **Website(s) or other Internet site(s)**
  *List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.*

  Nothing to report

- **Technologies or techniques**
  *Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.*

  Nothing to report

- **Inventions, patent applications, and/or licenses**
  *Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.*

Nothing to report

- **Other Products**

  *Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:*
  - *data or databases;*
  - *physical collections;*
  - *audio or video products;*
  - *software;*
  - *models;*
  - *educational aids or curricula;*
  - *instruments or equipment;*
  - *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
  - *clinical interventions;*
  - *new business creation; and*
  - *other.*

  Nothing to report

## 7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

**What individuals have worked on the project?**

*Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".*

Name: **Junhua Wang**
Project Role: Scientific Associate
Researcher Identifiers: ORCID 0000-0002-6110-3711 Nearest person month worked: 5.9
Contribution to Project: Prepare the experiments, perform and lead the experiments, perform the lab measurements, collect the samples, archive and record the project results, contribute to the reports.

Name: **Robert Rieben**
Project Role: PI
Researcher Identifiers: ORCID 0000-0003-4179-8891 Nearest person month worked: 1.2
Contribution to Project: Global supervision of the project.

Name: **Esther Vögelin**
Project Role: Co-PI
Researcher Identifier: ORCID: 0000-0003-4179-8891

Nearest person month worked: 1.2
Contribution to Project: Responsible for all surgical aspects of the project.

Name: **Nicoletta Sorvillo**
Project Role: Senior Research Assistant
Researcher Identifiers: none
Nearest person month worked: 2.1
Contribution to Project: Analyze the data obtained from both ex vivo and in vivo re-perfusion.

**Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**
*If there is nothing significant to report during this reporting period, state "Nothing to Report."*

*If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.*

Nothing to report

**What other organizations were involved as partners?**
*If there is nothing significant to report during this reporting period, state "Nothing to Report."*

*Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.*

*Provide the following information for each partnership:*
*Organization Name:*
*Location of Organization: (if foreign location list country)*
*Partner's contribution to the project (identify one or more)*

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

Nothing to report

## 8. SPECIAL REPORTING REQUIREMENTS

**COLLABORATIVE AWARDS:** *For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to* ***https://ebrap.org/eBRAP/public/index.htm*** *for each unique award.*

**QUAD CHARTS:** *If applicable, the Quad Chart (available on https://www.usamraa.army.mil/Pages/Resources.aspx) should be updated and submitted with attachments.*

An updated Quad Chart has been submitted.

## 9. APPENDICES: *Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.*