Call: HORIZON-HLTH-2025-01

(Cluster 1 - Health (Single stage - 2025))

Topic: HORIZON-HLTH-2025-01-TOOL-02

Type of Action: HORIZON-RIA

(HORIZON Research and Innovation Actions)

Proposal number: 101286237

Proposal acronym: APO2FOOT

Type of Model Grant Agreement: HORIZON Action Grant Budget-Based

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Proposal ID **101286237**

Acronym **APO2FOOT**

1 - General information

Fields marked * are mandatory to fill.

Topic	HORIZON-HLTH-2025-01-TOOL-02	Type of Action	HORIZON-RIA
Call	HORIZON-HLTH-2025-01	Type of Model Grant Agreement	HORIZON-AG

Acronym: APO2FOOT

Proposal title: A randomized, open label, observer-blinded clinical trial to evaluate safety and efficacy of APO-2 in comparison to Standard of Care in the treatment of diabetic foot ulcers

Note that for technical reasons, the following characters are not accepted in the Proposal Title and will be removed: < > " &

Duration in months: 60

Fixed keyword 1: Medical biotechnology

Fixed keyword 2: Regenerative medicine

Free keywords: *secretome angiogenesis Diabetic foot ulcers*

Abstract *

Diabetic foot ulcers (DFUs) are one of the most severe complications of diabetes, affecting nearly one million people in the EU every year. Chronic DFUs fail to heal within months, leading to infections, amputations, and a five-year mortality rate exceeding that of many cancers. Current standard care (blood sugar control, debridement, wound dressings, off-loading) is often insufficient, and available adjunctive therapies show limited efficacy, high costs, or lack regulatory approval. This situation creates an urgent medical, societal, and economic challenge for Europe's healthcare systems.
APO2FOOT will demonstrate the safety and efficacy of APO-2, a novel off-the-shelf secretome therapy, in a clinical phase II study. It will be the first effective and reimbursable treatment to accelerate healing of chronic diabetic foot ulcers and reduce amputations
APO2FOOT clinically validates APO-2, a novel, cell-free secretome therapy. Unlike cell-based treatments, APO-2 is stable, virus-inactivated, easily stored, and can be applied topically as an "off-the-shelf" product. Early clinical trials have shown that APO-2 accelerates wound healing by promoting angiogenesis, re-epithelialisation, and anti-inflammatory effects, resulting in faster and more complete wound closure. The project will conduct a multicentre, multinational, observer-blinded phase II clinical trial with 120 patients across 5 EU countries, comparing APO-2 to SoC . Key innovations include central wound adjudication, digital imaging tools, and harmonised treatment protocols, ensuring robust and reliable clinical data. By delivering conclusive evidence of safety and efficacy, APO2FOOT aims to establish APO-2 as the first effective, accessible, and reimbursable active treatment for chronic DFU. The expected impact is fewer amputations, improved patient QoL, reduced mortality, and substantial savings for healthcare systems, while positioning Europe at the forefront of secretome-based regenerative medicine.

Remaining characters 7

Has this proposal (or a very similar one) been submitted in the past 2 years in response to a call for proposals under any EU programme, including the current call? ○ Yes ◉ No

Please give the proposal reference or contract number.
Previously submitted proposals should be with either 6 or 9 digits.

Administrative forms

Proposal ID **101286237**

Acronym **APO2FOOT**

Declarations

Field(s) marked * are mandatory to fill.

1) We declare to have the explicit consent of all applicants on their participation and on the content of this proposal. * ☒

2) We confirm that the information contained in this proposal is correct and complete and that none of the project activities have started before the proposal was submitted (unless explicitly authorised in the call conditions). * ☒

3) We declare:
- to be fully compliant with the eligibility criteria set out in the call
- not to be subject to any exclusion grounds under the EU Financial Regulation 2018/1046
- to have the financial and operational capacity to carry out the proposed project. * ☒

4) We acknowledge that all communication will be made through the Funding & Tenders Portal electronic exchange system and that access and use of this system is subject to the Funding & Tenders Portal Terms and Conditions. * ☒

5) We have read, understood and accepted the Funding & Tenders Portal Terms & Conditions and Privacy Statement that set out the conditions of use of the Portal and the scope, purposes, retention periods, etc. for the processing of personal data of all data subjects whose data we communicate for the purpose of the application, evaluation, award and subsequent management of our grant, prizes and contracts (including financial transactions and audits). * ☒

6) We declare that the proposal complies with ethical principles (including the highest standards of research integrity as set out in the ALLEA European Code of Conduct for Research Integrity, as well as applicable international and national law, including the Charter of Fundamental Rights of the European Union and the European Convention on Human Rights and its Supplementary Protocols. Appropriate procedures, policies and structures are in place to foster responsible research practices, to prevent questionable research practices and research misconduct, and to handle allegations of breaches of the principles and standards in the Code of Conduct. * ☒

7) We declare that the proposal has an exclusive focus on civil applications (activities intended to be used in military application or aiming to serve military purposes cannot be funded). If the project involves dual-use items in the sense of Regulation 2021/821, or other items for which authorisation is required, we confirm that we will comply with the applicable regulatory framework (e.g. obtain export/import licences before these items are used). * ☒

8) We confirm that the activities proposed do not
- aim at human cloning for reproductive purposes;
- intend to modify the genetic heritage of human beings which could make such changes heritable (with the exception of research relating to cancer treatment of the gonads, which may be financed), or
- intend to create human embryos solely for the purpose of research or for the purpose of stem cell procurement, including by means of somatic cell nuclear transfer.
- lead to the destruction of human embryos (for example, for obtaining stem cells)

These activities are excluded from funding. * ☒

9) We confirm that for activities carried out outside the Union, the same activities would have been allowed in at least one EU Member State. * ☒

The coordinator is only responsible for the information relating to their own organisation. Each applicant remains responsible for the information declared for their organisation. If the proposal is retained for EU funding, they will all be required to sign a declaration of honour.

False statements or incorrect information may lead to administrative sanctions under the EU Financial Regulation.

2 - Participants

List of participating organisations

#	Participating Organisation Legal Name	Country	Role	Action
1	[redacted]	AT	Coordinator	
2	[redacted]	Austria	Partner	
3	Aposcience AG	AT	Partner	
4	[redacted]	FR	Partner	
5	[redacted]	Czechia	Affiliated	
6	[redacted]	AT	Partner	
7	[redacted]	AT	Partner	
8	[redacted]	DE	Partner	
9	[redacted]	Germany	Partner	
10	[redacted]	CH	Partner	
11	[redacted]	SK	Partner	
12	[redacted]	PT	Partner	
13	[redacted]	AT	Partner	

Organisation data

PIC	Legal name
999989976	[redacted]

Short name: MUW

Address

Street	[redacted]
Town	[redacted]
Postcode	[redacted]
Country	Austria
Webpage	[redacted]

Specific Legal Statuses

Legal person	yes
Public body	yes
Non-profit	yes
International organisation	no
Secondary or Higher education establishment	yes
Research organisation	yes

SME Data

Based on the below details from the Participant Registry the organisation is not an SME (small- and medium-sized enterprise) for the call.

SME self-declared status	01/04/2004 - no
SME self-assessment	unknown
SME validation	unknown

Departments carrying out the proposed work

Department 1

Department name [redacted] ☐ not applicable

☒ Same as proposing organisation's address

Street [redacted]

Town [redacted]

Postcode [redacted]

Country Austria

Links with other participants

Type of link	Participant

Main contact person

This will be the person the EU services will contact concerning this proposal (e.g. for additional information, invitation to hearings, sending of evaluation results, convocation to start grant preparation). The data in blue is read-only. Details (name, first name and e-mail) of Main Contact persons should be edited in the step "Participants" of the submission wizard.

Title Prof.

Gender ◯ Woman ◉ Man ◯ Non Binary

First name* [redacted]

Last name* [redacted]

E-Mail* [redacted]

Position in org. [redacted]

Department [redacted]

☒ Same as organisation name

☒ Same as proposing organisation's address

Street [redacted]

Town [redacted]

Post code 1090

Country Austria

Website [redacted]

Phone [redacted]

Phone 2 [redacted]

Other contact persons

First Name	Last Name	E-mail	Phone
[redacted]	[redacted]	[redacted]	+xxx xxxxxxxxx
[redacted]	[redacted]	[redacted]	+xxx xxxxxxxxx
[redacted]	[redacted]	[redacted]	+xxx xxxxxxxxx
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]

Researchers involved in the proposal

Title	First Name	Last Name	Gender	Nationality	E-mail	Career Stage	Role of researcher (in the project)	Reference Identifier	Type of identifier
[redacted]									
[redacted]									
[redacted]									
[redacted]									
[redacted]									

Role of participating organisation in the project

Project management	☒
Communication, dissemination and engagement	☒
Provision of research and technology infrastructure	☒
Co-definition of research and market needs	☐
Civil society representative	☐
Policy maker or regulator, incl. standardisation body	☐
Research performer	☒
Technology developer	☐
Testing/validation of approaches and ideas	☒
Prototyping and demonstration	☐
IPR management incl. technology transfer	☐
Public procurer of results	☐
Private buyer of results	☐
Finance provider (public or private)	☐
Education and training	☒
Contributions from the social sciences or/and the humanities	☐
Other If yes, please specify: (Maximum number of characters allowed: 50)	☐

List of up to 5 publications, widely-used datasets, software, goods, services, or any other achievements relevant to the call content.

Type of achievement	Short description (Max 500 characters)
Publication	*Review Journal Investigative Dermatology: Therapeutic Application of Cell Secretomes in Cutaneous Wound Healing. doi: 10.1016/j.jid.2023.02.019 Application of stem cells to chronic wounds emerged as a candidate therapy, however the mechanism of action remains unclear. In the last two decades, stem cell secretomes as well as PBMC secretome-based therapies have been expended. In this study, we review the modes of action of cell secretomes in wound healing.*
Publication	*Sci Rep. 2015 Nov 16:5:16662. doi: 10.1038/srep16662. Analysis of the Secretome of Apoptotic Peripheral Blood Mononuclear Cells: Impact of Released Proteins and Exosomes for Tissue Regeneration.* *This study aimed to characterize the secretome of PBMCs to investigate its biologically active components in vitro and vivo. Our results indicate that irradiation augments the release of proteins, lipid-mediators and extracellular vesicles into the PBMC secretome.*
Publication	*Cell Death Dis. 2019 Sep 30;10(10):729. doi: 10.1038/s41419-019-1974-6. Tissue-regenerative potential of the secretome of γ-irradiated peripheral blood mononuclear cells is mediated via TNFRSF1B-induced necroptosis.* *Our study demonstrates that the pro-angiogenic activity of the secretome of γ-irradiated PBMCs requires interplay of different PBMC subpopulations. These findings contribute to a better understanding of secretome therapies in regenerative medicine.*
Publication	*Eur Heart J. 2015 Mar 14;36(11):676-85. doi: 10.1093/eurheartj/ehs459. Mononuclear cell secretome protects from experimental autoimmune myocarditis.* *Cultured mononuclear cells (MNC) secrete immunomodulating factors which have canattenuate detrimental inflammatory in vivo. Experimental autoimmune myocarditis (EAM) is a CD4+ T cell-dependent model to determine the influence of MNC secretome on myocardial inflammation. In this work we show that PBMC secretome abrogated myocardial inflammation.*
Publication	*Review Apoptosis. 2016 Dec;21(12):1336-1353. doi: 10.1007/s10495-016-1292-8. Peripheral blood mononuclear cell secretome for tissue repair. Secreted paracrine factors are increasingly accepted to exert beneficial biological effects that promote tissue regeneration. The PBMC secretome includes a variety of proteins, lipids, microRNAs, and extracellular vesicles, such as exosomes. We discuss pre-clinical studies with apoptotic cell-based therapies and regulatory issues that have to be considered.*

List of up to 5 most relevant previous projects or activities, connected to the subject of this proposal.

Name of Project or Activity	Short description (Max 500 characters)
Clinical Phase I/II trial 1994, Clinic of Surgery	*Published in: Clinical Trial Eur J Vasc Surg. 1994 May;8(3):351-6. doi: 10.1016/s0950-821x(05)80155-0. Treatment of non-healing skin ulcers with autologous activated mononuclear cells. This study nvestigate whether cultured autologous mononuclear cells (MNC) effectively improve wound healing. MNC group augmented wound healing as compared to control. The work of Holzinger et al. is the first cell based therapy trial in chronic wound treatment that was reported.*
GMP production of Aposec	*Published in: Stem Cell Res Ther. 2020 Jan 3;11(1):9. doi: 10.1186/s13287-019-1524-2. Reproducibility of GMP-compliant production of therapeutic stressed peripheral blood mononuclear cell-derived secretomes, a novel class of biological medicinal products. Good manufacturing practice (GMP)-compliant production guarantees high batch-to-batch consistency, reproducible efficacy and stability.*

Phase I trial utilizing autologous Aposec	*Published in: Clinical Trial Sci Rep. 2017 Jul 24;7(1):6216. doi: 10.1038/s41598-017-06223-x. Safety and tolerability of topically administered autologous, apoptotic PBMC secretome (Aposec) in dermal wounds: a randomized Phase 1 trial (MARSYAS I). No therapy-related serious adverse events occurred in any of the participants, and both low- and high-dose treatments were well tolerated. Wound closure was not affected by Aposec therapy in this 7 day intervention trial. (together with APOSC)*
Phase I/II trial utilizing allogeneic Aposec	*Published in: Trials. 2021 Jan 6;22(1):10. doi: 10.1186/s13063-020-04948-1. Safety and clinical efficacy of the secretome of stressed PBMC in patients with diabetic foot ulcer-study protocol of the randomized, placebo-controlled, double-blind, multicenter, international phase II clinical is presented. The IMP Aposec, a novel, innovative drug, was tested in the phase I/II study MARSYAS II. Aposec therapy was be applied for 4 weeks (EOT). (together with APOSC)*
GMP Aposec and GLP Toxikology for human trials	*Published in: Sci Rep. 2019 Apr 3;9(1):5598. doi: 10.1038/s41598-019-42057-5. Toxicological testing of allogeneic secretome derived from peripheral mononuclear cells (Aposec). Regulatory authorities classify these secretomes as biological medicinal products and non-clinical safety assessment thus falls under the scope of ICH S6. Acute and repeated-dose toxicity studies evidenced that Aposec administered intravenously and subcutaneously was safe*

Description of any significant infrastructure and/or any major items of technical equipment, relevant to the proposed work.

Name of infrastructure of equipment	**Short description (Max 300 characters)**
MUW *Surgical Research Laboratory*	

Gender Equality Plan

Does the organization have a Gender Equality Plan (GEP) covering the elements listed below? (•) Yes ( ) No

Minimum process-related requirements (building blocks) for a GEP

- **Publication:** formal document published on the institution's website and signed by the top management
- **Dedicated resources:** commitment of human resources and gender expertise to implement it.
- **Data collection and monitoring:** sex/gender disaggregated data on personnel (and students for establishments concerned) and annual reporting based on indicators.
- **Training:** Awareness raising/trainings on gender equality and unconscious gender biases for staff and decision-makers.
- **Content-wise, recommended areas** to be **covered** and addressed via concrete measures and targets are:
    - work-life balance and organisational culture;
    - gender balance in leadership and decision-making;
    - gender equality in recruitment and career progression;
    - integration of the gender dimension into research and teaching content;
    - measures against gender-based violence including sexual harassment.

[redacted]

Address

Street	[redacted]
Town	[redacted]
Postcode	[redacted]
Country	Austria
Webpage	[redacted]

Specific Legal Statuses

Legal person	yes
Public body	no
Non-profit	no
International organisation	no
Secondary or Higher education establishment	no
Research organisation	no

SME Data

Based on the below details from the Participant Registry the organisation is an SME (small- and medium-sized enterprise) for the call.

SME self-declared status	14/09/2019 - yes
SME self-assessment	14/09/2019 - yes
SME validation	unknown

Departments carrying out the proposed work

No department involved

Department name *Name of the department/institute carrying out the work.* ☒ not applicable

☐ Same as proposing organisation's address

Street *Please enter street name and number.*

Town *Please enter the name of the town.*

Postcode *Area code.*

Country *Please select a country*

Links with other participants

Type of link	Participant

Main contact person

This will be the person the EU services will contact concerning this proposal (e.g. for additional information, invitation to hearings, sending of evaluation results, convocation to start grant preparation). The data in blue is read-only. Details (name, first name and e-mail) of Main Contact persons should be edited in the step "Participants" of the submission wizard.

Title Mr

Gender ○ Woman ○ Man ◉ Non Binary

First name* [redacted]

Last name* [redacted]

E-Mail* [redacted]

Position in org. [redacted]

Department [redacted]

☒ Same as organisation name

☒ Same as proposing organisation's address

Street [redacted]

Town [redacted]

Post code [redacted]

Country Austria

Website *Please enter website*

Phone *+xxx xxxxxxxxx*

Phone 2 *+xxx xxxxxxxxx*

Researchers involved in the proposal

Title	First Name	Last Name	Gender	Nationality	E-mail	Career Stage	Role of researcher (in the project)	Reference Identifier	Type of identifier	
[redacted]										
[redacted]										

Role of participating organisation in the project

Project management	☒
Communication, dissemination and engagement	☒
Provision of research and technology infrastructure	☐
Co-definition of research and market needs	☐
Civil society representative	☐
Policy maker or regulator, incl. standardisation body	☐
Research performer	☐
Technology developer	☐
Testing/validation of approaches and ideas	☐
Prototyping and demonstration	☐
IPR management incl. technology transfer	☒
Public procurer of results	☐
Private buyer of results	☐
Finance provider (public or private)	☐
Education and training	☒
Contributions from the social sciences or/and the humanities	☐
Other If yes, please specify: (Maximum number of characters allowed: 50)	☐

List of up to 5 publications, widely-used datasets, software, goods, services, or any other achievements relevant to the call content.

Type of achievement	Short description (Max 500 characters)
Service	[redacted]
Publication	
Service	
Service	

[redacted]

Description of any significant infrastructure and/or any major items of technical equipment, relevant to the proposed work.

Name of infrastructure of equipment	Short description (Max 300 characters)
Digital Project Management Tools	*Use of validated project management platforms (Asana, Teams, Miro) for collaborative planning, task tracking, and documentation.*
Data Collection & Reporting Templates	*Customizable Horizon Europe reporting templates ensuring consistency in progress reporting and KPI tracking.*
Secure Cloud Infrastructure	*GDPR-compliant storage and collaboration environment ensuring safe handling of project documents and partner data.*

Gender Equality Plan

Does the organization have a Gender Equality Plan (GEP) covering the elements listed below? ○ Yes ◉ No

Minimum process-related requirements (building blocks) for a GEP

- **Publication:** formal document published on the institution's website and signed by the top management
- **Dedicated resources:** commitment of human resources and gender expertise to implement it.
- **Data collection and monitoring:** sex/gender disaggregated data on personnel (and students for establishments concerned) and annual reporting based on indicators.
- **Training:** Awareness raising/trainings on gender equality and unconscious gender biases for staff and decision-makers.
- **Content-wise, recommended areas** to be **covered** and addressed via concrete measures and targets are:
    - work-life balance and organisational culture;
    - gender balance in leadership and decision-making;
    - gender equality in recruitment and career progression;
    - integration of the gender dimension into research and teaching content;
    - measures against gender-based violence including sexual harassment.

PIC	Legal name
870877835	*Aposcience AG*

Short name: APOSC

Address

Street	Dresdner Strasse 87/A 21
Town	Vienna
Postcode	1200
Country	Austria
Webpage	https://www.aposcience.at

Specific Legal Statuses

Legal person	yes
Public body	no
Non-profit	no
International organisation	no
Secondary or Higher education establishment	no
Research organisation	no

SME Data

Based on the below details from the Participant Registry the organisation is not an SME (small- and medium-sized enterprise) for the call.

SME self-declared status	26/06/2025 - no
SME self-assessment	unknown
SME validation	unknown

Departments carrying out the proposed work

No department involved

Department name *Name of the department/institute carrying out the work.* ☒ not applicable

☐ Same as proposing organisation's address

Street *Please enter street name and number.*

Town *Please enter the name of the town.*

Postcode *Area code.*

Country *Please select a country*

Links with other participants

Type of link	Participant

Main contact person

This will be the person the EU services will contact concerning this proposal (e.g. for additional information, invitation to hearings, sending of evaluation results, convocation to start grant preparation). The data in blue is read-only. Details (name, first name and e-mail) of Main Contact persons should be edited in the step "Participants" of the submission wizard.

Title Prof.

Gender ◯ Woman ◉ Man ◯ Non Binary

First name* **Hendrik Jan**

Last name* **Ankersmit**

E-Mail* **hendrik.ankersmit@aposcience.com**

Position in org. CEO

Department Aposcience AG

☒ Same as organisation name

☒ Same as proposing organisation's address

Street Dresdner Strasse 87/A 21

Town Vienna

Post code 1200

Country Austria

Website *Please enter website*

Phone 0043-664-2120557

Phone 2 0043-676-7346179

Other contact persons

First Name	Last Name	E-mail	Phone
Helmut	Hofbauer	helmut.hofbauer@aposcience.com	+xxx xxxxxxxxx
[redacted]	[redacted]	[redacted]	+xxx xxxxxxxxx

Researchers involved in the proposal

Title	First Name	Last Name	Gender	Nationality	E-mail	Career Stage	Role of researcher (in the project)	Reference Identifier	Type of identifier	
Prof	Hendrik Jan	Ankersmit	Man	Austria	hendrik.ankersmit@aposcience.com	Category A Top grade re	Leading	0000-0002-8761-3517	Orcid ID	
Prof	Michael	Mildner	Man	Austria	michael.mildner@aposcience.com	Category B Senior resea	Team member	0000-0002-6892-925X	Orcid ID	
Dr	Lisa	Auer	Woman	Austria	lisa.auer@meduniwien.ac.at	Category D First stage r	Team member	0009-0000-5991-1288	Orcid ID	
Dr	Helmut	Hofbauer	Man	Austria	helmut.hofbauer@aposcience.com	Category C Recognised	Team member	0000-0002-5139-2644	Orcid ID	
Ms	Melanie	Salek	Woman	Austria	melanie.salek@meduniwien.ac.at	Category D First stage r	Team member	0009-0000-2976-1880	Orcid ID	
Mr	Peter	Posch	Man	Austria	peter.posch@aposcience.com	Category D First stage r	Team member		Other ID	
Dr	Sylwia	Gazda-Miarecka	Woman	Austria	sylwia.gazda-miarecka@aposcience.com	Category D First stage r	Team member		Other ID	

Role of participating organisation in the project

Project management	☒
Communication, dissemination and engagement	☒
Provision of research and technology infrastructure	☒
Co-definition of research and market needs	☒
Civil society representative	☐
Policy maker or regulator, incl. standardisation body	☐
Research performer	☐
Technology developer	☒
Testing/validation of approaches and ideas	☒
Prototyping and demonstration	☒
IPR management incl. technology transfer	☒
Public procurer of results	☐
Private buyer of results	☐
Finance provider (public or private)	☐
Education and training	☒
Contributions from the social sciences or/and the humanities	☐
Other If yes, please specify: (Maximum number of characters allowed: 50)	☒

Qualified Person PP
Qality Management SGM

List of up to 5 publications, widely-used datasets, software, goods, services, or any other achievements relevant to the call content.

Type of achievement	Short description (Max 500 characters)
Publication	*Stem Cell Res Ther. 2020 Jan 3;11(1):9. doi: 10.1186/s13287-019-1524-2. Reproducibility of GMP-compliant production of therapeutic stressed peripheral blood mononuclear derived secretomes, a novel class of biological medicinal products. GMP-compliant production guarantees high batch-to-batch consistency, efficacy and stability. The results from this study provide for the first time the basis for selecting appropriate quality control parameters for GMP-compliant production of Aposec.*
Publication	*Sci Rep. 2019 Apr 3;9(1):5598. doi: 10.1038/s41598-019-42057-5. Toxicological testing of allogeneic secretome derived from peripheral mononuclear cells (Aposec). Regulatory authorities classify these secretomes as biological medicinal products and non- clinical safety assessment thus falls under the scope of ICH S6. Acute and repeated-dose toxicity studies evidenced that Aposec administered intravenously and subcutaneously was safe.*
Publication	*Blood Transfus. 2020 Jan;18(1):30-39. doi: 10.2450/2019.0249-18. Epub 2019 Feb 21.Viral safety of APOSECTM: a novel peripheral blood mononuclear cell derived-biological for regenerative medicine. Effectiveness of inactivation of model viruses by methylene blue/light treatment, lyophilisation, and gamma irradiation during the manufacturing was demonstrated. In this work we show that three manufacturing steps of APOSEC under GMP conditions was efficacious at reducing potentially present viruses.*
Other achievement	*Quality System CMO and Aposcience*
Other achievement	*Patent landscape Aposcience - see Exploitation in impact section*

List of up to 5 most relevant previous projects or activities, connected to the subject of this proposal.

Name of Project or Activity	Short description (Max 500 characters)
CD-Labor for Diagnosis and Regeneration	*Project through an international peer reviewed grant, supported by a private public partnership. Goal: development of PBMC Secretome in multiple preclincal disease models (https://www.cdg.ac.at/en/research-units/labor/cardiac-and-thoracic-diagnosis-regeneration) - 2.4 Mio Euro total grant amount. (2009-2015) (preclincial and phase I trial)*
FFG Project "Aposec - 852748&862068"	*Product charactirization and development of GMP production process in the GMP facility of CMO Blood bank Linz (BSZ-Linz)*
GMP certificate to produce APO-2, phase I-II-III	*Project to obtain AGES GMP certificate for the production of APOSEC for systemic and topical phase I,II,III trials*
Phase I trial utilizing autologous Aposec	*Published in: Clinical Trial Sci Rep. 2017 Jul 24;7(1):6216. doi: 10.1038/s41598-017-06223-x. Safety and tolerability of topically administered autologous, apoptotic PBMC secretome (Aposec) in dermal wounds: a randomized Phase 1 trial (MARSYAS I). No therapy-related serious adverse events occurred in any of the participants, and both low- and high-dose treatments were well tolerated. Wound closure was not affected by APOSEC therapy in this 7 day intervention trial. (together with MUW)*
Phase I/II trial in DFU allogeneic APO-2	*Published in: Trials. 2021 Jan 6;22(1):10. doi: 10.1186/s13063-020-04948-1.Safety and clinical efficacy of the secretome of stressed PBMC in patients with diabetic foot ulcer-study protocol of the randomized, placebo-controlled, double-blind, multicenter, international phase II clinical is presented. The IMP APOSEC, a novel, innovative drug, is tested in the phase I/II study MARSYAS II, where its efficacy to promote healing of diabetic foot ulcers will be determined. (together with MUW)*

Description of any significant infrastructure and/or any major items of technical equipment, relevant to the proposed work.

Name of infrastructure of equipment	Short description (Max 300 characters)

	Surgical Research Laboratory, Quality System, Administrative Offices at Med. University,

Gender Equality Plan

Does the organization have a Gender Equality Plan (GEP) covering the elements listed below? ○ Yes ◉ No

Minimum process-related requirements (building blocks) for a GEP

- **Publication:** formal document published on the institution's website and signed by the top management
- **Dedicated resources:** commitment of human resources and gender expertise to implement it.
- **Data collection and monitoring:** sex/gender disaggregated data on personnel (and students for establishments concerned) and annual reporting based on indicators.
- **Training:** Awareness raising/trainings on gender equality and unconscious gender biases for staff and decision-makers.
- **Content-wise, recommended areas** to be **covered** and addressed via concrete measures and targets are:
    - work-life balance and organisational culture;
    - gender balance in leadership and decision-making;
    - gender equality in recruitment and career progression;
    - integration of the gender dimension into research and teaching content;
    - measures against gender-based violence including sexual harassment.

PIC	Legal name
948646712	[redacted]

Short name: ECRIN

Address

Street	[redacted]
Town	[redacted]
Postcode	[redacted]
Country	France
Webpage	[redacted]

Specific Legal Statuses

Legal person	yes
Public body	yes
Non-profit	yes
International organisation	no
Secondary or Higher education establishment	no
Research organisation	yes

SME Data

Based on the below details from the Participant Registry the organisation is not an SME (small- and medium-sized enterprise) for the call.

SME self-declared status	08/02/2022 - no
SME self-assessment	unknown
SME validation	unknown

Departments carrying out the proposed work

Department 1

Department name: Management Office ☐ not applicable

☒ Same as proposing organisation's address

Street: [redacted]

Town: [redacted]

Postcode: [redacted]

Country: France

Links with other participants

Type of link	Participant

Main contact person

This will be the person the EU services will contact concerning this proposal (e.g. for additional information, invitation to hearings, sending of evaluation results, convocation to start grant preparation). The data in blue is read-only. Details (name, first name and e-mail) of Main Contact persons should be edited in the step "Participants" of the submission wizard.

Title Mrs

Gender ◉ Woman ○ Man ○ Non Binary

First name* [redacted]

Last name* [redacted]

E-Mail* [redacted]

Position in org. Clinical Project Manager

Department [redacted]

☒ Same as organisation name

☒ Same as proposing organisation's address

Street [redacted]

Town [redacted]

Post code [redacted]

Country France

Website *Please enter website*

Phone [redacted]

Phone 2 [redacted]

Other contact persons

First Name	Last Name	E-mail	Phone
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]

Researchers involved in the proposal

Title	First Name	Last Name	Gender	Nationality	E-mail	Career Stage	Role of researcher (in the project)	Reference Identifier	Type of identifier
[redacted]									

Role of participating organisation in the project

Project management	☒
Communication, dissemination and engagement	☒
Provision of research and technology infrastructure	☒
Co-definition of research and market needs	☐
Civil society representative	☐
Policy maker or regulator, incl. standardisation body	☐
Research performer	☐
Technology developer	☐
Testing/validation of approaches and ideas	☐
Prototyping and demonstration	☐
IPR management incl. technology transfer	☐
Public procurer of results	☐
Private buyer of results	☐
Finance provider (public or private)	☐
Education and training	☐
Contributions from the social sciences or/and the humanities	☐
Other If yes, please specify: (Maximum number of characters allowed: 50)	☐

List of up to 5 publications, widely-used datasets, software, goods, services, or any other achievements relevant to the call content.

Type of achievement	Short description (Max 500 characters)
Publication	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]

List of up to 5 most relevant previous projects or activities, connected to the subject of this proposal.

Name of Project or Activity	Short description (Max 500 characters)
PERMIT	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted] *)*

Description of any significant infrastructure and/or any major items of technical equipment, relevant to the proposed work.

Name of infrastructure of equipment	Short description (Max 300 characters)
ECRIN national scientific partners	*The European Clinical Research Infrastructure Network is a public, non-profit organisation that links scientific partners and networks across Europe to facilitate multinational clinical research. ECRIN's scientific partners are national networks that are composed of academic clinical trial units.*

Gender Equality Plan

Does the organization have a Gender Equality Plan (GEP) covering the elements listed below? (•) Yes ( ) No

Minimum process-related requirements (building blocks) for a GEP

- **Publication:** formal document published on the institution's website and signed by the top management
- **Dedicated resources:** commitment of human resources and gender expertise to implement it.
- **Data collection and monitoring:** sex/gender disaggregated data on personnel (and students for establishments concerned) and annual reporting based on indicators.
- **Training:** Awareness raising/trainings on gender equality and unconscious gender biases for staff and decision-makers.
- **Content-wise, recommended areas** to be **covered** and addressed via concrete measures and targets are:
    - work-life balance and organisational culture;
    - gender balance in leadership and decision-making;
    - gender equality in recruitment and career progression;
    - integration of the gender dimension into research and teaching content;
    - measures against gender-based violence including sexual harassment.

PIC	Legal name
999880657	[redacted]

Short name: MU

Address

Street	[redacted]
Town	[redacted]
Postcode	[redacted]
Country	Czechia
Webpage	[redacted]

Specific Legal Statuses

Legal person	yes
Public body	yes
Non-profit	yes
International organisation	no
Secondary or Higher education establishment	yes
Research organisation	yes

SME Data

Based on the below details from the Participant Registry the organisation is not an SME (small- and medium-sized enterprise) for the call.

SME self-declared status	02/03/2022 - no
SME self-assessment	unknown
SME validation	12/11/2008 - no

Departments carrying out the proposed work

No department involved

Department name *Name of the department/institute carrying out the work.*

☒ not applicable

☐ Same as proposing organisation's address

Street *Please enter street name and number.*

Town *Please enter the name of the town.*

Postcode *Area code.*

Country *Please select a country*

Links with other participants

Type of link	Participant

Researchers involved in the proposal

Title	First Name	Last Name	Gender	Nationality	E-mail	Career Stage	Role of researcher (in the project)	Reference Identifier	Type of identifier	
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]		[redacted]	Other ID	LinkedIn

Role of participating organisation in the project

Project management	☐
Communication, dissemination and engagement	☐
Provision of research and technology infrastructure	☒
Co-definition of research and market needs	☐
Civil society representative	☐
Policy maker or regulator, incl. standardisation body	☐
Research performer	☒
Technology developer	☐
Testing/validation of approaches and ideas	☐
Prototyping and demonstration	☐
IPR management incl. technology transfer	☐
Public procurer of results	☐
Private buyer of results	☐
Finance provider (public or private)	☐
Education and training	☐
Contributions from the social sciences or/and the humanities	☐
Other If yes, please specify: (Maximum number of characters allowed: 50)	☐

List of up to 5 publications, widely-used datasets, software, goods, services, or any other achievements relevant to the call content.

Type of achievement	Short description (Max 500 characters)
Service	*Clinical trial lead for ECRIN projects*
Publication	*Enhancing pragmatic competence in investigator-initiated clinical trials: structure and evaluation of the CONSCIOUS II training programme* *BMC Medical Education* *2025-04-08 | Journal article* *DOI: 10.1186/s12909-025-07054-5* *SOURCE-WORK-ID: cv-prod-id-4667847* *Part of ISSN: 1472-6920*
Publication	*Investigator initiated clinical trials (IICTs): a systematic search in registries comparing Czech Republic and Portugal in terms of funding policies and scientific outcomes* *2020-02-27 | Preprint* *DOI: 10.21203/rs.2.24789/v1*

List of up to 5 most relevant previous projects or activities, connected to the subject of this proposal.

Name of Project or Activity	Short description (Max 500 characters)
TTV Guide H2020	*A randomised and controlled trial to compare the safety, tolerability and preliminary efficacy between standard and Torque Teno virus-guided immunosuppression in stable adult kidney transplant recipients with low immunological risk in the first year after*

Description of any significant infrastructure and/or any major items of technical equipment, relevant to the proposed work.

Name of infrastructure of equipment	Short description (Max 300 characters)

Gender Equality Plan

Does the organization have a Gender Equality Plan (GEP) covering the elements listed below? (•) Yes ( ) No

Minimum process-related requirements (building blocks) for a GEP

- **Publication:** formal document published on the institution's website and signed by the top management
- **Dedicated resources:** commitment of human resources and gender expertise to implement it.
- **Data collection and monitoring:** sex/gender disaggregated data on personnel (and students for establishments concerned) and annual reporting based on indicators.
- **Training:** Awareness raising/trainings on gender equality and unconscious gender biases for staff and decision-makers.
- **Content-wise, recommended areas** to be **covered** and addressed via concrete measures and targets are:
    - work-life balance and organisational culture;
    - gender balance in leadership and decision-making;
    - gender equality in recruitment and career progression;
    - integration of the gender dimension into research and teaching content;
    - measures against gender-based violence including sexual harassment.

PIC	Legal name
970512452	[redacted]

Short name: BZL

Address

Street [redacted]

Town [redacted]

Postcode [redacted]

Country [redacted]

Webpage

Specific Legal Statuses

Legal person	yes
Public body	no
Non-profit	yes
International organisation	no
Secondary or Higher education establishment	no
Research organisation	yes

SME Data

Based on the below details from the Participant Registry the organisation is not an SME (small- and medium-sized enterprise) for the call.

SME self-declared status	10/01/1948 - no
SME self-assessment	unknown
SME validation	unknown

Departments carrying out the proposed work

Department 1

Department name [redacted] ☐ not applicable

☐ Same as proposing organisation's address

Street [redacted]

Town [redacted]

Postcode [redacted]

Country Austria

Links with other participants

Type of link	Participant

Main contact person

This will be the person the EU services will contact concerning this proposal (e.g. for additional information, invitation to hearings, sending of evaluation results, convocation to start grant preparation). The data in blue is read-only. Details (name, first name and e-mail) of Main Contact persons should be edited in the step "Participants" of the submission wizard.

Title [redacted] Gender ◉ Woman ○ Man ○ Non Binary

First name* [redacted] Last name* [redacted]

E-Mail* [redacted]

Position in org. [redacted]

Department [redacted] ☐ Same as organisation name

☐ Same as proposing organisation's address

Street [redacted]

Town [redacted] Post code [redacted]

Country [redacted]

Website [redacted]

Phone [redacted] *Phone 2* *+xxx xxxxxxxxx*

Researchers involved in the proposal

Title	First Name	Last Name	Gender	Nationality	E-mail	Career Stage	Role of researcher (in the project)	Reference Identifier	Type of identifier
[redacted]									

Role of participating organisation in the project

Project management	☐
Communication, dissemination and engagement	☒
Provision of research and technology infrastructure	☒
Co-definition of research and market needs	☐
Civil society representative	☐
Policy maker or regulator, incl. standardisation body	☐
Research performer	☒
Technology developer	☒
Testing/validation of approaches and ideas	☒
Prototyping and demonstration	☐
IPR management incl. technology transfer	☐
Public procurer of results	☐
Private buyer of results	☐
Finance provider (public or private)	☐
Education and training	☐
Contributions from the social sciences or/and the humanities	☐
Other If yes, please specify: (Maximum number of characters allowed: 50)	☒

contract manufacturing of IMP Aposec

List of up to 5 publications, widely-used datasets, software, goods, services, or any other achievements relevant to the call content.

Type of achievement	Short description (Max 500 characters)
Publication	*Stem Cell Res Ther. 2020 Jan 3;11(1):9. doi: 10.1186/s13287-019-1524-2. Reproducibility of GMP-compliant production of therapeutic stressed peripheral blood mononuclear derived secretomes, a novel class of biological medicinal products. GMP-compliant production guarantees high batch-to-batch consistency, efficacy and stability. The results from this study provide for the first time the basis for selecting appropriate quality control parameters for GMP-compliant production of Aposec.*
Publication	*Sci Rep. 2019 Apr 3;9(1):5598. doi: 10.1038/s41598-019-42057-5. Toxicological testing of allogeneic secretome derived from peripheral mononuclear cells (Aposec). Regulatory authorities classify these secretomes as biological medicinal products and non- clinical safety assessment thus falls under the scope of ICH S6. Acute and repeated-dose toxicity studies evidenced that APOSEC administered intravenously and subcutaneously was safe.*
Publication	*Blood Transfus. 2020 Jan;18(1):30-39. doi: 10.2450/2019.0249-18. Epub 2019 Feb 21. Viral safety of APOSECTM: a novel peripheral blood mononuclear cell derived-biological for regenerative medicine. Effectiveness of inactivation of model viruses by methylene blue/light treatment, lyophilisation, and gamma irradiation during the manufacturing was demonstrated. In this work we show that three manufacturing steps of APOSEC under GMP conditions was efficacious at reducing potentially present viruses.*
Publication	*Clinical Trial Sci Rep. 2017 Jul 24;7(1):6216Clinical Trial Sci Rep. 2017 Jul 24;7(1):6216. doi: 10.1038/s41598-017-06223-x. Safety and tolerability of topically administered autologous, apoptotic PBMC secretome (Aposec) in dermal wounds: a randomized Phase 1 trial (MARSYAS I). No therapy-related serious adverse events occurred in any of the participants, and both low- and high-dose treatments were well tolerated. Wound closure was not affected by APOSEC therapy in this 7 day intervention trial.*
Publication	*Trials. 2021 Jan 6;22(1):10. doi: 10.1186/s13063-020-04948-1. Safety and clinical efficacy of the secretome of stressed PBMC in patients with diabetic foot ulcer-study protocol of the randomized, placebo-controlled, double-blind, multicenter, international phase II clinical is presented. The IMP APOSEC, a novel, innovative drug, is tested in the phase I/II study. This therapeutic intervention lasted for 28 days.*

List of up to 5 most relevant previous projects or activities, connected to the subject of this proposal.

Name of Project or Activity	Short description (Max 500 characters)
Establishment of APOSEC GMP Process 2014-2025	
Production of GMP Aposec for Phase I- I/II trial	
Quality System (SOP) for GMP Aposec production	
Regular Inspections by regulatory affairs (AGES)	

Description of any significant infrastructure and/or any major items of technical equipment, relevant to the proposed work.

Name of infrastructure of equipment	Short description (Max 300 characters)
clean rooms	*800 m2 clean rooms (about 200 m2 grade C and B with laminar airflows of grade A)*

lyophilisator	*pilot dry freezer (Christ, Epsilon 2-10D)*
viral clearance equipment	*methylene blue/illumination (Macopharma, Macotronic B2)*
flow cytometry	*FACS Canto II (BD)*

Gender Equality Plan

Does the organization have a Gender Equality Plan (GEP) covering the elements listed below? ○ Yes ◉ No

Minimum process-related requirements (building blocks) for a GEP

- **Publication:** formal document published on the institution's website and signed by the top management
- **Dedicated resources:** commitment of human resources and gender expertise to implement it.
- **Data collection and monitoring:** sex/gender disaggregated data on personnel (and students for establishments concerned) and annual reporting based on indicators.
- **Training:** Awareness raising/trainings on gender equality and unconscious gender biases for staff and decision-makers.
- **Content-wise, recommended areas** to be **covered** and addressed via concrete measures and targets are:
    - work-life balance and organisational culture;
    - gender balance in leadership and decision-making;
    - gender equality in recruitment and career progression;
    - integration of the gender dimension into research and teaching content;
    - measures against gender-based violence including sexual harassment.

PIC	Legal name
887142504	[redacted]

Short name: ABF

Address

Street	[redacted]
Town	[redacted]
Postcode	[redacted]
Country	Austria
Webpage	[redacted]

Specific Legal Statuses

Legal person	yes
Public body	no
Non-profit	no
International organisation	no
Secondary or Higher education establishment	no
Research organisation	no

SME Data

Based on the below details from the Participant Registry the organisation is not an SME (small- and medium-sized enterprise) for the call.

SME self-declared status	22/01/2025	- no
SME self-assessment	31/12/2020	- yes
SME validation	unknown	

Departments carrying out the proposed work

Department 1

Department name [redacted] ☐ not applicable

☒ Same as proposing organisation's address

Street [redacted]

Town [redacted]

Postcode [redacted]

Country Austria

Links with other participants

Type of link	Participant

Main contact person

This will be the person the EU services will contact concerning this proposal (e.g. for additional information, invitation to hearings, sending of evaluation results, convocation to start grant preparation). The data in blue is read-only. Details (name, first name and e-mail) of Main Contact persons should be edited in the step "Participants" of the submission wizard.

Title [redacted] Gender ◯ Woman ◉ Man ◯ Non Binary

First name* [redacted] Last name* [redacted]

E-Mail* [redacted]

Position in org. [redacted]

Department [redacted] ☒ Same as organisation name

☒ Same as proposing organisation's address

Street [redacted]

Town [redacted] Post code [redacted]

Country [redacted]

Website [redacted]

Phone [redacted] *Phone 2* *+xxx xxxxxxxxx*

Researchers involved in the proposal

Title	First Name	Last Name	Gender	Nationality	E-mail	Career Stage	Role of researcher (in the project)	Reference Identifier	Type of identifier
[redacted]									
[redacted]									
[redacted]									
[redacted]									

Role of participating organisation in the project

Project management	☐
Communication, dissemination and engagement	☒
Provision of research and technology infrastructure	☐
Co-definition of research and market needs	☐
Civil society representative	☐
Policy maker or regulator, incl. standardisation body	☐
Research performer	☐
Technology developer	☐
Testing/validation of approaches and ideas	☒
Prototyping and demonstration	☒
IPR management incl. technology transfer	☐
Public procurer of results	☐
Private buyer of results	☐
Finance provider (public or private)	☐
Education and training	☒
Contributions from the social sciences or/and the humanities	☐
Other If yes, please specify: (Maximum number of characters allowed: 50)	☒

Clinical Trial Supply Services

List of up to 5 publications, widely-used datasets, software, goods, services, or any other achievements relevant to the call content.

Type of achievement	Short description (Max 500 characters)
Software	*Implemented warehouse management system (Navision) to track and trace investigational medicine products end-2-end at ABF*
Service	*Supporting clients with QP consultancy/ services.*
Service	*Provide project management services to accelarate clinical trials*

List of up to 5 most relevant previous projects or activities, connected to the subject of this proposal.

Name of Project or Activity	Short description (Max 500 characters)
Labeling services	*Labeling of investigational medical products in compliance with regulatory requirements - Label design, GMP printing of booklet, multilingual & tear off labels, re-labeling, return and destruction, temperature ranges: room temperature, 2-8C, -20C*
Packaging services	*Primary packaging of tablets & capsules, secondary packaging , complete traceability & quality control*
Distrubition / Logistics	*Shipping to study centers worldwide, storage of investigational medicine products, import & export,*
QP services	*EU Batch Certification, assistenence with batch expiry extension, QP release, consultancy*

Description of any significant infrastructure and/or any major items of technical equipment, relevant to the proposed work.

Name of infrastructure of equipment	Short description (Max 300 characters)
GMP Warehousing	*Storage facility and logistics facility to store medicines at 15-25 Celcius, 2-8 C, -20C, -80C, -150 C, Liquid Nitrogen (LN)*
GMP production facility	*Cleaning room for secondary and primary packaging and labeling*

Gender Equality Plan

Does the organization have a Gender Equality Plan (GEP) covering the elements listed below? (•) Yes ( ) No

Minimum process-related requirements (building blocks) for a GEP

- **Publication:** formal document published on the institution's website and signed by the top management
- **Dedicated resources:** commitment of human resources and gender expertise to implement it.
- **Data collection and monitoring:** sex/gender disaggregated data on personnel (and students for establishments concerned) and annual reporting based on indicators.
- **Training:** Awareness raising/trainings on gender equality and unconscious gender biases for staff and decision-makers.
- **Content-wise, recommended areas** to be **covered** and addressed via concrete measures and targets are:
    - work-life balance and organisational culture;
    - gender balance in leadership and decision-making;
    - gender equality in recruitment and career progression;
    - integration of the gender dimension into research and teaching content;
    - measures against gender-based violence including sexual harassment.

PIC	Legal name
999897729	[redacted]

Short name: TUD

Address

Street	[redacted]
Town	[redacted]
Postcode	[redacted]
Country	[redacted]
Webpage	[redacted]

Specific Legal Statuses

Legal person	yes
Public body	yes
Non-profit	yes
International organisation	no
Secondary or Higher education establishment	yes
Research organisation	no

SME Data

Based on the below details from the Participant Registry the organisation is not an SME (small- and medium-sized enterprise) for the call.

SME self-declared status	17/01/2022 - no
SME self-assessment	unknown
SME validation	11/06/1999 - no

Departments carrying out the proposed work

Department 1

Department name [redacted] ☐ not applicable

☐ Same as proposing organisation's address

Street [redacted]

Town [redacted]

Postcode [redacted]

Country [redacted]

Links with other participants

Type of link	Participant

Main contact person

This will be the person the EU services will contact concerning this proposal (e.g. for additional information, invitation to hearings, sending of evaluation results, convocation to start grant preparation). The data in blue is read-only. Details (name, first name and e-mail) of Main Contact persons should be edited in the step "Participants" of the submission wizard.

Title [redacted] Gender (•) Woman ( ) Man ( ) Non Binary

First name* [redacted] Last name* [redacted]

E-Mail* [redacted]

Position in org. [redacted]

Department [redacted] ☐ Same as organisation name

☐ Same as proposing organisation's address

Street [redacted]

Town [redacted] Post code [redacted]

Country Germany

Website [redacted]

Phone [redacted] *Phone 2* *+xxx xxxxxxxxx*

Other contact persons

First Name	Last Name	E-mail	Phone
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]

Researchers involved in the proposal

Title	First Name	Last Name	Gender	Nationality	E-mail	Career Stage	Role of researcher (in the project)	Reference Identifier	Type of identifier
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]		[redacted]	[redacted]
	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]			
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]			

Role of participating organisation in the project

Project management	☐
Communication, dissemination and engagement	☐
Provision of research and technology infrastructure	☒
Co-definition of research and market needs	☐
Civil society representative	☐
Policy maker or regulator, incl. standardisation body	☐
Research performer	☒
Technology developer	☐
Testing/validation of approaches and ideas	☒
Prototyping and demonstration	☐
IPR management incl. technology transfer	☐
Public procurer of results	☐
Private buyer of results	☐
Finance provider (public or private)	☐
Education and training	☐
Contributions from the social sciences or/and the humanities	☐
Other If yes, please specify: (Maximum number of characters allowed: 50)	☐

List of up to 5 publications, widely-used datasets, software, goods, services, or any other achievements relevant to the call content.

Type of achievement	Short description (Max 500 characters)

List of up to 5 most relevant previous projects or activities, connected to the subject of this proposal.

Name of Project or Activity	Short description (Max 500 characters)
IntelliLung (HE grant)	*Intelligent Lung Support for Mechanically Ventilated Patients in the Intensive Care Unit* *Relevance: TUD-KKS acts as Sponsor of the Clinical Study*
TTV Guide TX (H2020 grant)	*PERSONALISATION OF IMMUNOSUPPRESSION BY MONITORING VIRAL LOAD POST KIDNEY TRANSPLANTATION - A RANDOMISED CONTROLLED PHASE II TRIAL* *Relevance: TUD-KKS manages all German trial centers*
R-Link (H2020)	*Optimizing response to Li treatment through personalized evaluation of individuals with bipolar I disorder: the R-LiNK initiative* *Relevance: TUD-KKS manages all German trial centers*

Description of any significant infrastructure and/or any major items of technical equipment, relevant to the proposed work.

Name of infrastructure of equipment	Short description (Max 300 characters)
GMP-compliant Study software	*Ensures GMP-compliance*
Member of the TUD-internal sponsor committee	*Allows TUD to act as spnsor of the clinical study*

Gender Equality Plan

Does the organization have a Gender Equality Plan (GEP) covering the elements listed below? (•) Yes ( ) No

Minimum process-related requirements (building blocks) for a GEP

- **Publication:** formal document published on the institution's website and signed by the top management
- **Dedicated resources:** commitment of human resources and gender expertise to implement it.
- **Data collection and monitoring:** sex/gender disaggregated data on personnel (and students for establishments concerned) and annual reporting based on indicators.
- **Training:** Awareness raising/trainings on gender equality and unconscious gender biases for staff and decision-makers.
- **Content-wise, recommended areas** to be **covered** and addressed via concrete measures and targets are:
    - work-life balance and organisational culture;
    - gender balance in leadership and decision-making;
    - gender equality in recruitment and career progression;
    - integration of the gender dimension into research and teaching content;
    - measures against gender-based violence including sexual harassment.

PIC	Legal name
888146357	[redacted]

Short name: REG

Address

Street	[redacted]
Town	[redacted]
Postcode	[redacted]
Country	Germany
Webpage	[redacted]

Specific Legal Statuses

Legal person	yes
Public body	no
Non-profit	no
International organisation	no
Secondary or Higher education establishment	no
Research organisation	no

SME Data

Based on the below details from the Participant Registry the organisation is an SME (small- and medium-sized enterprise) for the call.

SME self-declared status	04/01/2022 - yes
SME self-assessment	unknown
SME validation	unknown

Departments carrying out the proposed work

No department involved

Department name *Name of the department/institute carrying out the work.* ☒ not applicable

☐ Same as proposing organisation's address

Street *Please enter street name and number.*

Town *Please enter the name of the town.*

Postcode *Area code.*

Country *Please select a country*

Links with other participants

Type of link	Participant

Main contact person

This will be the person the EU services will contact concerning this proposal (e.g. for additional information, invitation to hearings, sending of evaluation results, convocation to start grant preparation). The data in blue is read-only. Details (name, first name and e-mail) of Main Contact persons should be edited in the step "Participants" of the submission wizard.

Title [redacted] Gender ◯ Woman ◉ Man ◯ Non Binary

First name* [redacted] Last name* [redacted]

E-Mail* **dirk.sorgenfrey@regenold.com**

Position in org. [redacted]

Department [redacted] ☒ Same as organisation name

☒ Same as proposing organisation's address

Street [redacted]

Town [redacted] Post code [redacted]

Country Germany

Website *Please enter website*

Phone *+xxx xxxxxxxxx* *Phone 2* *+xxx xxxxxxxxx*

Researchers involved in the proposal

Title	First Name	Last Name	Gender	Nationality	E-mail	Career Stage	Role of researcher (in the project)	Reference Identifier	Type of identifier
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]			
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]			

Role of participating organisation in the project

Project management	☐
Communication, dissemination and engagement	☒
Provision of research and technology infrastructure	☒
Co-definition of research and market needs	☐
Civil society representative	☐
Policy maker or regulator, incl. standardisation body	☐
Research performer	☐
Technology developer	☐
Testing/validation of approaches and ideas	☒
Prototyping and demonstration	☐
IPR management incl. technology transfer	☐
Public procurer of results	☐
Private buyer of results	☐
Finance provider (public or private)	☐
Education and training	☐
Contributions from the social sciences or/and the humanities	☐
Other If yes, please specify: (Maximum number of characters allowed: 50)	☐

List of up to 5 publications, widely-used datasets, software, goods, services, or any other achievements relevant to the call content.

Type of achievement	Short description (Max 500 characters)
Publication	*Viral safety of APOSECTM: a novel peripheral blood mononuclear cell derived-biological for regenerative medicine. Gugerell A, Sorgenfrey D, Laggner M, Raimann J, Peterbauer A, Bormann D, Suessner S, Gabriel C, Moser B, Ostler T, Mildner M, Ankersmit HJ. Blood Transfus. 2020 Jan;18(1):30-39. doi: 10.2450/2019.0249-18. Epub 2019 Feb 21. PMID: 30865581 Free PMC article.*
Publication	*Reproducibility of GMP-compliant production of therapeutic stressed peripheral blood mononuclear cell-derived secretomes, a novel class of biological medicinal products. Laggner M, Gugerell A, Bachmann C, Hofbauer H, Vorstandlechner V, Seibold M, Gouya Lechner G, Peterbauer A, Madlener S, Demyanets S, Sorgenfrey D, Ostler T, Erb M, Mildner M, Ankersmit HJ. Stem Cell Res Ther. 2020 Jan 3;11(1):9. doi: 10.1186/s13287-019-1524-2. PMID: 31900195*
Publication	*Böhmert L, Voß L, Stock V, Braeuning A, Lampen A, Sieg H. Isolation methods for particle protein corona complexes from protein-rich matrices. Nanoscale Adv. 2020 Jan 9;2(2):563-582. doi: 10.1039/c9na00537d. eCollection 2020 Feb 18.PMID: 36133244*
Service	*regenold GmbH provides full-service regulatory affairs support, including strategic advice, preparation, and submission of dossiers (EMA, FDA, national authorities) for medicinal products, medical devices, and combination products. Expertise in Clinical Trial Applications (CTA), preparation of IMPD/IB, and regulatory liaison throughout the study lifecycle, including safety reporting and compliance. More than 30 years of experience supporting pharmaceutical, biotech, and medtech companies in EU*
Publication	*Voss L, Hoché E, Stock V, Böhmert L, Braeuning A, Thünemann AF, Sieg H. Intestinal and hepatic effects of iron oxide nanoparticles. Arch Toxicol. 2021 Mar;95(3):895-905. doi: 10.1007/s00204-020-02960-7. Epub 2021 Feb 8.PMID: 33554279*

List of up to 5 most relevant previous projects or activities, connected to the subject of this proposal.

Name of Project or Activity	Short description (Max 500 characters)
AI4HF	*Trustworthy Artificial Intelligence for Personalised Risk Assessment in Chronic Heart Failure: AI4HF Project ID 101080430 | Programme HORIZON AI4HF will develop the first trustworthy AI solutions for personalised risk assessment and management of HF patients. The project will build on a unique set of big data repositories, trustworthy AI methods, computational tools and clinical results from major EU-funded projects in cardiology.*

Description of any significant infrastructure and/or any major items of technical equipment, relevant to the proposed work.

Name of infrastructure of equipment	Short description (Max 300 characters)
Regulatory Affairs Management Systems	*Use of validated regulatory tracking and document management systems (e.g. regaffinity®- a software solution for streamlined regulatory workflows and documentation management.) for dossier preparation and submission.*
Secure IT & Compliance Tools	*ISO 27001-compliant IT infrastructure ensuring data security and compliance with GDPR for sponsor and patient data.*
Experienced Regulatory Network	*Access to a network of >70 European regulatory experts via EUCOPE and national affiliates for pan-European submissions.*

Gender Equality Plan

Does the organization have a Gender Equality Plan (GEP) covering the elements listed below? ○ Yes ◉ No

Minimum process-related requirements (building blocks) for a GEP

- **Publication:** formal document published on the institution's website and signed by the top management
- **Dedicated resources:** commitment of human resources and gender expertise to implement it.
- **Data collection and monitoring:** sex/gender disaggregated data on personnel (and students for establishments concerned) and annual reporting based on indicators.
- **Training:** Awareness raising/trainings on gender equality and unconscious gender biases for staff and decision-makers.
- **Content-wise, recommended areas** to be **covered** and addressed via concrete measures and targets are:
    - work-life balance and organisational culture;
    - gender balance in leadership and decision-making;
    - gender equality in recruitment and career progression;
    - integration of the gender dimension into research and teaching content;
    - measures against gender-based violence including sexual harassment.

PIC	Legal name
870452781	[redacted]

Short name: SGS

Address

Street	[redacted]
Town	[redacted]
Postcode	[redacted]
Country	Switzerland
Webpage	

Specific Legal Statuses

Legal person	yes
Public body	no
Non-profit	no
International organisation	no
Secondary or Higher education establishment	no
Research organisation	no

SME Data

Based on the below details from the Participant Registry the organisation is not an SME (small- and medium-sized enterprise) for the call.

SME self-declared status	24/07/2025 - no
SME self-assessment	unknown
SME validation	unknown

Departments carrying out the proposed work

Department 1

Department name [redacted] ☐ not applicable

☒ Same as proposing organisation's address

Street [redacted]

Town [redacted]

Postcode [redacted]

Country [redacted]

Links with other participants

Type of link	Participant

Main contact person

This will be the person the EU services will contact concerning this proposal (e.g. for additional information, invitation to hearings, sending of evaluation results, convocation to start grant preparation). The data in blue is read-only. Details (name, first name and e-mail) of Main Contact persons should be edited in the step "Participants" of the submission wizard.

Title [redacted] Gender ◯ Woman ◉ Man ◯ Non Binary

First name* [redacted] Last name* [redacted]

E-Mail* [redacted]

Position in org. [redacted]

Department [redacted] ☒ Same as organisation name

☒ Same as proposing organisation's address

Street [redacted]

Town [redacted] Post code [redacted]

Country Switzerland

Website *Please enter website*

Phone *+xxx xxxxxxxxx* *Phone 2* *+xxx xxxxxxxxx*

Other contact persons

First Name	Last Name	E-mail	Phone
Michael	Erb	[redacted]	+xxx xxxxxxxxx

Researchers involved in the proposal

Title	First Name	Last Name	Gender	Nationality	E-mail	Career Stage	Role of researcher (in the project)	Reference Identifier	Type of identifier
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]			

Role of participating organisation in the project

Project management	☐
Communication, dissemination and engagement	☐
Provision of research and technology infrastructure	☒
Co-definition of research and market needs	☒
Civil society representative	☐
Policy maker or regulator, incl. standardisation body	☐
Research performer	☒
Technology developer	☒
Testing/validation of approaches and ideas	☐
Prototyping and demonstration	☐
IPR management incl. technology transfer	☐
Public procurer of results	☐
Private buyer of results	☐
Finance provider (public or private)	☐
Education and training	☐
Contributions from the social sciences or/and the humanities	☐
Other If yes, please specify: (Maximum number of characters allowed: 50)	☐

List of up to 5 publications, widely-used datasets, software, goods, services, or any other achievements relevant to the call content.

Type of achievement	Short description (Max 500 characters)
Service	*SGS is a global leader in inspection, verification, testing, and certification, with extensive experience in conducting Phase I–IV clinical trials, including regulatory consulting and bioanalysis.*
Service	*SGS Clinical Research provides GCP-compliant Clinical Trial Management Services, including monitoring, data management, biostatistics, and medical writing.*
Dataset	*Proprietary clinical databases and validated EDC (Electronic Data Capture) systems ensuring data security, 21 CFR Part 11 compliance, and high-quality data integrity.*
Software	*SGS Life Science Analytics platforms for real-time clinical data analysis and reporting, supporting adaptive trial designs and fast decision-making.*
Other achievement	*Successful delivery of >300 clinical studies over the last decade, with a strong focus on rare diseases, infectious diseases, and immunology.*

List of up to 5 most relevant previous projects or activities, connected to the subject of this proposal.

Name of Project or Activity	Short description (Max 500 characters)
CoroPrevention(H2020)	*Personalized Prevention for Coronary Heart Disease CoroPrevention Project ID 848056 | Programme H2020 This program will establish a new economically sustainable personalized treatment practice applicable throughout Europe particularly to those regions where CHD prevention needs upgrading. The used protocols and technologies will carefully assessed by NICE using their standard evaluation methods that will allow independent expert opinions for different European authorities and decision makers.*
COVID-19 Vaccine Studies	*Managed several accelerated clinical trials for COVID-19 vaccine candidates with large patient cohorts, ensuring full regulatory compliance and rapid timelines.*
Advanced Therapy Trials	*Supported clinical trials for ATMPs (Advanced Therapy Medicinal Products), including GMP handling, logistics, and regulatory interactions.*

Description of any significant infrastructure and/or any major items of technical equipment, relevant to the proposed work.

Name of infrastructure of equipment	Short description (Max 300 characters)
Bioanalytical Laboratory	*GLP-accredited bioanalytical lab for PK/PD and biomarker analysis, equipped with LC-MS/MS and advanced immunoassay technologies.*
Data Management Systems	*Validated EDC, CTMS, and safety reporting systems enabling secure, efficient data handling and real-time insights.*
Secure IT Infrastructure	*ISO 27001-certified IT environment supporting safe processing and hosting of clinical trial data.*

Gender Equality Plan

Does the organization have a Gender Equality Plan (GEP) covering the elements listed below? ○ Yes ◉ No

Minimum process-related requirements (building blocks) for a GEP

- **Publication:** formal document published on the institution's website and signed by the top management
- **Dedicated resources:** commitment of human resources and gender expertise to implement it.
- **Data collection and monitoring:** sex/gender disaggregated data on personnel (and students for establishments concerned) and annual reporting based on indicators.
- **Training:** Awareness raising/trainings on gender equality and unconscious gender biases for staff and decision-makers.
- **Content-wise, recommended areas** to be **covered** and addressed via concrete measures and targets are:
    - work-life balance and organisational culture;
    - gender balance in leadership and decision-making;
    - gender equality in recruitment and career progression;
    - integration of the gender dimension into research and teaching content;
    - measures against gender-based violence including sexual harassment.

PIC	Legal name
948230485	[redacted]

Short name: KOL

Address

Street	[redacted]
Town	[redacted]
Postcode	[redacted]
Country	Slovakia
Webpage	[redacted]

Specific Legal Statuses

Legal person	yes
Public body	no
Non-profit	no
International organisation	no
Secondary or Higher education establishment	no
Research organisation	no

SME Data

Based on the below details from the Participant Registry the organisation is an SME (small- and medium-sized enterprise) for the call.

SME self-declared status	26/05/2025 - yes
SME self-assessment	26/05/2025 - yes
SME validation	unknown

Departments carrying out the proposed work

No department involved

Department name *Name of the department/institute carrying out the work.*

☒ not applicable

☐ Same as proposing organisation's address

Street *Please enter street name and number.*

Town *Please enter the name of the town.*

Postcode *Area code.*

Country *Please select a country*

Links with other participants

Type of link	Participant

Main contact person

This will be the person the EU services will contact concerning this proposal (e.g. for additional information, invitation to hearings, sending of evaluation results, convocation to start grant preparation). The data in blue is read-only. Details (name, first name and e-mail) of Main Contact persons should be edited in the step "Participants" of the submission wizard.

Title [redacted] Gender ◉ Woman ○ Man ○ Non Binary

First name* [redacted] Last name* [redacted]

E-Mail* [redacted]

Position in org. [redacted]

Department [redacted] ☒ Same as organisation name

☒ Same as proposing organisation's address

Street [redacted]

Town [redacted] Post code [redacted]

Country Slovakia

Website [redacted]

Phone [redacted] *Phone 2* *+xxx xxxxxxxxx*

Researchers involved in the proposal

Title	First Name	Last Name	Gender	Nationality	E-mail	Career Stage	Role of researcher (in the project)	Reference Identifier	Type of identifier	
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]			Other ID	

Role of participating organisation in the project

Project management	☐
Communication, dissemination and engagement	☒
Provision of research and technology infrastructure	☐
Co-definition of research and market needs	☐
Civil society representative	☐
Policy maker or regulator, incl. standardisation body	☐
Research performer	☒
Technology developer	☐
Testing/validation of approaches and ideas	☒
Prototyping and demonstration	☐
IPR management incl. technology transfer	☐
Public procurer of results	☐
Private buyer of results	☐
Finance provider (public or private)	☐
Education and training	☒
Contributions from the social sciences or/and the humanities	☐
Other If yes, please specify: (Maximum number of characters allowed: 50)	☒

Supplier Auditor
Quality Assurance Specialist

List of up to 5 publications, widely-used datasets, software, goods, services, or any other achievements relevant to the call content.

Type of achievement	Short description (Max 500 characters)
Service	*Performance of approx. 630 system audits/inspections of vendors, suppliers, and sites, on-site and remote in the pharmaceutical industry (such as chemical/biotechnology/plant sourced APIs active pharmaceutical ingredients, bulk ware manufacturers, all kinds finished dosage forms, incl. terminal-sterilized and aseptic forms, cell therapeutics, excipient manufacturers, distributors, packaging materials, IMP manufacturing, Quality Control labs, medical devices, freight forwarders.*
Service	*Quality Management consultancy: support for compilation & revision of Quality Assurance documentation; gap analysis of quality assurance systems;* *Assistance in preparation for regulatory/authority inspections by EU agencies; follow-up and CAPA support after EU GMP inspections;* *Supporting of EU regulatory inspections;* *Supplier qualifications for CMO, Drug product & drug substance manufacturer, laboratories, distribution, etc. Mock inspections, self-inspections; EU GMP upgrading of facilities*
Publication	*Active participation for the development of PIC/S Aide Memoire on the Inspection of APIs (PI 030-1)* *EU GMP (Good Manufacturing Practice) Aide Memoire*

List of up to 5 most relevant previous projects or activities, connected to the subject of this proposal.

Name of Project or Activity	Short description (Max 500 characters)
AGES Austrian Medicines and Medical Devices Agency	*GMP Inspector at AGES: conducting of GMP inspections of pharmaceutical companies, producers, wholesalers and distribution firms, domestic and abroad* *GMP = Good Manufacturing Practice for medicines*
AGES Austrian Medicines and Medical Devices Agency	*Authorized expert for the Austrian Federal Office for Safety in Health Care for GMP inspections*
Quality Assurance Manager	*Working as Qualified Person* *GMP, QA/QC compliance of production sites outside of Austria, Auditing of 3rd party supplier* *Batch release of products from 3rd party manufactures, Compilation of SOPs, Control procedures*

Description of any significant infrastructure and/or any major items of technical equipment, relevant to the proposed work.

Name of infrastructure of equipment	Short description (Max 300 characters)
Database GMP Berater Peithner AG	*Access to the GMP Berater Database for GMP (Good Manufacturing Practice) standards*

Gender Equality Plan

Does the organization have a Gender Equality Plan (GEP) covering the elements listed below? ○ Yes ◉ No

Minimum process-related requirements (building blocks) for a GEP

- **Publication:** formal document published on the institution's website and signed by the top management
- **Dedicated resources:** commitment of human resources and gender expertise to implement it.
- **Data collection and monitoring:** sex/gender disaggregated data on personnel (and students for establishments concerned) and annual reporting based on indicators.
- **Training:** Awareness raising/trainings on gender equality and unconscious gender biases for staff and decision-makers.
- **Content-wise, recommended areas** to be **covered** and addressed via concrete measures and targets are:
    - work-life balance and organisational culture;
    - gender balance in leadership and decision-making;
    - gender equality in recruitment and career progression;
    - integration of the gender dimension into research and teaching content;
    - measures against gender-based violence including sexual harassment.

PIC	Legal name
950531616	[redacted]

Short name: 2CABRAGA

Address

Street	[redacted]
Town	[redacted]
Postcode	[redacted]
Country	Portugal
Webpage	[redacted]

Specific Legal Statuses

Legal person	yes
Public body	no
Non-profit	yes
International organisation	no
Secondary or Higher education establishment	no
Research organisation	yes

SME Data

Based on the below details from the Participant Registry the organisation is not an SME (small- and medium-sized enterprise) for the call.

SME self-declared status	22/04/2013 - no
SME self-assessment	unknown
SME validation	unknown

Departments carrying out the proposed work

No department involved

Department name *Name of the department/institute carrying out the work.*

☒ not applicable

☐ Same as proposing organisation's address

Street *Please enter street name and number.*

Town *Please enter the name of the town.*

Postcode *Area code.*

Country *Please select a country*

Links with other participants

Type of link	Participant

Main contact person

This will be the person the EU services will contact concerning this proposal (e.g. for additional information, invitation to hearings, sending of evaluation results, convocation to start grant preparation). The data in blue is read-only. Details (name, first name and e-mail) of Main Contact persons should be edited in the step "Participants" of the submission wizard.

Title _____ Gender ◉ Woman ○ Man ○ Non Binary

First name* [redacted] Last name* [redacted]

E-Mail* [redacted]

Position in org. [redacted]

Department [redacted] ☒ Same as organisation name

☒ Same as proposing organisation's address

Street [redacted]

Town [redacted] Post code [redacted]

Country [redacted]

Website [redacted]

Phone [redacted] Phone 2 [redacted]

Other contact persons

First Name	Last Name	E-mail	Phone
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]

Researchers involved in the proposal

Title	First Name	Last Name	Gender	Nationality	E-mail	Career Stage	Role of researcher (in the project)	Reference Identifier	Type of identifier
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]			

Role of participating organisation in the project

Project management	☐
Communication, dissemination and engagement	☒
Provision of research and technology infrastructure	☒
Co-definition of research and market needs	☒
Civil society representative	☐
Policy maker or regulator, incl. standardisation body	☐
Research performer	☒
Technology developer	☐
Testing/validation of approaches and ideas	☒
Prototyping and demonstration	☐
IPR management incl. technology transfer	☐
Public procurer of results	☐
Private buyer of results	☐
Finance provider (public or private)	☐
Education and training	☒
Contributions from the social sciences or/and the humanities	☐
Other If yes, please specify: (Maximum number of characters allowed: 50)	☐

List of up to 5 publications, widely-used datasets, software, goods, services, or any other achievements relevant to the call content.

Type of achievement	Short description (Max 500 characters)
Publication	*Lopes V, Machado C, De Sousa Lages A. Autoimmune hypothyroidism and trastuzumab therapy: a rare association. Endocrinol Diabetes Metab Case Rep. 2023 Apr 25;2023(2):22-0412. doi: 10.1530/EDM-22-0412. PMID: 37096975; PMCID: PMC10241240.*
Service	*CR-Digital is a web-based platform designed to streamline the digitalization of clinical trials research related documents. Developed as part of a project led by 2CA-Braga, this innovative solution successfully transitioned to the market, culminating in its acquisition by an external company.*

List of up to 5 most relevant previous projects or activities, connected to the subject of this proposal.

Name of Project or Activity	Short description (Max 500 characters)
HfPT - Health from Portugal	*Project title: Health from Portugal* *Funding Program: European Union through Recovery and Resilience Plan* *Period: 1/11/2021 – 31/12/2025* *Role: Partner* *Budget for 2CA: 2.401.750,71€;*
Smart Health 4 All	*Project title: Smart medical technologies for better health and care* *Funding Program: Portugal 2020* *Period: 23/07/2020-30/06/2023* *Role: Partner* *Budget for 2CA:436.564,54€;*

Description of any significant infrastructure and/or any major items of technical equipment, relevant to the proposed work.

Name of infrastructure of equipment	Short description (Max 300 characters)
Clinical Academic Center	*Robust infrastructure that supports the development of clinical, translational research, including the conception, piloting and validation of innovative solutions as well as the execution of clinical trials, with direct access to healthy volunteers, patients, and an array of services, including 700*
MRI	*In-house 3 Tesla MRI dedicated to research projects*
TILT	*In-house TILT equipment that supports both clinical practice and research*
Sleep Laboratory	*Level 1 polysomnography with 2 rooms fully equipped, supporting clinical practice and research*
Fully equipped rooms	*CCAB's dedicated inpatient wing supports diverse clinical research, from pilot studies to Phase I-IV trials. Its fully equipped, quiet environment consolidates care, assessments, tests, and questionnaires in one location, enhancing patient comfort and ensuring seamless trial experiences.*

Gender Equality Plan

Does the organization have a Gender Equality Plan (GEP) covering the elements listed below? ○ Yes ◉ No

Minimum process-related requirements (building blocks) for a GEP

- **Publication:** formal document published on the institution's website and signed by the top management
- **Dedicated resources:** commitment of human resources and gender expertise to implement it.
- **Data collection and monitoring:** sex/gender disaggregated data on personnel (and students for establishments concerned) and annual reporting based on indicators.
- **Training:** Awareness raising/trainings on gender equality and unconscious gender biases for staff and decision-makers.
- **Content-wise, recommended areas** to be **covered** and addressed via concrete measures and targets are:
    - work-life balance and organisational culture;
    - gender balance in leadership and decision-making;
    - gender equality in recruitment and career progression;
    - integration of the gender dimension into research and teaching content;
    - measures against gender-based violence including sexual harassment.

PIC	Legal name
871206665	[redacted]

Short name: DAY

Address

Street	[redacted]
Town	[redacted]
Postcode	[redacted]
Country	Austria
Webpage	[redacted]

Specific Legal Statuses

Legal person	yes
Public body	no
Non-profit	no
International organisation	no
Secondary or Higher education establishment	no
Research organisation	no

SME Data

Based on the below details from the Participant Registry the organisation is an SME (small- and medium-sized enterprise) for the call.

SME self-declared status	02/06/2025 - yes
SME self-assessment	unknown
SME validation	unknown

Departments carrying out the proposed work

Department 1

Department name [redacted] ☐ not applicable

☐ Same as proposing organisation's address

Street [redacted]

Town [redacted]

Postcode [redacted]

Country Austria

Links with other participants

Type of link	Participant

Main contact person

This will be the person the EU services will contact concerning this proposal (e.g. for additional information, invitation to hearings, sending of evaluation results, convocation to start grant preparation). The data in blue is read-only. Details (name, first name and e-mail) of Main Contact persons should be edited in the step "Participants" of the submission wizard.

Title [redacted] Gender ◉ Woman ○ Man ○ Non Binary

First name* [redacted] Last name* [redacted]

E-Mail* [redacted]

Position in org. [redacted]

Department [redacted] ☐ Same as organisation name

☐ Same as proposing organisation's address

Street [redacted]

Town [redacted] Post code [redacted]

Country Austria

Website [redacted]

Phone [redacted] *Phone 2* *+xxx xxxxxxxxx*

Other contact persons

First Name	Last Name	E-mail	Phone
daniel	aletaha	daniel.aletaha@meduniwien.ac.at	+xxx xxxxxxxxx

Researchers involved in the proposal

Title	First Name	Last Name	Gender	Nationality	E-mail	Career Stage	Role of researcher (in the project)	Reference Identifier	Type of identifier
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]

Role of participating organisation in the project

Project management	☐
Communication, dissemination and engagement	☒
Provision of research and technology infrastructure	☐
Co-definition of research and market needs	☒
Civil society representative	☐
Policy maker or regulator, incl. standardisation body	☐
Research performer	☐
Technology developer	☐
Testing/validation of approaches and ideas	☐
Prototyping and demonstration	☐
IPR management incl. technology transfer	☐
Public procurer of results	☐
Private buyer of results	☐
Finance provider (public or private)	☐
Education and training	☐
Contributions from the social sciences or/and the humanities	☐
Other If yes, please specify: (Maximum number of characters allowed: 50)	☐

List of up to 5 publications, widely-used datasets, software, goods, services, or any other achievements relevant to the call content.

Type of achievement	Short description (Max 500 characters)
Software	[redacted] *is a digital platform designed to support patient recruitment in clinical trials. It allows treating physicians to access ongoing studies and register potentially eligible patients based on key inclusion criteria. With patient consent, study centres can then directly contact patients for eligibility screening. The platform supports multilingual use, and is fully compliant with GDPR and clinical data protection standards.*
Service	*Patient Recruitment:* [redacted] *provides a structured, user-friendly service to support patient recruitment in clinical trials by targeted pre-screening of patients by community doctors. Through a secure digital interface, treating physicians can review trial information, assess basic eligibility criteria, and register patients of interest, facilitating collaboration between community doctors and study centres.*
Service	*Clinical Trial Visibility:* [redacted] *increases the visibility of ongoing trials within the clinical community by making study opportunities accessible to treating physicians in a user-friendly interface tailored to physicians' specialties. Targeted outreach and digital alerts ensure that relevant studies reach appropriate specialists at the right time. This proactive visibility helps trials reach a broader network of clinicians beyond traditional study sites.*
Service	*Medical Community Engagement:* [redacted] *actively supports medical community engagement by building trusted networks between community physicians, study centres, and clinical research teams. The platform provides onboarding, training, and ongoing support to clinicians, enabling them to participate meaningfully in the clinical trial ecosystem. Through regular communication, feedback loops, and multilingual resources, P*[redacted] *fosters long-term collaboration and awareness.*

List of up to 5 most relevant previous projects or activities, connected to the subject of this proposal.

Name of Project or Activity	Short description (Max 500 characters)
STOP-PsA	*In this ongoing clinical trial investigating risk factors and therapeutic targets for early psoriatic arthritis, to date 71 out of 400 patients have been successfully recruited via PREFERRIX, enabled by pre-screening and registration of eligible patients by community-based physicians within the established network.*
JAK-SPARE	*In this completed clinical trial evaluating the safety and efficacy of Baricitinib in patients with newly diagnosed Polymyalgia Rheumatica, 3 out of 18 patients were successfully recruited via* [redacted]*, enabled by pre-screening and registration of eligible patients by community-based physicians within the established network.*
HORIZON HZN-1116	*In this ongoing early-phase study assessing HZN-1116 in patients with Sjögren-Syndrome, 1 out of 4 patients has been successfully recruited via* [redacted]*, supported by direct collaboration with a local physician within the established network.*
EU Funded Projects	[redacted] *staff bring extensive experience in supporting the planning, patient recruitment, site coordination and execution of EU-funded clinical trials in rheumatology (e.g. SQUEEZE and Autopix), making* [redacted] *a trusted partner in improving recruitment outcomes in clinical research across the EU.*

Description of any significant infrastructure and/or any major items of technical equipment, relevant to the proposed work.

Name of infrastructure of equipment	Short description (Max 300 characters)

Gender Equality Plan

Does the organization have a Gender Equality Plan (GEP) covering the elements listed below? ○ Yes ◉ No

Minimum process-related requirements (building blocks) for a GEP

- **Publication:** formal document published on the institution's website and signed by the top management
- **Dedicated resources:** commitment of human resources and gender expertise to implement it.
- **Data collection and monitoring:** sex/gender disaggregated data on personnel (and students for establishments concerned) and annual reporting based on indicators.
- **Training:** Awareness raising/trainings on gender equality and unconscious gender biases for staff and decision-makers.
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    - work-life balance and organisational culture;
    - gender balance in leadership and decision-making;
    - gender equality in recruitment and career progression;
    - integration of the gender dimension into research and teaching content;
    - measures against gender-based violence including sexual harassment.

3 - Budget

No.	Name of beneficiary	Country	Role	Personnel costs/€	Subcontracting costs/€	Purchase costs - Travel and substistence/€	Purchase costs - Equipment/€	Purchase costs - Other goods, works and services/€	Internally invoiced goods and services/€ (Unit costs- usual accounting practices)	Indirect costs/€	Total eligible costs	Funding rate	Maximum EU contribution to eligible costs	Requested EU contribution to eligible costs/€	Max grant amount	Income generated by the action	Financial contributions	Own resources	Total estimated income
1	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
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This proposal version was submitted by **georg MELZER-VENTURI** on **16/09/2025 11:42:07** Brussels Local Time. Issued by the Funding & Tenders Portal Submission System.

Administrative forms

Proposal ID **101286237**

Acronym **APO2FOOT**

11	[redacted]																		
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[redacted]																			

4 - Ethics & security

Ethics Issues Table

1. Human Embryonic Stem Cells and Human Embryos			Page
Does this activity involve Human Embryonic Stem Cells (hESCs)?	○ Yes	◉ No	
Does this activity involve the use of human embryos?	○ Yes	◉ No	
2. Humans			**Page**
Does this activity involve human participants?	◉ Yes	○ No	all
Are they volunteers for non medical studies (e.g. social or human sciences research)?	○ Yes	◉ No	
Are they healthy volunteers for medical studies?	○ Yes	◉ No	
Are they patients for medical studies?	◉ Yes	○ No	all
Are they potentially vulnerable individuals or groups?	○ Yes	◉ No	
Are they children/minors?	○ Yes	◉ No	
Are they other persons unable to give informed consent?	○ Yes	◉ No	
Does this activity involve interventions (physical also including imaging technology, behavioural treatments, etc.) on the study participants?	◉ Yes	○ No	all
Does it involve invasive techniques?	○ Yes	◉ No	
Does it involve collection of biological samples?	○ Yes	◉ No	
Does this activity involve conducting a clinical study as defined by the Clinical Trial Regulation (EU 536/2014)? (using pharmaceuticals, biologicals, radiopharmaceuticals, or advanced therapy medicinal products)	◉ Yes	○ No	all
Is it a clinical trial?	◉ Yes	○ No	all
Is it a low-intervention clinical trial?	◉ Yes	○ No	all
3. Human Cells / Tissues (not covered by section 1)			**Page**
Does this activity involve the use of human cells or tissues?	○ Yes	◉ No	
4. Personal Data			**Page**
Does this activity involve processing of personal data?	◉ Yes	○ No	all
Does it involve the processing of special categories of personal data (e.g.: genetic, biometric and health data, sexual lifestyle, ethnicity, political opinion, religious or philosophical beliefs)?	○ Yes	◉ No	
Does it involve profiling, systematic monitoring of individuals, or processing of large scale of special categories of data or intrusive methods of data processing (such as, surveillance, geolocation tracking etc.)?	○ Yes	◉ No	
Does this activity involve further processing of previously collected personal data (including use of preexisting data sets or sources, merging existing data sets)?	○ Yes	◉ No	
Is it planned to export personal data from the EU to non-EU countries?	○ Yes	◉ No	
Is it planned to import personal data from non-EU countries into the EU or from a non-EU country to another non-EU country?	○ Yes	◉ No	
Does this activity involve the processing of personal data related to criminal convictions or offences?	○ Yes	◉ No	

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5. Animals			Page
Does this activity involve animals?	○ Yes	◉ No	

6. Non-EU Countries			Page
Will some of the activities be carried out in non-EU countries?	○ Yes	◉ No	
In case non-EU countries are involved, do the activities undertaken in these countries raise potential ethics issues?	○ Yes	◉ No	
It is planned to use local resources (e.g. animal and/or human tissue samples, genetic material, live animals, human remains, materials of historical value, endangered fauna or flora samples, etc.)?	○ Yes	◉ No	
Is it planned to import any material (other than data) from non-EU countries into the EU or from a non-EU country to another non-EU country? For data imports, see section 4.	○ Yes	◉ No	
Is it planned to export any material (other than data) from the EU to non-EU countries? For data exports, see section 4.	○ Yes	◉ No	
Does this activity involve low and/or lower middle income countries, (if yes, detail the benefit-sharing actions planned in the self-assessment)	○ Yes	◉ No	
Could the situation in the country put the individuals taking part in the activity at risk?	○ Yes	◉ No	

7. Environment, Health and Safety			Page
Does this activity involve the use of substances or processes that may cause harm to the environment, to animals or plants.(during the implementation of the activity or further to the use of the results, as a possible impact) ?	○ Yes	◉ No	
Does this activity deal with endangered fauna and/or flora / protected areas?	○ Yes	◉ No	
Does this activity involve the use of substances or processes that may cause harm to humans, including those performing the activity.(during the implementation of the activity or further to the use of the results, as a possible impact) ?	○ Yes	◉ No	

8. Artificial Intelligence			Page
Does this activity involve the development, deployment and/or use of Artificial Intelligence-based systems?	○ Yes	◉ No	

9. Other Ethics Issues			Page
Are there any other ethics issues that should be taken into consideration?	○ Yes	◉ No	

I confirm that I have taken into account all ethics issues above and that, if any ethics issues apply, I will complete the ethics self-assessment as described in the guidelines How to Complete your Ethics Self-Assessment ☒

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Ethics Self-Assessment

Ethical dimension of the objectives, methodology and likely impact

Data protection: personalised data will be securely managed and stored at the individual centres according to the general data protection regulation (GDPR) guideline. Data analysis will exclusively be performed on pseudonymised data. Only authorisised persons have access to the data. Publication of patient data will only be performed in an anonymised way.

Clinical trial management: all clinical studies (including study protocols and measures) will be designed and performed in agreement with ICH-GCP guidelines and the Declaration of Helsinki. Patients will receive all information on the studies benefits and risks and have to sign informed consent forms prior to study enrollment. Trial subjects will have an additional insurance covering potential harm resulting from the study participation.

During the trial we will be taking photographs of the open wound to assess the wound healing. All pictures are stored in an secure file system approve for storing clinical trial data.

Remaining characters 4015

Compliance with ethical principles and relevant legislations

Activities relating to Ethics and Governance will be led by the Ethics Coordinator (MUW) and supported by an external Ethics Advisor (Contractor).
Tasks to ensure that all national and international regulatory and ethical requirements are fulfilled will be performed in cooperation with the Clinical Study Management Team. Contact with all project partners is crucial for communication of critical information from project management to partners and vice versa.
Research and innovation have improved our lives in many ways. However, science and technology sometimes create new risks and ethical dilemmas. They can generate controversy and not solve problems or create new issues of concern. RRI responds to different challenges by engaging all stakeholders (researchers, governments, citizens, etc) through inclusive and participatory methodologies. To be effective, participation takes place at all stages of the process and at all levels (from program planning to design, implementation and evaluation). Research and innovation will address societal challenges and align with the values, needs and expectations of the public.

Ethics Objective: to ensure that all national and international regulatory and ethical requirements are fulfilled for the project.

Specifically, the central objectives are: (a) to ensure that the study protocol and measures are in agreement with ICH-GCP and the Declaration of Helsinki; (b) to ensure continuous ethical scrutiny of activities in the project; (c) to provide advice for patient consenting and inclusion regarding the randomised approach; (d) to ensure – via the external EGC – independent external supervision and advice regarding the ethical, legal and safety aspects of the clinical studies; (e) to monitor the procedures in place for the study to ensure safeguard of patients' interests and entitlements; (f) to review data safety measures and ensure compliance with the current EU data protection legislation; (g) to provide a framework that enables project partners to address ad hoc ethical and legal issues competently and swiftly.

Support to WP3. Lead: MUW Ethics Department- Activities relating to Ethics will be led by the Ethics Coordinator and supported by external Ethics Independent Advisors.

[redacted]

Application (CTA) dossier (master regulatory document) that will be submitted to the IRBs and competent authorities as necessary for assessment and feedback, according to European and national legislation. National ECRIN scientific partners will adapt the CTA to national requirements, and translate it into the respective languages of the study partners. The CTA will include the clinical study protocols and accompanying documents (e.g. informed consent, case report form [CRF], investigator's brochure), which will be reviewed by all clinical centres and receive final approval by the EGC.
Task C): Data protection [Task leader: Ethics Coordinator; MUW; Months 1 to 60]: This task will monitor all aspects of data protection. Ethics Coordinator will check whether data are treated according to EU regulation 2016/679 (e.g. pseudonymisation, security measures to prevent unauthorised access and sampling according to data minimisation principles). The Sponsor ensures that the investigators will permit data-related monitoring and audits, providing direct access to source data/documents.
Task D): _H - Requirement No. 1
Task E)_POPD- Requirement no 3

Milestones:- Ethical approval of the trial (MS4)

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Security issues table

1. EU Classified Information (EUCI)[2]			Page
Does this activity involve information and/or materials requiring protection against unauthorised disclosure (EUCI)?	○ Yes	◉ No	
Does this activity involve non-EU countries which need to have access to EUCI?	○ Yes	◉ No	
2. Misuse			**Page**
Does this activity have the potential for misuse of results?	○ Yes	◉ No	
3. Other Security Issues			**Page**
Does this activity involve information and/or materials subject to national security restrictions? If yes, please specify: (Maximum number of characters allowed: 1000)	○ Yes	◉ No	
Are there any other security issues that should be taken into consideration? If yes, please specify: (Maximum number of characters allowed: 1000)	○ Yes	◉ No	

Security self-assessment

Please specify: (Maximum number of characters allowed: 5000)

Remaining characters 5000

[2]According to the Commission Decision (EU, Euratom) 2015/444 of 13 March 2015 on the security rules for protecting EU classified information, "European Union classified information (EUCI) means any information or material designated by an EU security classification, the unauthorised disclosure of which could cause varying degrees of prejudice to the interests of the European Union or of one or more of the Member States".

[3]Classified background information is information that is already classified by a country and/or international organisation and/or the EU and is going to be used by the project. In this case, the project must have in advance the authorisation from the originator of the classified information, which is the entity (EU institution, EU Member State, third state or international organisation) under whose authority the classified information has been generated.

[4]EU classified foreground information is information (documents/deliverables/materials) planned to be generated by the project and that needs to be protected from unauthorised disclosure. The originator of the EUCI generated by the project is the European Commission.

5 - Other questions

Essential information to be provided for proposals including clinical Trials / studies / investigations

Clinical study means, for the purpose of this document, any systematic prospective or retrospective collection and analysis of health data obtained from individual patients or healthy persons in order to address scientific questions related to the understanding, prevention, diagnosis, monitoring or treatment of a disease, mental illness, or physical condition. It includes but it is not limited to clinical studies as defined by Regulation 536/2014 (on medicinal products), clinical investigation and clinical evaluation as defined by Regulation 2017/745 (on medical devices), performance study and performance evaluation as defined by Regulation 2017/746 (on in vitro diagnostic medical devices).

Are clinical studies / trials / investigations included in the work plan of this project? (●) Yes ( ) No

Please upload the dedicated annex 'Essential information for clinical studies / trials / investigations' (a Word template is provided under 'download templates' in the up-load section for Part B and Annexes).

This document should include the relevant information of each clinical study / trial / investigation included in the work plan of this project.

Please give a short title, an acronym or a unique identifier to each clinical study / trial / investigation, to be used as a reference/ identifier in the other parts of the proposal.
APO2FOOT

APO2FOOT: A randomised, open label, observer-blinded clinical trial to evaluate safety and efficacy of APO-2 in comparison to Standard of Care in the treatment of Diabetic Foot Ulcers

#@APP-FORM-HERIAIA@#

List of participants

Partic. No.	Participant organisation name (Short name)	Country	Type
1 (Coord)	[redacted]	AT	UNI
2		AT	SME
3	Aposcience AG (APOSC)	AT	IND
4		FR	SME
5		CZ	UNI
6		AT	NGO
7		AT	IND
8		DE	UNI
9		DE	SME
10		CH	IND
11		SK	SME
12		PT	UNI
13		AT	SME

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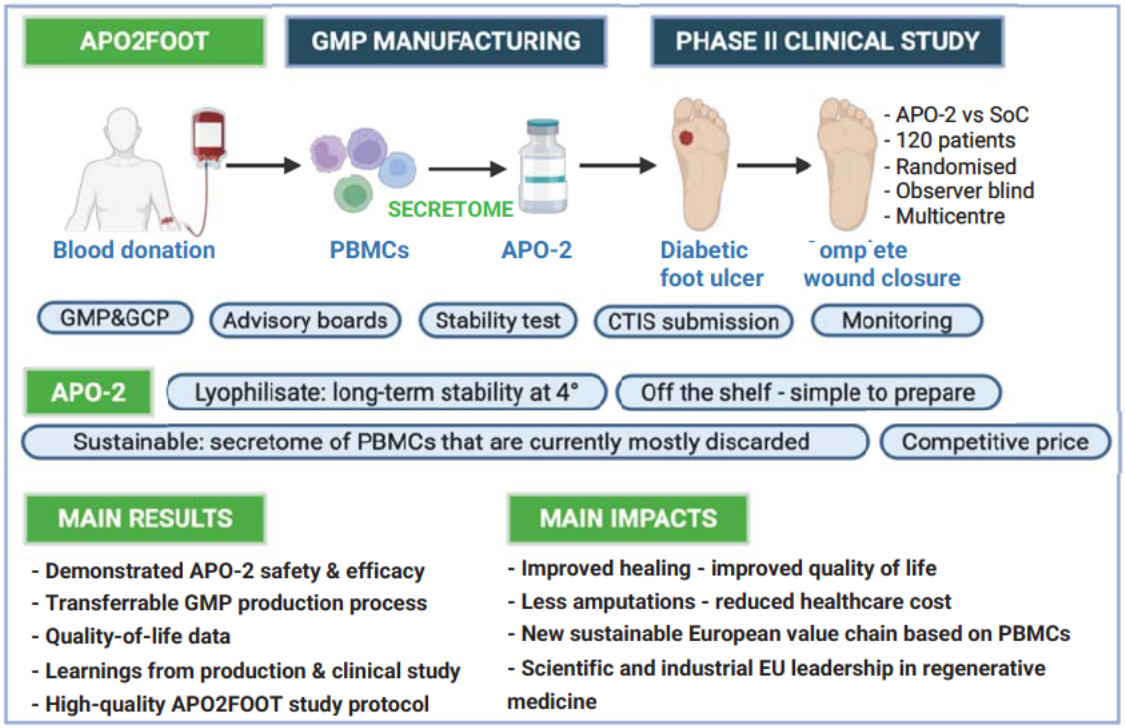
List of abbreviations

(c)DMP: (clinical) Data Management Plan	**IMPD**: Investigational Medicinal Product Dossier
ATMP: Advanced Therapy Medicinal Products	**IP**: Intellectual Property
BASG: Austrian national health authority	**IWGDF**: International Working Group on the Diabetic Foot
CMO: Contract Manufacturing Organisation	**KPI**: Key Performance Indicator
CRO: Clinical Research Organisation	**MoA**: Mode of Action
CSP: Clinical Study Protocol	**MS**: Milestone
CSR: Clinical Study Report	**PAB**: Patient Advisory Board
CTIS: Clinical Trials Information System	**PAD**: peripheral artery disease
DEC: Dissemination, Exploitation, Communication	**PBMC**: Peripheral Blood Mononuclear Cells
DFU: Diabetic Foot Ulcer	**PI**: Principal Investigator
DSMB: Data Safety and Monitoring Board	**QMS**: Quality Management System
DSUR: Development Safety Update Report	**QoL**: Quality of Life
EGC: Ethics and Governance Council	**SAB**: Scientific Advisory Board
eCRF: electronic Case Report Form	**SoC**: Standard of Care
EoS: End of Study	**SOP**: Standard Operating Procedure
EoT: End of Treatment	**SO**: Specific Objective
GCP: Good Clinical Practice	**SUSAR**: Suspected Unexpected Serious Adverse Reaction
GLP: Good Laboratory Practice	**TPP**: Target Product Profile
GMP: Good Manufacturing Practice	**U**: Units of APO-2
ICH: International Commission of Harmonization	**WP**: Work Package
IMP: Investigational Medicinal Product	

Executive Summary



CHALLENGE/NEED: **Diabetic foot ulcers (DFUs)** are one of the most severe complications of diabetes, affecting nearly **one million people in the EU every year**. Chronic DFUs fail to heal within months, leading to infections, **amputations**, and a five-year mortality rate exceeding that of many cancers. Current standard care (blood sugar control, debridement, wound dressings, off-loading) is often insufficient, and available adjunctive therapies show limited efficacy, high costs, or lack regulatory approval. This situation creates an **urgent medical, societal, and economic challenge** for Europe's healthcare systems.

SOLUTION: APO2FOOT will demonstrate the safety and efficacy of APO-2, a novel off-the-shelf secretome therapy, in a clinical phase II study. It will be the first effective and reimbursable treatment to accelerate healing of chronic diabetic foot ulcers and reduce amputations

APO2FOOT clinically validates **APO-2**, a novel, cell-free secretome therapy derived from **stressed peripheral blood mononuclear cells** (PBMCs- white blood cells). Unlike cell-based treatments, APO-2 is a **stable** Investigational Medicinal Product (IMP), **virus-inactivated** and can be stored for years. This "off-the-shelf" product can be **easily prepared and applied** in DFU patients. Preclinical studies and early clinical trials (MARSYASI/II) have shown the multiple modes of action (MoA) of APO-2: it accelerates **wound healing by promoting angiogenesis**. APO-2 production will comply with Good Manufacturing Practice (**GMP**) requirements. A rigorously designed, multicentre, multinational, observer-blinded **phase II clinical trial** with 120 patients across five EU countries, comparing APO-2 plus standard of care (SoC) to SoC alone, will be conducted. Key innovations of APO2FOOT will include a) a well described **GMP process** of APO-2 production that can be easily **transferred** to other production sites and b) the execution of a meticulously planned DFU study that meets the **highest clinical standards**. After delivering evidence that APO-2 is safe and efficacious, APOSC, the sponsor, will continue with a pivotal trial. APO2FOOT aims to establish **APO-2 as the first effective, accessible, and reimbursable active treatment for chronic DFU**.

KNOWLEDGE BOX: during Aposec development, different types (sources, production processes) of Aposec have been used. Here a short overview:
Aposec: is the general name given to the secretome of stressed peripheral blood mononuclear cells (PBMC-white blood cells) – the APOptotic SECretome.
APO-1: human Aposec for intravenous application
APO-2: human Aposec produced under GMP for topical application

VISION: The expected health impacts are **fewer amputations**, improved patient **quality of life**, reduced mortality, and substantial **savings for healthcare** systems, while positioning Europe at the forefront of secretome-based **regenerative medicine**. The project exemplifies how combining non-profit production capacities with pharmaceutical and clinical expertise can achieve both societal benefits and economic sustainability. The use of PBMC as starting material highlights the **resource-efficient** and innovative character of this approach.

1. Excellence

#@REL-EVA-RE@#

1.1. Objectives and ambition

#@PRJ-OBJ-PO@#

1.1.1. Specific objectives (SO) and key performance indicators (KPIs)

The specific objectives (SOs) of APO2FOOT are related to the **scope** and activities of the Topic (**Pertinence**). A detailed work plan implemented through work packages (WPs) ensures the SOs are **achievable**, while results and KPIs make them **measurable and verifiable**.

SO1: Ensure the project stays on time and within budget while achieving its objectives

Pertinence: SO1 fulfils the scope of "***pave the way*** *to secretome-based therapies that are safe, efficacious, and regulatory-approved for human use*" through timely and efficient project management.

Challenge: effective monitoring of budget and quality; bridging the gap between science and clinical practice;

Achievable: is ensured through effective overall management of APO2FOOT, covering governance, administrative and financial management, risk management and data management, and communication between partners. The advisory boards (Scientific-SAB; Patient-PAB; Data Safety and Monitoring Board - DSMB) will help ensure high scientific, ethical, and regulatory standards.

SO2: Deliver sufficient stable, and trial-ready APO-2, ensure GCP compliance of study centres and enable post-project scale-up of the GMP production process

Pertinence: this SO mainly addresses "*establishment of a* ***fully GMP-conform production*** *process*" and "***manufacturing*** *protocol for the selected secretome… mode of administration, final* ***formulation***" … while ensuring "***compliance*** *with all requirements of the relevant competent authorities*", and also contributes to *"All activities that are necessary to ensure regulatory and ethical approvals… full* ***characterization*** *…****quality*** *assurance"*

Challenge: Full documentation of GMP production; ensure GCP-compliance of many diverse study sites.

Achievable: GMP-grade APO-2 will be produced and tested (stability, packaging, etc). We will prepare product-relevant documents for the submission of the clinical trial, audit suppliers and ensure Good Clinical Practice (GCP) compliance of study centres. The GMP production process will be described in detail for post-project tech transfer.

Results & KPIs: Sufficient APO-2 for clinical trial and stability study produced according to GMP. Stability confirmed. Product-related documents for CTIS submission. Scalable GMP production process for APO-2.

SO3: Guarantee the ethical integrity and regulatory compliance of the APO2FOOT clinical study by adhering to all applicable national and international standards and obtaining full regulatory approval

Pertinence: SO3 answers to *"All activities that are necessary to ensure* ***regulatory and ethical approvals****"* and contributes to "***compliance*** *with all requirements of the relevant competent authorities*"

Challenge: Manage variations in ethics procedures between study countries.

Achievable: we will establish an independent Ethics & Governance Council to define an Ethics & Governance Framework for APO2FOOT. The regulatory/ethical section of the study application will be prepared, as well as Quality of Life (QoL) and pain assessment questionnaires. Quality by Design (QbD)-based risk principles will be implemented.

Results & KPIs: Ethics & Governance Framework; QoL and pain assessment questionnaires; regulatory/ethics-related documents for CTIS submission.

SO4: Deliver a high-quality Phase II trial proving APO-2's safety and efficacy in DFU with 120 patients by 2030, to initiate a Phase III trial

Pertinence: SO4 addresses "*Conduct of an interventional randomised controlled* ***clinical trial*** *comprising phase 1 and phase 2 to generate scientific evidence* ***demonstrating safety and efficacy***" and "*deliver…* ***documentation*** *needed for the conduct of the clinical trial*"

Challenge: Prepare flawless regulatory dossier to avoid questions that would cause delays; Consistent study conduct across sites.

Achievable: this is the core of APO2FOOT: implement in the clinical trial, from preparing and submitting the clinical study application, initiating study sites, and recruiting patients, to conducting the trial with rigorous monitoring, pharmacovigilance, and data management. Adverse event reporting and statistical analysis will confirm safety and efficacy; a futility evaluation will decide about continuation into phase III (post-project)

Results & KPIs: Clinical study application and approval; 120 randomised patients finish the trial; 3 Safety Update Reports; Clinical Study Report or decision to continue into Phase III (post-project)
SO5: Maximise the visibility, uptake, and long-term impact of APO2FOOT through strategic dissemination, effective communication, and well-planned exploitation
Pertinence: SO5 contributes to the scope of "***pave the way*** *to secretome-based therapies*" through outreach and communication activities. **Challenge**: balance openness with intellectual property (IP) protection and confidentiality. **Achievable**: We develop and implement a comprehensive dissemination, exploitation, and communication (DEC) strategy and design a project website, social media channels and tailored materials to reach out to both expert and non-expert audiences. Results will be disseminated and exploitation prepared plans to support APO-2 marketing.
Results & KPIs: 3 DEC plans; Exploitation plan; Project website and social media channels

Pertinence: The Topic lists several additional requirements/activities which we all fulfil:
"*deliver …the documentation needed for the* ***GMP-conform production***": this has been achieved prior to APO2FOOT: APO-2 production has been GMP-conform for many years, an up-to-date description for later upscaling will be prepared in WP2.
"*The selection of a secretome-based therapy whose main* ***mechanism of action*** *has been elucidated in in-vitro and/or in-vivo;… have been characterised and its/their therapeutic activity should already have been demonstrated in relevant pre-clinical models*." All these activities have been conducted, please see the methodology (Section 1.2) and Annex on clinical studies for details.
"*All types of diseases, dysfunctions or health impairments may be targeted, preference should be given to conditions that affect* ***larger patient populations****…*". This is clearly addressed by targeting chronic DFU, which affects approx. 600,000 patients in the EU per year (see also ambition section below)[1].
"*Participation of* ***SMEs*** *is strongly encouraged and if an exploitation strategy is developed, it should commit to a* ***first deployment in the EU***": Of the 13 beneficiaries, 5 are European SMEs ([redacted]). They will gain valuable experience related to clinical trials and regulatory issues related to a biological (see also Section 3.2.). As to the exploitation strategy (Section 2.2.6), the aim is to achieve marketing approval first in Austria, followed by mutual recognition procedures in other European countries.

1.1.2. Ambition and R&I maturity

MAIN AMBITION: APO-2 aims to be the first and only adjunct treatment with an active substance for chronic DFU with solid clinical data confirming high efficacy in Europe. With the APO2FOOT study we aim to achieve that the International Working Group on the Diabetic Foot (IWGDF) changes its Wound Healing Guideline to include APO-2 as an adjunctive intervention to SoC in chronic DFU, in cases where SoC alone fails based on strong and reliable results in the clinical study.

INTRO: In the following pages we explain, 1) why we focus on chronic DFU; 2) describe SoC, defined as moist wound treatment without active ingredients, 3) which treatments with active ingredients are authorised in Europe and why they have not been successful in the market, 4) which treatments are authorised in the USA and elsewhere, but not in Europe, 5) which of the products authorised in Europe are actually available to patients in Europe by taking a look at current European reimbursement practices; 6) Summary of active wound healing products in Europe. In 7) we present an outlook – listing ongoing clinical studies on DFU, showing that there is also no parallel development with comparable potential – and 8) explain why other secretome therapies have not yet reached the phase of clinical examination. All of this confirms that APO-2 is unrivalled by combining **high efficacy in a comparatively short treatment period, ease of use, long shelf life / stability and competitive pricing**. Finally, there is a secondary ambition of APO2FOOT concerning the study itself: setting a gold-standard for DFU studies.

1) Chronic diabetic foot ulcer (DFU) – a disease of high medical need:

- **Prevalence of diabetes**: About 33.7 million adults (20-79 years) in the EU have diabetes (9.7% of the adult population).[2]

[1] Nearly 1 million patients in the EU suffer from DFU per year (Sämann et al. 2008, doi: 10.1111/j.1464-5491.2008.02435.x); 60-70% of DFUs become chronic (Armstrong et al. 2023, doi: 10.1001/jama.2023.10578) ≥ 600,000 patients.
[2] IDF Diabetes Atlas 11th Edition – 2025, Factsheet "Diabetes in Europe – 2024".

- In the **EU**, 2.9% of diabetics suffer from a **DFU** per year (**978,000 DFU patients**), 0.8% of diabetics suffer from an acute DFU at any timepoint (=right now) (270,000 patients).[3] In the USA, 1.6 million people are affected by DFU each year; worldwide 18.6 million people are estimated to suffer from DFU in any given year.[4]
- DFU that does not heal within **8 weeks** are considered **chronic** – this is the target of APO-2[5]
- 30-40% of the DFU heal at 12 weeks, but 23% of patients have a non-healed DFU after 12 months.[6]
- The **probability** to develop a DFU during a diabetic's lifetime is **19-34% (lifetime prevalence**).[7]
- If a DFU is left untreated it can progress to soft tissue infection, gangrene and limb loss (**amputation**)[8]
- 40-60% of all non-traumatic amputations (=amputations not resulting from accidents) are due to DFU.[9]
- The 5-year **mortality** rate of a person with DFU is 30%; the 5-year mortality rate of a person with an above-foot amputation exceeds 70%.[10]

The target group of APO2FOOT are patients with chronic DFU. We aim to make DFU heal faster to reduce the risk of amputation.

Usually, patients with DFU are **first treated with SoC**, and only in cases where SoC alone fails, **adjunct therapies** are recommended **for consideration**. The average SoC treatment success rates are exemplified in an Italian study: 583 patients treated with SoC according to guidelines were followed for 10 years (2009-19). 79.6% of wounds healed in a mean time of 7.6 months; 6.9% did not heal at all and 13.5% of the patients' experienced amputations.[11]

2) Standard of Care (SoC):

SoC consists of the following essential elements:[12]

- Blood sugar control and treatment of internal underlying diseases;
- Infection control;
- Debridement of non-vital tissue parts;
- Effective off-loading of pressure;
- Therapy of vascular diseases when indicated;
- Wound dressings which absorb exudate and preserve a moist wound environment without any active ingredient[13]

Recommendation E29 of the German Association for Wound Healing and Wound Treatment: "*Wound dressings or products **without any active components** should be used to cover and treat wounds without clinical signs of infection*."[14]

3) Adjunct treatments authorised in Europe

In cases **where SoC alone has no effect**, the International Working Group on the Diabetic Foot (IWGDF) does recommend some wound care products as adjunct treatments, which are expected to **actively promote wound healing**, based on a thorough review of clinical evidence[15]. Mostly, theses adjunct therapies are recommended **for consideration only** because of a) **weak results** in clinical studies and/or b) the **non-reliability** of the methodology and results of the clinical studies. The following adjunct treatments to SoC with active ingredients for chronic DFUs are **recommended for consideration** by the IWGDF and **authorised in Europe, but they have limited therapeutic effectiveness and have not been successful in the market and are not being used widely:**

• 3C Patch (formerly LeucoPatch): autologous leucocyte-platelet-fibrin patch from patient's own blood, authorised as medical device class IIa. IWGDF considers this clinical study as the **only high-quality study** with low risk of bias. Results: complete wound closure in 20 weeks in 34% (45/132) vs 22% (29/134) in the control with weekly

[3] Sämann A et al. 2008, doi: 10.1111/j.1464-5491.2008.02435.x.; de.statista.com
[4] Zhang Y et al. 2020, doi: 102337/dc19-1614.
[5] "Lokaltherapie schwerheilender und/oder chronischer Wunden...", S3-Leitlinie der Deutschen Gesellschaft für Wundheilung und Wundbehandlung. Version 2.2, 31.10.2023, Glossary.
[6] Armstrong DG et al. 2023, doi: 10.1001/jama.2023.10578.
[7] Praxisempfehlungen der Deutschen Diabetes Gesellschaft. S2, Nov. 2024, p. 349.
[8] Ndosi M et al. 2018, doi: 10.1111/dme.13537.
[9] Köhler G et al. Diabetische Neuropathie und diabetischer Fuß (Update) (=Guidance of the Austrian Diabetes Society), Wiener Klinische Wochenschrift (2023) 135 (Suppl 1): S. 164-183; p. 173.
[10] Armstrong DG es al. 2020, doi: 10.1186/s13047-020-00383-2.
[11] Gazzaruso C et al. 2020, doi: 10.1007/s12020-020-02431-0.
[12] S2-Leitlinie of the German Diabetes Society, 2024, doi: 10.1055/a-2312-0739.
[13] See also: Guidelines on interventions to enhance healing of foot ulcers in people with diabetes. IWGDF 2023 update.
[14] "Lokaltherapie schwerheilender und/oder chronischer Wunden...", S3-Leitlinie der Deutschen Gesellschaft für Wundheilung und Wundbehandlung. Version 2.2, 31.10.2023.
[15] IWGDF: Guidelines on interventions to enhance healing of foot ulcers in people with diabetes, IWGDF 2023 update.

application. Disadvantages: **complicated, time-consuming** (>20 min/treatment, requires **special device** and centrifuge), risk of anaemia, not established in market, no reimbursement in EU health systems, no documented use in AT/DE. Dose of active secretome much lower than in APO-2.

• Sucrose-octasulfate (SOS) impregnated dressing: conditional use – not for all wounds, authorised as class IIb device. IWGDF considers the clinical study (EXPLORER) with low risk of bias but only **moderate evidence**. Results: complete wound closure after 20 weeks in 48% (60/126) vs 30% (34/114) in the control, 2 weekly applications. [16] Disadvantages: results achieved only after 20 weeks, 20% wound **infections** in SOS group, moderate effect. APO-2 aims at a comparable or higher wound closure rate already after 12 weeks.

• Kerecis Omega3 is a fish skin substitute and has marketing authorisation as a class III (=high risk) medical device in Europe, under Medical Device Directive (93/42/EEC) which expired in 2022, not yet under the new Medical Device Regulation (2017/745). Results: 44% wound closure vs 26% in the control at 16 weeks, 255 patients. The product is also mentioned below in the section of adjunct treatments outside Europe because it is reimbursed by Medicare in the USA. However, although authorised in Europe, according to Coloplast Annual Report 2024, Kerecis Omega3 has been sold up to date **exclusively in the USA**.

• Hyperbaric oxygen therapy: patients inhale pure oxygen at high pressure in a chamber for 45–60 min. IWGDF found 3 double-blind and 15 unblinded studies; 2 double-blind studies had low bias but contradictory results. German insurance reimburses this therapy under restrictive conditions.[17] Results: A meta-analysis of 11 studies found wound closure in 46% (148/321) vs 23% (75/323) from 14 days **to 12 months**.[18] Disadvantages: **resource-intensive**, requires the chamber, available only in specialised centres, daily therapies for 4–6 weeks, high compliance needed.

• Topical oxygen therapy: [19] applied directly to wound. Approaches: continuous oxygen (NATROX O2, OxyGeni, EpiFlo); cyclical/low pressure extremity chambers (TWO2, O2-TopiCare, O2Boot, O2Sacral); haemoglobin spray (Granulox); continuous-release oxygen dressings (OxyBand, OxyGenesis, Oxyzyme/hydrogel). For most of these therapies, there is no clinical evidence. IWDGF found 3 double-blinded and 7 non-blinded clinical studies. 2 double-blinded trials were at low risk of bias, but only one had statistically significant results. The evidence was of **low certainty**, with desirable effects rated as moderate. Results: Granulox[20]: 25% (5/20) healed in 4 weeks vs 5% (1/15) in the control. TWO2: 41.7% (15/36) healed in 12 weeks vs 13.5% (5/37) in the control. Disadvantages: Only some therapies are marketed/available in some countries e.g. O2-TopiCare in Austria; topical oxygen therapy in 5 wound care centres; Granulox is authorised in Europe as class III medical device. In June 2025 Germany started an evaluation of the cost-benefit ratio of topical oxygen therapy.

APO-2 EFFICACY COMPARISON/AMBITION: as detailed above, there are few clinical studies in DFU mentioned by the IWGDF as of high quality and low bias, of products authorised in the EU: **LeucoPatch**[26] study, **sucrose-octasulfate dressing**[27] and the **Kerecis fish-skin** study[28]. Kerecis was tested on especially deep wounds (penetrating to the bone and joint and amputation wounds), so this is a **different target** to APO2FOOT.
LeucoPatch achieved 34% vs 20% ulcers healed in **20 weeks**, sucrose-octasulfate dressing 48% vs 30% healed ulcers in 20 weeks. Based on the results of the previous APO-2 clinical study MARSYAS I/II, we are confident that APO-2 will be **more effective** AND heal most chronic DFUs already after 12 weeks, so **much faster.**

Currently, the IWGDF does NOT recommend for consideration as adjunct therapies the following, due to insufficient clinical evidence or clinical studies with high risk of bias: as routine adjunct therapy for DFU: antiseptic or antimicrobial dressings, honey (or bee-related products), topical phenytoin, dressings based or topical applications impregnated with herbal remedies, cellular skin substitute products, acellular skin substitute products, autologous skin graft, skin substitute products, autologous platelet therapy, any other cell therapy, growth factor therapy, any pharmacological agents promoting perfusion and angiogenesis, pharmacological agents that supplement vitamins and trace elements, pharmacological agents that stimulate red cell production, and Negative Pressure Wound Therapy for non-surgically-related foot wounds.

4) Adjunct treatments outside Europe

There is a long list of wound healing products with active ingredients as adjunct treatments to SoC for chronic DFUs, that have **no marketing authorisation in Europe,** or only in some limited countries and are thus not available to most European patients. They have in common that they are highly unlikely to obtain market authorisation in Europe because of the EU regulatory system (except Kerecis fish skin, which is authorised):

[16] Edmonds M et al. 2018, doi: 10.1016/ S2213-8587(17)30438-2.
[17] IQWig Report 382, 20.04.2016.
[18] Sharma R et al. 2021, doi: 10:1038/s41598-021-81886-1
[19] Frykberg R et al. 2023, doi: 10.12968/jowc.2023.32.Sup8b.
[20] Hunt SD et al. 2016, doi: 10.3402/dfa.v7.33101

• **Skin Substitutes and Biologicals**: there are currently 235 such products in the USA which are covered by Medicare and Medicaid. Of these, after revision of the clinical evidence, **only 17 will be covered from 2026 on** due to doubts concerning therapeutic effectiveness: Affinity; AmnioBand, guardian; Apligraf; DermACELL, awm, pourous; Derma-Gide; Dermagraft; Epicord; Epifix; FlexHD or AllopatchHD; Grafix stravix prime pl; GraftJacket; Integra or Omnigraft dermal regeneration template, MariGen shield; NuShield; Oasis wound matrix; PriMatrix; Theraskin; Kerecis Omega3 (fish skin – see EU-authorised products).[21] Efficacy: IWGDF considered the following results at low risk of bias: All numbers present investigational therapy vs SoC; endpoint: complete wound closure; number of patients: Affinity: 60% vs 38% at 12 weeks, 76 patients; AmnioBand, guardian: 85% vs 25% at 12 weeks, 40 patients; AmnioBand, guardian: 85% vs 33% at 12 weeks, 80 patients; Epicord: 70% vs 48% at 12 weeks, 155 patients; Epifix 70% vs 50% at 12 weeks, 110 patients; Theraskin 76% vs 36% at 12 weeks, 50 patients. A potential bias of all studies lies in the unknown selection criteria for chronic wounds – non-chronic wounds lead to higher wound closure rates. Regulatory situation**:** They are **highly unlikely to obtain marketing authorisation in the EU** in the future due to different regulatory pathways: In the US, they are regulated as medical devices. In the EU, they would be classified as **advanced therapy medicinal products** (ATMPs), presenting a huge regulatory hurdle. Exceptions: Apligraf is authorised as medicinal product in Switzerland since 2015: It became the first so-called "transplant product" to receive marketing approval; Kerecis fish skin - see EU-authorised products.

• **Growth factor therapies** containing recombinant human epidermal growth factor (**rhEGF**). This includes Heberprot-P (Heber Biotech); Easyef (Daewong-Pharmaceuticals) and Regen-D (Bharat Biotech). Efficacy: data is easily available only for Heberprot-P: Endpoint: tissue granulation covering ≥50% of the ulcer: 83% vs 40%, 101 patients; endpoint complete wound closure: in 85% in 44 days (20 patients).[22] In 2016, a clinical study was completed but no results were published. A phase III study was announced to start in 2024 in the USA but has not yet started. Regulatory situation**:** Heberprot-P is authorised in 26 countries, e.g. Turkey and Russia. It is administered by injection into the wound (intralesional injection). Easyef is only authorised in India, Thailand, and the Philippines. Regen-D is only authorised in India and Thailand.

• **Growth factor therapy** containing a recombinant form of human platelet-derived growth factor-BB (**PDGF-BB):** Regranex (Smith & Nephew). Efficacy: Regranex 100µg/g vs Regranex 30 µg/g vs SoC: 50% vs 36% vs 35% in 20 weeks, 395 patients[23]. It was authorised in Europe until 2012, then voluntarily withdrawn for commercial reasons. It still is authorised in the USA but had a black box warning 2008-2018 because of increased cancer incidence.

5) Availability and reimbursement of active wound healing products in European countries

The therapies listed in 3) - wound therapies to be considered as adjunct therapies - have active ingredients **authorised** in Europe, but most are **not available** to the patient. E.g., 3C Patch cannot be requested by the patient in the public health system, only some private wound care centres or wound care professionals might offer it. The same is true for hyperbaric and topical oxygen therapy. A report by the European Wound Management Association (EWMA) on **reimbursement systems for wound care products in 13 selected European countries** gives insight into which products are currently really **available** on the European market: The categories mentioned in this report are: (i) essential wound care products (basic dressings, bandages, etc.), (ii) advanced wound care products (hydrocolloids, alginates, hydrogels, foam dressings), (iii) active dressings (antimicrobial dressings, honey and silver dressings) (IV) compression therapy, disinfectants and NPWT. Turkey is the only country where wound care products containing extracellular matrix elements are mentioned, but only for burn treatment. To sum up, **National European health care systems restrict themselves to SoC** (covering the wound **without active ingredient**) or venture to include a few active substances like honey and silver (antimicrobial effect). The same is true for Austria: The Österreichische Gesundheitskasse (ÖGK) covers only products for wound cleaning and dressing; active ingredients are limited to honey, silver and activated carbon, which are not recommended by the IWGDF.[24]

6) Summary of the situation of active wound healing products in Europe

Above we have presented a 2-step argument: (1) most wound care products with active ingredients authorised somewhere in the world are not authorised in Europe; (2) of the few products authorised in Europe, some are not available to patients in practice. (Ad 1) Most potential therapies are not competing with APO-2 because they are currently not authorised in Europe and are unlikely to ever be authorised in Europe for regulatory reasons. (Ad 2) The only authorised therapy with active ingredients in Europe that would be comparable to Aposec is 3C Patch

[21] Local Coverage Determination of the Centers for Medicare & Medicaid Services L35041 for services performed after 01.01.2026.
[22] Fernández-Montequín J et al. 2009, doi: 10.1111/j.1742-481X.2009.00641.x.
[23] https://www.ema.europa.eu/en/documents/scientific-discussion/regranex-epar-scientific-discussion_en.pdf
[24] ÖGK, Ingrid Kern-Komolka: Wichtige Wundversorungsprodukte und ihre Wirkweisen. August 2020.

(LeucoPatch) but this product has not established itself on the European market and is not generally available to patients because wound care practices do not have it in store/have the device and do not offer it to patients. Kerecis fish skin, also a medical device like 3C Patch, is in theory available in Europe, but not sold there – all revenue is derived from the US.[25] This general situation of **lack of wound care products with active ingredient** is reflected in European health insurance systems which only reimburse dressing materials and, from the products with active substances, antimicrobial dressings with silver or active carbon and honey products – products not recommended by the IWGDF for chronic DFU.

In summary, there are currently no marketed, available, competing products to APO-2 in Europe.

7) Parallel developments/studies for chronic DFU with active substances

There is a range of clinical trials for parallel developments, some with promising results, but none combines all **advantages of APOSEC: high efficacy in a comparably short treatment period, easy to apply, comparatively cheap, potentially available in high amounts**. Ongoing or recently finished studies in Europe include:

IMP	Study Status/Results	APO-2 advantage / Comments
AUP1602-C: gene therapy product (ATMP): genetically modified *L. lactis* producing FGF-2, IL-4 and CSF-1 (cytokines)	EU CT No. 2022-502048-10-00 (Phase II, 60 patients). Results: Complete wound closure after 20 weeks: 69% vs 37%. No. 2024-519317-66-00 (Phase IIa, 12 patients): authorised 05/2025.	APO-2 is expected to achieve similar wound closure results but in **12 weeks instead of 20 weeks**.
Ozempic (semaglutide) in a pen - already authorised for diabetes treatment.	No. 2023-504913-65-01 (Phase II, 52 patients): recruitment pending	**Expensive**. It exerts various effects (e.g. weight loss) and might be effective in wound healing.
Autologous **bone marrow-derived stem cells***	No 2024-512873-29-00 (Phase II, 110 patients); 2024-519687-40-00 (Phase II/III, 60 patients): recruitment pending	For patients with end-stage limb-threatening peripheral artery disease (PAD). Bone-marrow cells are extracted in a **painful procedure** and injected.*
Allogeneic **adipose-tissue-derived mesenchymal stem cells***	No. 2023-505961-10-00 (Phase I/II, 30 patients): ongoing; No. 2024-518533-29-00 (Phase II, 105 patients): recruiting started; No. 2024-516342-19-00 (Phase II, 45 patients): recruitment pending;	The therapeutic effect of stem cells is mediated via the secretome*. Stem cells are **more expensive,** cannot be **stored** easily, and they **cannot undergo virus reduction treatment** like APO-2.
Udonitrectag: a small molecule drug acting like nerve growth factor (NGF)	No. 2024-519405-35-00; (Phase I/II, 24 patients), recruitment pending	Induces differentiation in endothelial cells, **one mechanism** of wound healing; APO-2 has multiple MoAs and will thus probably be much more effective.
Dakin's solution: a 50/50 mixture of 1% NaClO and 2% $NaHCO_3$	No. 2024-512612-22-00 (Phase III, 202 patients): recruiting since 12/2021	This is a disinfectant to clean wounds but it is also **cytotoxic** which can affect wound healing negatively.
TP-102: cocktail of 5 lytic bacteriophages against *S. aureus*, *P. aeruginosa*, and *A. baumannii*	No. 2023-507716-13-00 (Phase IIb, 80 patients): recruitment pending; No. 2022-500541-24-00 (Phase I/II, 60 patients): ongoing since 07/2024	This treatment is specifically intended for **infected** ulcers.

* **Stem cell** therapies have similar MoAs as Aposec, but their production and administration are **complex, costly**, and carry risks such as potential tumorgenicity[26]. This makes them less practical than using the secretome. Numerous clinical studies are ongoing e.g. with adipose stem cells, but the stem cell approach is more suited to small-scale, hospital-based treatments for severe cases rather than broad application in routine DFU therapy.

In addition, there are several **internation (non-EU) studies** listed in the clinicaltrial.gov-database:

ON101 Cream: *Plectranthus amboinicus* and *Centella asiatica* extract	NCT01898923: Phase III, 236 patients: Complete wound closure after 16 weeks: 61% vs Aquacel dressing 35%
ENERGI-F703 Gel: contains adenine	NCT02672436: Phase II, 141 patients: Complete wound closure in 14 weeks: 36% vs 26%; NCT05930210: Phase III, 230 patients: ongoing.

[25] Coloplast Annual Report 2023/24

[26] Tseng S-L et al. 2024, doi: 10.4239/wjd.v15.i6.1162.

PEP-TISSEEL Kit: lyophilised apheresed platelets in plasma (PEP) and a fibrin sealant (TISSEEL)	NCT06319287: Phase I/II, 184 patients; NCT06793748: Phase II, 40 patients: ongoing.
Vendaje / BR-AM / BR-AC: dehydrated human amnion graft	3 clinical studies, no phase assignation, with 100, 60 and 60 patients: NCT06150209, NCT06565156, and NCT06511596; ongoing.
SkinTE: autologous heterogeneous skin construct: healthy full-thickness skin harvested from the patient	NCT03881254: no phase assignation, 100 patients; Interim analysis after 50 patients: complete wound closure after 12 weeks: 72% vs 32%; NCT06140303: Phase III, 100 patients, ongoing
HOMSC200: 1 injection of human oral mucosal stem cells	NCT06003530: phase I, 21 patients; complete wound closure 53% vs 33% after 18 months
ProgenaMatrix: Human keratin matrix	NCT06292026: no phase assignation, 100 patients, since 03/2022.

At present, there is **too little publicly available information** on most ongoing trials to realistically assess their future potential. Even if one of them were to demonstrate convincing efficacy and obtain marketing authorisation in Europe, the clinical need and market size are sufficiently large to accommodate several therapies. In this context, APO-2 offers clear competitive advantages, including a comparatively short treatment duration, ease of application, and its long shelf life and stability.

8) Parallel secretome developments for wound healing in general

A recent comprehensive review analysed preclinical studies that used secretome derived from multiple sources.[27] APO-2 derived from PBMC is the only secretome in clinical development and confirmed safety and Phase I/II efficacy data. So APOSEC is the clear frontrunner in the field of GMP secretome therapy in the dermatological field **- by far the most clinically advanced secretome therapy for wound healing**.

SECONDARY AMBITION: setting a gold-standard for DFU Studies

One of the reasons for the current situation of authorised products in DFU care is that evidence from randomised clinical studies conducted until now is considered to be insufficient: they are of low quality and/or high bias. **Standardising clinical studies in DFU is difficult**: Concerning wound dressings, the IWGDF summed up: "*We identified **50 published RCTs** related to our interventions …. **All but four studies reviewed were considered at high or moderate risk of bias***."[28] The IWGDF criticised:

- the duration of treatment and follow-up period varied widely (24 hours to 34 weeks);
- many studies provided limited description of the ulcer and patient characteristics; they typically recruited superficial ulcers or non-infected ulcers;
- most studies recruited individuals without peripheral artery disease (PAD) or with mild PAD;
- there is a significant lack of clear descriptions of SoC provision including the type and quality of offloading provided;
- there is a lack of description of the type and impact of any additional supportive interventions undertaken, such as revascularisation.

We are convinced that the cause for the unsatisfactory quality of the clinical studies in DFU is not simply negligence on the part of the sponsors but the fact that clinical studies in DFU are extremely **difficult to standardize**, and that anyone undertaking a clinical study in DFU has to put **extra effort into the design, methodology and description of the clinical study.** This is the ambition of APO2FOOT. By involving a **SAB** highly experienced in DFU studies early on, but also **other practitioners, nurses, patient representatives** etc, APO2FOOT will set a new standard on how to develop and implement DFU studies and serve as an example for others in the future. This will be achieved not only by publishing and promoting the study protocol but also **sharing the learnings** and any potential for further improvements after the study.

Research & Innovation maturity

Over the past decade, APO-2 has progressed from laboratory discovery and preclinical validation in murine and porcine wound models to first-in-human studies (MARSYAS I/II), establishing safety and initial efficacy. The **production of APO-2** is currently at **technology readiness level (TRL)** 5 - technology validated in a relevant environment: a semi-automated GMP production process has been set up at [redacted] and APO-2 stability confirmed. APO2FOOT will produce several batches of APO-2, thus **demonstrating** the GMP manufacturing process – **TRL6**. By the end of the project, APO-2 will be available as a fully GMP-certified, stable, and reproducible IMP together

[27] Bormann et al., 2023, doi: 10.1016/j.jid.2023.02.019
[28] Guidelines on interventions to enhance healing of foot ulcers in people with diabetes. IWGDF 2023 update.

with detailed plans for further upscaling and automation. The **clinical work** in APO2FOOT **starts at TRL 6** - prototype demonstration in a relevant environment: APO-2 has undergone early clinical testing. APO2FOOT will advance this significantly by conducting a multicentre, multinational, phase II clinical trial in DFU patients. This demonstration in an **operational environment** and readiness for a pivotal phase III trial to support marketing authorisation lifts the clinical readiness of APO-2 to **TRL 7**.

#§PRJ-OBJ-PO§#

1.2. Methodology

#@CON-MET-CM@# #@COM-PLE-CP@#

The challenge - Diabetic foot ulcer (DFU)

Chronic wounds are defined as not healing within 12 weeks and represent an **enormous unmet clinical need**, which is mainly treated with moist wound dressings (see Chapter 1.1.2). If closure of the wound is delayed, consequences include extended hospitalisation, decreased quality of life, infections of the bone, amputations or even death. Ulcers are an unmet challenge to wound care professionals, consuming an increasing share of healthcare resources around the globe with severe impact on quality of life of patients. Due to increasing incidence of diabetes and demographic changes patient numbers are expected to rise. In developed countries, the prevalence rate for chronic nonhealing wounds is 1-2% of the general population.[29]

DFU are a leading cause of hospitalisation. They have a severe impact on the quality of life of patients as they fail to heal over extensive time periods and frequently become infected. Up to **75% of all amputations** in developed nations are a consequence of DFU. Thus, the clinical challenge is to provide a therapy that ensures wound closure within 12 weeks. APOSC chose DFU as a suitable first indication to be treated with APO-2 because of its intense medical need, market size and lack of therapeutic strategy. No active wound care product has become standard of care, which provides an enormous opportunity for a treatment with APO-2: it is a novel and affordable therapeutic approach to restore the internal regenerative capacity for wound healing. Importantly, **topical application** of APO-2 conforms well with the current standard of care. Moreover, the therapeutic endpoints are well established and can be evaluated within relatively short observation periods with simple and inexpensive diagnostic measures.

DFU provides a priority indication for development of a novel secretome-based regenerative therapy under the guidelines set by the regulatory bodies: (i) unmet medical need; (ii) simple topical administration; (iii) European market worth € 1 Bn €.

The challenge: cutaneous wound healing in diabetes refers to the tightly regulated, multistage processes that reestablish the physiological skin barrier and its homeostatic functions. In diabetic patients, hyperglycemia, insulin resistance, excessive free fatty acids and high levels of advanced glycation end products (AGEs) are responsible for systemic inflammation, augmented atherosclerosis and peripheral neuropathy. The latter causes impaired neurological feedback loops that lead to poor gait. Over time, hyperkeratosis of the compressed skin will form a hematoma and break due to neuropathy and increased plantar load, eventually developing into a DFU that is difficult to heal. Paramount mechanisms in the genesis of DFU are peripheral artery disease (PAD); peripheral neuropathy; germ infection; and immune cell dysfunction (Figure 1).

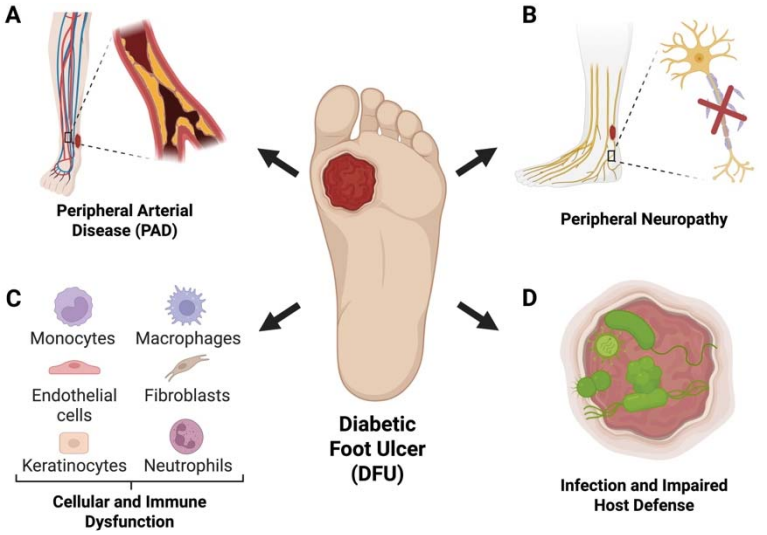


Figure 1: The four main aspects of DFU formation.

Figure 2 shows the 4 main processes needed for wound healing, all of which are impaired in DFU patients. There are several changes in the **primary cellular biological functions** in patients with DFUs. Immune cells, keratinocytes, fibroblasts, endothelial cells, and different cytokines are involved in the healing of DFUs. In the inflammatory stage, infiltration monocytes/macrophages in the wound are essential for the transformation of the wound from a proinflammatory to an anti-inflammatory environment[30]. However, in DFU patients the macrophages continue to maintain a proinflammatory state. In diabetic wounds, phagocytosis, neutrophil degranulation, and the anti-infective

[29] Nussbaum et al., 2018, doi: 10.1016/j.jval.2017.07.007; Srivastava et al., 2024, doi: 10.3390/polym16091280; Smith+Nephew, Annual Report 2024; Markets and Markets, 2025, Advanced Wound Care Market Size, Growth, Share & Trend Analysis

[30] Wynn et al., 2016, doi: 10.1016/j.immuni.2016.02.015

effects of reactive oxygen species peptides are disrupted[31]. Excessive infiltration and activation at the site of tissue injury mediate tissue damage by releasing cytokines and proteases and regulating the adaptive immune response[32].

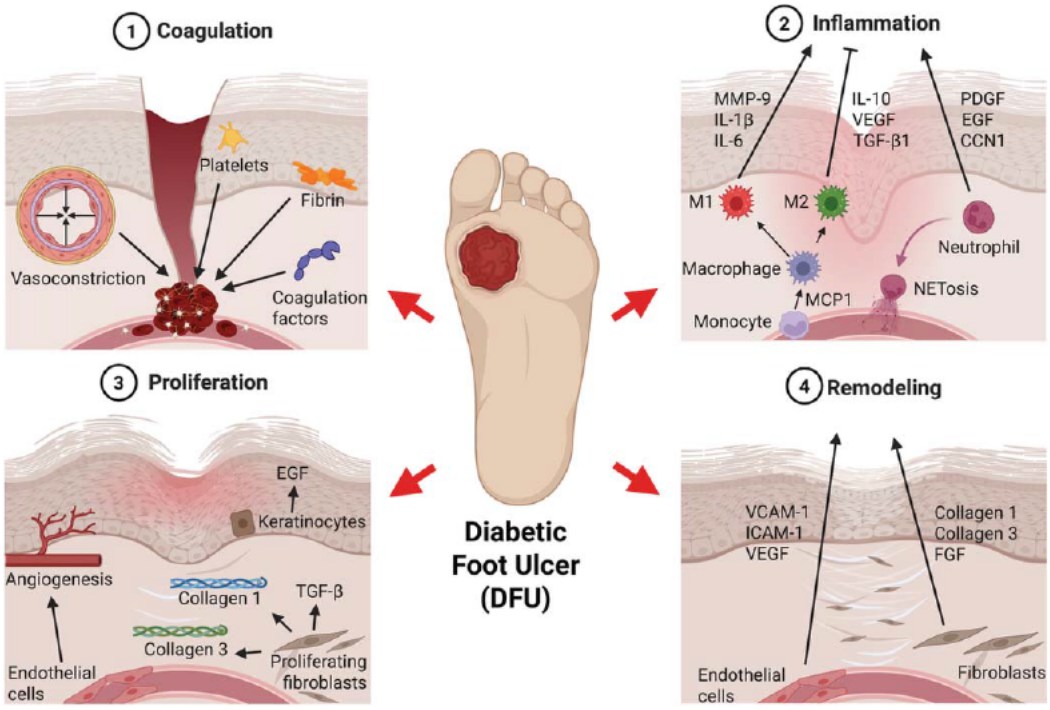


Figure 2: The process of wound healing needs to go through several stages: (1) Coagulation: platelet activation, vasoconstriction, clotting factor; (2) Inflammation: neutrophil activation, monocyte/M1/M2 macrophage activation; (3) Proliferation: angiogenesis, extracellular matrix reorganisation; re-epithelialisation; (4) Remodelling: fibroblast, endothelial cell formation.

Moreover, the expression of neutrophil protein arginine deiminase (PAD)-4 is upregulated by hyperglycemia. When neutrophils invade the wound, their ability to secrete neutrophil extracellular traps (NETs) is inhibited, resulting in delayed wound healing[33]. Impaired re-epithelialisation due to decreased keratinocyte and fibroblast migration in DFU[34] is associated with decreased angiogenesis and alterations of the extracellular matrix[35]. In most patients DFUs are commonly superinfected by *Stahpylococcus aureus*, *Streptococcocus* spp., *Pseudomonas* or fungal pathogens[36]. This mixed infection in addition to immune cell dysfunction (leukopenia), and inflammatory response disorder (persistent inflammation of macrophages) are causing a vicious cycle that propagates wound extension.

The secretome – Aposec (APO-2) is a biological medicinal product: regulatory implications

It is scientifically accepted that the therapeutic effects of **stem cell therapies**, which are **advanced therapy medicinal products** (ATMPs), derive from the **secretome** of transplanted cells. The secretome of ATMPs was identified as a key factor for accelerating cutaneous wound healing. APOSC pioneered secretome derived from peripheral blood mononuclear cells (PBMC - white blood cells) as an **exclusive proprietary technology**, which represents a potential game-changer in regenerative medicine.

Apoptotic cells have long been known to release a complex mixture of substances, including vesicles, proteins and lipids. We have proven in preclinical and clinical trials that the secretome of cultured, stressed PBMCs augments wound healing. To identify the **active components** within the secretome, we proved experimentally that no single fraction provides the complex regenerative potency (see MoA below for details). Rather, the regenerative capacity resides in exosomes, proteins and lipids as the major biologically active components of APO-2 (Figure 3). APO-2 content is the secretome derived from PBMC and medical grade albumin and insulin (from the growth medium). Thus, **APO-2 is the lyophilisate of PBMCs secretome and the totality of APOSCs proprietary AM2 growth medium**. This **cell-free** product can be produced and stored as an "**off-the-shelf lyophilised** product" with a proven **stability of 2 years at +4°C**.

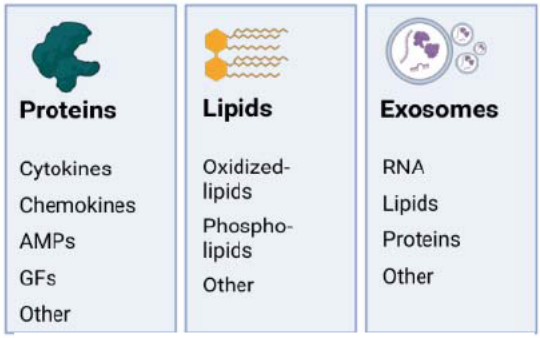


Figure 3: The secretome of APO-2 contains proteins, lipids and exosomes.

[31] Kolaczkowska et al., 2013, doi: 10.1038/nri3399
[32] Caielli et al., 2012, doi: 10.1016/j.coi.2012.09.008
[33] Wong et al., 2015, doi: 10.1038/nm.3887
[34] Catrina et al., 2016, doi: 10.1002/dmrr.2742
[35] Eming et al., 2014, doi: 10.1126/scitranslmed.3009337
[36] Baig et al., 2022, doi: 10.3390/life12071054

Based on this analysis, a robust **manufacturing process** for Aposec has been established in cooperation with [redacted], according to Good Manufacturing Practice (GMP) (Figure 4). **Blood donors** packed blood cells are separated into the plasma fraction, red blood cell fraction and buffy coat (PBMCs, platelets). Currently, approximately 70% of all buffy coats are **discarded** in all blood donation centres worldwide. Under GMP conditions PBMCs are separated, irradiated and cultured for 20-24 hours. After depletion of cellular compartments, the secretome is filtered, and the first **viral clearance** of the secretome is performed (methylene blue, cold light). Thereafter, the PBMC secretome is **lyophilised** and terminal sterilisation of the lyophilisate in primary packaging (glass vial) is performed. 1 vial contains 50 Units (U) of APO-2, where 1U is the secretome derived from $1x10^6$ PBMC.

Regulatory authorities classify Aposec as a **biological medicinal product** (Directive 2001/83/EC) and not as an ATMP (Regulation EC No 1394/2007), see Figure 5 for the regulatory differences between an ATMP and Aposec.

Thus, the non-clinical development strategy (**toxicology program**) to support the proposed phase II clinical study is primarily based on the regulatory requirement of ICH

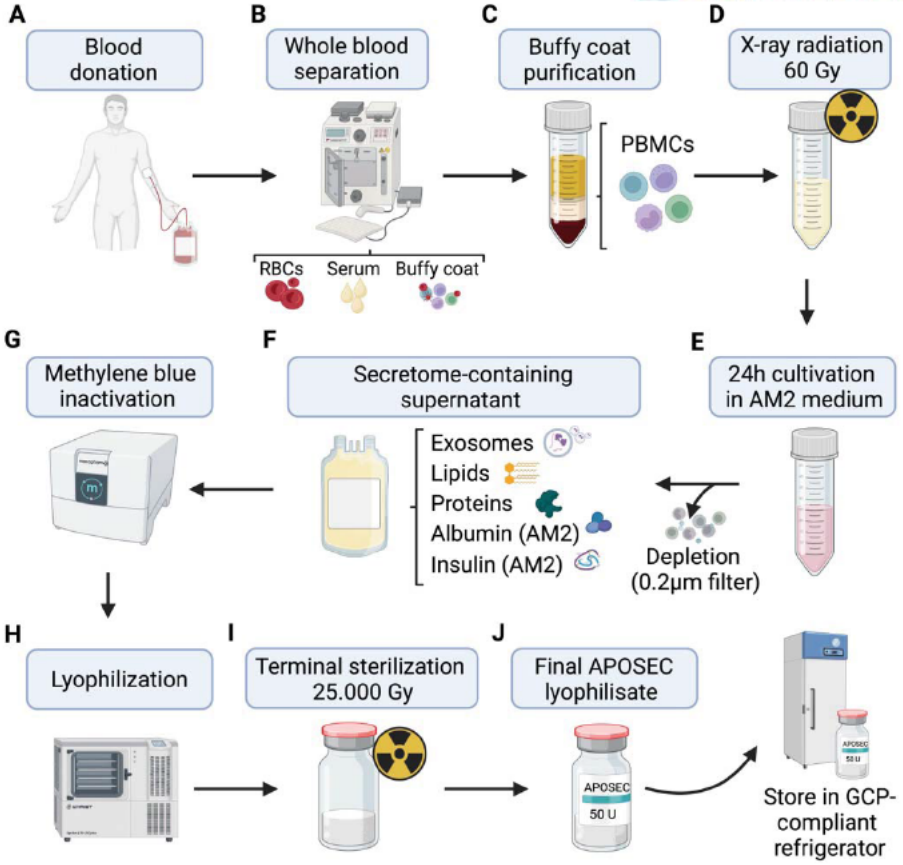


Figure 4: APO-2 GMP production: (A) Whole blood is collected from multiple healthy donors. (B) Using a Macopress Smart (Macopharma), the blood is fractionated into approximately 200–250 mL serum, 200–250 mL packed red blood cells, and ~50 mL buffy coat per 450 mL donation. (C) The buffy coat fraction is centrifuged again to remove e.g residual red blood cells, yielding a purified cell fraction. (D) PBMCs within the purified buffy coat are irradiated with 60 Gy X-rays. (E) Irradiated PBMCs are cultured for 24 h in AM2 medium, followed by depletion of cells and debris through filtration with a 0.2 µm filter. (F) The resulting secretome-containing supernatant is transferred into sterile storage bags as intermediate product. Exosomes, lipids, and proteins are released by PBMCs, while insulin and albumin are integral components of the AM2 medium. (G) Methylene blue inactivation is performed using the Macopharma system. (H) The product is subsequently lyophilised in a freeze-dryer (Lyophilisator, Martin Christ, Epsilon 2-10 LSCplus). (I) The lyophilisate is aliquoted into sterile vials (50U APO-2 per vial) and subjected to terminal sterilisation with 25,000 Gy. (J) The final APOSEC lyophilisate is stored in a GCP-compliant refrigerator until clinical use.

	ATMP	APOSEC (biological medicinal product)
Drug product	**Living cells:** somatic cells or tissue engineered cells	**Cell free**: cell-derived paracrine factors
Mode of action	Mediated by more than one active ingredient	Mediated by multiple paracrine factors
Starting material	Human cells / tissue; origin: autologous / allogenic	Human PBMCs; origin: autologous / allogenic
Viral clearance	Not possible	**Possible**

Figure 5: Differences between an ATMP and a biological medicinal product. Aposec is a so called "cell free product" contain only factors produced by living cells.

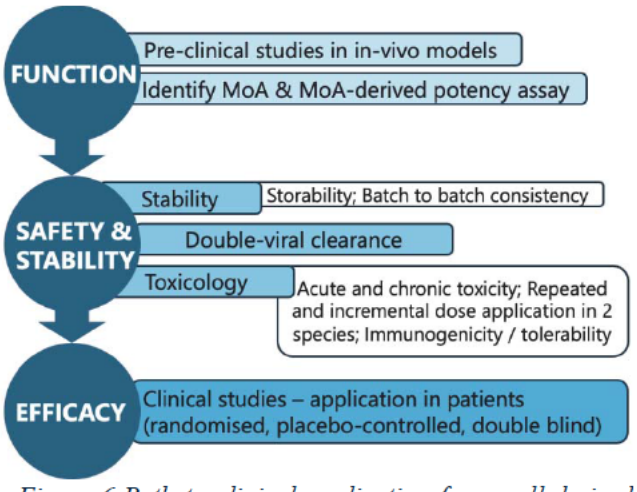


Figure 6:Path to clinical application for a cell-derived biological. Mandatory regulatory elements to achieve market authorisation.

S6 (R1) "Preclinical safety evaluations of biotechnology-derived pharmaceuticals" (EMA/CHMP/ICH/731268/1998) and, where relevant, on ICH guideline M3 (R2) "Non-clinical safety studies for the conduct of human clinical trials and marketing authorisation for pharmaceuticals" (EMA/CMPM/ICH/286/1995). The path to clinical application of a cell-derived biological is summarised in Figure 6. In short, all suppliers involved in the manufacture of Aposec were qualified by the APOSC **quality management system** (QMS), and all analytical tests are carried out and documented according to validated standard operating procedures (SOPs). **Batch to batch consistency** is shown by in-process controls and validated potency assays. Furthermore, regulatory authorities are inspecting the Contract Manufacturing Organisation (CMO) of Aposec, [redacted], regularly. APOSC holds an **operating licence** to produce Aposec as an investigational medicinal product (IMP) for intravenous and topical application for phase I, II, III clinical studies (13924124). In our project we consider collaboration with the **JRC** for **pre-normative regulatory science** to support the validation of our assays, ensuring they are fit for regulatory purpose and aligned with EU-wide standards.

The "raw material" for APO-2

Peripheral blood mononuclear cells (PBMCs), the source of APO-2, are obtained from **buffy coats**, a by-product of **blood donations.** Buffy coats are the leukocyte-rich fraction that remains after donated whole blood is centrifuged into plasma, red blood cells, and the thin intermediate layer of PBMC and platelets. A whole blood donation (450 ml) is usually separated into 200-250 mL of plasma, 200-250 ml of red blood cells and 50ml buffy coat bag. Most buffy coats are currently discarded. This fraction is a rich source of PBMCs (incl. lymphocytes (T cells, B cells, NK cells) and monocytes) which can be isolated under GMP conditions and used to generate the secretome. Repurposing buffy coats to produce APO-2 not only establishes a **safe and abundant cell source** but also **creates value** from a material that would otherwise be considered biomedical waste, highlighting the resource-efficient character of the approach.

Availability of 'raw material' blood: for 1 batch APO-2 (1000 vials) the buffy coats of approx. 90 blood donations are required. Austria: In 2024, the Red Cross in Austria collected 331,312 blood donations (private communication), adjusting for blood groups and age of donors (to reduce risk of transmissible spongiform encephalopathy-TSE) 150,000 buffy coats could be used to produce 1,260,000 vials of APO-2. Approximately 35,000 DFU could be treated with that, depending on the wound size (~1-1.5 vials per treatment for up to 12 weeks). This is 4x what would be needed to treat every DFU in Austria (~ 9000 DFU per year in Austria: 479,000 diabetics / 2.9% with DFU per year / 65% of that chronic). EU: Following this logic, APO-2 only from buffy coats from Germany would allow to treat 61% of all chronic DFU patients in the entire EU. In summary, **each country has 3-4 more buffy coats available** (currently discarded) for APO-2 than needed if every chronic DFU was treated with APO-2.

Identification of APOSEC MoA in vitro

Based on the preclinical observation that Aposec augments wound healing in vivo (see Annex on clinical studies - 1.2.1 for relevant publications and results)) we examined which MoA are potentially responsible for these experimental observations. Details are described in Chapter 1.2.1.2 of the Annex on clinical studies. In summary, we evidenced that **Aposec has multiple MoAs**: angiogenesis[37], re-epithelialisation[38], induction of cytoprotection and up-regulation of anti-apoptotic gene product in cell assays[39] and a favourable alteration of extracellular matrix generation after wound healing[40]. On the cellular level we have evidenced that Aposec prevents platelet aggregation[41], stabilises granulocytes[42], prevents dendritic cell maturation[43], stabilises basophils and mast cells[44], prevents monocyte activation[45], augments M1/M2 differentiation[46] and attenuates T-cell activation in vitro[47]. In addition, we have shown that Aposec induces nitric oxide (NO) in human endothelial cells in vitro and causes in a dose dependent manner

[37] Mildner et al., 2013, doi: 10.1371/journal.pone.0060103; Hacker et al., 2016, doi: 10.1038/srep25168; Hacker et al., 2020, doi: 10.1002/btm2.10186; Haider et al., 2015, doi: 10.1016/j.expneurol.2015.03.013
[38] Mildner et al., 2013, doi: 10.1371/journal.pone.0060103; Hacker et al., 2016, doi: 10.1038/srep25168; Vorstandlechner et al., 2023, doi: 10.3390/pharmaceutics15041065
[39] Mildner et al., 2013, doi: 10.1371/journal.pone.0060103; Altmann et al., 2014, doi: 10.12688/f1000research.4219.2; Haider et al., 2015, doi: 10.1016/j.expneurol.2015.03.013; Lichtenauer et al., 2011, doi: 10.1007/s00395-011-0224-6
[40] Vorstandlechner et al., 2023, doi: 10.3390/pharmaceutics15041065
[41] Hoetzenecker et al., 2012, doi: 10.1007/s00395-012-0292-2
[42] Klas et al., 2022, doi: 10.3390/antiox11081559
[43] Laggner et al., 2020, doi: 10.1016/j.ebiom.2020.102774
[44] Laggner et al., 2022, doi: 10.1016/j.ebiom.2022.104093
[45] Panahipour et al., 2020, doi: 10.3390/ijms21134694
[46] Haider et al., 2015, doi: 10.1016/j.expneurol.2015.03.013
[47] Hoetzenecker et al., 2013, doi: 10.1093/eurheartj/ehs459

vasodilation in coronary arteries ex vivo[48]. Finally, Aposec activates proangiogenic and anti-proteolytic processes in naïve PBMC, is capable to protect the endothelial barrier[49] and contains antimicrobial peptides[50].

Content PBMC subfractions and mode of action: As described above (Figure 3), the Aposec secretome contains lipids, proteins and exosomes[51] and is derived from PBMC (mononuclear cells: T-cells, monocytes, granulocytes, B-cells, NK-cells and stem cells) that are separated and cultured for 20-24 hours. The proprietary culture medium contains clinical grade albumin and insulin. Thus, albumin and insulin (excipient) and the PBMC secretome become an integral part of our IMP Aposec. We identified in our preclinical work that angiogenesis induced by Aposec is the main MoA for wound healing. To prove whether cellular subfractions of PBMC do induce the same effect, we investigated whether a) secretome of T helper (CD4), T cytotoxic (CD8), NK cells (CD56), monocyte (CD4) versus total PBMC secretome had different abilities to induce angiogenesis in vitro; b) whether fractions of PBMC secretome (lipid, protein, exosome) induced angiogenesis in vitro as compared to Aposec. We have conclusively shown that **only the "total Aposec preparation" exerts its full biologic activity** in validated potency assays mimicking angiogenesis (see below)[52].

Aposec potency assay mimics the main MoA and correlates with angiogenesis in vitro: to prove **batch to batch consistency and shelf-life stability** we had to develop two validated potency assays that functionally **mirror angiogenesis** in vitro. AP-1 promotor activity and the phosphorylation of HSP27 **correlate with experimental tube formation** in vitro, a surrogate marker for angiogenesis. Aposec induces AP1/HSP27 activation in a dose-dependent fashion in two validated cell-based biological systems. These potency assays correlate with tube formation assays which allow to assess in vitro the angiogenic properties of compounds by measuring the ability of endothelial cells to form capillary-like tubular structures. Furthermore, we proved that inhibition of AP-1 and HSP27 abrogates Aposec induced tube formation and, thus, showed **specificity** of our potency assays in relation our main MoA of Aposec. These experiments were essential in the approval process of MARSYAS II study for the German national health authority, the Paul Ehrlich Institute (EudraCT.:2018-001653-27).

Previous trials

Pre-clinical studies of systemic application of Aposec in vivo

The preclinical development of APO-1 (systemic Aposec) and APO-2 (topical Aposec) is described in a range of scientific publications. In summary, APO-1 was shown to be effective in acute and latent acute myocardial infarction[53]; myocarditis[54]; spinal cord injury[55]; and stroke[56].

Pre-clinical studies of Aposec in wound healing in vivo

APO-2 as therapeutic IMP for wound healing was **initiated in 2010**. In a first iteration we examined **murine PBMC secretome** without radiation. We demonstrated convincingly that this secretome of PBMC augmented wound healing and induced neo-angiogenesis in a murine model in vivo[57]. We showed that **Pre-APO-2** (human / laboratory grade) blended with Alginate gel induced angiogenesis and improved skin quality in a **porcine burn model**[58] and APO-2 augmented wound healing in a murine diabetic wound model[59]. In addition, we have evidenced the beneficial effect of APO-2 blended with fibrin in a rodent epigastric flap model[60] and demonstrated that our APO-2 IMP is an option to beneficially modify murine skin scarring after acute wounding[61]. For details, please see the Annex on clinical studies, Chapter 1.2.1 – below one example of a key experiment regarding angiogenesis is presented:

Aposec augments angiogenesis in a porcine acute wound model: Increased numbers of CD31+ and ASMA cells were observed in wounds treated with Pre-APO-2 (laboratory-grade APO-2)[62] **:** To investigate the capacity of Pre-

[48] Hoetzenecker et al., 2012, doi: 10.1007/s00395-012-0292-2
[49] Copic et al., 2022, doi: 10.3390/pharmaceutics14081600
[50] Kasiri et al., 2016, doi: 10.1111/eci.12667
[51] Beer et al., 2016, doi: 10.1007/s10495-016-1292-8
[52] Wagner et al., 2018, doi: 10.1038/s41598-018-36928-6; Simader et al., 2019, doi: 10.1038/s41419-019-1974-6
[53] Lichtenauer et al., 2011b, doi: 10.1007/s00395-011-0224-6; Pavo et al., 2014, doi: 10.1016/j.biomaterials.2013.12.071
[54] Hoetzenecker et al., 2015, doi: 10.1093/eurheartj/ehs459
[55] Haider et al., 2015, doi: 10.1016/j.expneurol.2015.03.013
[56] Altmann et al., 2014, doi: 10.12688/f1000research.4219.2
[57] Mildner et al., 2013, doi: 10.1371/journal.pone.0060103
[58] Hacker et al., 2016, doi: 10.1038/srep25168
[59] Wagner et al., 2018, doi: 10.1038/s41598-018-36928-6
[60] Hacker et al., 2020, doi: 10.1002/btm2.10186
[61] Vorstandlechner et al., 2023, doi: 10.3390/pharmaceutics15041065
[62] Hacker et al., 2016, doi: 10.1038/srep25168

APO-2 and Livosec (secretome of non-stressed PBMC) to **induce angiogenesis in vivo**, we harvested punch biopsies at the corner of the wounds. We found a strong increase in CD31+ cells in the wounds treated with Pre-APO-2: the number of CD31+ cells was almost twice as high as in the other groups. To support these findings, we performed an additional staining for the mature blood vessel marker alpha smooth muscle Actin (ASMA) and found a significant increase in ASMA+ cell numbers in Pre-APO-2 treated wounds compared to the control groups (Figure 7.)

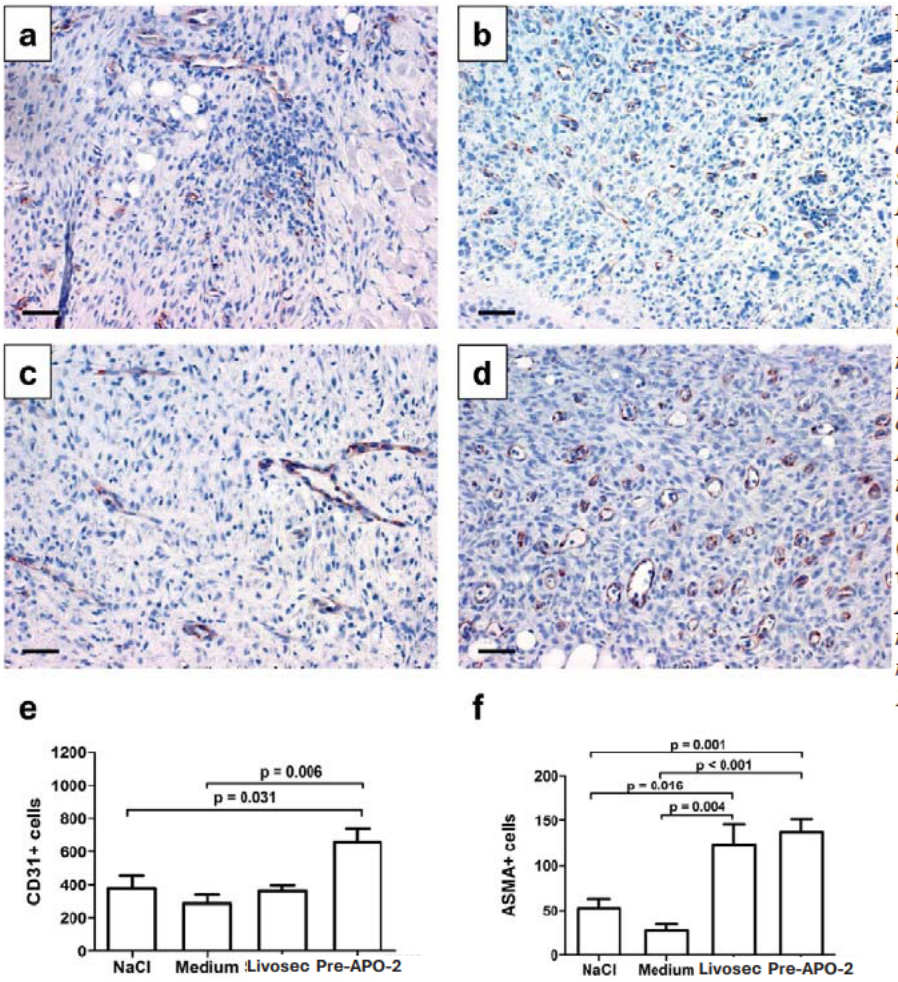


Figure 7: *Increased numbers of CD31+ and ASMA cells were observed in wounds treated with Aposec. Punch biopsy sections taken on day 5 were stained for the angiogenesis marker CD31. Representative samples of the NaCl (a), medium (b), Livosec (secretome of non-stressed PBMC) (c) and Pre-APO-2 (non-GMP) (d) treated wounds are shown. 200× magnification, scale bar: 50 µm. The quantification of CD31+ cells was performed on four randomly selected sections per wound. The numbers correspond to the total amount of cells over four sections. (e) Treatment with Pre-APO-2 led to a significant two-fold increase in CD31+ cells compared to the control groups. (f) Mature blood vessels (ASMA+ cells) were more frequent in the wounds treated with both Livosec and Pre-APO-2 compared to the control groups, respectively. Error bars indicate SEM. n = 6. (Adapted Fig. 4 of Hacker et al., 2016, 10.138/srep25168)*

Clinical trials utilising Aposec

MARSYAS I: The clinical development of APO-2 was initiated in a **phase I** study (NCT02284360) at MUW with **autologous GMP Aposec** in a **7-day acute wound model study**[63]. Autologous GMP Aposec (PBMC sourced from the proband) was utilised for the treatment of an acute wound. Thus, the regulatory risk was negligent. In summary:

Model	Effects	Application	PBMC source
Phase I: Acute wound model, 7d intervention study	Safe, well tolerated	**Autologous APO-2** blended with Alginat gel, wound by punch biopsy	Autologous (human) GMP Aposec, (12.5 Units (U)/cm^2 or 25 U/cm^2)

MARSYAS I/II: The clinical development of APO-2 was continued using allogenic (human) GMP Aposec in a **28-day phase I/II trial in DFU** patients[64]. Note: The concentration of 12.5/25/50 U/ml corresponds to the dose 6.25/12.5/25U/cm^2. For details, please see Annex on clinical studies Chapter 1.2.1.1. Summary:

Model	Effects	Application	PBMC source
Phase I/II: DFU wound, double-blind, randomised, multinational, placebo-controlled, 28d intervention study	Safe, well tolerated; efficacy in % reduction of DFU wounds, complete wound closure	**Allogenic APO-2** blended with Alginate gel, wound diameter: 0.8-8cm^2	Allogen (human) GMP Aposec (6.25 / 12.5 / 25 U/cm^2)

[63] Simader et al., 2017, doi: 10.1038/s41598-017-06223-x
[64] see NCT04277598 and Gugerell et al., 2021, doi: 10.1186/s13063-020-04948-1

Results: the final study report includes two Full Analysis Sets (FAS): a) n=122: all randomised patients; b) n=94: all randomised patients after central wound adjudication with true 0.8cm^2 - 8cm^2. Both data sets are shown in the Clinical Study Report for maximum transparency[65]. We consider the FAS=94 results to be relevant for clinical interpretation, and this is the base for the sample size calculation of APO2FOOT. The **primary endpoint** of this study was **wound reduction** after End of Treatment (EoT, a 4-week treatment), shown in Figure 8. Already with 4 weeks of treatment, **some wounds closed completely** (Figure 9, left). This percentage increased until End of Study (EoS) - 4-week treatment + 8 weeks follow-up with SoC (Figure 9, right). This indicates that the 4-week intervention had a conditioning effect on wounds that led to **complete wound closure** after the treatment had stopped.

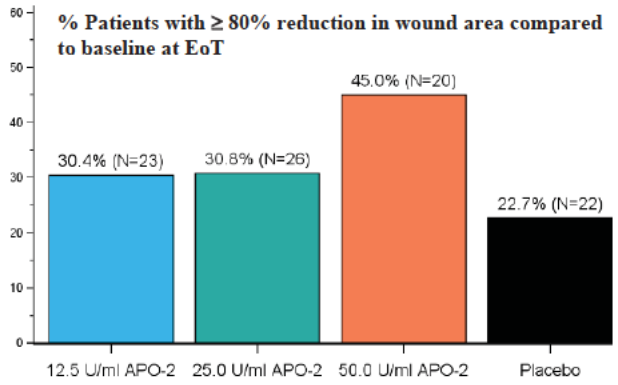


Figure 8: Primary endpoint of MARSYAS I/II: % wound reduction in patients in FAS N= 94 – after central wound adjudication.

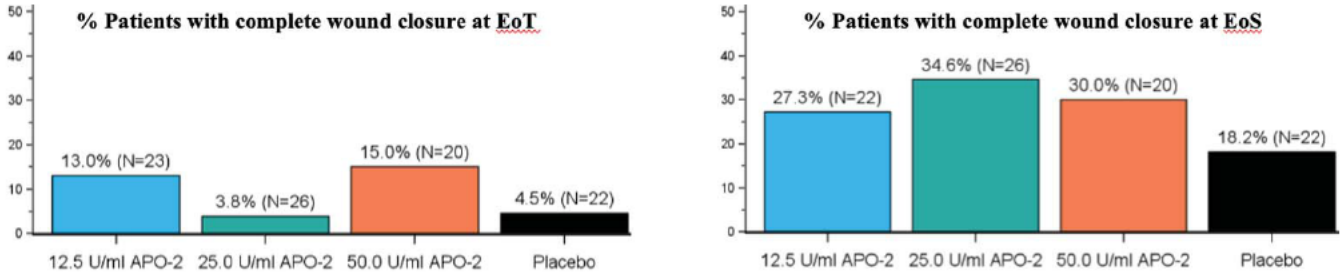


Figure 9: % of patients with complete wound closure after treatment with APO-2. Left: End of Treatment (EoT) - 4 weeks treatment; Right: End of Study (EoS): 8-week follow-up with SoC after treatment.

Conclusion: MARSYAS I (autologous GMP Aposec) and MARSYAS I/II (allogeneic GMP Aposec) have shown **safety and tolerability** of topically applied Aposec blended with Alginate gel in different dosages. In Marsyas I/II we learned that Aposec at a concentration of 50U/ml (= 25U/cm^2) led to wound reduction or complete wound closure in patients with wound sizes adjudicated as 0.8 - 8cm^2. The sample size calculation for APO2FOOT is based on complete wound closure after 4 weeks in FAS=94.[66]

Learnings from MARSYAS I/II - see Annex on clinical studies Chapter 1.2.1.1 for details. The MARSYAS I/II study was performed as multi-national, placebo controlled double blind investigational study in DFU. This study utilised APO-2 blended with Hydrogel Alginate (NUGEL™). The **control** arm consisted of culture medium blended also with Hydrogel Alginate (NUGEL™). This measure was taken to allow "**blinding**" of the IMP substance as the perfect "placebo" arm. This focus for the 'perfect' placebo in the design of MARSYAS II led to **challenging** consequences: (1) our IMP had to be prepared by the pharmacy and was then distributed to the clinical study site. (2) The NUGEL™ used as placebo is not an approved SoC. In our planned **APO2FOOT** trial, we altered our approach completely. This phase II study (and potentially, phase III post-project) will be more related to the **real-life situation**: The APO-2 lyophilisate will be brought to the clinical sites and stored in a monitored fridge at +4°C. When a study patient is randomised to APO-2 treatment (vs SoC group), APO-2 will be resuspended with physiologic saline and applied on an Alginate fleece that is placed on the DFU wound. Since the TTP has also changed slightly, we will perform this trial with one dose (25 U/cm^2).

Good Clinical Practice (GCP), Good Laboratory Practice (GLP) and Good Manufacturing Practice (GMP) conformity

GCP, GLP and GMP norms are **mandatory** for the development of secretome-based IMP. APOSC has invested significant effort in complying with regulatory requirements (see overview in Figure 10) and undergoes regular inspections by the Austrian national **health authority** (BASG). The company originated as a spin-out of the Medical University of Vienna (MUW) and was founded as a privately owned stock corporation in 2008 (FN: 308089 y, ATU64127689 FG: Wien). In 2019 APOSC was registered as pharmaceutical company (according to §63 of the Austrian Medicines Act, including issue of a trade law licence), which is a **legal prerequisite to initiate clinical trials**.

[65] MARSYAS II Synopsis of Clinical Study Report – Aposcience AG
[66] Gugerell et al., 2021, doi: 10.1186/s13063-020-04948-1, MARSYAS II Synopsis of Clinical Study Report – Aposcience AG

- APOSC holds a **GMP certificate** (No. 482918-102482868) for the manufacture of Aposec as IMP for the clinical phases I, II, III for topical as well as systemic application, issued by BASG on 24.06.2024 (valid to 23.06.2026). The first GMP certificate was issued on 16.07.2019.
- APOSC also holds a **Manufacturer's Authorisation** (**13924124**) issued by the BASG on 30.03.2021, for the manufacture and distribution of IMPs for the clinical phases I, II and III.

Aposec is produced by our CMO [redacted] – which is defined in a technical agreement. APOSC maintains a **pharmaceutical quality system** (QMS) that is guaranteed by a full time Quality Assurance (QA) employee. According to the Austrian Ordinance AMBO 2009, § 7. (1) *"Every operation of a pharmaceutical manufacturer, [...] as well as every operation that controls pharmaceuticals must continuously and uninterruptedly have at least one Qualified Person [Sachkundige Person]."* A Qualified Person has been employed at APOSC since 2019. In accordance with ICH/GMP requirements, we developed potency assays related to angiogenesis, as well as physicochemical evaluations, to a) guarantee batch to batch comparability/consistency and b) performed stability programs for Aposec. In addition, we executed extensive GLP toxicology testing that included a repeated dose toxicology program in minipigs (2018: 4 weeks, for phase I/II trial; 2025: 12 weeks for phase II/III trial). See Annex on clinical studies Chapter 1.2.1.1 "Safety of APO-2" for details.

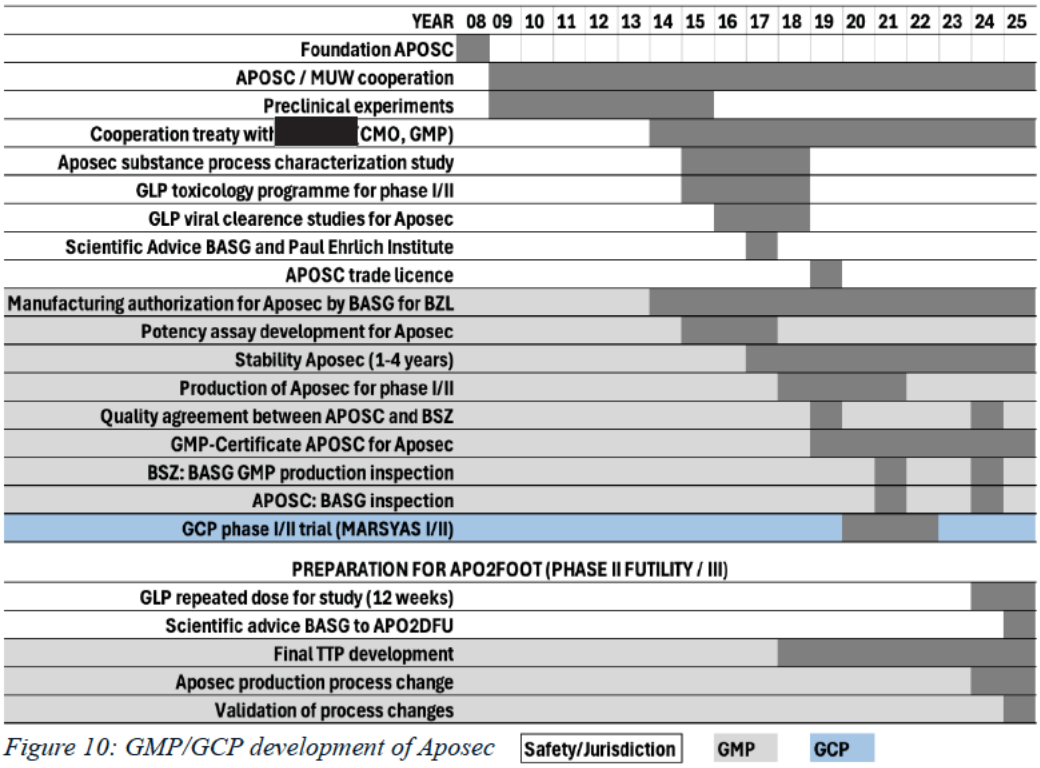


Figure 10: GMP/GCP development of Aposec

In accordance with ICH/GMP requirements, we developed potency assays related to angiogenesis, as well as physicochemical evaluations, to a) guarantee batch to batch comparability/consistency and b) performed stability programs for Aposec. In addition, we executed extensive GLP toxicology testing that included a repeated dose toxicology program in minipigs (2018: 4 weeks, for phase I/II trial; 2025: 12 weeks for phase II/III trial). See Annex on clinical studies Chapter 1.2.1.1 "Safety of APO-2" for details.

All GMP/GLP/GCP activities agreed with the recommendations gathered in **two scientific advice sessions** with the national health authorities of Austria (BASG) and Germany (Paul Ehrlich Institute-PEI), held in 2017. As secretome-based pharmaceutical products (including their regulatory categorisation) were unknown by this time, we approached the national agencies in Europe most experienced in the development of ATMPs and biologics for regulatory product development advice. Both agencies concluded that the **secretome should be developed as a biological according to ICH Q6B**. The MARSYAS I/II clinical trials (2019-2023) complied with GCP regulations (see previous chapter).

In the period 2018-2025 we defined our final target product profile (TPP). Furthermore, in 2024 and 2025, we approved within the frame of our QM system **process changes of Aposec production** (change of source of irradiation, and culture medium) and performed an additional 12-week repeated dose toxicology programme in minipigs (Phase II Futility/III trial). An additional scientific advice meeting with BASG for a phase II Futility/III programme will be held in Q3/2025.

Since 2016, APOSC has developed a QMS and is working according to written procedures (SOPs). [redacted], the CMO for Aposec production, is also a GMP-certified organisation with a GMP certificate valid for the manufacture of biologicals. It was agreed with the Austrian health authority BASG that the head of quality control lab and the Qualified Person at [redacted] are employed by APOSC to guarantee optimal control of the production process. Cooperation agreements concerning the development and manufacture of Aposec between APOSC and [redacted] are in place since 2014, and a Technical Agreement, to fulfil GMP requirements, is effective since 2024.

GMP/GCP activities planned in APO2FOOT:

In addition to all these achievements, APO2FOOT will include a range of GMP/GCP activities as show in Figure 11. **GMP** includes production of Aposec for stability studies, and for the clinical trial phase II; the stability programme of the Aposec target product profile (TPP), the Aposec intermediates and the AM2 proprietary culture medium;

APOSC and [redacted] will be inspected by BASG; vendor audits. **CGP activities** include a) GCP CETIS application for APO2FOOT; b) the phase II clinical trial itself with the final target product profile: 120 patients; observer-blinded randomised trial, topical application, 2x week, 12-week duration of intervention – see below and Annex on clinical studies for details.

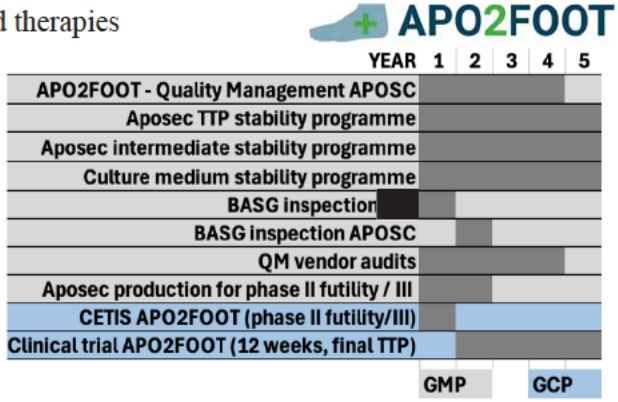


Figure 11: GMP/GCP activities in APO2FOOT

The clinical study phase II APO2FOOT

Only a **summary** is shown here, for details and **inclusion/exclusion** criteria please see the Annex on clinical studies. APO2FOOT is a **multi-centre, multi-national, observer-blinded** clinical study in patients with DFU (Wagner I–II classified wounds), conducted in Austria, Germany, Czech Republic, Italy, and Portugal. The trial is designed as a phase II/III programme: phase II is included in this EC-funded project, while a **potential phase III continuation** will be financed by APOSC. The phase II part will **enrol a total of 134 patients** randomised 1:1 to receive either Aposec plus Standard of Care (SoC) or SoC alone. The sample size is based on data from the MARSYAS II study (Full analysis set-FAS=94 with central wound adjudication) with **10% dropout**. Patients will undergo a 5–7-week **screening period** to ensure chronicity of wounds (≤30% change in wound size). The maximum wound size eligible is 4 cm². Treatment will consist of **2 applications per week** for 12 weeks (24 treatments or until complete closure), followed by a 3-month **follow-up**. Aposec will be resuspended in NaCl and applied to an alginate fleece, which is placed on the wound. The comparator is SoC as defined in the S2 guideline of the German Wound Association. The clinical study physician will determine wound size and is responsible for patient randomisation and end-of-treatment measurement. **Quality of Life** will be assessed at randomisation, End of Treatment, and End of Study using a validated questionnaire. Only one dose (25 Units/cm² wound size) will be tested. **The primary endpoint is complete wound closure** after 12 weeks of treatment. After completion of phase II, a futility analysis will be performed by the DSMB: if the data suggest sufficient efficacy, the study will proceed into phase III; otherwise, the trial will be stopped. Phase III would further evaluate complete wound closure with an estimated additional 110–130 patients, bringing total patient numbers to a level sufficient for submission of a marketing authorisation application.
Study centres: 2 study centres are beneficiaries (MUW, 2CAB), other study centres will be subcontracted – see list in the Annex on clinical studies.

On a **practical level**, APO2FOOT is organised as follows:
- CTIS submission of clinical study application: [redacted]
- IMP manufacture: [redacted]
- IMP release testing: [redacted] and subcontractor [redacted]
- IMP release by the Qualified Person of APOSC
- IMP storage and distribution to study sites (shipments): [redacted]
- Purchase and distribution of study materials (e.g., NaCl, Alginate fleece, Mepilex) to study sites: [redacted]
- Purchase and distribution of off-loading shoes to study centres: [redacted]
- Order system: Study centres order IMP and study materials from [redacted]
- APO2FOOT study management by [redacted]
- APO2FOOT study monitoring by [redacted] (in German speaking countries) and by [redacted] (in CZ, IT and PT)
- GCP audits and co-monitoring of study sites: APOSC
- Management of study site costs and refund of patient travel costs: [redacted]

1.2.1. Linked research and innovation

Beneficiaries have been and are involved in multiple projects relevant to APO2FOOT, the most important ones are listed below:

Project	Input (data, results, networks, etc) / Relevance and link
Christian Doppler Laboratory, 2009-2015: Diagnosis and regeneration of heart and thoracic diseases; (APOSC, MUW)	**Preclinical** research: APO-1/2 is effective in regenerative medicine: myocardial infarction, myocarditis, stroke, spinal cord injury, wound healing and first secretome based therapy in acute wounds
APOSEC, 2015-2017, Austrian Research Promotion Agency (FFG) grant to APOSC	Product and process **characterisation** studies, GMP production of APO-1/2, Regulatory compliance, Scientific Advice, Toxicology;

APOSEC to clinic, 2017-2019, Vienna Business Agency grant to APOSC	Product and process characterisation studies, GMP production of APO-1/2, Regulatory compliance, Scientific Advice, Toxicology;
[redacted]	[redacted] and [redacted] are partners, bringing relevant experience in **regulatory** tasks, risk-based quality **management**, data management, project management and **communication/dissemination** related to clinical trials into APO2FOOT.
[redacted]	[redacted] was partner gaining valuable **regulatory** experience concerning clinical trials, especially for Germany, helping APO2FOOT trials in Germany.
[redacted]	[redacted] gains experience in the **validation** of innovative healthcare solutions, supporting the translation of advanced technologies into **practice** – similar to APO2FOOT, which helps ensuring results have both scientific impact and practical relevance.

APO2FOOT has already **established links** to other research/innovation e.g. through the **SAB** (see Annex on clinical studies 2.1.3), several of its members participated in a previous high-quality DFU study. After project start we will reach out to the **other projects funded** under this topic to explore **cooperation** potential (see T1.6).

The importance of improving treatment for DFU patients is reflected when searching for "DFU" in CORDIS, revealing 19 EC-funded projects in Horizon2020 and Horizon Europe. The difficulty of translating research into practice also becomes evident, as none of these projects so far led to an authorised therapy or clinical trial (see 1.1.2 Ambition for authorised treatments and clinical trials).

1.2.2. Interdisciplinarity aspects

APO2FOOT is inherently interdisciplinary, bringing together expertise from biomedical **science**, **clinical** medicine, **regulatory** affairs, pharmaceutical **manufacturing**, and industry. At its core, testing of APO-2 as an IMP requires the integration of GMP (production at [redacted] under APOSC oversight), and GCP in the phase II clinical study (coordinated by [redacted] and [redacted]). This convergence ensures a seamless pipeline **from laboratory to patient treatment.** The project mobilises diverse disciplines to achieve its objectives. Physician-scientists and clinical investigators contribute knowledge of DFU pathology and trial execution while regulatory experts from [redacted] guide regulatory compliance, ensuring **quality and safety**. Pharmaceutical engineers and quality managers at APOSC and [redacted] provide the expertise needed to establish validated manufacturing, stability testing, and batch-to-batch comparability. Meanwhile, [redacted] and [redacted] ensure rigorous study design, data management, and **adherence** to ethical and governance requirements. Importantly, interdisciplinarity in APO2FOOT extends beyond the scientific-technical domain. Diabetes **patient organisations** and **advisory** boards (see Annex on clinical studies – 2.1.3 and T1.5) bring real-world experience and societal perspectives into the project, ensuring that the therapy is developed with end-user needs in mind. This **ecosystem approach**—linking clinical practice, regulatory science, manufacturing, quality management, and patient advocacy—ensures that APO2FOOT can advance a safe, effective, and scalable regenerative therapy for DFU, ultimately aligning scientific innovation with clinical and societal impact.

Social sciences and humanities: The inclusion of social sciences is not compulsory for this topic and APO2FOOT does not require dedicated social science research to achieve its objectives. Nonetheless, the project integrates relevant societal aspects by involving a Patient Advisory Board to capture patient perspectives, implementing quality-of-life and pain assessments in the trial, and ensuring inclusive outreach and communication. These measures ensure that patient needs and societal concerns are considered without requiring a separate social science component.

1.2.3. Sex and gender

In the medical area, sex is key characteristic to be considered. A study[67] with 1,771 patients in Belgium about sex differences in DFU found that the **majority of patients were male** (72%). Also, ulcers in men were deeper, more frequently displaying probe to bone, and more frequently deeply infected. Twice as many men as women had systemic infections. Men demonstrated a higher prevalence of previous lower limb revascularisation and smoking was more common in men. Women had a 26% higher chance of healing without major amputation. A meta-study comprising 9 studies with 4,399 DFU patients found that **being male was one of the major risk factors of DFU** in diabetes mellitus patients[68]. Similarly, the American Diabetes Association summed up sex differences for DFU as follows: (i) Incidence of DFU is approximately 1.5 times higher in men than in women with diabetes; (ii) Incidence of amputation is 1.4 -3.5 times higher in men; (iii) Men with diabetes have a higher prevalence of peripheral neuropathy and

[67] Vanherwegen AS et al. 2023, doi: 10.1371/journal.pone.0281886.
[68] Hu X et al. 2023, doi: 10.22514/jomh.2024.002.

peripheral arterial disease (the main causes for DFU), as well as cardiovascular disease, which together account for a majority of sex differences in DFU risk. Regarding potential underlying causes for the sex differences in DFU, they noted that compliance with therapeutic footwear is similar between the sexes, but women are more likely to perform recommended self-care and foot care. In summary, while the numbers above differ, they all indicate that DFU is more of a problem for men. It seems that men care less for themselves, and with age they enter a general state of health which is worse than that of women and thus more prone to the development of DFU. However, DFU is not only associated with being male but also with race, ethnicity, socioeconomic status and geography: in the USA, poor black or Hispanic adults from rural regions experience DFU, DFU-related amputation and DFU-caused death more frequently than middle class urban white adults.[69]

In the **patient recruitment** for APO2FOOT we will not be able to take sex into consideration because our focus must be on recruiting sufficient patients of any sex as fast as possible to complete the clinical study: the **inclusion and exclusion criteria** and the selection of only chronic wounds limit the pool of recruitable patients. Including sex as and additional criterium (trying to achieve a balance of female/male participants) would probably slow down recruitment significantly, endangering the timeline of the project. Because of higher and more severe incidence of DFU in men, we **anticipate a ratio of 70% male and 30% female** patients. We expect APO-2 to be **similarly effective** in men and women, but differences between the sexes in the healing duration of chronic wounds after APO-2 treatment could be possible due to differences in the tissue and vascularity between men and women. However, the sample size of the APO2FOOT study, or even the combined phase II/III study, will probably most likely be too small to be able to study sex differences and evaluate wound healing in men and women separately. The appropriate period for this kind of studies is **phase IV** when APO-2 is already authorised for marketing. Still, we intend to perform such a separated evaluation according to sex for the combined phase II/III study. If significant differences in the healing rates between men and women are found, methods need to be developed how this difference in the tissue/extracellular matrix/vascular situation between the sexes can be studied.

Another aspect related to sex are the **blood donations** forming the basis for APO-2 production. Currently, at [redacted], 60% of donors are male and 40% female. This ration shifts to 80% male / 20% female when it comes to the actual buffy coats that are used for APO-2 production, because of safety selection criteria (e.g. age - see safety description in the Annex on clinical studies; and discarding lipemic ones (excess lipids that can interfere with lab testing.).

Inclusive Outreach: APO2FOOT will systematically integrate sex and gender considerations into all communication and outreach activities to ensure inclusiveness and impact. We will design inclusive materials, where language and visuals avoid stereotypes and reflect diversity in gender, age, ethnicity, and professional roles. Outreach strategies will be adapted to different audiences, acknowledging that information needs and preferred channels may vary across genders. Participation and engagement will be tracked with attention to gender balance, with adjustments made if imbalances arise. This approach ensures project messages are accessible and resonate broadly, strengthening the societal impact of the project.

1.2.4. Open science including data management

[redacted]

[69] Mc Dermott K et al. 2023, doi: 10.2337/dci22-0043; Armstrong DG et al. 2023, doi: 10.1001/jama.2023.10578.

compliance with Open Science principles and the safeguarding of participants' rights, while maximising the visibility and long-term accessibility of our results.

[redacted]

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2. Impact

#@IMP-ACT-IA@#

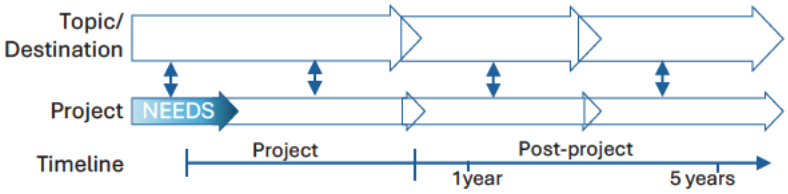
APO-2 is **groundbreaking** not only in its area of application, aiming to be the first **therapy** for chronic DFU with an active ingredient in Europe, that makes a real difference; we aim to be trailblazers for a whole new branch in pharmaceutical research and development: (1) providing a new **gold-standard for clinical trials** in DFU (GCP-compliant) and (2) serve as a template for EU-based **GMP-production** of secretome therapies. In summary:

APO2FOOT paves the way for the first marketed secretome-based therapy, transforming diabetic foot ulcer care, reducing amputations and healthcare costs, and establishing Europe as the global leader in secretome-based treatments.

This main impact will be achieved through **3 impact pathways**, each focusing on one of the 3 main impact areas: societal, economic and scientific:

2.1. Project's pathways towards impact, and scale & significance

Each impact pathway is triggered by the **needs** of a target group, which are addressed with project **results**. These results are aligned with one or several **expected outcomes** of the topic, all of which we are addressing. We disseminate and exploit our results, thus generating specific **project outcomes** approx. 1-year post-project. Our **long-term impacts** (after approx. 5 years) are grouped into societal, economic and scientific impacts, with the most important ones listed first. Each pathway contributes to 1 **expected impact** as listed in the Destination we are addressing. **Requirements and barriers** to achieve these impacts are listed separately below, as most of them are relevant for several impact pathways.



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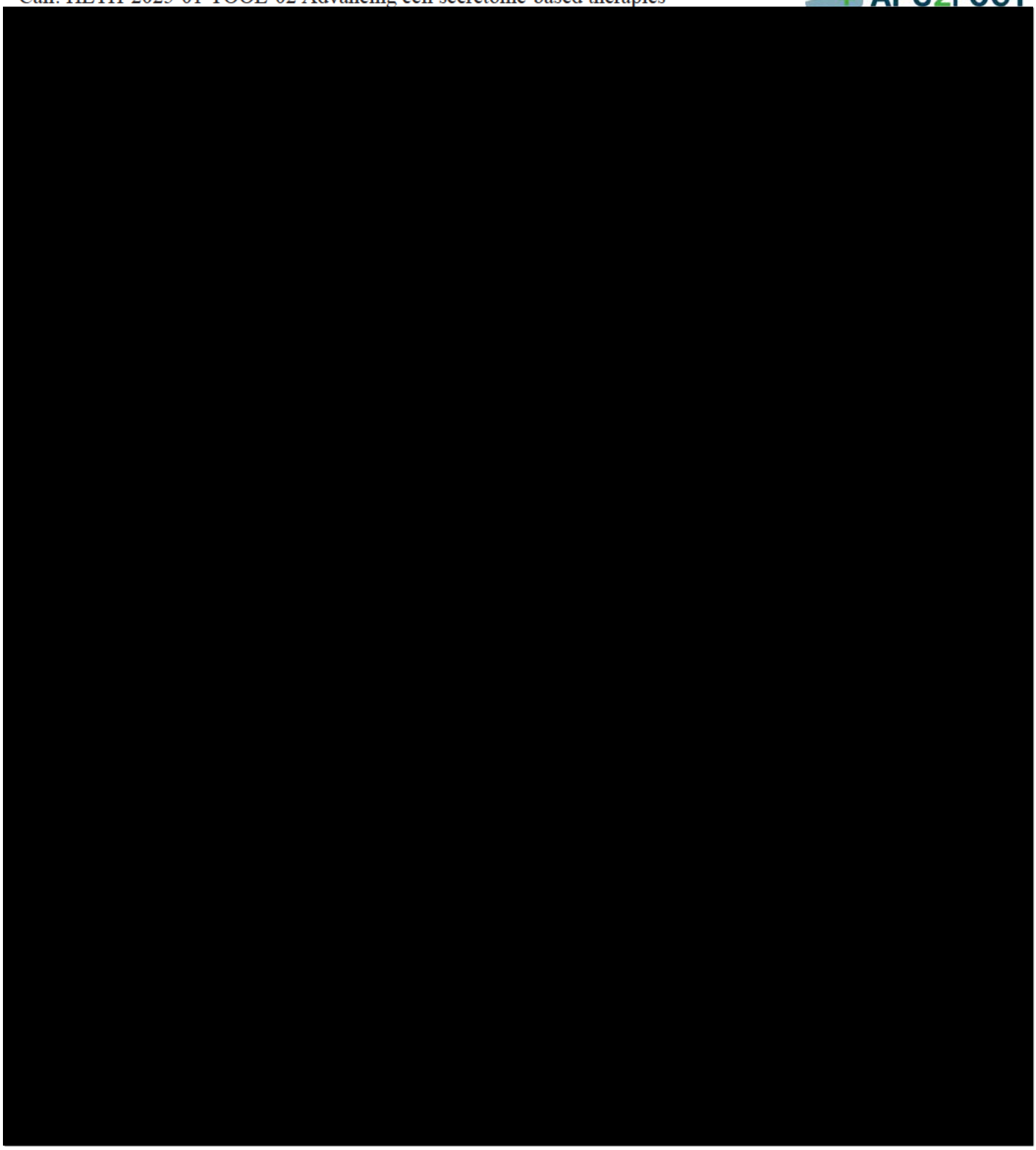
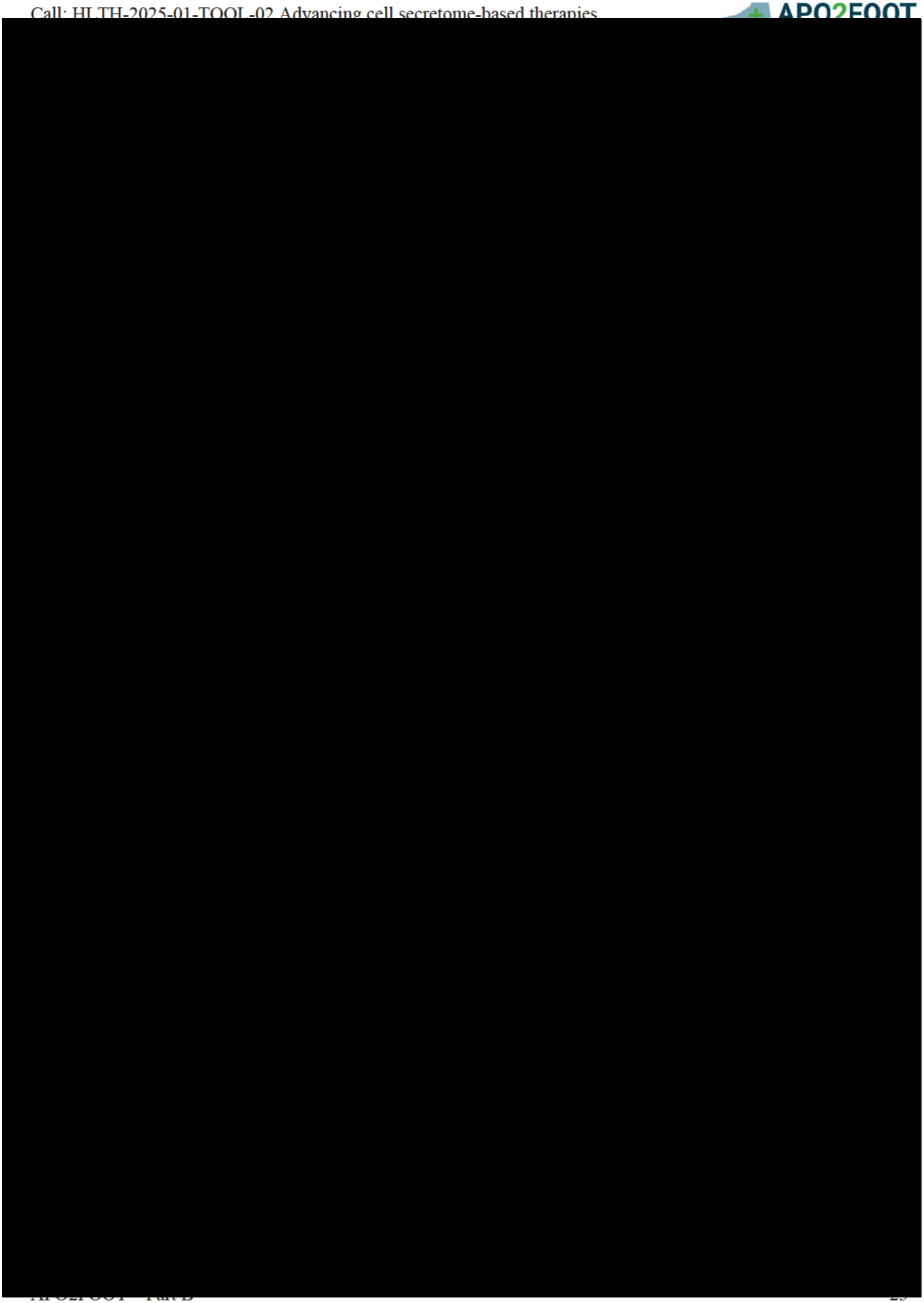
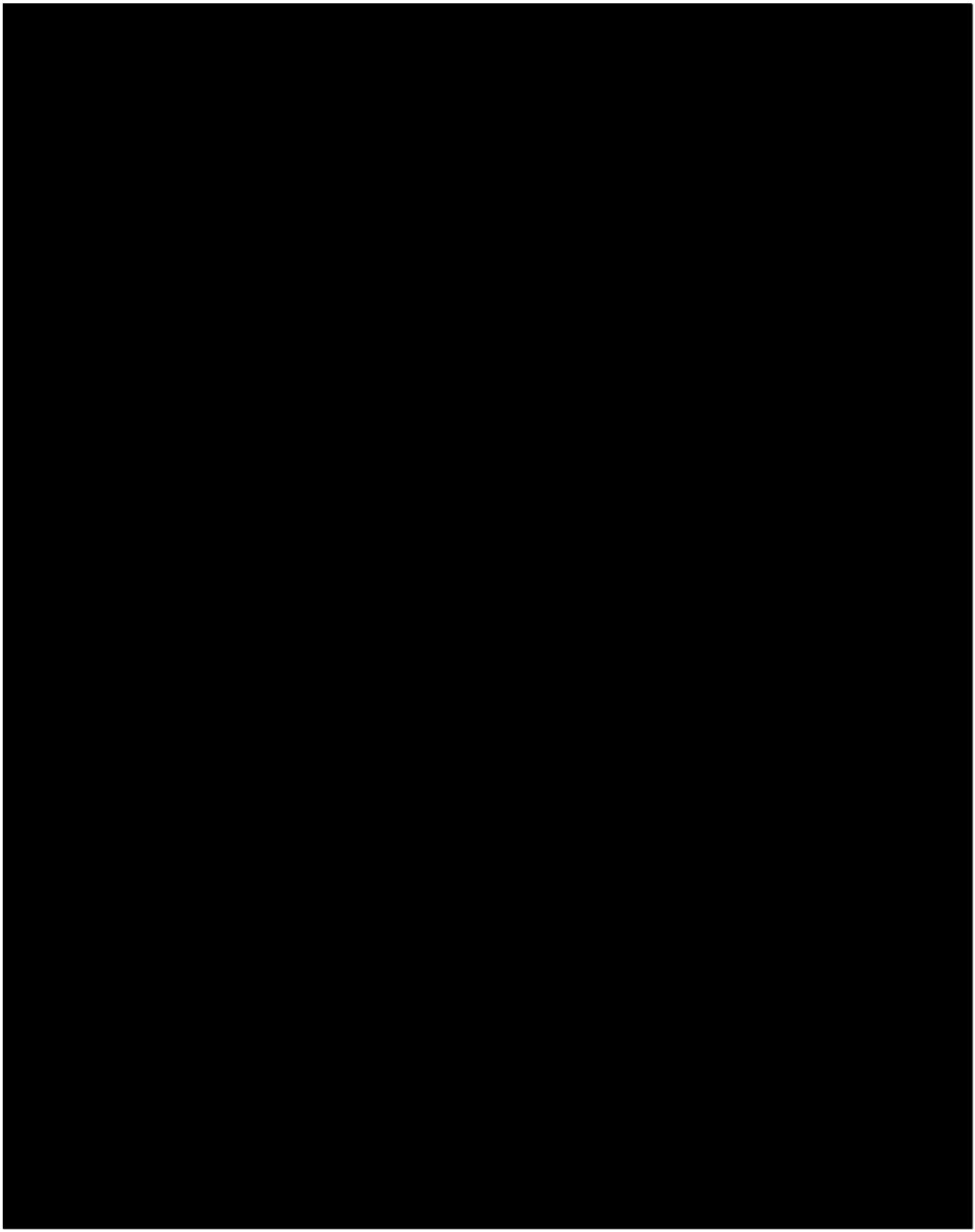


[70] Nearly 1 million patients in the EU suffer from DFU per year (Sämann et al. 2008, doi: 10.1111/j.1464-5491.2008.02435.x); 60-70% of DFUs become chronic (Armstrong et al. 2023, doi: 10.1001/jama.2023.10578) ≥ 600,000 patients.
[71] https://www.ispor.org/conferences-education/conferences/past-conferences/ispor-europe-2024/program/program/session/euro2024-4016/141609
[72] https://www.europarl.europa.eu/doceo/document/E-9-2022-002065_EN html
[73] Cost of amputation over two years (in DE) is ~ €32,000 – see previous reference- x 19,000 patients ≈ € 600 million
[74] Kähm K et al. 2018, doi: 10.2337/dc17-1763; https://cordis.europa.eu/project/id/777661/reporting;
[75] IDF Diabetes Atlas 11th Edition – 2025, Factsheet "Diabetes in Europe – 2024"; Praxisempfehlungen der Deutschen Diabetes Gesellschaft. S2, Nov. 2024, p. 349.
[76] Armstrong DG es al. 2020, doi: 10.1186/s13047-020-00383-2.





[redacted]

[77] https://www.gesundheitswirtschaft.at/weiterhin-kritische-blutlagerstaende-in-oesterreich/; https://de.wikipedia.org/wiki/Blutspende

[redacted]

[redacted]

[redacted]

2.2.5. Exploitation

Exploitation success is backed by APOSC's strong track record as IP owner and clinical trial sponsor:
Founded in 2008 APOSC operates under Austrian corporate law. The company history has two phases: **(a) Preclinical research (2009–2015)**: A cooperation treaty between MUW and 2 family offices secured private funding of research in the department of surgery. An additional €2 .5 million grant from the Christian Doppler Society (international peer review) supported work relating to the PBMC secretome therapy. We were able to show that Aposec reduces hypoxia-induced inflammation and promotes tissue regeneration in models of acute myocardial infarction, stroke, spinal cord injury, myocarditis, and wound healing. A GMP facility was identified ([redacted] to produce APO-1/2. In 2015 a Phase I trial confirmed safety and tolerability of autologous APO-2 in wound healing. **(b) Allogeneic APO-2 development (2015–2023)**: Regulatory guidance from BASG (AT) and PEI (DE) helped us to ensure compliance with ICH guidelines when developing GMP Aposec. In 2019, a multinational Phase I/II trial in DFU was launched in four European countries, showing that Aposec was safe, well tolerated, and improved healing in DFU. **In summary**, APOSC proved it can obtain (a) robust preclinical data, (b) strong IP protection (see below); (c) regulatory approval (already two clinical trials utilising "APO-2 secretome therapy"), and (d) necessary funds via public private partnership - €18.3M private and € 7M public funding were secured (e.g. from Christian Doppler Laboratory, Austrian Research Promotion Agency (FFG), Wiener Wirtschaftsagentur, Forschungsprämie of the Austrian Ministry of Finance.)

Post-project activities for exploitation of APO2FOOT results: If this clinical study is successful, the following steps will be needed post-project for up-scaling and to reach as many patients as possible:

Marketing Authorisation: APOSC aims to attain marketing authorisation for APO-2 as an adjunct therapy to SoC for chronic DFU. The APO2FOOT phase II study will be completed by a phase III (pivotal trial) financed by APOSC. If the results are significant, APOSC will first submit a national marketing authorisation in Austria. Additional marketing authorisations in other European countries will follow through a mutual recognition procedure.
GMP production capacities: APOSC will expand GMP manufacturing capacities at [redacted] and other facilities of the Red Cross in Austria and Germany to meet market demand. Up to now, manufacturing capacities at [redacted] are limited to development and clinical study-scale. Talks about further cooperation with the [redacted] have already begun (see [redacted] to the Annex on clinical studies). This is needed to secure the raw material (buffy coats) and to establish additional production facilities for Aposec.
Reimbursement in Europe: We will initiate talks with national health insurances for reimbursement of APO-2. In this process all data concerning clinical evidence of APO-2 and its cost-effectiveness will be reviewed. However, our aim is not restricted to obtaining this reimbursement – gaining recognition in national healthcare insurance reimbursement programs also is a huge push for Aposec to establish itself in the market.
A key partner of APOSC for exploitation is [redacted]: they are in a key position to enable tech transfer to other blood donation centres, as well as by scaling up production in their own facilities.

Automation and further upscaling: **Production capacities** at the [redacted] pilot-plant: production needs to be further upscaled and automated, to enter other markets. The current bottleneck is the number of available clean rooms and schooled personnel. [redacted] can currently release approximately 7,500 vials (50U each) of APO-2 per year (APO2FOOT will require approx. 2,500-3,000 vials), which could be increased to max. 63,500 vials, if they use all available clean rooms. APOSC will seek partnering with organisations related to the pharmaceutical industry, especially those that work with blood and have experience with GMP (blood donation centres, plasma companies) to bring production of APO-2 to a large scale. A successful and well-organised APO2FOOT phase II study will attract interest from large pharmaceutical companies in Europe, India, China and the USA. This needs to be paired with **automation** of the production process, e.g. an automated system for the separation of PBMC cells is currently only available at lab scale (see also requirements 2.1.1).

APO-2 for other indications: In parallel APO-2 will be tested by APOSC for additional indications, such as venous leg ulcer, chronic wounds in patients with peripheral artery disease, burn wounds and skin affections related to inflammation (e.g. toxic degenerative eczema, atopic dermatitis).

Market: In 2025, the global advanced wound care market (AWCM) is estimated at about 12.5 billion USD growing to about **15 billion USD** in 2030 at a compound annual growth rate (**CAGR) of 3.7%**.[78] Others foresee even higher growth rates of up to 6%.[79] This market comprises all medicinal products and medical devices, with or without active ingredients, aiming at preserving a moist wound environment – the state of the art in current wound care. It comprises the following product categories: wound dressings, devices, biological skin substitutes, and topical agents. The largest players are Solventum (US, 15%), Smith & Nephew (UK, 14%), Mölnycke (SE, 10%), and ConvaTec (UK, 6%).[80] Many companies share the rest of the market - market concentration is low. Interestingly, **European companies lead** this market. By product type, wound dressings account for about 45%, devices for 35%, other active wound care products account for only 20%.[81] The most important growth factor is the aging population – diabetes mellitus is associated with old age. According to the WHO, the world population aged 60 years and older is expected to increase from 1.1 billion in 2023 to 1.4 billion by 2030. In the EU, the percentage of people aged +65y is expected to be growing from 21.2% in 2022 to 30.5% in 2070.[82]

Price of APO-2: with the current, largely manual, not up-scaled/automated production process, the costs of goods (COG) of APO-2 at [redacted] per vial, which contains 50U. The **cost per treatment then depends on the wound size**: on average we assume that 1-1.5 vials will be needed per treatment (1 vial is sufficient for 2 cm^2, the size of the wound will decrease during the treatment). Assuming 12 weeks of treatment and 2 treatments per week, this would mean total cost (only considering COG) of € 2,136 - 3,200. The price of APO-2 at market introduction will not only depend on COG of APO-2. Additional costs will have to considered: overhead costs of APOSEC, profit margin, production volume and R&D costs will be factored in. COG will massively decrease when upscaling and automation is possible. **Partnering with the non-profit** [redacted] for GMP production of APO-2 keeps manufacturing costs low, allowing these savings to be passed on and making our therapy more affordable. As no other secretome therapies have reached the market, this cannot be compared to similar treatments, and only few treatments of chronic DFU are available in the EU. The Kerecis fish skin has reached the EU market as a medical device and costs on average € 1,257 (€139,69[83] per 1,75x1,75 cm^2, 8 applications), but no national insurance in the EU has endorsed reimbursement. While not directly relevant for the EU, prices in the USA can be used as comparisons. For a range of skin substitutes prices are published online.[84] For a similar wound size as assumed in the APO-2 calculation above, cost of therapies differs widely, ranging from $ 1,100 (Apligraf) to $ 31,000 (Novachor).

Beyond its therapeutic efficacy, stability and ease of use, APO-2 is also expected to be competitive on price, since its current cost of goods is already moderate and will decrease substantially with upscaling and automation, positioning it below many alternative wound therapies in the EU and US market.

2.2.6. Intellectual property (IP) management

IP management is crucial for successful marketing and thus exploitation of a novel medicinal product. In APO2FOOT all relevant IP, that forms the basis of our project is **owned by APOSC**. The Aposec platform technology is described in 1.2 Methodology.

[78] Mordor Intelligence; MarketsandMarkets
[79] Smith & Nephew, Annual Report 2024, p. 48.
[80] Smith & Nephew, Annual Report 2024, p. 48.
[81] globalgrowthinsights.com
[82] European Commission, 2024 Ageing Report, p. 333.
[83] https://www.medizinfuchs.at/kerecis-deutschland-gmbh html
[84] https://www.organogenesis.com/pdf/2024-Q4-Hotsheet-Physician-Office.pdf

APOSC IP strategy: the **portfolio of granted patents** has helped the company to establish its credibility as forerunner in the field of "secretome therapy" in the EU. All developments related to Aposec were actively discussed with a patent attorney. In 2008 APOSC was granted priority for the Aposec platform technology and the treatment of medical indications of unmet need (intravenous and topical application). All patents are managed by Dr. Andreas Pföstl (KOPAS[85]) since 2008. **APOSC was the first company ever to receive a PBMC secretome patent** in national relevant jurisdictions. The production of Aposec ("product is the process") and its potential indications are an integral part of APOSCs IP management. Related to **"the process is the product"**, additional patents were secured relating to the potency assay, AM2 culture medium (proprietary medium for Aposec generation), and the pharmaceutical composition Aposec/Alginate combination for wound healing.

The names "**Aposcience"** and "**Aposec"** are registered trademarks since 2009. We are certain that APOSC does not infringe any other patents related to secretome therapy: Two **Freedom to Operate (FTO)** analysis were performed by IP law firms in autumn 2014. Furthermore, the APOSC patent attorney performs regular patent **landscape reviews (latest in 2023). Currently no other company is** infringing the patent families of APOSC. The production of the APO-1/2 IMP was developed and financed in a highly regulated space (APOSC and [redacted]).

In respect to further **indications**, we have secured the IP for the treatment of allergic reactions (skin), attenuation of scarring (skin), as well as prevention of systemic vascular leak. An overview of APOSCs IP is shown below in a recent IP landscape reported by Schwarz & Partner from July 2025:

Patent	Number	Date	Expiry	Countries
APO-1 (Ischemia)	EP2379086B1	18.12.2008	2028	AT, AU, BE, BR, CA, CH, CN, CZ, DE, DK, EP, ES, FR, GB, HU, IT, JP, NL, NZ, PL, RU, SE, SK, TR, US, ZA
APO-2 (Wound & topical)	EP2379085B1	18.12.2008	2028	AT, AU, BR, CA, CH, CN, DE, EP, ES, FR, GB, HU, IT, JP, NL, NZ, PL, RU, SE, TR, US, ZA
Potency Assays for Aposec	EP3729079B1	20.12.2017	2037	AT, AU, BE, BR, CA, CN, CH, CZ, DE, DK, EP, ES, FR, GB, HU, IN, IT, JP, KR, NL, PL, RU, SA, SE, SK, TR, US
Allergy	EP4081246B1	23.12.2019	2039	AT, AU, BE, BR, CA, CH, CN, CR, DE, DK, ES, FR, GB, HU, IN, IT, NL, JP, KR, PL, RU, SE, SK, TR, US
Scars	EP4322969A1	13.04.2021	2041	AU, BR, CA, CN, EP, IN, JP, KR, RU, US
Vascular leak	EP4452284A1	20.12.2021	2041	BR, CA, EP, JP, US
AM2 Culture Medium	PCT/EP2025/05904	03.04.2024	2044	European patent filed
Skin lesions	EP24198778.3	05.09.2024	2044	European patent filed

All inventors of patents are current and past employees of MUW and APOSC (project leader: Prof. Ankersmit (MUW, CEO APOSC), Prof. Mildner (MUW, CSO APOSC), Dr. Gugerell (former MUW, APOSC). To pursue maximal scientific transparency all scientific content was published open access (https://www.aposcience.at/news/key-publications/) after peer review. As all underlying IP is owned by APOSC, all data generated by APO2FOOT will be owned by APOSC for exploitation. This will be detailed in the Consortium Agreement before the start of the project. MUW and APOSC have a cooperation treaty which was signed in 2008.

Of relevance to European society: APOSC has financed 15 MD/PhD careers at MUW in the last 16 years (2009-2025). Most of their work related to preclinical and GMP relevant APO-1/2 product development.

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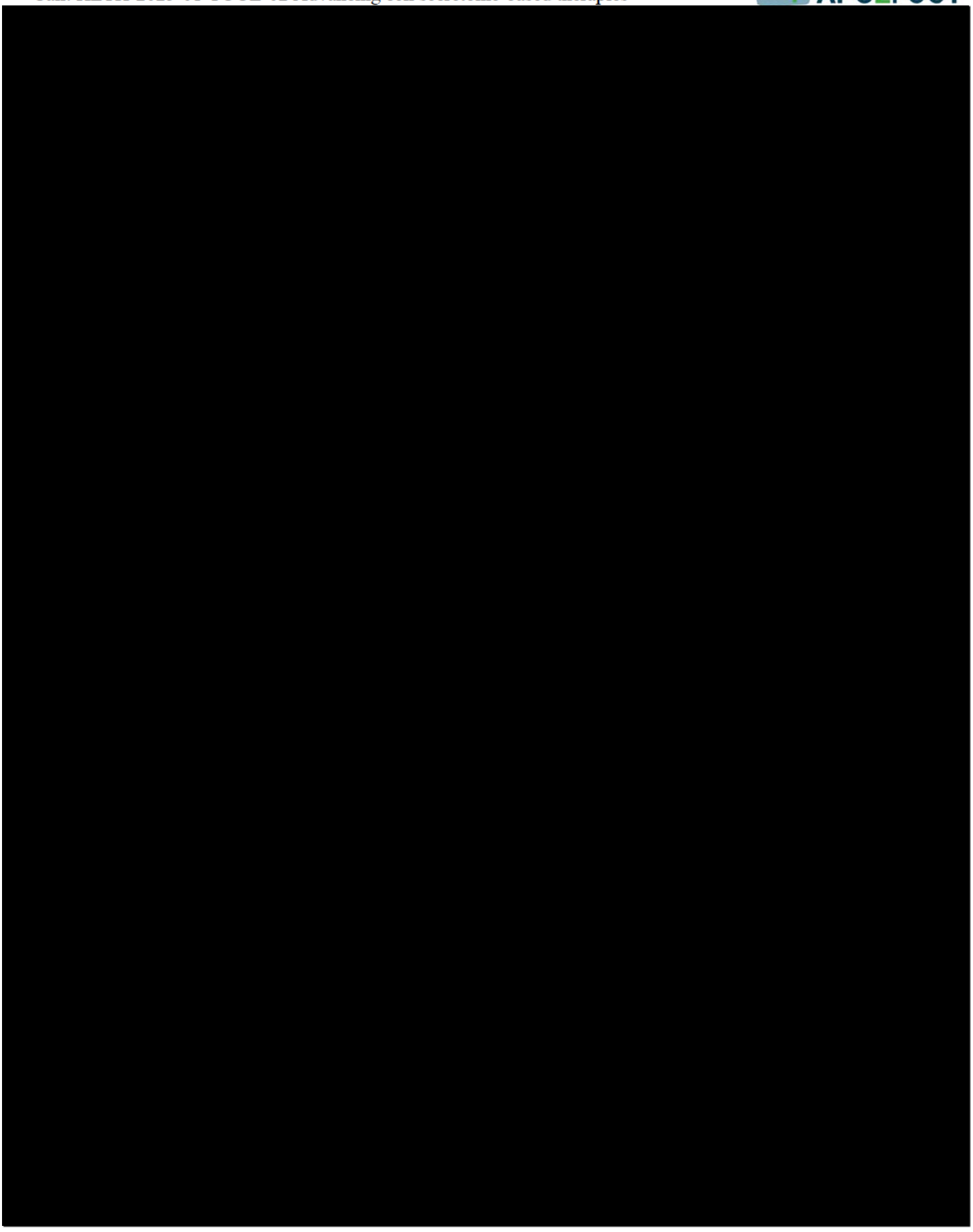
2.3. Summary

[redacted]



3. Quality and efficiency of the implementation

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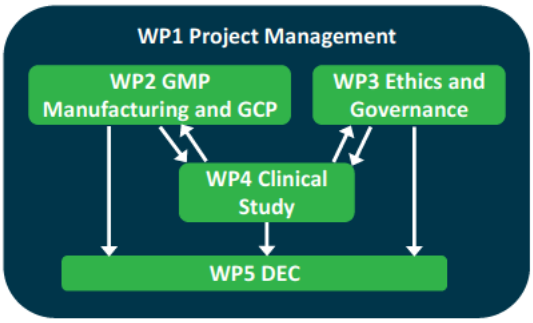
3.1. Work plan and resources

The APO2FOOT project is structured into five **interlinked** work packages (WPs – see Pert chart), each working towards one specific objective, and subdivided into tasks to group activities. They are designed to ensure a logical flow from governance and production, through regulatory preparation, to clinical validation and impact. **WP1** covers overall coordination, project management and data management, ensuring efficient governance and timely delivery of results. **WP2** establishes and validates the GMP production process for APO-2, providing the stable IMP required for clinical testing in WP4, and ensures GCP is followed in WP4. **WP3** ensures ethical integrity and regulatory compliance and advises the trial. **WP4** implements the multicentre phase II clinical trial, which is the core of the project, generating safety and efficacy data. Finally, results from all WPs feed into **WP5** to maximise visibility, dissemination, exploitation and communication, ensuring that the results reach key stakeholders and prepare for post-project scale-up.



The **timing** of the WPs and tasks reflects their activities (see Gantt below). Management activities (WP1) are needing during the whole project duration. The DEC WP also runs for the whole duration, but the focus shifts over time, with more communication at the start, while later dissemination and exploitation of results become more important. WP2 includes both short, early activities like preparation of product-related documents for WP4 to submit the study to CTIS, and activities with a long duration like stability studies running for the whole project duration. Ethics activities (WP3) start in M1 to also feed into the CTIS submission, and accompany the clinical study itself, thus finishing with "last patient out" in M50. The core WP of the clinical study runs from M1 when study management processes are established and documents prepared and submitted to CTIS, followed by study initiation, implementation and the final step of result analysis.

Gantt:

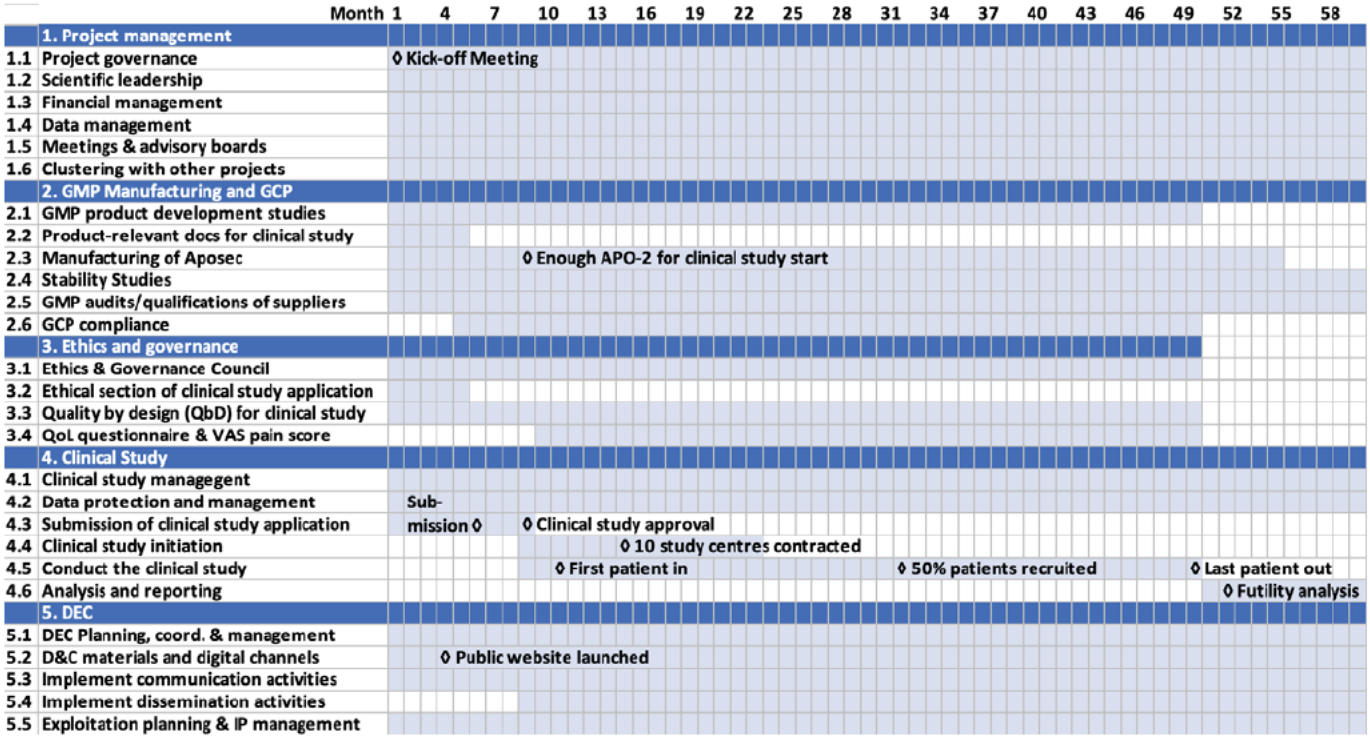


Table 3.1a: List of work packages (WPs) incl. Lead participant (LEAD) and person months (PM)

WP No	WP Title	LEAD No	LEAD short name	PM	Start month	End month
1	Project Management	1	[redacted]	61	1	60
2	GMP Manufacturing and GCP	3	APOSC	147	1	60
3	Ethics and Governance	1	[redacted]	56	1	50

4	Clinical Study	8	[redacted]	298	1	60
5	Exploitation, Dissemination and Communication	2	[redacted]	94	1	60

Table 3.1b: Work package (WP) description. Task leaders indicated in bold.

WP number	1	Lead partner: MUW	Start	1
WP title	Project management		End	60

01 [redacted]: 30PM	02 [redacted]: 20PM	03 APOSC: 6PM	08 [redacted]: 5PM

Objective (SO1): Ensure the project stays on time and within budget while achieving its objectives.
WP1 will be led by the project coordinator Univ.-Prof. Dr Hendrik Ankersmit, MBA ([redacted]), with support of the administrative manager [redacted], MA ([redacted]) as the project management team. WP leaders also play an important role in technical management and coordination within their WPs, e.g. preparing the periodic activity reports and finalising deliverables. **Sub-objectives** of this WP are:

- Keep the project within budget and schedule by monitoring progress, risks and cost
- Ensure quality of project results (deliverables) to maximise impact
- Coordinate clustering activities with other projects funded in the same call
- Manage advisory boards: PAB, SAB and DSMB, cooperate closely with the EGC - see WP3
- Coordinated, FAIR data management aligned with regulatory requirements and clinical trial needs (WP4)

Description of work:

T1.1 Project governance (M1-M60) [redacted]
This task covers the administrative coordination and overall governance of APO2FOOT, led by the project management team [redacted] as coordinator, [redacted] for administrative management). This includes communication with the EC and management of amendments to the grant- and consortium agreement, if needed. A detailed **Project Manual** (D1.1) will be prepared to define internal processes such as decision-making, conflict resolution, reporting, quality assurance, risk management, and financial administration. All activities will be closely aligned with meetings organised via T1.5. The General Assembly will act as the only decision-making body, with one vote per beneficiary.

T1.2 Scientific leadership: progress, deliverables, risk management and quality assurance (M1-M60) [redacted], APOSC, [redacted]
This task ensures effective scientific and technical coordination to implement WPs. Supervised by [redacted] WP leaders are responsible for their WPs, supported by task leaders. Internal **activity plans** will be created and continuously updated. These, together with **milestones**, are the basis for progress monitoring and will be summarised in 6-monthly written **progress reports** that feed into the formal periodic EC reports. **Risks** will be reviewed at least every 3 months for timely implementation of mitigation measures. To ensure **quality**, deliverables will undergo internal peer review before submission to the EC.

T1.3 Financial management (M1-M60) [redacted]
MUW, supported by [redacted], will provide **templates, guidelines, and training** on financial rules and reporting obligations, and support beneficiaries in preparing their individual cost reports and review them prior to EC submission. **Internal mid-period reporting** will support budget tracking and allow for early detection of financial deviations. Payments received from the EC will be distributed to partners as defined in the consortium agreement.

T1.4 Data management (M1-M60) [redacted], supported by all partners, will set up and implement procedures to manage research data for the **whole project** in line with the **FAIR** principles. This is on top of specific trial data management in WP4. A Data Management Plan (**DMP** - D1.2, M6) will specify data types, sources, metadata standards, storage, access, and reuse. It will guide data collection, curation, and preservation across WPs, aligning with the DEC plan and open science strategy. The DMP will include quality assurance and documentation protocols, and will be updated at least yearly, with a formal revision in M30 (D1.3) and a final version in M60 (D1.4) focusing on post-project data availability.

T1.5 Meetings, sub-teams and advisory boards (M1-M60) [redacted] APOSC
For smooth implementation of APO2FOOT different types of meetings with different beneficiaries / sub-teams will be organised (see 3.2 for graphical representation): **Consortium Meetings** with all project partners will be held every 6 months, alternating between in-person and online meetings, to discuss current project status and challenges. The first of these meetings is the in-person kick-off meeting in Vienna in M1. A **Steering Board** consisting of WP leaders will meet online at least every 2 months to discuss interplay between the WPs. Meetings of the Clinical Study Team are organised through T4.1, as well as an 'Investigator Meeting' with all study centres. APO2FOOT is strengthened with **4 types of external, independent advisory boards**: The independent Data & Safety Monitoring Board (**DSMB**) will meet first before study start to review the study design and sign the DSMB Charter, follow study progress, and after unblinding (M50) conduct a futility analysis and recommend continuation (post-project),

modification, or termination of the trial. The Scientific Advisory Board (**SAB**) will meet repeatedly in the preparatory phase of the clinical study to discuss study design, in- and exclusion criteria, off-loading and study materials. The participants are listed in the Annex on clinical studies 2.3.2. This task closely collaborates with T3.2 organising the Ethics and Governance Council (**EGC**) and includes setting up a Patient Advisory Board (**PAB**) with diabetes patient organisations from at least 2 countries, to consider patients' wishes and concerns. First meetings of both EGC and PAB will take place around M3 to integrate their recommendations in the clinical study design.

T1.6 Clustering: Collaboration with parallel secretome projects (M1-M60) [redacted]

We foresee clustering activities with other projects funded in the **same topic** and other relevant projects on wound healing and secretome. To coordinate these joint activities efficiently, we will contact the coordinators of the related projects after project start to establish a common cooperation **cluster** for regular exchange. Activities will include joint workshops for **knowledge exchange** and common **D&C efforts** to maximise our impact. Online coordination meetings will be held every six months to ensure alignment and identify opportunities for joint activities in the most meaningful and resource-efficient manner.

Deliverables: D1.1 Project Manual (M3-R-SEN) D1.3 Mid-project DMP (M30-DATA-PU)
D1.2 First DMP (M6-DATA-PU) D1.4 Final DMP (M60-DATA-PU)

WP number	2					Lead partner: APOSC		Start	1
WP title	GMP Manufacturing and GCP							End	60
03 APOSC: 24PM	06 [redacted]: 60PM	07 [redacted]: 7PM	08 [redacted]: 5PM	09 [redacted]: 6PM	10 [redacted]: 35PM	11 [redacted]: 10PM			

🎯**Objective (SO2): Deliver sufficient stable, and trial-ready APO-2, ensure GCP compliance of study centres and enable post-project scale-up of the GMP production process**

Please note that our GMP work packages comprise only a selection of our full GMP work program. Parts of this process (revalidation of analytical and comparability studies) and the implementation of the manufacturing process changes into the pharmaceutical development program of APO-2 will be completed before the project.

The WP consists in requalifying our suppliers, updating all documents and the APO-2 product specifications, stability studies, etc. The documents created resulting from our GMP work will be used for post-project **technology transfer** of APO-2 to other production sites and for the marketing authorisation application. WP2 will be led by APOSC with support of [redacted] and [redacted]. [redacted] will implement the actual production of all APO-2 product for the trail. All GMP studies need to be conducted by certified laboratories (e.g., partner [redacted], through qualified contractors). **Sub-objectives**:

- Compile product-relevant documentation for regulatory authorities and investigators
- Validate GMP-compliance of manufacturing and testing processes
- Produce, package, and label GMP batches of APO-2 for the clinical trial and stability testing
- Confirm stability and batch-to-batch efficacy of APO-2 batches
- Implement quality assurance through supplier audits and GCP oversight of study sites

Description of work

T2.1 GMP product development studies (M1-M50) APOSC, [redacted]

The studies will be conducted by APOSC and [redacted], advised by REG, with the support of appropriate contractors who are able to offer validated test methods for the required tests. These are mainly relevant for **patient safety** and have to be completed before the start of phase III. They include: Nitrosamines: risk assessment and confirmatory testing; Endotoxins: testing of all batches and additional method validation; Extractables/leachables: testing of glass vials (primary packaging) and writing of final reports; Container closure integrity of primary packaging (glass vials, stoppers, caps): testing with dye-immersion test and method validation (e.g., vacuum decay); Sterility: process validation. Results will be summed up in D2.3.

In this task we consider collaboration with the **JRC**, leveraging its expertise in pre-normative regulatory science to support the validation of assays, ensuring they are fit for regulatory purpose and aligned with EU-wide standards.

T2.2 Product-relevant documents for the clinical study dossier (M1-M5) APOSC, [redacted]

Using the MARSYAS II document as a starting point, the Investigational Medicinal Product Dossier (**IMPD**) and the Investigator's Brochure (**IB**) will be prepared (D2.1). These documents will include the results of studies which prove that APO-2 still has comparable quality after production process changes (comparability studies) and the results of GMP product development studies undertaken since the MARSYAS II study. This also includes first results of Task 2.1 as available at that time. APOSC, in cooperation with [redacted] and [redacted], will prepare the IMPD - a detailed description of the quality of the IMP which allows the competent authorities to decide on its suitability for a clinical study. (The IMPD has the same structure as the Common Technical Document (CTD) which is used for the marketing authorisation application, and the CTD will be created from the IMPD.). The Investigator's Brochure, an abbreviated

description of the IMP for the principal investigators (PIs) to enable them estimate to the risk of the IMP for the study participants, will be prepared by APOSC and [redacted].

T2.3 Manufacturing of Aposec (M1-M55) [redacted], APOSC, [redacted] will **produce APO-2** in their established GMP production line, this will be then released by the Qualified Person of APOSC. For the clinical trial, 5 batches of APO-2 (including 10% of safety margin) will be needed, the first 2 batches until start of the clinical study (MS5 in M9). 3 more batches of APO-2 will be produced for ongoing stability studies (T2.4). In parallel, the secondary **packaging** - cardboard boxes with inlays for APO-2 vials - will be designed and produced. GCP compliant labels for primary packaging and secondary packaging of APO-2 will be designed and printed according to Clinical Trials Regulation 536/2014. **Production SOP and SOP of specifications** will be updated based on the production and summed up in D2.5. This contributes to T5.5 and makes the GMP-compliant technology easy to transfer to other production sites. **Learnings** will feed into a publication prepared in T5.4.

T2.4 Stability studies (M1-M60) APOSC, [redacted]
While we can submit the clinical study using stability data from APO-2 in MARSYAS II, this needs to be followed with stability studies of the "new APO-2" - produced according to the new manufacturing process (change of growth medium and irradiation source). According to ICH Q5C, stability information should be provided for at least 3 batches. In addition, the Austrian Ordinance for Pharmaceutical Companies (AMBO 2009, §28 - "Ongoing Stability Program") requires a stability study with 1 batch of product per year for the period of the clinical study – 3.5 years in our case. In total, 5 batches will be needed: 1 batch will be produced before/outside this project, 4 batches in T2.3. Thus, the stability studies can start before the APO2FOOT project, to have first data as early as possible.
The following stability studies are planned: (1) **Stability studies** with 3 batches at 3 temperature conditions (-20 / +5 / +25 °C) for up to 4 years (process validation batches). The first batch will start before the project; the 2nd and 3rd batches will cover the first 2 years of the clinical study as "ongoing stability studies". (2) 2 additional "ongoing" stability studies over 4 years: one per year, covering the 3rd and 4th year of the clinical study. Continuation of these studies post-project will be financed by APOSC. (3) **Freeze-thaw** study with 3 batches for 2 weeks to simulate stress conditions: as Aposec lyophilisate cannot be stored at 45°C because of protein degradation, stress conditions, as required for stability studies according to ICH Q5C, stress can only be simulated by freeze-thaw studies. (4) **Intermediate** stability study at -20 °C for 4 years. This determines the period in which the intermediate of Aposec (frozen secretome of PBMC) can be stored safely before the final product is produced.
In addition, [redacted] will carry out Aposec-specific and validated **potency assays.** Subcontractor [redacted] has previously developed physico-chemical tests for Aposec and will carry these out in APO2FOOT. This includes stability studies with Aposec lyophilisate, and freeze-thaw studies. [redacted] will also store the samples at different temperatures. [redacted] will store the Aposec intermediates and is responsible for the coordination of the intermediate stability studies. Interim results are summed up in D2.2 with final results presented in D2.4.

T2.5 GMP audits, (re-)qualifications of suppliers (M1-M60) [redacted], APOSC
It is a regulatory requirement that all suppliers of GMP relevant goods and services (starting materials, primary packaging, analytical assay services) need to be **qualified and audited** (if qualification is not possible without audit) by APOSC. This will be done according to the APOSC **audit plan** for the following suppliers: (i) [redacted] (beneficiary [redacted]) (AT): our pharma distributor for the clinical study; (ii) [redacted] (beneficiary [redacted]) (AT): CMO producing APO-2 for the clinical study and performing release testing; (iii) [redacted] (DE): supplier of the glass vials, stoppers and caps that serve as primary packaging for Aposec lyophilisate. Possibly, other companies in the supply chain of [redacted] need to be qualified too, because the vials sold by MediPac are produced and prepared by other companies; (iv) [redacted] (AT): supplier for terminal sterilisation of APO-2; (v) [redacted] (DE): contract manufacturer of the AM2 growth medium; (vi) [redacted] (beneficiary [redacted]) and (vii) [redacted] (DE): laboratories for Aposec release testing and stability assays; (viii) [redacted] (AT): contract laboratory for container closure integrity testing.

T2.6 GCP compliance (M4-M50) APOSC
APOSC will take on **sponsor oversight** responsibility according to ICH E6(R3) to assure the compliance of the study sites with the clinical study protocol (CSP) and data safety. This activity will be performed by a subcontractor. [redacted] and [redacted] will be audited before the start of the clinical study to check that all processes, systems (e.g. electronic Case Report Form - eCRF) and documents (SOPs and plans) required for the clinical study are in place. All **study centres** will be visited, either for a GCP audit or in the form of a co-monitoring (=the GCP auditor visits the study centre together with the monitor of either [redacted] or [redacted] performing the monitoring work in the clinical study.) Study centres that need support in designing/updating GCP study SOPs will be supported by the subcontractor. This task also includes support for PIs without valid GCP-certificate so they can take a GCP course and/or renew their certificate. The main emphasis is on perfect GCP preparation for the clinical study; however, assistance will also be granted to study centres undergoing GCP inspections by competent authorities.

Deliverables: D2.1 Report on IMPD and Investigator's Brochure (M5-R-SEN)	D2.3 Product development studies (M50-R-SEN)
D2.2 Interim stability report (M26-R-SEN)	D2.4 Final stability report (M50-R-SEN)
	D2.5 Report of GMP production (M55-R-SEN)

WP number	**3**	**Lead partner: MUW**	**Start** **1**
WP title	**Ethics and Governance**		**End** **50**
01 [redacted]: 20PM	03 APOSC: 15PM	04 [redacted]: 6PM	05 [redacted]: 2PM
07 [redacted]: 3PM	08 [redacted]: 8PM	09 [redacted]: 2PM	

Objective (SO3): Guarantee the ethical integrity and regulatory compliance of the APO2FOOT clinical study by adhering to all applicable national and international standards and obtaining full regulatory approval.

This WP will be led by [redacted] in close cooperation with the EGC and the Clinical Study Management Team (APOSC, [redacted]) and critical information communicated to all beneficiaries and study centres, and vice versa.

Sub-objectives:

- Establish and apply a robust ethical and governance framework
- Fulfil all regulatory and ethical requirements for the clinical study application
- Apply a Quality by Design (QbD) approach to the APO2FOOT clinical trial

Description of work

T3.1 Ethics and Governance Council (EGC) (M1-M50) [redacted], APOSC, [redacted] will set up the EGC and coordinate EGC meetings. 2 ethics experts have confirmed their participation ([redacted] as in-house ethics expert, [redacted] as external / contracted expert. The EGC will participate in the kick-off **meeting** and invited to all consortium meetings. Prior to inclusion of the first patient in the study, an initiation meeting of the EGC will take place, with its conclusions and recommendations presented in D3.1. A summary will be shared on the project website. The work of the independent EGC will result in an Ethics and Governance Framework document (D3.3), which will **guide and inform** the work of all partners in APO2FOOT. This framework is intended to be a living document that evolves as the project progresses.

T3.2 Regulatory/ethical section of clinical study application (M1-5) [redacted], APOSC, [redacted]
This task ensures that all national and international regulatory and ethical requirements are fulfilled in the submission of the clinical study application. [redacted] will generate **Part II** of the clinical study application documents (D3.2) for T4.3, it contains the documents specific for ethical issues. This includes, among others, the Informed Consent Form (**ICF**) for the study participants, the information on the suitability of study sites and study personal involved, the arrangements for the recruitments of subjects, arrangements for compensation of study subjects and investigators and an appropriate study insurance; registration in public clinical study databases.
Informed Consent Form and other relevant clinical study documents (e.g. manuals, training material) will be translated from English (master document) into the respective languages of the study centres by local partners or subcontractors where we do not have local partners.

T3.3 Quality by design (QbD) - risk analysis of the APO2FOOT clinical study (M1-M50) APOSC, [redacted], [redacted]
The new GCP guideline, ICH E6(R3), requires QbD – "building quality into the clinical studies" instead of testing/analysing quality only at the end. This means that a **thorough risk analysis** will be done before the study, evaluating all eventualities that could impact patient safety or data reliability and to define risk-mitigation or contingency measures (summed up in D3.4). The starting point is the project risk table (Table 3.1e) with a clear focus on the clinical trial and going much more into detail. APOSC, supported by the other members of the Clinical Study Team, will develop such a QbD risk analysis. This risk analysis is not part of the clinical study application dossier (Part I or II); it is an **internal** document which is part of the quality management system (QMS) of APOSC, but which is shared with the other members of the Clinical Study Team to facilitate risk communication and management. This is a **living document** and will be updated as needed while the study is running.

T3.4 Quality of Life (QoL) questionnaire and VAS pain score (M10-M50) APOSC, [redacted]
A **validated wound QoL questionnaire** will be licenced and used to assess quality of life of the patients (developed by Prof. Dr Matthias Augustin, D3.4). It will be applied at randomisation (visit 3), End of Treatment (EoT - visit 27) and end of follow-up (visit 30). The results of the QoL Questionnaire will be presented in the clinical study report (CSR) of the phase II study or, if the study is continued into phase III, in the CSR of the completed phase II/III study. Furthermore, a **VAS pain score** will be used to monitor wound pain of the patients. The VAS score will be used at all visits from visit 1 to visit 30 (the last visit of the follow-up period) with exception of visit 2 (in the middle of the run-in period). Results will be summed up in a final ethics report (D3.5, M50).

Deliverables: D3.1 First EGC and PAB report (M3-R-PU) D3.4 Report on setting up QbD (M12-R-PU)
D3.2 Public version of the Informed Consent Form (M5-R-PU) D3.5 Final ethics report (M50-R-PU)
D3.3 Ethics and Governance Framework (M9-R-PU)

WP number	4				Lead partner: TUD		Start	1
WP title	Clinical Study						End	60
01 [redacted]: 40PM	02 [redacted]: 10PM	03 APOSC: 60PM	04 [redacted]: 20PM	05 [redacted]: 14PM	07 [redacted]: 7PM			
08 [redacted]: 50PM	09 [redacted]: 10PM	12 [redacted]: 75PM	13 [redacted]: 12PM					

Objective (SO4): Deliver a high-quality Phase II trial proving APO-2's safety and efficacy in DFU with 120 patients by 2030, to initiate a Phase III trial

This will be achieved through submission of the clinical study; initiation of the clinical study; conduct the clinical study (mainly through subcontracts, [redacted] and [redacted] as partner); analysis and reporting of study results, in line with the **sub-objectives**:

- Ensure efficient clinical study governance and coordination
- Prepare and submit a complete clinical study application package and obtain approval of the study
- Implement secure and compliant management and protection of clinical data
- Initiate and operationalise study sites effectively
- Recruit sufficient patients
- Conduct the clinical trial with rigorous monitoring
- Analyse and report study outcomes

Please note: If the APO2FOOT study yields positive results leading to a recommendation by the DSMB to continue the study into phase III, there will be no Clinical Study Report (CSR). This will only be written in case the DSMB recommends finalisation of the clinical study – see T4.6

Description of work

T4.1 Clinical study management (M1-M60) [redacted] APOSC, [redacted]

The task includes **management and monitoring** of all the following activities in this WP, through the **Clinical Study Team** (APOSC, [redacted] - see Management Structure in 3.2). The team will meet online every second week or more often, if necessary, e.g. while initialising the clinical study centres. It will be advised by the SAB, the EGC and the PAB. The Clinical Study Team will coordinate **preparatory** work (planning of recruitment, preparation of documents, study site initiation), **implementation** of the clinical study (monitoring visits, study materials supply, pharmacovigilance), and **completion** of the study (unblinding of the sponsor, data analysis and preparation for the futility analysis by the DSMB).

[redacted] will setup and manage the Trial Master File (**TMF**) - the collection of official documents of the clinical study.

T4.2 Data protection and management for the clinical trial (M1-M60) [redacted], APOSC, [redacted]

Data management: In cooperation with APOSC, [redacted] will develop a **clinical DMP (cDMP)** for eCRF of the clinical study (D4.2). The cDMP preserves the integrity of the data by outlining how data should be collected, checked, validated, processed, and stored throughout the trial. [redacted] will also set up the **eCRF** - the electronic platform where the study centres enter the patient data, which is then regularly signed by the PIs. Access to the eCRF is restricted by means of username and password. The eCRF provides functions for query handling, medical coding, serious adverse event review and PI signature. The eCRF will undergo plausibility checking of the data entered and is the basis of a data validation plan created by [redacted], which describes the exact scope of data quality checks for plausibility and completeness. [redacted] and APOSC will also create a **Communication Plan**, which describes the communication channels between the organisations and roles within the clinical trial (e.g., orders of study sites for IMP or study materials to the pharma distributor [redacted] information on the IMP shipments from [redacted] to the study site to the sponsor, etc.), so that every party receives only the information necessary to perform its task and that the blinding of the study is maintained. Furthermore, [redacted] and APOSC will prepare a **Pharmacovigilance Plan** which defines annual pharmacovigilance reporting in Development Safety Update Reports (DSUR) (1 per study year, D4.4, D4.6, and D4.7) as well as immediate Suspected Unexpected Serious Adverse Reaction (SUSAR) reporting of unexpected adverse reactions. DSURs will be submitted yearly via CTIS.

Data protection: [redacted] will ensure that all data entered in the eCRF are treated according to EU regulation 2016/679 (e.g., pseudonymisation, security measures to prevent unauthorised access, sampling according to data minimisation principles). APOSC will ensure in the study site contracts that all study centres permit data-related monitoring and audits, providing direct access to source data/documents. Furthermore, APOSC will set up a dedicated e-mail address and designate a data protection officer with corresponding qualification who is responsible for data protection enquiries from patients concerning the clinical study.

Wound measurement will be performed through wound photography and standardised as much as possible. A separate wound photography and measurement SOP will describe the process in detail. In short, study centres will be provided with iPhones equipped with good cameras (identical hardware for all study centres). The study team will take photos of the wound (plus labels, ID and a centimetre scale) before and after debridement and upload these photos to the eCRF. Wound measurement will be performed by the PI with ImageJ and the results entered in the eCRF. Wound photography and measurement training will be done by [redacted] and [redacted] at the study initiation visit and will also be given special attention during monitoring visits. Wound photographs will be blinded to observers (no indication whether APO-2 or SoC is administered), be sharp and readable, respect the patient's dignity, and preserve data protection by safe upload of pseudonymised photos to the eCRF.

T4.3 Submission of clinical study application (M1-M9) APOSC, [redacted]

This task collects relevant documents from other tasks and prepares additional needed documents to submit the clinical study in CTIS. The submission package has two parts:

Part I includes all documents relevant for the whole trial, those documents which are evaluated by the competent authorities, e.g. related to the quality of the IMP (IMPD), the Investigator's Brochure (see T2.2) and the CSP prepared in this task by [redacted] under the lead of APOSC and discussed with [redacted] and [redacted] (using MARSYAS II CSP as template).

Part II with country specific documents is prepared in T3.2 - D3.3. This includes e.g. the Informed Consent Form, information on the clinical study centres, insurance. This will be assessed by the Ethics Commissions of the respective countries where the clinical study takes place.

Further documents according to Annex I of Reg. 536/2014 are: cover letter, EU application form, documentation relating to compliance with GMP, and content of the labelling of the IMP. The submission of the clinical study application package to CTIS will be handled by [redacted] and APOSC (MS3, M6). In the evaluation process the sponsor APOSC may receive requests from the authorities for clarifications. Approval is expected in M9 (MS4), when also the D4.1, the Study Initiation Package will be submitted (registration number of the clinical study, a public final version of the CSP and regulatory and ethics approvals).

T4.4 Clinical study initiation (M9-M23) [redacted], APOSC, [redacted]

This includes the following activities: (i) Drafting and negotiating **contracts** with the study sites (MUW supported by [redacted]), aiming for signature of 10 contracts in M15 (MS7); (ii) **'Investigator Meetings'** (in batches as study centres are contracted) of the Clinical Study Team with all PIs of the study sites will be organised before the start of the trial at the respective sites, to discuss details of the study design and boost recruitment motivation (summed up in D4.3); (iii) Preparation of further clinical study-relevant **documents**, such as the Statistical Analysis Plan (by [redacted] and [redacted]), training manuals (by [redacted] and [redacted]), and GCP SOPs (by APOSC as the sponsor). (iv) Study centre **initiation visits** by [redacted] in AT and DE, [redacted] in CZ, IT and PT will provide the study centres with the study centre binders and with trainings for the study teams on the study design, IMP handling and application, wound measurement with the ImageJ software, and use of the QoL questionnaire and VAS pain score. (iv) Shipments of initial **packages of IMP** and study materials to the study centres and providing the study centres with order forms for IMP and study materials ([redacted]).

T4.5 Conduct the clinical study (M9-M50) [redacted] APOSC, [redacted]

Supervised by T4.1, this task includes: (i) Study site **monitoring**: online monitoring of eCRFs, scheduling and conduct of monitoring visits or of co-monitoring with a representative of the sponsor, and the review of monitoring visit reports. Monitoring reports will be drafted by [redacted] (for AT and DE) and [redacted] (for CZ, IT and PT) and discussed with APOSC. (ii) **Adverse event** handling (including serious adverse event reporting). Yearly DSURs will be drafted by [redacted] and discussed with and completed by APOSC. [redacted] will submit them to CTIS. (iii) **SUSAR** reporting: SUSARs must be reported to the EudraVigilance System within 7 days if life-threatening or 15 days if not life-threatening. This will be done by [redacted] in cooperation with APOSC and the PI of the study site where the SUSAR appeared according to a Pharmacovigilance Plan; (iv) **Medical coding** [redacted]); (v) **Supply of study materials**: [redacted] will store Aposec (IMP) at +2 to +8 °C at its premises under GMP conditions and purchase and store other study materials (syringes, alginate gauze, dressings). Study materials will be shipped to study centres by qualified couriers. A system of forms for ordering and receipt of temperature-controlled shipment of IMP and study materials will be set up and run by [redacted] (vi) **Conducting the study**: For the study centres that are beneficiaries ([redacted]), this task includes the activities as a study centre, other study centres will be subcontracted – see list in the Annex on clinical studies. (vii) Constant **monitoring of recruitment** progress [redacted], APOSC) is needed to meet clinical study timelines. [redacted] will prepare monthly status reports (about regulatory status, study site contracts, study site imitations or closures, monitoring visits, and patient recruitment) which serve as a tool for study management. We aim for "first patient in" – MS6 in M11, 50% of patients recruited by M32 (MS8, D4.5) and last patient out in M50 (MS9). Monthly updates to the study sites about patient recruitment serve transparency. In Austria, the **recruitment process** will additionally be enhanced by the digital platform [redacted] for clinical

recruitment operated [redacted] allows **outpatient physicians** to search for appropriate clinical studies for their patients and to preselect patients according to inclusion criteria published on the platform. [redacted] will use their network to prompt use of the platform to boost recruitment. In cases of lack of commitment of a study centre or lack of suitable patients, the closure of the concerned centre and the initiation of another, alternative study centre will be considered.

T4.6 Analysis and reporting (M51-M60) [redacted], APOSC, [redacted]
This includes the following activities: (i) **Database lock**, unblinding and transfer of results to the sponsor by [redacted]; (ii) **Statistical analysis** according to the Statistical Analysis Plan (created by [redacted]) and preparation of the data for the Futility Analysis by [redacted] in cooperation with APOSC and [redacted]; (iii) **Futility Analysis** and recommendation by the IDMC to continue the clinical study into phase III (post project) or end it. (iv) Writing of the **Clinical Study Report** in case of negative recommendation of the IDMC by [redacted] and APOSC, supported by [redacted] (D4.8); (v) **Posting** the summary of study results in the EU Clinical Trials Register via CTIS by [redacted] (D4.9).

Deliverables: D4.1 Study initiation package (M9-R-PU)	D4.6 DSUR 2 (M33-R-SEN)
D4.2 Clinical DMP (M9-R-SEN)	D4.7 DSUR 3 (M45-R-SEN)
D4.3 Investigator meeting report (M21-R-PU)	D4.8 Futility Analysis Report or CSR (M52-R-PU)
D4.4 DSUR 1 (M21-R-SEN)	D4.9 Report on the status of posting of results of the clinical study (M60-R-PU)
D4.5 Midterm recruitment report (M32-R-PU)	

WP number	5		Lead partner: [redacted]			Start	1
WP title	Dissemination, Exploitation and Communication					End	60
01 [redacted]: 15PM	02 [redacted]: 36PM	03 APOSC: 20PM	04 [redacted]: 2PM	06 [redacted]: 6PM	07 [redacted]: 2PM		
08 [redacted]: 2PM	09 [redacted]: 2PM	10 [redacted]: 2PM	11 [redacted]: 2PM	12 [redacted]: 3PM	13 [redacted]: 2PM		

Objective (SO5): Maximise the visibility, uptake, and long-term impact of APO2FOOT through strategic dissemination, effective communication, and well-planned exploitation
This WP will ensure targeted dissemination (D) of scientific data and project communication (C) to diverse stakeholders, including patients, and exploitation (E) of the data to foster the clinical implementation of APO-2. If the APO2FOOT clinical study phase II is successful in the futility analysis, the clinical study will be continued as a phase III study financed by APOSC. The aim of the entire phase II/III clinical package is to (1) lay the base for marketing admission application, (2) generate data to convince national health insurance systems that APO-2 is therapeutically more effective and less costly in comparison to other wound healing products on the market and that it would therefore be advantageous for European healthcare systems to refund DFU treatment with APO-2.
This WP builds on the draft DEC plan in Chapter 2.2 to maximise APO2FOOT's impact and is led by [redacted] in close cooperation with the core DEC team ([redacted] APOSC). This will be achieved through the following **subobjectives**:

- Develop and manage a comprehensive DEC strategy, based on open science principles
- Provide high-quality tools, materials, and digital channels, targeted to specific stakeholders
- Implement targeted dissemination and communication activities
- Ensure long-term use and impact of APO2FOOT results through IP protection and exploitation plans for post-project upscaling of production

Description of work
T5.1 DEC Planning, coordination, and management (M1-60) [redacted] APOSC, all
Elaborating on Chapter 2.2, we jointly develop a comprehensive **DEC strategy and action plan** (D5.1, M5). It will define APO2FOOT's visual identity, detail the target groups and key messages, and appropriate channels tailored to different audiences. It will include SOPs, e.g. for publications, KPIs, and timelines for implementation. Internal coordination will ensure alignment across WPs, while result management procedures will safeguard sensitive results. The DEC plan will be a **living** document, updated formally in M20 and M40 (D5.3, D5.4) to reflect project progress.

T5.2 Design, implement, and maintain D&C materials and digital channels (M1-60) [redacted] APOSC
Based on the strategy defined in T5.1 and the needs of activities in T5.3 and T5.4, EUTEMA sets up **channels** and creates and updates **materials**, together with the DEC team. This includes the project **website** as a central information hub (1st version in M4 –MS2), with a blog for original content, and **social media** accounts (e.g. LinkedIn, Bluesky, YouTube). Materials (flyers, posters, factsheets, newsletters, videos, press releases) will be developed, tailored to different audiences. [redacted] A coordinates content production and publication, all partners will contribute content and provide translations where needed.

T5.3 Implement communication (C) activities (M1-M60) [redacted], all

This task implements targeted C **activities** as planned in T5.1, using tools/channels from T5.2, to engage stakeholders and raise awareness e.g. about DFU. In addition to continuous outreach, C is structured to address both **expert and non-expert audiences** through e.g. press releases and public science events. **Patient organisations** will be directly addressed by audience-targeted communication. Regular newsletters will increase communication outreach and not only inform about the project but also parallel developments; project partners will actively amplify activities through their networks. All activities will be monitored through KPIs to ensure visibility, reach, and impact (see Chapter 2.2 for details).

T5.4 Implement dissemination (D) activities (M9-M60) APOSC, [redacted], all
This task will focus on the dissemination of the project **results** and **learnings**, as detailed in the DEC plan. Project dissemination will start later than communication as first results become available. First content for dissemination are the clinical study protocol (part of D4.1) and detailed learnings from the previous study MARSYASI/II (D5.2). The GMP-production process of APO-2 is also a major advance worth disseminating: a publication will be prepared summing up **learnings** from establishing and implementing GMP-compliant production of a secretome therapy (preprint as D5.5) Most activities will be designed around dissemination of results of the clinical study (WP4) to be presented at scientific meetings in year 5 and submitted to peer-reviewed international scientific journals. This will include a publication similar to D5.2, presenting learnings of the APO2FOOT clinical study. Finally, a **whitepaper** about secretome-based therapies will be prepared together with SAB members to contribute to evidence-based policy making (D5.6).

T5.5 Exploitation planning and IP management (M1-M60) APOSC, [redacted]
This task ensures that all results, not only the clinical study results, are identified, managed, and used post-project. Procedures will be established to manage results in line with the grant- and consortium agreement, including internal review processes to **prevent premature disclosure** through D activities. Building on Chapters 2.2.5 and 2.2.6, results owners will assess TRL and develop, and update exploitation plans, document emerging IP, and decide on IP management, inc. freedom-to-operate (FTO) analysis. Final **exploitation plans** will include results ownership lists and information on taken or planned **IP protection** activities (D5.7). An important part of this document will be plans how to **scale up and increase automation** of production post-project, developed together with key partners (e.g. [redacted] and other potential production sites – see LoI), based on D2.5.

Deliverables: D5.1 Initial DEC Plan (M5-R-SEN)
D5.2 Learnings from the previous study MARSYASI/II (M12-DEC-PU)
D5.3 First DEC plan update (M20-R-SEN)
D5.4 Second DEC plan update (M40-R-SEN)
D5.5 Learnings: GMP production of a secretome (M55-DEC-PU)
D5.6 Whitepaper about secretome-based therapies (M60-DEC-PU)
D5.7 Exploitation plan (M60-R-SEN)

Table 3.1c: List of Deliverables: lead participant (Lead), dissemination level (DL) and delivery date (DD) in months.

No.	Deliverable name	Short description	WP	LEAD	Type	DL	DD
D1.1	Project Manual	internal, for all beneficiaries	1	[redacted]	R	SEN	3
D1.2	First DMP	for FAIR data management	1	[redacted]	DATA	PU	6
D1.3	Mid-project DMP	update of D1.2	1	[redacted]	DATA	PU	30
D1.4	Final DMP	post-project data availability	1	[redacted]	DATA	PU	60
D2.1	Report on IMPD and Investigator's Brochure	summary, actual documents for T4.3	2	APOSC	R	SEN	5
D2.2	Interim stability report	Aposec after 2 years storage	2	APOSC	R	SEN	26
D2.3	Product development studies	a summary of all reports	2	APOSC	R	SEN	50
D2.4	Final stability report	Aposec after 4 years storage	2	APOSC	R	SEN	50
D2.5	Report of GMP production	summary, actual document for T5.4	2	APOSC	R	SEN	55
D3.1	First EGC and PAB report	about the inaugural meeting	3	[redacted]	R	PU	3
D3.2	Public version of the Informed Consent Form	incl. non-sensitive data	3	APOSC	R	PU	5
D3.3	Ethics and Governance Framework	for the whole study	3	[redacted]	R	PU	9
D3.4	Report on setting up QbD	clinical trial risk analysis	3	APOSC	R	PU	12
D3.5	Final ethics report	incl. QoL results	3	APOSC	R	PU	50
D4.1	Study initiation package	incl. public version of the CSP	4	APOSC	R	PU	9
D4.2	Clinical DMP	cDMP for eCRF	4	[redacted]	R	SEN	9
D4.3	Investigator meeting report	summary report	4	APOSC	R	PU	21

No.	Deliverable name	Short description	WP	LEAD	Type	DL	DD
D4.4	DSUR 1	from 1st year of the trial	4	[redacted]	R	SEN	21
D4.5	Midterm recruitment report	confirming 50% recruitment	4	APOSC	R	PU	32
D4.6	DSUR 2	from 2nd year of the trial	4	[redacted]	R	SEN	33
D4.7	DSUR 3	from 3rd year of the trial	4	[redacted]	R	SEN	45
D4.8	Futility Analysis Report or CSR	depending on futility result	4	APOSC	R	PU	52
D4.9	Report on the status of posting of results of the clinical study	end of trial	4	APOSC	R	PU	60
D5.1	Initial DEC Plan	incl. SOPs	5	[redacted]	R	SEN	5
D5.2	Learnings from the previous study MARSYASI/II	Preprint publication	5	APOSC	DEC	PU	12
D5.3	First DEC plan update	planning for Period 2	5	[redacted]	R	SEN	20
D5.4	Second DEC plan update	planning for Period 3	5	[redacted]	R	SEN	40
D5.5	Learnings: GMP production of a secretome	Preprint publication	5	APOSC	DEC	PU	55
D5.6	Whitepaper about secretome-based therapies	Together with SAB members	5	APOSC	DEC	PU	60
D5.7	Exploitation plan	Incl. production upscaling & automation planning	5	APOSC	R	SEN	60

While there is a limited list of public deliverables, due to confidentiality restrictions and the commercial exploitation potential of most results, all deliverables will include a **public summary** that can be used for communication and dissemination activities.

Table 3.1d: List of milestones (MS): Due date (DD) given in months (M)

MS	MS name	Related WPs	DD	Means of verification
1	Kick-off Meeting in Vienna (in person)	all	1	All beneficiaries attended
2	Public website launched	5	4	Website online
3	Submission of the clinical study in CTIS	4	6	CTIS Notification of submission
4	Approval of the clinical study	4	9	CTIS Notification of approval
5	APO-2 produced to start the clinical study (2 batches)	2, 4	9	First vials are delivered
6	First patient in	4	11	Notification by [redacted]
7	Study contracts signed with 10 study centres	4	15	10 signed contracts available
8	50% of patients recruited	4	32	D4.6 submitted
9	Last patient out	4	50	Notification by [redacted]
10	Results of Futility Analysis by DSMB	4	52	Short report

Table 3.1e: Critical risks for implementation incl. WPs involved. While the project is built on a solid plan and an experienced consortium, certain risks may arise. Overall risk management is part of T1.2, including regular reviews (every 3 months), updates to the risk table, and clear responsibilities for monitoring and response. In addition, T3.3 employs **QbD** for detailed risk analysis and management of the clinical trial. Most-relevant potential risks are listed below; mitigation includes both preventive measures and reactive contingency measures. Likelihood and severity are rated as low (L), medium (M), or high (H).

#@RSK_MGT_RM@#

[redacted]

Description of risk (likelihood / severity)	WPs	Proposed risk-mitigation measures	Contingency measures
[redacted]			

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Table 3.1f: Summary of staff effort: PM number of WP leader in bold.

	WP1	WP2	WP3	WP4	WP5	Total PM per Participant
01 [redacted]	**30**		**20**	40	15	**105**
02 [redacted]	20			10	**36**	**66**
03 APOSC	6	**24**	15	60	20	**125**
04 [redacted]			6	20	2	**28**
05 [redacted]			2	14		**16**
06 [redacted]		60			6	**66**
07 [redacted]		7	3	7	2	**19**
08 [redacted]	5	5	8	**50**	2	**70**
09 [redacted]		6	2	10	2	**20**
10 [redacted]		35			2	**37**
11 [redacted]		10			2	**12**
12 [redacted]				75	3	**78**
13 [redacted]				12	2	**14**
Total PM	**61**	**147**	**56**	**298**	**94**	**656**

Table 3.1g: 'Subcontracting costs' items: most are planned in the budget of APOSC i) to continue previously established contractual relationships; ii) because APOSC is the owner of APO-2; iii) because APOSC is the sponsor of the study.

Part # / Short Name	Cost (€)	Description of tasks and justification
03-APOSC	[redacted]	[redacted] GCP external audit for all sites (for T2.6)
03-APOSC	[redacted]	Clinical Study patient recruitment and Study implementation fee (for T4.5) for external wound clinics as subcontractors: 134 Patients recruited (120 patients + 10% drop-out safety margin) at [redacted] Up to **21 study sites** will be engaged as subcontractors, as detailed in Section 3.2.1 of the Clinical Studies Annex, following standard practices to prevent any conflict of interest. The list of sites is provisional, as the exact number to be involved will depend on the observed patient recruitment rates and the commitment of the participating sites. Each site will be responsible for screening, recruiting, and treating patients, as well as providing the relevant data entry and query management by study nurses, and the costs of clinical procedures. Sites will receive [redacted], covering part of the costs for clinicians' time, eligibility assessments, data entry, query management, and clinical procedures. Compensation will be performance-based and directly linked to the number of patients successfully recruited at each site.
03-APOSC	[redacted]	[redacted] Physico-chemical tests for Aposec (drug) substance (Stability tests) (for T2.4)
04-[redacted]	[redacted]	The local Clinical Study Coordinator for the **study sites in Italy** is a subcontractor of [redacted], implementing the following activities: Regulatory and ethical support (Initial and follow up throughout study duration), Local monitoring, Local project (study) management, Local Pharmacovigilance (linked to Task 3.2 and WP4)

Table 3.1h: 'Purchase costs' items (travel and subsistence, equipment and other goods, works and services)

03 [redacted]	Cost (€)	Justification
Other goods, works, services	[redacted]	Rental of 22 devices @ 25k€ each for 4 years incl. cloud storage ([redacted]) for precise Blood pressure measurement in feet/ankle (Certificate in Ethical Annex) Clinical Studies Insurance
Remaining purchase costs	[redacted]	(<15% of personnel costs)
Total	[redacted]	

07 [redacted]	Cost (€)	Justification
Other goods, works, services	[redacted]	24k Multilanguage booklet for patient information on the trial; 150k€ GCP/GMP conform shipment cost
Remaining purchase costs	[redacted]	(<15% of personnel costs)
Total	[redacted]	

08 [redacted]	Cost (€)	Justification
Travel and subsistence	[redacted]	In addition to consortium meetings and DEC event 30+ trips to Study sites
Other goods, works, services	[redacted]	61k€ Data software license fees- GCP conform data management system (electronic case Report Form) to collect the trial data. SOCRAMATE 62k€ fees for Ethics Committee & BOB Bundesoberbehörde (Competent authority)
Remaining purchase costs	[redacted]	(<15% of personnel costs)
Total	[redacted]	

11 [redacted]	Cost (€)	Justification
Travel and subsistence	[redacted]	In addition to consortium meetings and DEC event 5 extra trips for GMP audits- to save costs trips will be combined with Meetings and DEC activities
Remaining purchase costs	[redacted]	(<15% of personnel costs)
Total	[redacted]	

13 [redacted]	Cost (€)	Justification
Travel and subsistence	[redacted]	In addition to consortium meetings and DEC -recruitment travel 30 national trips a €500
Remaining purchase costs	[redacted]	(<15% of personnel costs)
Total	[redacted]	

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3.2. Capacity of participants and consortium as a whole

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[redacted]

Partner	Unique contribution and role
01 [redacted]	Research infrastructure, Coordinator, WP1 and WP3 lead
02 [redacted]	Management support, outreach coordination, WP5 lead
03 APOSC	Owner APO-2, trial sponsor, WP2 lead
04 [redacted]	Monitoring (IT/PT), translations
05 [redacted]	Local monitoring & CZ translations
06 [redacted]	GMP manufacturing
07 [redacted]	Storage, distribution
08 [redacted]	Clinical study management, monitoring (AT/DE), WP4 lead
09 [redacted]	Regulatory guidance
10 [redacted]	Analytical assays
11 [redacted]	GMP audits
12 [redacted]	Lead study centre - study implementation
13 [redacted]	Recruitment support

Figure 14: Management and governance of APO2FOOT

Resources and infrastructure: APO2FOOT depends on a range of resources and infrastructure, including specialised personnel, and all partners have the necessary certifications (GMP/GCP/GLP): [redacted] is a GMP certified pharmaceutical company; it contributes the pharmaceutical development documents contained in its QMS and the Qualified Person who releases APO-2 product for use in the clinical study. [redacted], as the GMP certified manufacturer of APO-2, provides the GMP production facility, trained production personnel, and the GMP compliant APO-2 production protocol. [redacted] research facilities (laboratories) and office space and personnel for the (financial) management of the study and the project as a whole. [redacted] (and [redacted]) are GMP certified laboratories offering validated potency assays ([redacted]) and physico-chemical assays [redacted]) for analytical testing of APO-2. [redacted] has all necessary know-how and resources to set up the clinical trial management (eCRF, eTMF, etc.); as a network with many national partners, [redacted] is indispensable for this multinational study centre network ([redacted]); [redacted] has all resources needed for GMP audits [redacted] possesses resources as a clinical study centre with the background of the infrastructure of a large University, and [redacted] offers its electronic platform and contacts to physician practices for patient recruitment.

External boards: As the expertise of the consortium partners is predominantly of the technical and regulatory kind, the consortium reached out to external experts to secure expertise in the fields of ethics, patient concerns, gender issues and clinical trial design. The **EGC** and the **PAD** will advise the consortium on ethical issues and patient concerns. APOSC has installed the **SAB** to discuss inclusion/exclusion criteria, endpoint definitions, the visit schedule, and options to standardise study procedures. Finally, the **DSMB** will meet regularly to watch adverse events, care for the safety of the study participants and express a recommendation to end the APO2FOOT study or to continue it into phase III in the futility analysis.

Financial background of APOSC: as the product owner of APO-2 and sponsor of the clinical study, APOSC is the one partner impossible to replace in APO2FOOT. Thus, its financial **stability** is of importance, giving other beneficiaries the certainty that the project will not only be implemented, but also results exploited post-project. APOSC is a private stock corporation with 2 main shareholders: the family offices of [redacted] and of [redacted] Together they own >90% of APOSC. **Univ.-Prof. Dr Hendrik Ankersmit**, CEO of APOSC, is also a shareholder of APOSC. The shareholders of APOSC are interested in **long-term business development** that helps patients suffering from DFU.

Industry / SMEs involvement: 7 out of 13 beneficiaries are for-profit organisations, 5 of those SMEs ([redacted], [redacted]). APOSC is considered industry only because of its shareholder structure. APO2FOOT will strengthen their expertise related to clinical trials and regulatory issues but also increase their visibility. This will help them to attract customers in the future.

Subcontracting: (1) most study centres for APO2FOOT will be subcontracted. This is to keep flexibility in this key area of the project. It allows to contract the centres only when the final study protocol is (nearly) finished and thus expectations can be better described. Also, should a centre substantially underperform in patient recruitment or struggle with working according to GCP, contracts could also be terminated and other study centres contracted instead. (2) Physico-chemical testing of the APO-2 will be done by [redacted], as described above. (3) GCP audits will also be performed by an external auditor.

Gender: Proposal development was led by one man (Prof. Ankersmit, [redacted]/APOSC) and one woman ([redacted]), together with a core team of 3 women ([redacted]) and two men ([redacted] APOSC; [redacted]).

***Disclaimer**: AI helped to optimise readability and shorten some texts. All texts were reviewed afterwards. Graphical abstract and Figures 1, 2, 3, 4, 13 created with BioRender.com*

#§CON-SOR-CS§# #§PRJ-MGT-PM§#

1. Description of the clinical study

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KNOWLEDGE BOX: over the course of the development of Aposec, many different types (different sources, different production processes) of Aposec have been used. Here a short overview: **Aposec**: the general name given to the secretome of stressed peripheral blood mononuclear cells (PBMC) – the APOptotic SECretome. In the discovery phase we utilized laboratory manufactured Aposec: **Livosec**: secretome of non-stressed PBMC, laboratory grade **Pre-APO-2**: secretome of stressed PBMC, laboratory grade **APO-2**: human Aposec produced under GMP for topical application **(APO-1**: human Aposec for intravenous application)	**ABBREVIATIONS** CRO: Clinical Research Organisation DFU: Diabetic Foot Ulcer DSMB: Data & Safety Monitoring Board EoS: End of Study EoT: End of Treatment FAS: Full Analysis Set GCP: Good Clinical Practice GLP: Good Laboratory Practice IMP: Investigational Medicinal Product ICH: International Commission of Harmonization PBMC: Peripheral Blood Mononuclear Cells SAB: Scientific Advisory Board SoC: Standard of Care TPP: Target Product Profile
Beneficiaries involved in the clinical trial: [redacted] Aposcience AG (APOSC) [redacted]	

1.1. Title, acronym, unique identifier of the clinical study

Acronym: APO2FOOT

Title: A randomised, open label, observer-blinded clinical trial to evaluate safety and efficacy of APO-2 in comparison to Standard of Care (SoC) in the treatment of diabetic foot ulcers (DFU).

Unique identifier: not yet available

1.2. Study rationale

INTRO: Based on our preclinical and clinical developmental data we are convinced that APO-2 (the secretome of stressed PBMC for topical application) is poised to be the first "**off the shelf**" active investigational medicinal product (IMP) for the successful treatment of DFU. Aposec was originally developed for the treatment of internal inflammatory diseases such as acute myocardial infarction, myocarditis and spinal cord injury (APO-1 = Aposec for the treatment of internal diseases, intravenous application). The decision to develop the stressed PBMC secretome for the indication of diabetic wound healing was based on an **unmet need for the treatment of DFU**, leading to the development of APO-2 for topical application. The APO-1/APO-2 IMP production recipe are identical.

This APO2FOOT clinical trial builds upon our **previous work** relating to a) relevant preclinical wound models (see 1.2.1); b) the establishment of a GMP production site for APO-1/2 according to guidelines for product development set by the ICH (International Commission of Harmonization - see main proposal 1.2); c) execution of MARSYAS I/II, a clinical phase I and I/II trial in diabetic foot ulcer (DFU) (see 1.2.1.1.); and the d) establishment of quality management system which is mandatory for the pharmaceutical company APOSC. APO2FOOT aims to augment GMP readiness for market approval of APO-2 and to execute a **phase II trial in DFU** that competes with the highest standards in clinical research. The rationales explained below are mainly derived from the MARSYAS I/II clinical trial (EudraCT No. 2018-001653-27):

Rationale for the treatment duration: MARSYAS I/II was the first secretome based study to evaluate whether allogeneic APO-2 augments wound healing as compared to placebo. We showed that a 1 month-treatment period is too short for complete wound closure. APO2FOOT will apply APO-2 for a 3-month treatment period (which is backed by a corresponding repeated dose toxicology study performed in 2024) with complete wound closure as our chosen unambiguous primary endpoint. This is also in line with most other clinical studies in DFU that also cover a treatment period of 3 months (12 weeks). This will allow for comparability with other published clinical reports.

Rationale for the screening period: In the MARSYAS II study, we applied a screening phase of 2-4 weeks, with a maximum change in ulcer size of 30% for patient to be included in the study. This screening period turned out to be too short to filter for chronic, hard-healing wounds in DFU. Therefore, in the APO2FOOT study, we plan to apply a screening phase of 5-7 weeks to identify DFU wounds which are true chronic wounds.

Rationale for the study duration: The screening period of 5-7 weeks and the treatment period of 12 weeks (End of Treatment, EoT) add up to 17-19 weeks (end of study, EoS). Additionally, we want to investigate whether wounds healed during the study remain closed after EoT. Therefore, we plan a follow-up phase of another 12 weeks, resulting in a total study period of 29-31 weeks.

Rationale for the study endpoint: Our primary endpoint is complete wound closure. This is a hard clinical endpoint with no potential of interpretation. This endpoint also allows for comparability with other clinical trials in DFU and is accepted as a measure for market approval.

Rationale for the treatment frequency: In the APO2FOOT study, we will treat patients 2 times a week for 12 weeks. This better aligns the treatment frequency with SoC wound dressing change.

Rationale for the dose: The dose that will be applied in APO2FOOT is 25 Units of Aposec/cm^2 of wound; this dose is equivalent to the high dose (50 Units of Aposec/mL) in the MARSYAS II study. The decision for this dosing unit was based on the Full Analysis Set (FAS) results of our completed MARSYAS II study. 45% of patients treated with the high dose of APO-2 achieved a ≥80% reduction of wound size in comparison to 22% in the placebo group after 4 weeks (EoT). Correspondingly, complete wound closure was attained in 15% of patients with the high dose of APO-2 versus 4.5% in the placebo group at EoT (see FAS of those patients with adjudicated 0.8cm^2-8cm^2 – see 1.2.1.1). The dose of 25 Units of APO-2/cm^2 is safely backed by our recently executed Good Laboratory Practice (GLP) repeated dose toxicology study in minipigs.

Rationale for the wound size to be treated: In the APO2FOOT study, we intend to treat wounds ranging from 0.8 to 4 cm^2. In the MARSYAS II study, we included wounds from 0.8 – 8 cm^2. We have realised in MARSYAS II study that wounds larger than 4 cm^2 were not found at the MARSYAS I/II clinical study sites.

Rationale for the inclusion/exclusion criteria: We are looking for patients with diabetes 1 or 2 with a diabetic wound who (1.) are capable of agreeing to informed consent and adhering to the treatment procedure, who (2.) do not suffer from severe peripheral arterial disease which (for ethical reasons) results in a revascularisation procedure during the course of our clinical study which induces a bias into the clinical study, who (3.) do not suffer from an additional severe disease which confounds the results of the clinical study, and who (4.) do not take medication which confounds the results of our clinical study. For details, see Chapter 1.4.

1.2.1. Extent and evaluation of current knowledge directly linked to the scientific question(s) to be answered by the clinical study

To contextualise the rationale behind APO2FOOT and the design of the clinical trial, we are summing up key studies on PBMC for wound healing in general, and key experiments performed with different types of Aposec (the PBMC secretome) that led to the clinical trials described in 1.2.1.1.

PBMC therapy in chronic wound healing: The first documented PBMC cell therapy in chronic wound healing was published by Holzinger et al. in 1994[1]. Holzinger treated patients with large ulcers (5.14 ± 2.7 cm2) 2x weekly utilising autologous stimulated mononuclear cells which were stimulated with phytohemaglutinin (PHA). After 24 hrs of culture, PBMCs were washed and resuspended. This PBMC cell suspension was applied upon the chronic wound. The control group (30 patients) received tissue culture medium without PBMC. Results: Complete wound healing was achieved in 30 of 33 patients (92%) within 60 days vs 16 patients in the control group. It is noteworthy that patients had their chronic wound for 9.2 ± 4.0 months before the study was initiated.
A similar approach was chosen by a Danish company Reapplix Inc[2]. They invented a medical device with the name of LeucoPatch. The DFU patient donates 18ml of blood. This is centrifuged in a filter container, a disc comprising leucocytes (PBMC), platelets and fibrin is formed, which is then used as patch that covers the wound. This very well perceived clinical study with 269 patients evidenced that this LeucoPatch formulation was able to augment complete wound closure after 20 weeks of treatment versus SoC: In the LeucoPatch group, 45 (34%) of 132 ulcers healed within 20 weeks vs 29 (22%) of 134 ulcers in the SoC group. Adverse events were comparable; the most common serious adverse event was foot infection: 24 events in the LeucoPatch group and 20 events in the standard care groups. Note: both studies utilised autologous PBMC (cell) compartments to augment wound healing. The Holzinger study was one of the first ever published cell therapy studies with a comparative control arm. The LeucoPatch study examined a medical device to separate PBMC and place those on patients' wounds.

PBMC secretome: In comparison to the approaches described above, APO-2 contains the secretome of stressed PBMC and is cell-free. Between 2008 and 2023, numerous preclinical experiments were performed which proved the therapeutic efficacy of Aposec. Aposec contains many active ingredients (exosomes, lipids, proteins) and thus exerts multiple therapeutic effects. The results of our preclinical work in acute wound models supported our path to clinical development, first in mouse to gain first evidence, then in porcine wound models: The scientific interpretation of porcine preclinical wound models has a greater credibility than murine and rodent models in wound healing, because porcine and human skin biology are similar. All our work regarding APO-2 development is published open access (2013-2014).
The following publications support the development of **APO-2 for the indication "skin wound"**:

- Mildner et al.: Secretome of Peripheral Blood Mononuclear Cells Enhances Wound Healing. PLOS ONE, 2013, 10.1371/journal.pone.0060103; (Livosec).
- Hacker et al. Paracrine Factors from Irradiated Peripheral Blood Mononuclear Cells Improve Skin Regeneration and Angiogenesis in a Porcine Burn Model. Sci Rep. 2016, doi: 10.1038/srep25168; (Livosec vs. pre-APO-2).
- Hacker et al. The secretome of stressed peripheral blood mononuclear cells increases tissue survival in a rodent epigastric flap model. Bioeng Transl Med 2020, doi: 10.1002/btm2.10186; (APO-2).
- Wagner et al. Different pro-angiogenic potential of γ-irradiated PBMC-derived secretome and its subfractions. Sci Rep. 2018, doi: 10.1038/s41598-018-36928-6; (APO-2).
- Vorstandlechner et al. The Secretome of Irradiated Peripheral Mononuclear Cells Attenuates Hypertrophic Skin Scarring Pharmaceutics 2023, doi: 10.3390/pharmaceutics15041065; (APO-2).

[1] Holzinger et al., 1994, doi: 10.1016/s0950-821x(05)80155-0
[2] Game et al., 1998, doi: 10.1016/S2213-8587(18)30240-7

In addition, the following publications address immune-mediated skin inflammation (murine model):

- Laggner M et al. Therapeutic potential of lipids obtained from γ-irradiated PBMCs in dendritic cell-mediated skin inflammation. EBioMedicine. 2020, doi: 10.1016/j.ebiom.2020.102774; (APO-2).
- Laggner M et al. The secretome of irradiated peripheral blood mononuclear cells attenuates activation of mast cells and basophils. EBioMedicine. 2022, doi: 10.1016/j.ebiom.2022.104093; (APO-2).

Preclinical path to APO-2 in murine and porcine wound models – Key Results

Livosec containing emulsions enhance wound healing in mice in vivo[3]: To study the capacity of secretome derived from PBMC to promote skin wound healing we used the full thickness punch wound model in C57BL/6J mice. We generated emulsions containing either lyophilised Livosec, lyophilised medium or physiological NaCl-solution. Six mm punch biopsies were set on the backs of C57BL/6J mice and treated daily with the different emulsions for 3 days. This confirmed that Aposec enhances wound healing in mice (Figure 1).

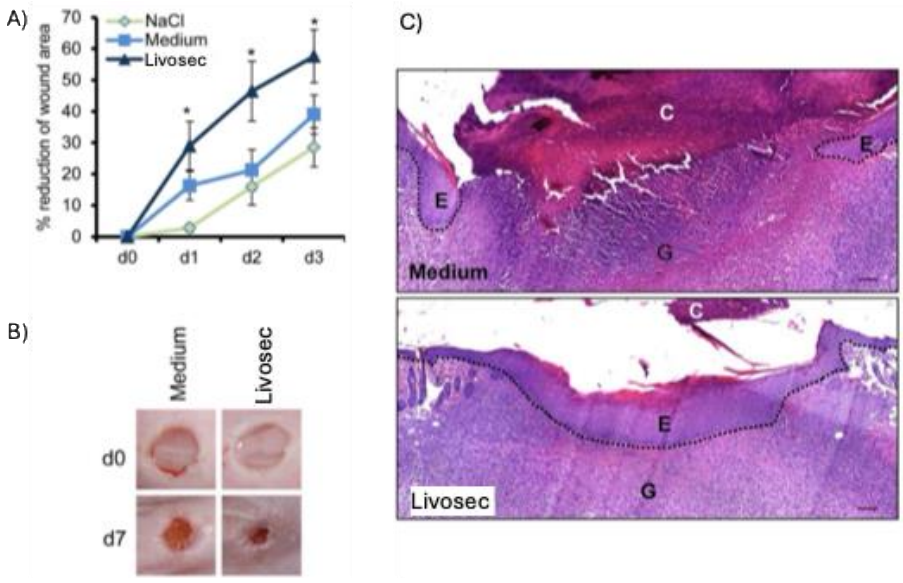


Figure 1: Aposec leads to enhanced wound closure and re-epithelialisation. (A) Wound areas were measured during the first 3 days after wounding. Treatment with Livosec significantly enhanced wound closure. Error bars represent one standard deviation calculated from 15 animals for each set of values (: $p<0.05$). (B) Representative photographs from mouse wounds (n=15 from each group) immediately after wounding and at day 7 after wounding are shown. (C) H&E staining of wounds treated with medium or Livosec 7 days after wounding is shown. While medium treated wounds still show a thick crust and little re-epithelialisation, Livosec treated wounds are fully re-epithelialised. C=crust, E=newly formed epidermis, G=granulation tissue. (Adapted Fig. 1 of Mildner et al., 2013, 10.1371/journal.pone.0060103*

[3] Mildner et al., 2013, doi: 10.1371/journal.pone.0060103.

Livosec shows strong angiogenic properties in vivo and in vitro[4]

Since a crucial event during wound healing is the sprouting of newly formed blood vessels into the wounded tissue, we examined the efficacy of Livosec to induce neo-angiogenesis in vivo. Skin wounds, harvested seven days after wounding, were stained for CD31, a specific marker for blood vessels. Results are shown in Figure 2.

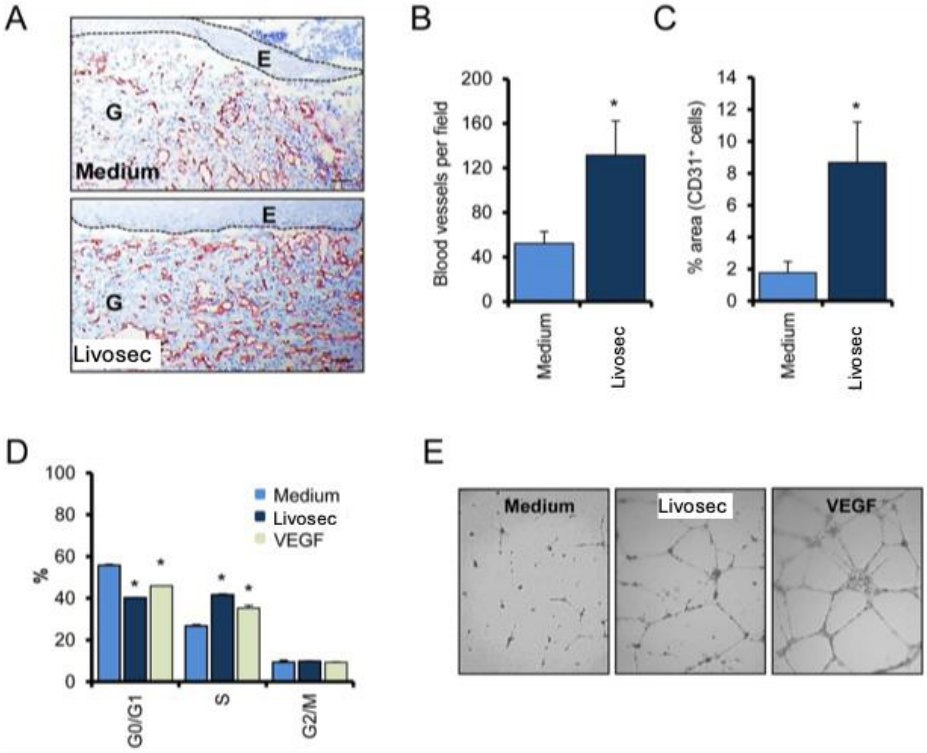


Figure 2: Livosec induces formation of new blood vessels. (A) Representative CD31 stainings of wounds underneath the original wound edge are shown. Scale bars: 50 µm (B) The numbers of CD31 positive cells underneath the newly formed epidermis were evaluated. The graph represents the mean of 15 animals in each group (: p<0.01). (C) The area of the granulation tissue taken up by CD31+ cells was evaluated. The graph represents the mean of 15 animals in each group. (D) Cell cycle analysis of Livosec treated microvascular EC shows a strong increase in proliferating cells. VEGF treatment served as positive control. One representative experiment of three each done in triplicates is shown (*: p<0.01). (E) A tube formation assay is shown. Compared to medium alone Livosec strongly induced tube formation in a matrigel assay. One representative experiment of three is shown. (Adapted Fig. 3 of Mildner et al., 2013, 10.1371/journal.pone.0060103)*

[4] Mildner et al., 2013, doi: 10.1371/journal.pone.0060103.

Pre-APO-2 blended with alginate gel improves skin quality and epidermal differentiation in a porcine burn model and is superior to Livosec, the secretome of unstressed PBMC cells[5]

In a next step, Pre-APO-2 and Livosec were tested in a porcine wound model, which is superior to murine wound skin models because of higher similarity to human skin. Burn wounds pose a serious threat to patients and often require surgical treatment. Skin grafting aims to achieve wound closure but requires a well-vascularised wound bed. We performed a porcine burn model, excised the wound and covered the wound with an autologous split skin graft and treated the animal with Pre-APO-2/ Livosec /controls blended with alginate gel 3 times within 10 days (study period). To evaluate the differentiation of the newly formed epidermal layer, we performed immunohistochemical staining for the late differentiation marker keratin-10. Images were taken of the wound margins to compare the pre-existing epidermis to the re-epithelialised areas. The differentiation of the newly formed epidermis was markedly progressed in the wounds treated with Pre-APO-2. The pre-existing and newly formed epidermis had minimal differences. A similar effect was observed in wounds treated with Livosec. However, in the control wounds, keratin-10 staining was minimal, indicating enhanced regeneration of the epidermal layer over the wound beds after application of the PBMC secretomes. This data indicated for the first time that Pre-APO-2 was significantly better than Livosec in the realm of this porcine acute wound model. Results are shown in Figure 3.

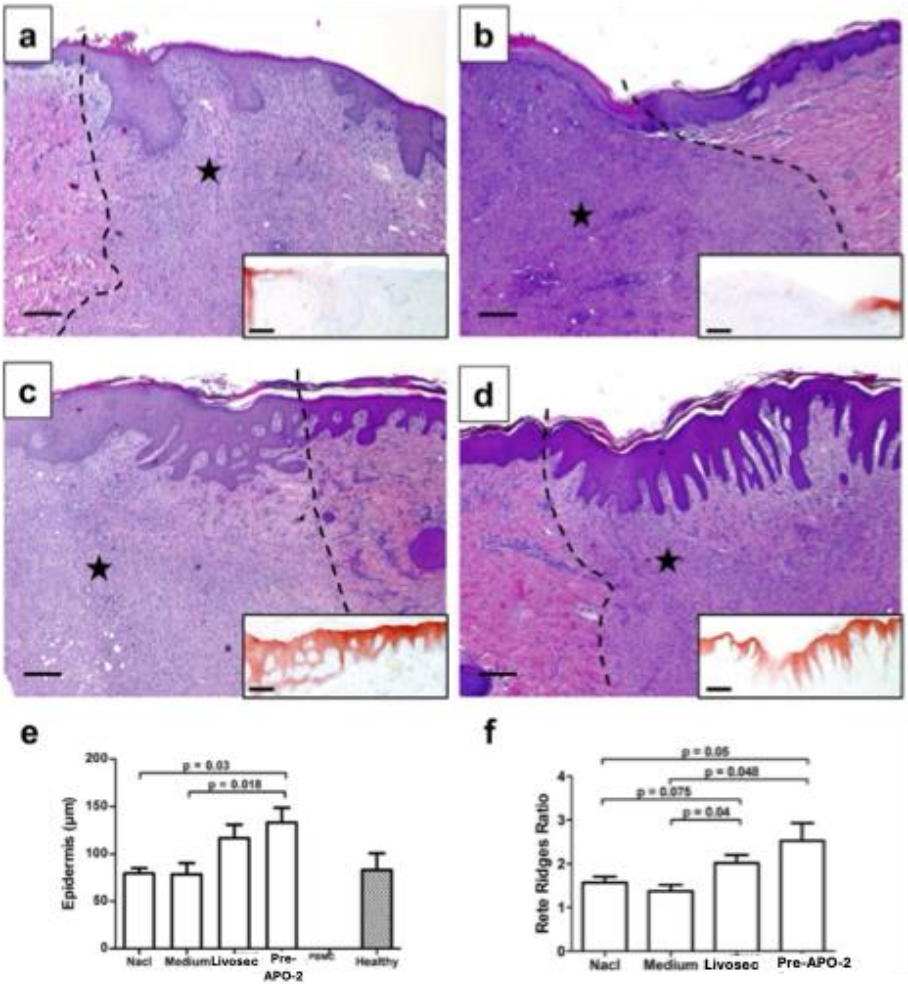


Figure 3: Secretome treatment improves skin quality and epidermal differentiation. Representative H&E staining of the wound edges taken from areas treated with NaCl (a), medium (b), Livosec (c), and Pre-APO-2 (d). The small inserted sections show the corresponding stainings for the epidermal differentiation marker keratin-10. A progressed epidermal differentiation was observed after treatment with Livosec and Pre-APO-2 compared to the control groups. The asterisk (*) indicates the wounded side; the other side shows the healthy, unburned skin. 100× magnification, scale bar: 100 µm. (e) The epidermal thickness was markedly increased in the Pre-APO-2 group. (f) The development of rete ridges – as indicated by a higher ratio between the length of the inner and outer epidermal border – was significantly increased in wounds treated with either Livosec or Pre-APO-2 compared to NaCl and medium controls. Error bars indicate SEM. n = 6. Healthy skin: n = 4. (Adapted Fig. 3 of Hacker et al., 2016, 10.138/srep25168)

[5] Hacker et al., 2016, doi: 10.1038/srep25168

Increased numbers of CD31+ and ASMA cells were observed in wounds treated with Pre-APO-2 in a porcine acute wound model. This data indicates that Aposec augments angiogenesis.[6]

To investigate the capacity of Livosec and Pre-APO-2 to induce angiogenesis in vivo, we harvested punch biopsies at the corner of the wounds. We found a strong increase in CD31+ cells in the wounds treated with Pre-APO-2: the number of CD31+ cells was almost twice as high as in the other groups. To support these findings, we performed an additional staining for the mature blood vessel marker alpha smooth muscle Actin (ASMA) and found a significant increase in ASMA+ cell numbers in Pre-APO-2 treated wounds compared to the control groups. Results are shown in Figure 4.

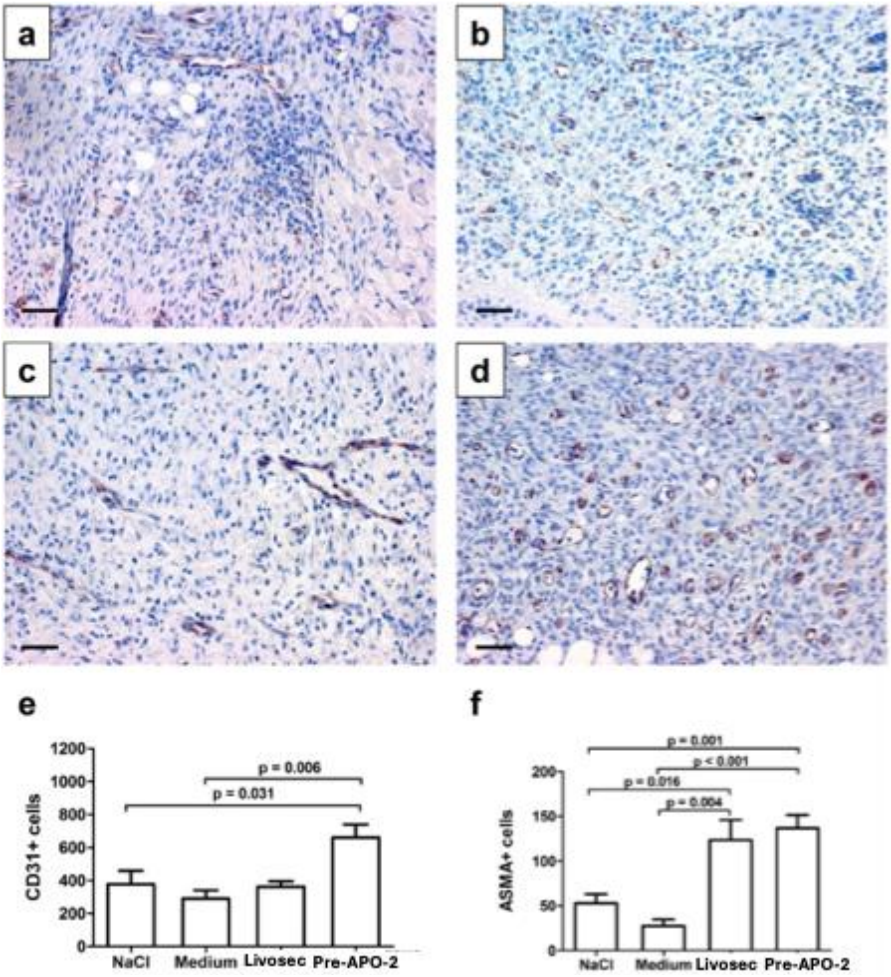


Figure 4: Increased numbers of CD31+ and ASMA cells were observed in wounds treated with Aposec. Punch biopsy sections taken on day 5 were stained for the angiogenesis marker CD31. Representative samples of the NaCl (a), medium (b), Livosec (c) and Pre-APO-2 (d) treated wounds are shown. 200× magnification, scale bar: 50 µm. The quantification of CD31+ cells was performed on four randomly selected sections per wound. The numbers correspond to the total amount of cells over four sections. (e) Treatment with Pre-APO-2 led to a significant two-fold increase in CD31+ cells compared to the control groups. (f) Mature blood vessels (ASMA+ cells) were more frequent in the wounds treated with both Livosec and Pre-APO-2 compared to the control groups, respectively. Error bars indicate SEM. n = 6. (Adapted Fig. 4 of Hacker et al., 2016, 10.138/srep25168)

[6] Hacker et al., 2016, doi: 10.1038/srep25168

APO-2 enhances wound healing in diabetic mice.[7]

In these experiments, we examined whether human APO-2 produced under GMP conditions at the [redacted] suffices to augment wound healing in a diabetic mouse model. As can be seen in Figure 5, APO-2 blended with an alginate gel augmented wound healing in a relevant preclinical wound model.

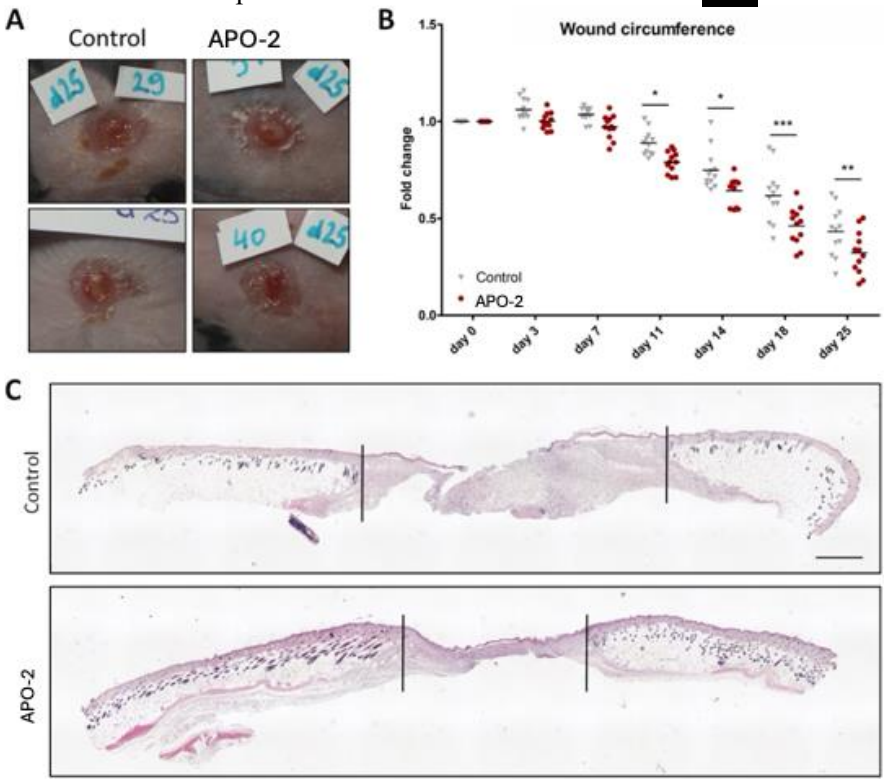


*Figure 5: (A) Representative photographs from wounds in diabetic mice (n=12 for each group) on day 25. The wounds were topically treated with APO-2. Drug vehicle treatment served as a control. (B) Wound circumferences were measured on days 0 (initial wound), 3, 7, 11, 14, 18, and 25 after wounding. Starting from day 11 post-wounding, APO-2 significantly reduced the wound circumference. *p<0.05; **p<0.01; ***p<0.001. (C) H&E staining of a representative wound section from APO-2 and control-treated mice 25 days post-wounding. Vertical lines indicate the edges of the wound section. Scale bar = 1 mm. (Adapted Fig. S5 of Wagner et al. 2018, 10.1038/s41598-018-36928-6)*

1.2.1.1. Outcomes (efficacy, safety) of completed and number of ongoing clinical studies utilising the same intervention in the same indication (including review of public registers)

APOSC is the proprietary owner of APOSEC platform technology. The "product is defined by the process". The patent of APO-1/2 generation was filed in 2008 and our technical development of the stressed PBMC secretome from bench to bedside had occurred in two stages: a) preclinical development; b) GMP production of APOSEC (2015-ongoing) and c) Good Clinical Practice (GCP) study utilising the APO-2 prototype in a phase I/II trial in DFU patients. There are no other ongoing trials that examine the secretome therapy in DFU.

MARSYAS I: First in human clinical trial utilising autologous GMP APO-2 in an acute wound model

MARSYAS I, a phase I clinical study utilising autologous APO-2: From October 2014 to May 2015, a clinical study phase I (MARSYAS I) was performed as academic single centre study to evaluate the safety and efficacy of autologous GMP APO-2 (EudraCT-Number: 2013-000756-17 / ClinicalTrials.gov Identifier: NCT02284360). Three doses of APO-2 versus placebo were examined. APO-2 verum was blended with a commercially available alginate gel (NUGEL®). The placebo in this trial was an alginate gel. Outcome: No therapy-related serious adverse events occurred in any of the participants, and both low- and high-dose treatments were well tolerated. Wound closure was not affected by APO-2 therapy due to the short study period (7 days). The synopsis of the Clinical Study Report of the MARSYAS I study is published on the Aposcience website: https://www.aposcience.at/news/key-publications/.

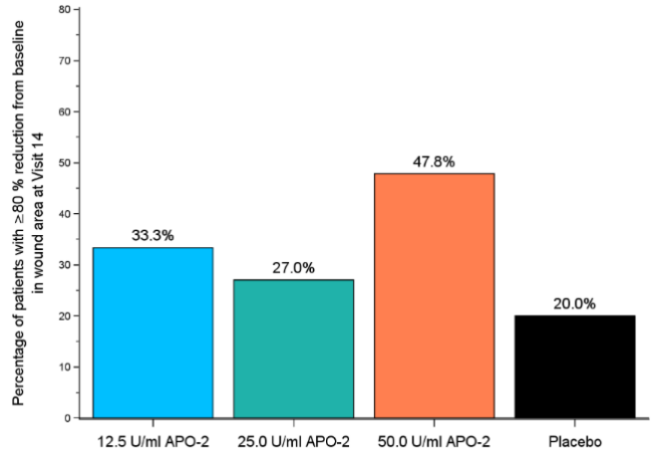
MARSYAS II: phase I/II clinical study: First in human clinical trial – MARSYAS I/II utilising allogenic GMP APO-2 in DFU patients

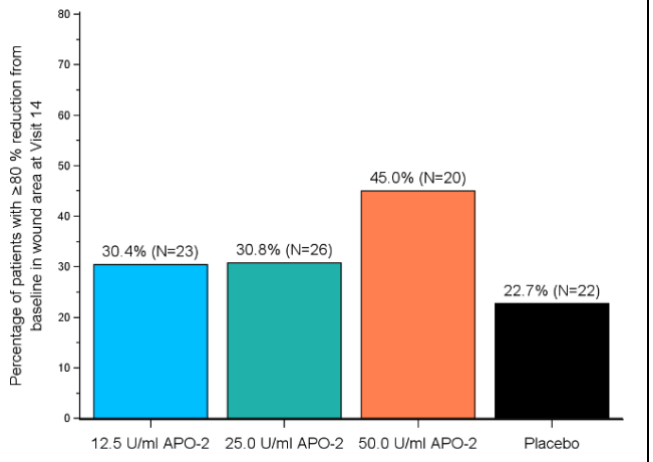
From Nov. 2020 to Dec. 2023, a clinical study phase I/II (MARSYAS II) was performed to evaluate the safety and efficacy of allogeneic APO-2 in three doses versus placebo in a randomised, double-blind controlled clinical trial in DFU patients (EudraCT No. 2018-001653-27 / Clinicaltrials.gov-Identifier NCT04277598). MARSYAS II was performed in 12 study centres in 4 European countries (Austria, Germany, Czech Republic and Poland). The regulatory body of Austria (BASG), Czeck Republic (SUKL), Poland (URPL) and Germany (PEI) approved the clinical study after examination of the Investigational Medicinal Product Dossier. The clinical study was monitored by a Clinical Research Organisation (CRO) and complied with Good Clinical Practice (GCP). The primary endpoint of MARSYAS II was wound reduction in %. The study results were evaluated according to the study plan set prior to the unblinding of MARSYAS II. Below we reported in our final study results: a) the full analysis set (FAS) of

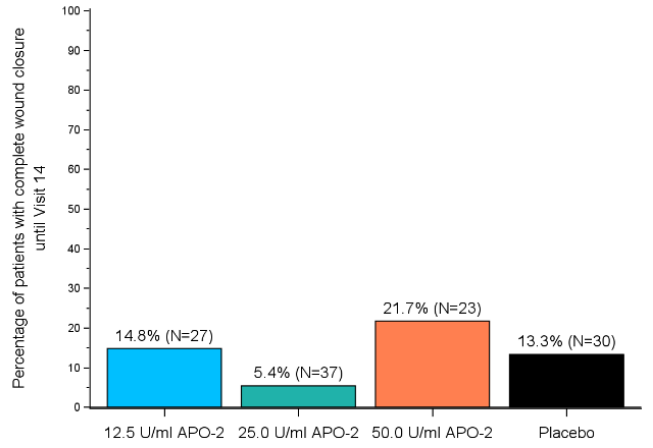
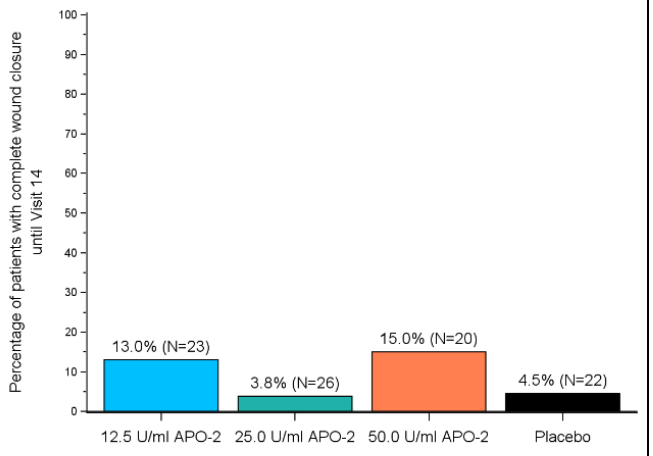
[7] Wagner et al., 2018, doi: 10.1038/s41598-018-36928-6

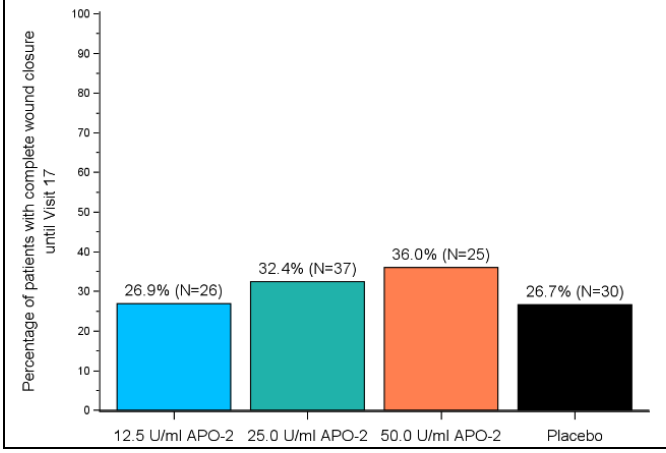
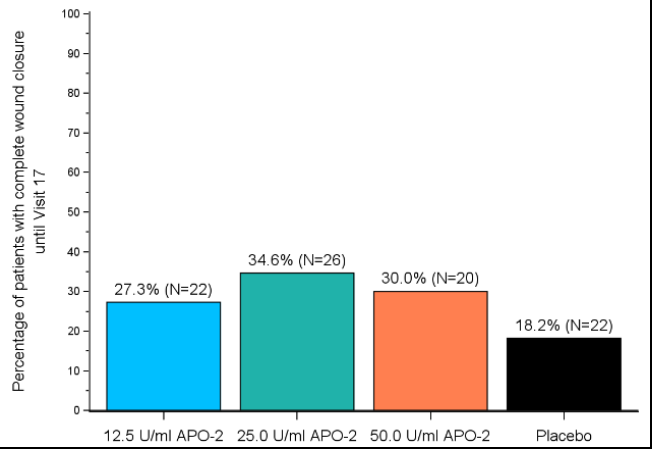
MARSYAS II (n=122). In a second analysis we included those wounds with an adjudicated wound size of 0.8cm^2 to 8cm^2 FAS (n=94) (post hoc). Important note: Full analysis set (FAS) and per protocol set (PPS) analysis of MARSYAS II had the same findings and thus support the conclusions depicted below.

The results below represent on the left the FAS n=122 (complete data set of MARSYAS II study) and FAS n=94 of those patients with adjudicated wound area ≥0.8cm^2-8cm^2 on the right.

<table>
<tr><th>FAS of MARSYAS II (n=122) - complete data set</th><th>FAS of MARSYAS II (adjudicated wound area ≥0.8cm2-8cm2) (n=94)</th></tr>
<tr><td colspan="2">Patients with ≥80% reduction from baseline in wound area after 4 weeks of treatment (EoT)</td></tr>
<tr><td> 

48% of patients treated with the high dose of APO-2 achieved a ≥80% reduction in wound size in comparison to 20% in the placebo group. Placebo vs high dose p<0.048. Post hoc analysis.</td><td> 

45% of patients treated with high dose of APO-2 achieved a ≥80% reduction in wound size in comparison to 23% in the placebo group. Post hoc analysis.</td></tr>
<tr><td colspan="2">Complete wound closure after 4 weeks of treatment (EoT)</td></tr>
<tr><td> 
</td><td> 

These values are the basis for the sample size calculation of APO2FOOT.</td></tr>
<tr><td colspan="2">Complete wound closure after 12 weeks (EoS)</td></tr>
<tr><td> 
</td><td> 
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The results of the MARSYAS II trial were promising because of the overall good healing rates in comparison to other published clinical trials in DFU. However, multiple confounders were identified in this initial phase I / II clinical study. These are presented in the table below.

Learnings from MARSYAS I/II

MARSYAS I/II	Improvements in APO2FOOT phase II / Futility
Simplicity / Target product profile (TPP)	
A **pharmacy** was needed at every study site to prepare the IMP - APO-2 concentrate blended with Alginate. Regulatory requirements (Annex 13, EU GMP Guide) determined this need in order guarantee a double blind, placebo controlled.	APO2FOOT is an observer-blind study. For the final TPP, APO-2 will be resuspended with physiological saline and applied on an alginate fleece / DFU wound. **No pharmacy** is required for this "ready to make" process at the study site.
Prototype APO-2: APO-2 lyophilisate was reconstituted with physiologic saline at the contract manufacturer facility (concentrate). The prototype APO-2 was manufactured utilising GMP Cell Genix DC Medium and gamma irradiation.	**Final APO-2** IMP: APO-2 lyophilisate production with AM2 medium and photon irradiation. At the clinical site the APO-2 lyophilisate will be resuspended with physiologic saline and applied on an alginate fleece which is placed on the DFU wound.
The eKare Insight Device was utilised in MARSYAS II study to **document wound area**. The study physicians judged this device as impractical (problems with device updates, loose contact to the camera, and over- and underestimation of wound size)	In APO2FOOT we will provide study sites with IPhones and anonymous patient labels to photograph the wound with a ruler. A separate SOP will be written: The wound will be photographed before debridement / cleaning, after debridement (if deemed necessary). The wound tracing is put on a white sheet of paper and photographed, including a cm marker and the inner aspect of the permanent marker line will represent the actual margin of the ulcer.
Study design	
The **primary endpoint** was wound reduction in %. It proved to be challenging to measure DFU wound size objectively. 30% of adjudicated wound size (measured by the study physicians) did not correspond to the eKare Insight Measurements. This is why two analysis sets are presented in the MARSYAS II outcome report (FAS=122 vs FAS=94 - adjudicated wound size, 0.8cm^2 - 8cm^2).	The primary endpoint of APO2FOOT will be **complete wound closure** after 12 weeks. This is much less ambiguous than % wound reduction. Complete wound closure is the accepted measure for market approval by FDA[8].
The **treatment period of 4 weeks** (EoT) was too short for complete wound closure. Marsyas I/II was designed to observe safety, tolerability, and efficacy of APO-2.	APO2FOOT is designed to show increased complete wound closure **after 12 weeks** with APO-2 treatment versus SoC.
Initial **inclusion criteria** (as suggested by the MARSYAS II scientific advisory board-SAB) were too narrow. This resulted in very low patient recruitment in 2019-21. After consultation with experts in the field we extended our inclusion criteria and allowed the inclusion of peripheral artery disease (PAD).	The inclusion criteria are being set by the current SAB and will correspond to our modified inclusion criteria of MARSYAS II (Version 4) – see Chapter 1.4. Our currently established SAB has a strong standing in the DFU community.
The **screening phase** of 2-4 weeks (suggested by the MARSYAS II SAB) was possibly too short to exclude non-chronic wounds.	In APO2FOOT the screening phase will last **5-7 weeks**. This is the current standard in well-performed international DFU studies.
No standardised **off-loading**.	Standardised off-loading shoes will be provided to standardise optimal wound care.

[8]https://www.fda.gov/regulatory-information/search-fda-guidance-documents/chronic-cutaneous-ulcer-and-burn-wounds-developing-products-treatment

MARSYAS I/II	Improvements in APO2FOOT phase II / Futility
Inclusion – Exclusion Criteria defined by ankle brachial index (ABI) was shown to be a suboptimal nominator to identify DFU patients	Will be exchanged for toe brachial index (TBI) pressure which will be determined by a validated automated system (see 3.3)
Monitoring / documentation / advisors	
The **SAB** included only national experts, with no prior experience in DFU studies.	International experts with phase III DFU trial experience were identified (and contracted) by APOSC to guided us in the APO2FOOT study design
Wound size determination for inclusion into the clinical study (randomisation) was performed by the PI and was documented by the eKare Insight Device measurement. CRO did not monitor wound size measurement by study physicians.	Wound size measurement will be done as described in a study handbook: with photo documentation, ruler, anonymous patient label, and week of APO-2 treatment. Correspondingly, SoC will be documented. CRO will monitor wound size measurement.
Screening of potential patients was not documented – documentation started only after initial screening of the patients. Consequence: lacking data to document site activity and patient collective.	The screening phase will be better documented and reasons of non-inclusion of patients will be reported in APO2FOOT.
Lack of **monitoring** of the wound photo documentation by the CRO. Reason: missing interface between photo documentation (cloud storage) and eCRF.	There will be an interface between the photo documentation and CRO (validated cloud storage).
Insufficient co-monitoring of the sponsor allowed partially wrong measurements at the randomisation visit.	APOSC will hire a GCP auditor to control all study sites (co-monitoring by APOSC will be **intensified**)
MARSYAS I/II study treatment was the first PBMC secretome therapy study. The trial duration was determined by the repeated dose toxicology study in minipigs. (3 times per week, for 4 weeks, dose range of 12.5-25U/kg body weight in a minipig model)	In 2023-24 we completed our GLP 3-month repeated dose toxicology programme with the final APO-2 IMP. In this toxicology study we tested APO-2 with the regimen 2 times per week, for 12 weeks, dose range of 12.5-25U/kg body weight in a minipig model. This APO-2 repeated dose trial in minipigs correspond to our Phase II study design. Conclusion: APO-2 evidenced no adverse reactions and allows our proposed APO2FOOT clinical trial utilising 25U/cm^2 wound size.

Safety of APO-2 has been thoroughly tested. In summary, (1) APO-2 has a very good **viral safety** profile based on 2 viral reduction steps in the GMP Aposec production process; (2) APO-2 has proven to be safe in **toxicology** studies and (3) in 2 **clinical trials** (MARSYAS I and MARSYAS II study); (4) **Prion safety** of APO-2 is guaranteed by including only blood donors younger than 45 years.

Ad 1.) Viral safety: 2 orthogonal steps of viral reduction are implemented in the GMP APO-2 production process: a) Viral reduction by **methylene blue** treatment and b) terminal sterilisation with **gamma irradiation** (25,000 Gray). The effectivity of these viral reduction steps has been evaluated in two GLP studies with Charles River Laboratories Germany GmbH (Cologne) in 2017 (Charles River reports K1/A56/17 and K2/56/17) and results published.[9]
Tests were run for the following viruses: bovine viral diarrhoea virus (BVDV) (enveloped virus), human immunodeficiency virus type 1 (HIV-1) (enveloped virus), pseudorabies virus (PRV) (enveloped virus), hepatitis A virus (HAV) (non-enveloped virus), and porcine parvovirus (PPV) (non-enveloped virus). It turned out that viral reduction by methylene blue treatment is mainly effective against enveloped viruses. Gamma irradiation contributes to viral reduction of enveloped viruses and to a lesser degree of non-enveloped viruses. The **lyophilisation** step, which was also examined, also has a reductive effect on the viability of several enveloped viruses. The calculated cumulative mean log10 viral factor potency of all three methods is 13.4 for BVDV, 12.0 for PRV, 13.6 for HIV-1, 5.4 for HAV and 2.7 for PPV. (Explanation: 1 log10 means 90% of the viruses are inactivated; 2 log10 means 99% of the viruses are inactivated, 3 log10 means 99.9% of the viruses are inactivated. We achieved 2.7 to 13.4 log10. Porcine parvovirus (PPV) could only be reduced with 2.7 log10. Please note: the starting material for APO-2 are blood

[9] Gugerell A et al., 2019, doi: 10.2450/2019.0249-18

donations (buffy coat). All blood donors are tested for Parvovirus B1. A potential blood donation would be discarded when this virus is detected.)

Ad 2.) Toxicology: An extensive toxicological study program (=non-clinical safety studies) was conducted for APO-2. The following list presents the studies conducted and their results. Please note that APO-2 does not pose the risk of sudden and strong immunological reactions such as anaphylactic shocks or cytokine storms as may be the case with other biological medicinal products. Executed GLP Toxicology program were performed between 2015-2025.

- **Single dose toxicity**: Acute Toxicity Study of APO-2 by single intravenous administration to CD® **rats** (Study No. 33201/1) – Results: no signs of toxicity were revealed by injection at a dose of 50, 250 and 500 Units/kg body weight.
- **Safety pharmacology**: Neuropharmacological screening in rats according to IRWIN following intravenous administration of APO-2 (Study No. 33202/1) – Results: 17 of 40 neuropharmacological parameters were influenced in a dose-related but transient manner at doses of 250 or 500 Units/kg body weight.
- **Local tolerance**: Skin sensitisation: local lymph node assay: BrdU-ELISA of APO-2 in CBA/JN mice – according to OECD guideline 442B (Study No. 33203/1) – Results: Administration of a dose of up to 250 Units/kg body weight did not reveal any skin sensitising properties.
- **Single dose toxicity**: Single Dose Toxicity Study with allogenic APO-2 after intravenous bolus injection to beagle dogs (Study No. 33749) – Results: No sign of toxicity or signs of local intolerance were revealed by injection at a dose of 250 Units/kg body weight.
- **Repeated dose toxicity**: 2-week dose range finding study of allogenic APO-2 by intravenous slow bolus administration 3 times weekly to CD® rats (Study No. 33647) – Results: No test-related findings in clinical signs after injection at a dose of 50, 250 or 500 Units/kg body weight.
- **Repeated dose toxicity**: Dose range-finding study with allogenic APO-2 by s.c. bolus injection to beagle dogs (Study No. 33649) – Results: No toxicities were observed after 3 subcutaneous bolus injections at a dose of 33 Units/kg body weight.
- **Repeated dose toxicity**: 4-week toxicity study of allogenic APO-2 by intravenous slow bolus administration 3 times weekly to mice (Study No. 34946): No toxicities were observed in this study in which mice received 13 injections at a dose of 500 Units/kg body weight.
- **Repeated dose toxicity**: 4-week toxicity study of allogenic APO-2 in Aachener minipigs with s.c. administration 3 times weekly (Study No. 34873) – No toxicities were observed in this study in which minipigs received 13 subcutaneous injections at a dose of 33.3 Units/kg body weight.
- **Repeated dose toxicity**: 2-week toxicity study of allogenic APO-2 by repeated twice weekly intravenous infusion to cynomolgus **monkeys** followed by a 2-week treatment-free period (Study No. 35015) – Results: toxicity was observed in 2 male monkeys at a dose of 350 Units/kg. However, these results were related to the vasodilating effects of APO-2. For intravenous treatment 2x weekly with APO-2, a No Adverse Effect Level (NOAEL) of 200 Units/kg was determined.

5 of these studies (1. Neuropharmacological screening, 2. Skin sensitisation, 3. Acute toxicity study, 4. 4-week repeated dose toxicity study in mice, 5. 4-week repeated dose toxicity study in minipigs) were published.[10]

For APO2FOOT, in 2024, a 3-month repeated dose toxicology was performed in minipigs, followed by a 14-day recovery period was initiated with APOSEC™ (APO-2). This executed GLP study (Study No. 1027-364.6482) evidenced no adverse reactions. Results: 2 times weekly subcutaneous injection of APO-2 at a dose of 12.5 and 25 Units/kg body weight revealed slight to moderate transient non-adverse local symptoms (oedema and erythema formation) with low incidence. Conclusion: **This positive toxicology study is an absolute regulatory precondition to perform our APO2FOOT study.**

Ad 3) Clinical trials: Safety and Tolerability of APO-2– study results of the MARSYAS II study:
APO-2 was safe and well tolerated in patients. No safety issues were reported in the final report of the clinical study (MARSYAS II Synopsis of Clinical Study Report – Aposcience AG)

Ad 4.) Prion safety was taken seriously in the development of APO-2. Prions are the cause for bovine spongiform encephalitis (BSE), and they can be transmitted by blood donations. An expert opinion of the Leader of the Austrian Reference Center for Human prion diseases and Member of the Steering-Committee of the European CJD Surveillance Network, Dr Gabor G. Kovacs, PhD, was obtained. Dr Kovacs concluded that only 1 of 4 forms of the disease, variant Creutzfeldt-Jakob Disease, is thought to be transmitted by blood transfusion, and that this form of

[10] Wuschko et al., 2019, doi: 10.1038/s41598-019-42057-5

disease can be excluded **by excluding blood donations from persons older than 45 years** as a starting material for the production of APO-2.

Stability studies: In contrast to many biological medicinal products, APO-2 lyophilisate is stable and stays biologically active for up to 5 years when stored at -80 ºC and at -20 ºC and for 2 years at +4°C. This has been confirmed in the Stability Report of APO-2 lyophilisate TD001a: The APO-2 lyophilisate (batches 131 and 150, manufactured in 2018) were tested for stability for 60 months (5 years; 02/2019 - 02/2024).
Results: All parameters tested remain stable when samples are stored at -20 °C and -80 °C for up to 60 months and +4°C for 24 months. APO-2 was also undergoing a GLP stress test: APO-2 degraded only at the temperature condition of +40 ºC and 75% relative humidity after 3 months (colour change, declined biological activity in the potency assays), probably due to denaturing of proteins, which happens at +40 ºC. Thus, this stress test assured the specificity of our potency assay.

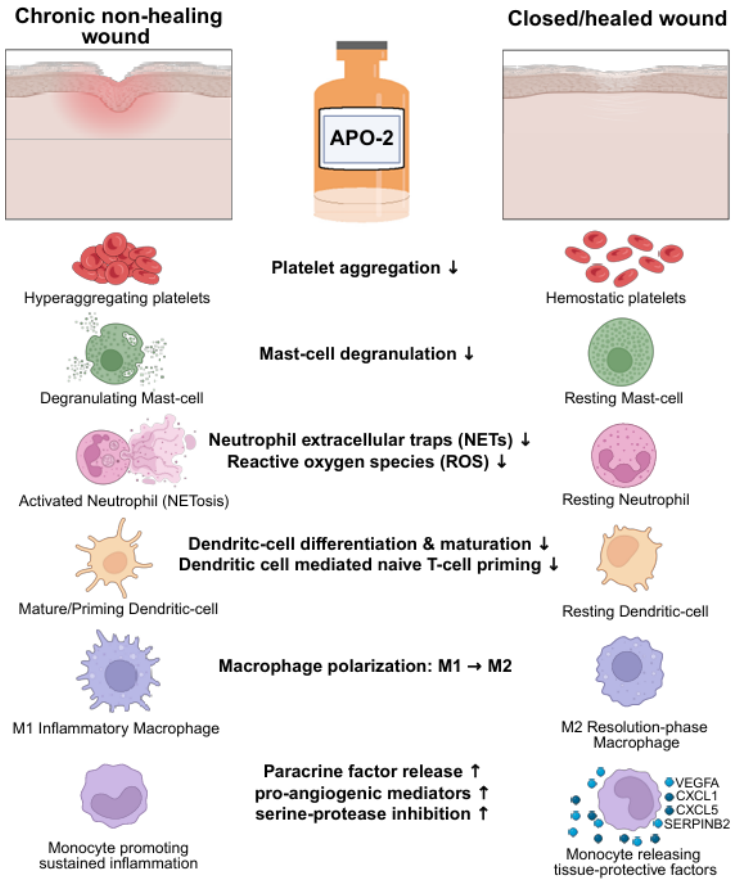
1.2.1.2. Level of evidence related to the mechanism of action of the intervention in the planned clinical study population

Based on the preclinical observation that APO-2 augments wound healing in vivo we examined which Modes of Action (MoA) are potentially responsible for these experimental observations:

Livosec induces angiogenesis in vitro and in vivo	Mildner et al., 2013, doi: 10.1371/journal.pone.0060103
Livosec induces re-epithelialisation in vitro and in vivo	Mildner et al., 2013, doi: 10.1371/journal.pone.0060103
Livosec induces cytoprotection and anti-apoptotic gene product in vitro	Mildner et al., 2013, doi: 10.1371/journal.pone.0060103
Pre-APO-2 and Livosec attenuate T-cell activation in vitro and vivo	Hoetzenecker et al., 2013 doi: 10.1093/eurheartj/ehs459
Pre-APO-2 and APO-2 induces angiogenesis in vitro and in vivo	Hacker et al., 2016, doi: 10.1038/srep25168 Haider et al., 2015, doi: 10.1016/j.expneurol.2015.03.013 Hacker et al., 2012, doi: 10.1002/btm2.10186
Pre-APO-2 and APO-2 induces re-epithelialisation in vitro and in vivo	Hacker et al., 2016, doi: 10.1038/srep25168 Vorstandlechner et al., 2023, doi: 10.3390/pharmaceutics15041065
APO-2 induces cytoprotection and anti-apoptotic gene product in vitro	Altmann et al., 2014, doi: 10.12688/f1000research.4219.2 Haider et al., 2015, doi: 10.1016/j.expneurol.2015.03.013
Pre-APO-2 induces favourable alteration of extracellular matrix generation after wound healing in vitro and in vivo	Vorstandlechner et al., 2023, doi: 10.3390/pharmaceutics15041065
Pre-APO-2 prevents platelet aggregation in vitro and vivo	Hoetzenecker et al., 2012, doi: 10.1007/s00395-012-0292-2
APO-2 stabilises granulocyte activation and prevents NETs secretion in vitro	Klas et al., 2022, doi: 10.3390/antiox11081559
APO-2 prevents dendritic cell maturation in vitro and vivo	Laggner et al., 2020, doi: 10.1016/j.ebiom.2020.102774
APO-2 prevents mast cell and basophil activation in vitro and vivo	Laggner et al., 2022, doi: 10.1016/j.ebiom.2022.104093
APO-2 prevents monocyte activation in vitro	Panahipour et al., 2020, doi: 10.3390/ijms21134694
APO-2 augments M1/M2 differentiation in vitro	Haider et al., 2015, doi: 10.1016/j.expneurol.2015.03.013
Pre-APO-2 augments vasodilation and nitric oxide (NO) generation in vitro and coronary artery ex vivo	Hoetzenecker et al., 2012 doi: 10.1007/s00395-012-0292-2
APO-2 activates proangiogenic and anti-proteolytic processes in whole blood and is capable to protect the endothelial barrier in vitro	Copic et al., 2022, doi: 10.3390/pharmaceutics14081600
APO-2 contains antimicrobial peptides (AMP) and induces AMP generation in vivo	Kasiri et al., 2016, doi: 10.1111/eci.12667

The immunomodulatory effects of APO-2 that promote wound healing are shown in Figure 6.

Figure 6: Immunomodulatory effects of APO-2 contributing to healing of chronic non-healing wounds – which constitute the Modes of Action (MoA of APO-2. On the left, the cellular compartments / mechanisms underlying chronic, non-healing wounds are depicted. The positive effects of APO-2 are described in the center, leading to the closed/healed wound shown on the right. APO-2 has proven in vitro effects on all cellular compartments of the immune system relevant in wound healing



1.3. Objective(s) of the clinical study

APO2FOOT will be the second clinical study with the final APO-2 IMP. The APO-2 formulation in MARSYAS I/II was a "prototype". APO-2 was manufactured with Cell Grow Medium (CellGenix®) and gamma irradiation. In the APO2FOOT study APOSC has modified the APO-2 formulation to become a) independent form the provider of the excipient, and b) we had to switch the initial stress from gamma to photon irradiation. In an extensive comparability study, we have shown that our final APO-2 drug product reaches the same relevant specifications as the APO-2 prototype. In the APO2FOOT study we thrive to a) finalise GMP development of APO-2; b) execute a clinical DFU study that can compete with the highest standards of clinical trial design in DFU; c) utilise APO-2 as secretome therapy versus SoC in DFU; d) potentially offer physicians/clinicians a simple and easy to handle effective IMP with a low cost of good (COG).

Objectives	**Endpoints**
Primary	
To investigate the efficacy of APO-2 in patients with DFU	Proportion of patients with complete wound closure during the 3-month treatment phase
Secondary	
To further investigate the efficacy of APO-2	• Wound size at baseline, and 1, 2, 3, 4, 6, 8 and 12 weeks after first application of the investigational medicinal product (IMP) • Recurrence rate of the ulcer during 3-month follow-up period • Evaluation of wound pain by visual analogue scale at baseline, at every treatment visit, and 4, 6, 8 and 12 weeks after first application of IMP • Evaluation of quality of life using Wound QoL questionnaire at baseline, 4, and 12 weeks after first application of IMP • Proportion of patients with complete wound closure during the 3-month follow up phase
To investigate the safety and tolerability of APO-2	Proportion of patients with treatment-emergent adverse events (TEAEs), TEAEs related to the IMP, serious TEAEs related to the IMP, and TEAEs leading to premature discontinuation of the IMP

1.4. Characteristics of the study population

Number of patients (total and for each treatment) planned

To ensure that 120 patients complete the trial, we plan to enrol 134 participants, accounting for an anticipated dropout rate of up to 10%. This will result in 67 patients per group (APO-2 vs. SoC). See also sample size calculation below.

Inclusion/exclusion - Justification

The inclusion/exclusion criteria of APO2FOOT **are based on** first advice from the SAB and high-quality phase III studies in DFU that were published recently. These are**:**

- Kerecis Omega3 Wound Plus SOC vs. SOC Alone in Treating Severe Diabetic Foot Ulcers and Forefoot Amputations[11]
- LeucoPatch in the Management of Hard-to-heal Diabetic Foot Ulcers[12]

Note: physicians that participated in the study design of the Kerecis phase III study in DFU are members of the APO2FOOT SAB (see Chapter 2.1.3).

Inclusion criteria: Patients meeting the following criteria will be considered for inclusion into the trial:

- Patients aged **18-80 years** (inclusive)
- Patients with Type I or Type II **diabetes** with a glycosylated haemoglobin (HbA1c) of ≤12%, obtained at enrolment or within 30 days prior to study enrolment
- Patients who have a **chronic** wound diagnosed as DFU, according to the investigator
- Estimated foot ulcer surface **area** between ≥0.8 cm^2 and ≤4 cm^2 as measured after debridement
- **Foot ulcer** Wagner grade I – II or ARMSTRONG grade I-A (superficial, non-infected, non-ischemic wound not involving tendon, capsules, or bone) or II-A (non-infected, non-ischemic wound penetrating to tendon or capsule but not to the bone or joint)
- A patient with more than one DFU may be included in the study but only one ulcer will be selected for the investigational treatment based on investigator judgment as far as the ulcer meets the inclusion criteria (the largest ulcer fitting the inclusion criteria will be selected as index ulcer)
- No acute progressive peripheral arterial occlusive disease (PAOD) defined as a toe-brachial index (TBI) ≥ 0.5, and a toe systolic pressure of at least 50.0 mmHg (or ankle systolic pressure of at least 70.0 mmHg if toe-pressure is not measured). Please note: Our SAB advised us to utilise TBI and toe systolic pressure since multiple scientific reports have claimed superiority to ABI[13]
- Patient must adhere to **off-loading** of the ulcer area (in mobile patients, adherence to off-loading footwear during the study is mandatory)
- Patient is able to give written informed consent prior to study start and to comply with the study requirements
- Women of childbearing potential agree using adequate birth control methods during the study

Exclusion criteria

- History of anaphylaxis, known hypersensitivity to sodium alginate, propylene glycol, methylene-blue or chicken-egg
- Target ulcer is over a deformity (such as Charcot deformity) that interferes with off-loading based on investigator's opinion
- Index wound duration of >3 years without intermittent healing
- Clinical evidence of ulcer bed infection or patients requiring intravenous (IV) antibiotics to treat the index wound infection at time of randomisation
- Current evidence of osteomyelitis (infection involving bone), cellulitis (extensive [>2 cm], distant from ulceration or rapidly progressive [including lymphangitis]), or other evidence of infection[14] including pus drainage from the

[11] https://clinicaltrials.gov/study/NCT04257370; Dardari et al., 2024, doi: 10.1056/EVIDoa2400171
[12] https://clinicaltrials.gov/study/NCT02224742; Game et al., 2018, doi: 10.1016/S2213-8587(18)30240-7
[13] Mayr et al., 2019, doi: 10.1024/0301-1526/a000808; Sonter et al., 2015, doi: 10.7547/0003-0538-105.3.201
[14] A wound is infected when at least two of these items are present: local swelling or induration; erythema >0.5 cm around the wound; local tenderness or pain; local increased warmth; purulent discharge; and no other cause(s) of an inflammatory response of the skin (e.g., trauma, gout, acute Charcot neuro-osteoarthropathy, fracture, thrombosis, or venous stasis) applies. A positive microbiological swab test result of the targeted wound alone is not considered a wound infection.
Osteomyelitis: infection involving bone.
Cellulitis applies if extensive (>2 cm), distant from ulceration or rapidly progressive (including lymphangitis) - according to the Guidelines on the diagnosis and treatment of foot infection in persons with diabetes (IWGDF 2019 update)

wound site, or documented history of osteomyelitis at the target wound location during the 8 weeks preceding the screening visit

- Major uncontrolled medical disorder(s) such as severe uncontrolled leg oedema, concurrent medication, or other issue that renders the patient unsuitable for participation in the study, including but not limited to: comorbid condition with an estimated life expectancy of ≤12 months, haemoglobin A1c (Hba1c) >12 % at screening, patients on dialysis, patients with severe pulmonary (requiring home oxygen, uncontrolled COPD Gold III/ IV) or cardiovascular conditions (heart failure NYHA IV, uncontrolled hypertension systolic BP by repeated measurement >180 mmHg)
- Raynaud disease or any other severe peripheral microvascular disease, current diagnosis of vasculitis
- Patients with peripheral artery disease (PAD) who
  - have not been assessed by vascular imaging as per SoC or
  - have acute peripheral artery occlusion of the index extremity or
  - have PAD Fontaine Stage III and IV or
  - have PAD with planned revascularisation during the upcoming 6 months or
  - had angioplasty for re-perfusion in the lower extremity with target ulcer for 3 months preceding the screening visit
- Dermatologic comorbid disease (e.g., pyoderma gangrenosum, vasculopathy or vasculitic ulcers), history of Systemic Lupus Erythematosus with elevated anti-DNA antibody titres, Buerger's disease (thromboangiitis obliterans)
- Patient currently treated for an active malignant disease or prior diagnosis of an active malignant disease who is disease free for less than 1 year. Treatment with anticancer therapy (chemotherapy, immunotherapy, radiotherapy, targeted therapy or gene therapy) within 3 months before the first administration of investigational product or at any time during the study.
- Patient with history of malignancy within the wound; history of radiation therapy to the wound region
- Patients who have undergone wound treatments with growth factors, dermal substitutes, or other biological therapies within the last 30 days or during the study
- Patients who received oral or parenteral corticosteroids, immunosuppressants, or cytotoxic agents within 30 days preceding the first study drug administration, or plan to use these medications during the study period
- Patients who are pregnant or breastfeeding
- Mental condition rendering the patient (or the patient's legally acceptable representative[s]) unable to understand the nature, scope and possible consequences of the study
- Patients who are incarcerated, including prisoners or patients compulsorily detained for treatment of either a psychiatric or physical (eg, infectious disease) illness
- Therapy with another investigational agent within thirty days of screening, or during the study
- Patients who are considered by the investigator to have a significant disease, which can impact the study; patients who are considered not suitable for the study by the investigator
- Employee at the study site, spouse/partner or relative of any study staff (eg, investigator, sub-investigators, or study nurse) or relationship to the sponsor

Randomisation criteria

- Foot ulcer surface area between ≥0.8 cm^2 and ≤4 cm^2 as measured and assessed by study physician; a central adjudication is planned.
- Wound area has not changed by more than 30% between screening visit and randomisation visit (at least 31 days) confirmed by a central read-out
- Randomisation will be stratified according to gender (m/f) and age (<65 years / ≥ 65 years)

Assessments

Wound imaging and wound size measurement will take place at clinical study site utilising a standardised photo documentation system that will function according to a handbook and SOP. Photo documents will be sent to eCRF and allocated to study number. The randomisation and inclusion criteria will be based on the results from a central adjudication assessment.

Sex distribution

Most published DFU clinical studies have the preponderance of male participation. This is also expected in APO2FOOT. Please see sex/gender in the excellence section and 3.2 below.

1.4.1. Details on sample size and power calculation

Sample size: A sample size for a **combined Phase II/III** study was calculated to take into account potential multiple testing in the potential case of an extension of the Phase II study to a Phase III study.
Sample size calculation was performed for a two-sided group sequential design test for two proportions (with efficacy and non-binding futility stopping). Equal sample sizes in the treatment groups were planned, and computation for 80 % power was performed, with a significance level of 0.05. The assumed response rates (complete wound closure) of 15% for the treatment group and 4.5% for the control group (10.5% response difference) were used, based on results from the MARSYAS II trial (FAS 0.8 cm^2 - 8 cm^2). Applying the O'Brien Fleming design, the two-sided alpha level for final analysis is 0.0465. Accordingly, a total sample size of 240 patients (120 per treatment group) at final analysis is sufficient. Taking into account a **drop-out rate of up to 10 %** (drop-out rate in the MARSYAS II study was 4 %), a total of 268 participants (134 per treatment group) should be enrolled if the study is expanded to the Phase III study. In the first instance, the Phase II study will include 50 % of this sample size: a total of **134 participants**, i.e. 67 per treatment group. Once 134 participants have been successfully enrolled, recruitment is stopped and after 120 participants have completed the 12-week treatment phase, an interim analysis is performed (in case more than 14 participants dropped out, the interim analysis is performed after all remaining of the 134 enrolled participants completed the 12-week treatment period). This concludes the end of the Phase II APO2FOOT study.
Only after evaluation of the interim results, decision is made if the study is extended to Phase III, and if the sample size needs to be re-estimated based on these interim results.

Interim analysis: A 2-stage adaptive design with O'Brien and Fleming shaped boundaries is applied for this study. After Phase II, an interim analysis will be performed after 134 patients have completed the 12-week treatment phase (including dropouts) or at least 120 to check for futility, safety, and efficacy, and to perform a sample size re-estimation based on unblinded data. Based on the results of this interim analysis, the study may be stopped for futility or safety or efficacy, or the sample size may be adapted, and the study will be extended to Phase III, enrolling the additional patients to achieve the re-estimated sample size. The stop for futility is non-binding, if the observed responder rate in the APO-2 group at interim analysis is equal to or smaller than the responder rate in the SoC group.

Statistical analysis: The primary endpoint proportion of patients with complete wound closure during the 3-month treatment phase will be evaluated on the full analysis set. The primary endpoint will be analysed confirmatory using a two-sided test. The primary analysis will be performed on the full analysis set with an appropriate multiple imputation method to account for missing data. All other endpoints will be analysed exploratorily.

1.5. Design of the clinical study

APO2FOOT will be a controlled, randomised, observer-blind, multicentre Phase II study in patients with diabetic foot ulcers.

Study design: Patients will be screened for eligibility in a **screening visit**. Patients not fulfilling all in- and exclusion criteria are not enrolled and reported as screening failures. Eligible patients are treated according to SoC, and four to five weeks later, they come to the site for the next wound assessment (V2). If a participant still meets all eligibility criteria and the wound area is not reduced/enlarged by >30% as compared to the screened visit, the participant is scheduled for the baseline visit. At the **baseline visit** (V3), results of wound size measurements are determined by the study physician the participant is **enrolled** into the study and **randomly assigned** to one of the treatment groups, and all baseline activities are performed. Patients not entering the randomised treatment phase are reported as baseline- failures. In the following, the participant returns at least twice a week to the site for wound management and efficacy and safety assessments. All efficacy and safety assessments will be performed by blinded rater, whereas the wound management itself will be performed by an unblinded investigator or qualified designee. After twelve weeks of continuous treatment, the **end-of-treatment** visit (V27) is performed, including assessment of the primary outcome measure (complete wound closure). Thereafter, the participant will attend on-site follow-up visits in week 17 (one month after EoT - V28) and week 21 (V29). Final **follow-up** visit will be performed at week 25 (6 months after baseline, 3 months after EoT – V30), to allow for assessment of long-term recurrence. Participants terminating the study (or being withdrawn by the investigator) prior to the end of treatment (V27) are reported as dropouts. Participants not attending the final visit (V30) are reported as lost-to-follow-up.
Patients will be **randomised** in a 1:1 ratio to 2 treatment groups with one group receiving APO-2 25 U/cm^2 intervention and one group receiving accepted SoC within intervention period. After our intervention period we will treat both groups with accepted SoC. The patients will be treated for 3 months with 2 applications per week (at least 2 days apart) until complete wound closure or for a maximum of 3 months. The randomisation and inclusion are based on wound measurements confirmed by a central read-out.

A 2-stage group sequential design with O'Brien and Fleming shaped boundaries is applied, with an Interim Analysis performed and evaluated by the independent DSMB after approximately 132 randomised patients have completed Visit 27 (End of treatment) – see previous chapter. Based on the results of this interim analysis, the study may be stopped for futility or safety or efficacy, or the sample size may be adapted to pursue with a pivotal trial that will be financed by APOSC

Standard of care (SoC) treatment

The SoC management of DFU includes:

1. Standard treatment of type 1 and 2 diabetes;
2. Frequency of wound management/wound dressing change: at the investigator's discretion, but at least twice a week
3. Removal of necrotic, infected or callus tissue by soft debridement (frequency: at the investigator's discretion);
4. Wound cavities are filled with a bacterial count-reducing dressing at the investigator's discretion
5. Covering bandage: polyurethane foam dressing for an improved local pressure distribution and with exudate-absorbing characteristics
6. Offloading/pressure relief (the patient must wear individually fitted diabetic shoes or follow orthopaedic procedures);

In case of wound infections, an antibiotic treatment is applied (at the investigator's discretion). A wound is infected when at least two of these items are present: local swelling or induration; erythema >0.5 cm around the wound; local tenderness or pain; local increased warmth; purulent discharge; and no other cause(s) of an inflammatory response of the skin (e.g., trauma, gout, acute Charcot neuro-osteoarthropathy, fracture, thrombosis, or venous stasis) applies. A positive microbiological swab test result of the targeted wound alone is not considered a wound infection.

Investigational treatment

The investigational treatment will be applied **instead of the bacterial count-reducing agent** dressing described under point 4 of the SoC treatment outlined above. The remaining points of the SoC applies also to the investigational treatment. The investigational product, APO-2, will be diluted with 0.6 mL 0.9% saline solution. One vial contains 50 U. A volume of 0.3 ml APO-2 suspension will be applied per cm^2 alginate gaze (this corresponds to 25U/cm2).

Previous and concomitant medication

During the study, SoC may not be changed. The following treatments will be **prohibited** in this study:

- Oral or parenteral corticosteroids of >20 mg/week within the past 4 weeks before screening and during the study (intranasal or inhaled steroids for allergies/asthma or COPD are allowed)
- Immunosuppressive or cytotoxic agents within 30 days preceding the first study drug administration and during the study period
- Wound treatments with growth factors, dermal substitutes, or other topical biological therapies within the last 30 days of screening and during the study duration
- Treatment with anticancer therapy (chemotherapy, immunotherapy, radiotherapy, targeted therapy or gene therapy) within 3 months before the first administration of investigational product or at any time during the study.
- Biologically or chemically active dressings (eg, with growth factors, antibiotics)

1.6. Type of intervention

APO-2 is a biological medicinal product. A biological medicinal product is defined as a product where "the active substance of which is a biological substance. A biological substance is a substance that is produced by or extracted from a biological source…" (Dir. 2001/82/EC, 3.2.1.1 of Part I of Annex I). APO-2 was also classified as a biological medicinal product by the Austrian regulatory authority BASG in the scientific advice session of 20 April 2017.

APO-2 is **not a biotechnological product**. "Biotechnology" is defined as "The use of living organisms to create or modify products, including medicines." (https://www.ema.europa.eu/en/glossary/biotechnology). APO-2 lacks the usual manufacturing process elements of biotechnologically manufactured products: upstream, downstream and purification. Note: Many EMA guidelines are written for biological/biotechnological products (because most of the biological products manufactured are biotechnological products). The application of these guidelines to APO-2 is to be based on case-to-case basis.

APO-2 is also **not an advanced therapy medicinal product** (ATMP) because:

- It does not contain any cells (=it is no somatic cell therapy medicinal product)
- It does not contain any tissue (=it is no tissue engineered product)
- It does not contain or consist of any recombinant nucleic acid (=it is no gene therapy medicinal product).

APO-2 is **not a blood product**. APO-2 is the secretome of stressed PBMC, derived from blood donors. The reason for that, as put in words by the Austrian regulatory authority BASG, is that with the secretome of white blood cells, something **new and different from blood** comes into existence which had not been in the blood before. Relevance: blood products require batch release by the Official Medicinal Control Laboratory, OMCL, of the regulatory Authority (in our case BASG). The regulatory body of Austria (BASG) and the Paul Ehrlich Institute in Germany were incremental in guiding APOSC to develop APO-2. 4 national health authorities (Poland, Germany, Austria, and Czeck Republic) have endorsed the clinical execution of MARSYS II. We believe that APOSCIENCE is the first company that utilised a PBMC derived allogeneic secretome in a clinical trial.

1.7.Description and timing of study procedures

Overview of the visit schedule: assignment of the visits (V) to days of patient study participation and to clinical study phases. Statement: Individual patients participate in the study for 213 (±5) days from the first screening visit till the final follow-up visit. However, study results of patients who participate till day 123 (End of Treatment-Visit) will also be included in the primary endpoint evaluation of the clinical study.

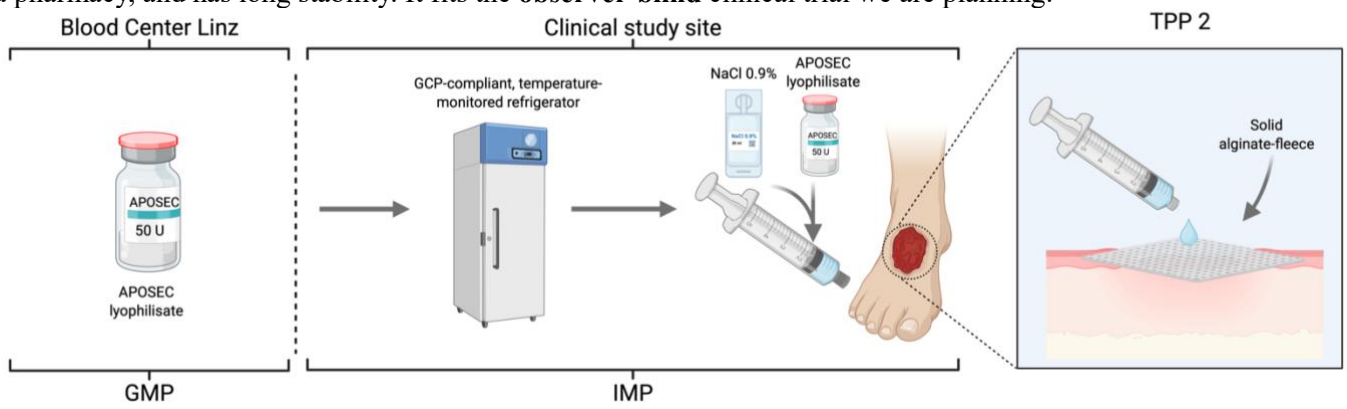
Periods	**Screening**	**Wound control**	**Baseline**	**Treatment phase**	**EoT**	**Follow-up**		
Visits	**V1**	**V2**	**V3**	**V4-V26**	**V27**	**V28**	**V29**	**V30**
Assessments/Study days	**W-6**	**W-1 (+/- 4 days)**	**Week 1, D1**	**23 visits in 12 weeks^**	**Week 13 ***	**Week 17 (+/-7 days)**	**Week 21 (+/-7 days)**	**Week 25 (+/-7 days)**
Informed consent	X							
In/exclusion criteria	X		X					
Randomisation criteria			X					
Demographics	X							
Medical history	X							
Physical examination	X		X	X	X	X	X	X
Vital signs	X		X	X	X	X	X	X
Clinical laboratory	X		X		X			
Urine pregnancy test	X		X					
SoC wound management	X	X						
Randomisation			X					
IMP/SoC treatment			X	X				
Wound assessment	X	X	X	X	X	X	X	X
Assessment of wound closure				X	X	X	X	X
Digital wound size measurement	X	X	X	X	X	X	X	X
Chronic wound quality of life questionnaire (QoL)			X		X			X
Pain (VAS)	X		X	X	X	X	X	X
Neurological assessment of foot			X	X	X	X	X	X
Adverse events	X	X	X	X	X	X	X	X
Concomitant medication	X	X	X	X	X			

V = Visit. *Or after complete wound closure. ^ with a minimal distance of 48h between two treatments

2. Preparedness status – Technical readiness level

APO-2 production: We consider the technical readiness level (TRL) of APO-2 GMP production at level 6- prototype demonstration in a relevant environment: we are using a pilot plant to produce APO-2 at the [redacted]. All production processes of APO-2 are controlled by a strict quality system of APOSC and [redacted]. Multiple inspections by the Austrian Regulatory Body were executed. No severe findings were reported and APOSC and [redacted] are allowed to produce APO-2 according to GMP – see main document Chapter 1.2. The production process still needs to be significantly upscaled and optimised for large-scale production after a successful pivotal trial. APOSC is actively working on business models to guarantee upscaling of production:
Currently, APO-2 cannot be produced for the mass market in the European Union. Working with the RedCross, or their affiliates, would allow production of APO-2 for the whole European Union. The raw material for APO-2 production are buffy coats that are a side product in all blood donation centres worldwide. To meet potential demand, APOSC is already working actively with several Red Cross units to establish new production sites (see LoI German Red, Hessen, Baden Würtemberg/Prof. Tonn and the Vienna Red Cross). Calculations show that donations from Austria and Germany alone could cover all DFU treatments in the EU. APOSC deems its proprietary platform technology as real asset to the European Union in reference to sustainability and prevention of a waste product, while generating huge benefits for DFU patients and the health system.

APO-2 TPP development path: APO2FOOT will utilise the final TPP of APO-2 in DFU. We will investigate whether APO-2 is in its final drug formulation/application is more effective than accepted SoC in DFU treatment.
The **APO2FOOT TPP** will be user friendly: it can be used off-the-shelf by physician directly, without the need for a pharmacy, and has long stability. It fits the **observer-blind** clinical trial we are planning:



APO2FOOT TPP: APO-2 lyophilisate will be produced at [redacted], released by a Qualified Person and sent as lyophilisate to the study site via a pharma distributor. APO-2 will be stored in a monitored +4°C refrigerator. After randomisation of a study patient, the APO-2 lyophilisate will be resuspended with physiologic saline and applied on a commercially available alginate fleece which will be placed on the DFU wound. The dosage will correlate to measured cm^2 of DFU wound ($25U/cm^2$). Since we are planning a controlled, randomised, observer-blinded, multicentre Phase II study in patients with DFU, this ready to use procedure is easy to implement and accepted by Annex 13, EU GMP Guide.

MARSYAS I/II TPP: This is a major step forward from the TPP of APO-2 in MARSYAS I/II: Regulatory requirements (Annex 13, EU GMP Guide) determined a more complex pharmaceutical manufacturing to allow a **double blind**, placebo-controlled study in DFU. In short: The APO-2 lyophilisate was produced by [redacted] and had to undergo an additional manufacturing step - the resuspension with physiologic saline. The APO-2 concentrate was sent to the clinical study site pharmacy. After randomisation of a DFU study patient the pharmacist mixed the APO-2 concentrate with an alginate gel and distributed the verum/placebo (with a labelled syringe) to the study physician to apply it.

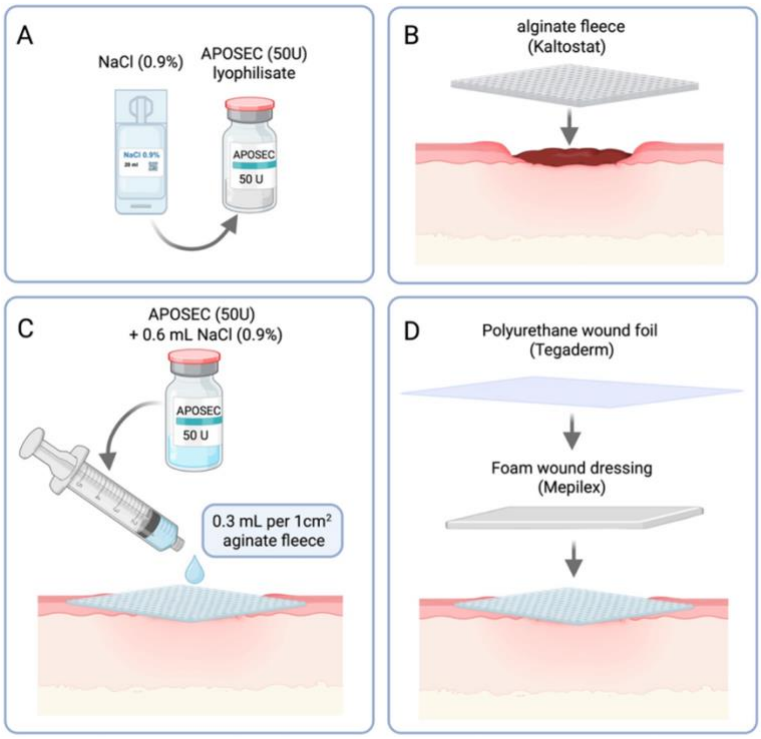


Figure 7: Schematic representation of the target product profile (TPP) for topical APOSEC (APO-2) application. (A) Lyophilised APOSEC (50U) is reconstituted in 0.6 mL NaCl (0.9%). (B) An alginate fleece (Kaltostat) is applied to the wound bed. (C) The alginate fleece is soaked with 0.3 mL/cm² reconstituted APOSEC (i.e. 25U/cm²). (D) The APOSEC-loaded alginate fleece is covered with a foam wound dressing (Mepilex) and sealed with a transparent polyurethane wound foil (Tegaderm).

Conclusion: The final TPP of APO-2 to be tested in APO2FOOT is a significant step forward compared to the prototype TPP that was tested in MARSYAS II: off the shelf, simple preparation, easy application, long shelf-live, while complying with Annex 13, EU GMP Guide (Figure 7).

APO-2 clinical testing

APO2FOOT is expected to evidence superiority of APO-2 versus SoC. After a DSMB-monitored futility analysis, APOSC is aiming to continue the clinical study as pivotal study, to achieve marketing authorisation in Europe. Only the Phase II trial is part of this grant application.

2.1.Development of the clinical study protocol

APOSC is set to reach all goals defined in the design of a clinical study in DFU. We have successfully completed a phase I/II intervention treatment with APO-2. This study met the standards of a monitored GCP clinical study. Our **experienced** team has a proven track record in the design of an investigational study protocol (IMPD), Investigator Brochure (IB) and we are familiar with many more clinical study documents, including the Statistical Analysis Plan, Standard Operational Procedures and Training Manuals for the Study Sites. All these documents from the already conducted clinical study (owned by APOSC) will serve as **templates** for the preparation of the documents to be submitted for APO2FOOT. They will, of course, require revisions concerning the current development stage and dosage form of the IMP APO-2.

The APO2FOOT clinical trial must comply with the novel requirements set by CTIS (Clinical Trial Regulation EU No. 526/2014). We plan to complete the preparation of the clinical study documents and their submission within the first year of APO2FOOT. In summary, [redacted], APOSC and [redacted]. we will be able to write the clinical study documents for the APO2FOOT study based on already existing knowledge and experience.

Currently we are preparing the **Briefing Book** for our third scientific advice with BASG. Like our previous scientific advice in 2017, we will present our APO2FOOT trial design to the authorities (22nd of October 2025). According to their suggestions we will modify the trial design. This scientific advice will be prepared by [redacted] – a beneficiary of APO2FOOT. Key personnel of [redacted] are familiar with APOSC developments since 2014.

The **study protocol** is already under development and is guided by a most renown **SAB** – see 2.3.2. [redacted], a regulatory consultant for clinical trials, will also accompany the creation of the clinical study protocol.

2.1.1. Scientific advice from regulatory and health technology assessment bodies

APOSC was the **first** company to execute a **clinical trial with a PBMC secretome** therapy in the EU and the first to present the "secretome concept" to the Austrian (BSAG) and German Regulatory Body (PEI). Both regulatory bodies **provided scientific advice** in 2017 (BASG/20 April 2017; Paul-Ehrlich-Insitut (PEI) December 2017). Their advice served as key input for the further development of APO-2 in the following years. Most relevant was a substantial deficiency letter from the PEI when we applied for our MARSYAS II trial in Germany. Based on notes by the German regulatory body we had to show that our validated potency assays truly mimicked angiogenesis. After successful demonstration of the relationship between AP-1 and HSP-27 potency assays and experimental tube formation (including blocking experiments) our MARSYAS I/II trial was endorsed by PEI (see 1.2 in the main document). MARSYAS II was approved in in all four national jurisdictions where MARSYAS II patients were recruited: Poland, Czeck Republic, Austria and Germany.

In preparation for APO2FOOT, a further **scientific advice meeting** will take place with the Austrian health authority BASG in October 2025. The briefing book is currently being written by the experts of [redacted]. Essential questions in this meeting will cover:

- the final dosage form and TPP of APO-2
- clinical trial design
- toxicology program
- chemistry, manufacturing, control (CMC) relevant APO-2 questions.

These will be key inputs for the final study protocol of APO2FOOT.

2.1.2. Clinical efficacy, safety, and methodological guidelines (including guidelines on statistics)

The following **guidelines** will apply when developing the **study protocol** and in the **execution** of APO2FOOT:

- World Medical Association: WMA Declaration of Helsinki – Ethical Principles for Medical Research Involving Human Participants
- Deutsche Gesellschaft für Wundbehandlung [German Association for the Treatment of Wounds]: Lokaltherapie schwerheilender und/oder chronischer Wunden aufgrund von arterieller Verschlusskrankheit, Diabetes mellitus oder chronischer venöser Insuffizienz (S3 Leitlinie, Version 2.2, 31.10.2023) [Local therapy of hard-to-heal and/or chronic wounds due to arterial occlusive disease, diabetes mellitus or chronic venous insufficiency (S3 Guidance, Version 2.2., 31.10.2023)]
- Clinical Trials Regulation (EU) No. 536/2014
- ICH E2A-E2F - Pharmacovigilance
- ICH E6(R3) – Good Clinical Practice
- ICH E8(R1) – General Considerations for Clinical Trials
- ICH E9 – Statistical Principles for Clinical Trials

2.1.3. Involvement of citizens / patients, carers in drawing up the clinical study protocol

In addition to consortium members, we are cooperating with the following **experts or organisations** in the planning of the APO2FOOT study, which have already contributed to the APO2FOOT study design described above:

- [redacted], Austria, **Nurse** practitioner: TPP development
- [redacted]: Distribution of DFU knowledge/**patient advocacy**
- [redacted]: **nurse** practitioners: Team Med – Metabolic Disorders and Nephrology - [redacted]
- [redacted]t: **nurse** practitioners: [redacted]: development of TPP APO2FOOT
- [redacted], Czech Republic: development of TPP APO2FOOT
- [redacted], Austria: development of TPP APO2FOOT
- [redacted], Austria: development of TPP APO2FOOT
- [redacted], Germany, and Dr Elisabeth Krippl, Austria, are involved in discussing the Clinical Study Protocol of APO2FOOT (in addition to the SAB-see below)

For APO2FOOT, we have started establishing a **patient advisory board** (PAB) – see Chapter 2.3.2 below.
A **Scientific Advisory Board (SAB)** is already guiding development of the study protocol (see 2.3.2)

2.2. Regulatory intelligence to ensure timely regulatory approval and ethics clearance of the clinical study in all jurisdictions where its implementation is planned

2.2.1. How the consortium will ensure access to regulatory expertise necessary to get advice on, and management of, regulatory affairs activities in all concerned jurisdictions?

For the writing of **regulatory documents** like the Investigational Medicinal Product Dossier (which needs to be updated), and the Clinical Study Protocol / Investigator Brochure, we are collaborating with [redacted] both senior Experts of [redacted] (beneficiary [redacted]). [redacted] specialises in comprehensive regulatory strategies, market access, and product development services and is a beneficiary of APO2FOOT.

For the submission of the clinical study in the European Clinical Trial Information System (**CTIS**) under the Clinical Trial Regulation EU No. 536/2014, the [redacted] (beneficiary [redacted]) will be our responsible partner. [redacted] has plenty of regulatory knowledge and experience to fulfil this task. According to the European Clinical Trials Regulation EU No. 536/2014, the clinical trial will be submitted centrally via CTIS in 5 countries: Austria, Germany, Czech Republic, Italy and Portugal.
The [redacted] will also assist in designing the **pharmacovigilance** processes for APO2FOOT. Furthermore, [redacted] will support the consortium in drawing up important clinical documents like the **Coordination Plan** and the **Statistical Analysis Plan**.

With the **European Clinical Research Infrastructure Network** ([redacted]), we have a further clinical research organisation in our consortium. [redacted] will be consulted, when it comes to regulatory questions and drawing up regulatory documents.

2.2.2. How the consortium will ensure access to ethics expertise necessary to get advice on current proceedings and documentation requirements of all concerned ethics committees?

The study will be submitted via CTIS by experienced personnel of [redacted]. According to Regulation EU No. 536/2014 the ethics committees are now directly linked to CTIS and will receive their part of the clinical trial application. APOSC will establish an Ethics and Governance Committee. Interaction with this Committee will be guided by a SOP (QM APOSC). That guarantees a documentation process between the sponsor APOSC and relevant national ethics committees and study sites.

2.3. How the scientific and operational governance of the clinical study will be ensured?

2.3.1. Please give details about the sponsor(s) (name, type of entity, seat or country of residence).

Aposcience AG, clinical stage spin-out of the Medical University of Vienna, Dresdner Strasse 87/A 21, 1200 Vienna, Austria. Aposcience possesses a Good Manufacturing Practice (GMP) Certificate for the manufacturing of Aposec as an IMP for the clinical phases I, II and III valid till 03 April 2027 (www.aposcience.com).

2.3.2. Please describe the composition, the role and the functioning of the planned board(s), governing bodies.

In addition to the management teams/board concerning the whole project, a **Clinical Study Team** consisting of [redacted] will meet every two weeks to discuss current issues of the clinical study.
The clinical trial will be supported by several boards made up of **external experts**:

We have installed a **Scientific Advisory Board** (SAB), which will guide us in the design of APO2FOOT study to meet the highest standards of clinical investigation. Many of our experts have been incremental in the design of previous Phase III DFU trials. They have already been involved in these first steps of defining the study protocol, e.g. advising on inclusion/exclusion criteria and off-loading:

- [redacted], Germany – [redacted] is chief physician of the [redacted]; he participated with his team in [redacted].

- [redacted], together with co-authors [redacted], is author of the standard work on [redacted], [redacted] was recommended by [redacted], another participant in [redacted] study, and is an expert in off-loading of DFU wounds.
- [redacted] is director of the Clinics for Vascular Surgery of the [redacted]; he participated with his team in [redacted] as one of four German study centres.
- [redacted], private surgical practice in [redacted], Germany – [redacted] also participated in [redacted] study as attending physician of the clinics [redacted] is an expert on blood vessels and complements [redacted] expertise in off-loading.
- [redacted] – [redacted] participated with her team in the MARSYAS II study and was invited to the SAB for her recommendations to us concerning off-loading footwear and other aspects relevant for clinical studies in DFU.

We plan to install a **Data & Safety Monitoring Board** (DSMB) which will follow our progress in the APO2FOOT study. The composition, roles and functioning of the DSMB will be described in the DSMB charter.

Ethics & Governance Committee: two independent ethics experts have confirmed their participation in APO2FOOT:

- [redacted] (in-house ethics expert)
- [redacted] (external / contracted)

Patient advisory board (PAB): will work in parallel to the SAB, to bring in patient perspective and bridge the gap between the study and patients. At later stages, they will be involved in communication activities. For Austria, the ÖDV (Österreichische Diabetikervereinigung) has confirmed participation, a patient organisation from at least one more trial country will be included. Corresponding associations will be identified in Germany, Czeck Republic, and Italy.

3. Operational feasibility

3.1. Please describe how the availability of the intervention(s) (including comparators) is secured throughout the entire implementation phase

Manufacturing: IMP production will be ensured by CMO [redacted] can easily produce IMP for APO2FOOT. Calculated production capability are 26-29 batches per year. Currently we are anticipating that APO2FOOT will need approximately 10 batches. The Qualified Person of APOSC will release APO-2 according to the GMP standards set by our CMO (project partner) and APOSC. The availability of our IMP is of no risk. Previous APO-2 GMP stability assays have demonstrated a long and lasting stability of APO-2 lyophilisate (up to 4 years). Moreover, our pharma distributor [redacted] has gained a most excellent track record in providing trial IMP to all European MARSYAS I/II study sites. Comparator treatment in DFU is readily available for study physicians.

Packaging, logistics, storage: The IMP APO-2 lyophilisate will be shipped packaged in 10R glass vials (primary packaging) and cartons with inlays (secondary packaging, 24 glass vials per carton) at +2 - 8 ºC. We do already have good stability data for Aposec lyophilisate stored in 20R glass vials: APO-2 proved to be stable at +4ºC for 2 years (which is exceptionally long for a biological medicinal product). The shipments will be organised and coordinated by beneficiary [redacted], a pharma distributor - and will be carried out by couriers qualified by [redacted]. The temperature of the IMP shipments will be controlled by temperature loggers. [redacted] will be responsible for storage of IMP (at +4 ºC) and study materials (at room temperature) as well as for handling orders for IMP and study materials coming in from the study centres. Furthermore, [redacted] will design (together with the clinical CRO and the sponsor) study-related labels. According to GCP all items that are used in APO2FOOT must be **labelled** by [redacted] (pharma distributor). These labels must be approved by APOSC qualified person (QP).

Comparator: As our clinical study APO2FOOT compares verum (APO-2) versus SoC - moist wound treatment with saline solution - shipment and storage of the comparator do not pose a problem for our clinical study. NaCl is shipped at room temperature.

3.2. Please describe how the study population will be recruited

Technical question and rational for physiologic parameters that determine patient selection: In MARSYAS II, we experienced major problems with patient recruitment; in the clinical study APO2FOOT, we will implement the lessons learned in the MARSYAS II trial. The main cause for low recruiting numbers lay in the fact that inclusion/exclusion criteria did not correspond to the real-life condition of DFU patients. Initially, members of the SAB advised us to include only DFU patients that had a near to perfect blood perfusion. Because of low recruiting we revised our clinical study protocol that was accepted by all four national authorities. In Version 4 of our trial document, we accepted patients with mild peripheral artery disease (PAD), Fontaine Stage I and II. This intervention increased the recruitment numbers of MARSYAS II.

APOSC will establish an online **platform** where all initiated study sites will get access. There, all centres will receive constant updates on recruiting numbers. Moreover, also screening numbers will be shared with the participating centres.

APOSC will also partner with [redacted]. This Austrian digital platform for **clinical trial recruitmen**t is operated by the company [redacted]. It is an innovative tool that connects hospitals and outpatient physicians with clinical study centres, allowing doctors to view study opportunities and register interested patients. In this study, treating physicians will access [redacted] through a secure web interface, where the trial is displayed together with the key eligibility criteria. Potential participants will be pre-screened against these predefined criteria and entered into the system by their treating physicians. Entries that meet the criteria will be forwarded to the study centre, where suitable patients will be invited for formal screening.

Sex and Gender relevance for recruiting:
In patient **recruitment** for APO2FOOT we will not take sex into consideration but focus exclusively on our inclusion/exclusion criteria, to reach the needed number of patients, regardless of their sex. We expect APO-2 to be similarly effective in men and women, but because of higher and more severe incidence in men, we anticipate a ratio of 70% male and 30% female patients.

3.2.1. How many clinical sites will contribute to the recruitment of the study population in which countries? Are these clinical sites part of an established clinical trial network? Please also describe the selection criteria of the clinical sites.

In the MARSYAS II study it was difficult for some study centres in hospitals to recruit patients fitting the inclusion/exclusion criteria. Patients with more superficial wounds were treated in outpatient settings whereas only the difficult cases were treated in hospitals.
Therefore, it will be of utmost importance to reach out via specialised recruiting offices. There is no established clinical trial network for DFU trial as in other fields e.g. oncology. In the planning of APO2FOOT our SAB introduced to APOSC many centres that do have a track record in participating in phase II/III DFU studies. The following centres (hospitals, outpatient wards) were contacted by APOSC and have committed themselves to participate in APO2FOOT study.

A Feasibility Questionnaire was prepared and sent out to potential study centres in Q2 of 2025. This questionnaire checked their experience with clinical studies and asked for expected recruitment numbers that meet our inclusion/exclusion criteria. In the following table, we present the potential study centres we have identified for APO2FOOT, all of whom have filled out the feasibility questionnaire and sent it back to APOSC (see Chapter 3.2.3 for results). Except for [redacted] who is beneficiary, all other study centres will be subcontracted (see main proposal document Table 3.1.g).

No.	Country	City	Study Centre	Principal Investigator	Type of Study Centre
1	Austria	[redacted]	[redacted]	[redacted]	[redacted]
2	Austria	[redacted]	[redacted]	[redacted]	[redacted]
3	Austria	[redacted]	[redacted]	[redacted]	[redacted]

No.	Country	City	Study Centre	Principal Investigator	Type of Study Centre
4	Austria				
6	Austria				
5	Austria				
7	Germany				
8	Germany				
9	Germany				
10	Germany				
11	Germany				
12	Germany				
13	Czech Republic				
14	Czech Republic				
15	Portugal				
16	Portugal				
17	Portugal				
18	Portugal				
19	Portugal				
20	Italy				

No.	Country	City	Study Centre	Principal Investigator	Type of Study Centre
21	Italy	[redacted]	[redacted]	[redacted]	[redacted]

3.2.2. Will recruitment of the study population be of competitive nature between the clinical sites? (Please describe how underperformance of individual clinical sites in recruitment will be managed.)

Recruitment will be competitive. Recruitment targets will be agreed with study centres individually. In case of underperformance, study sites may be closed, and additional sites may be recruited.

APO2FOOT includes a dedicated agency as partner to increase recruitment numbers. [redacted] is an Austrian digital platform for clinical trial recruitment, operated by the company [redacted]. It is an innovative tool that connects hospitals and outpatient physicians with clinical study centres, allowing doctors to view study opportunities and register interested patients. In this study, treating physicians will access [redacted] through a secure web interface, where the trial is displayed together with the key eligibility criteria. Potential participants will be pre-screened against these predefined criteria and entered into the system by their treating physicians. Entries that meet the criteria will be forwarded to the study centre, where suitable patients will be invited for formal screening.

3.2.3. What evidence supports the ability of the individual clinical sites to recruit the required number of study participants within the planned timeline

Some of the study centres detailed in 3.2.1 have previously worked successfully with project beneficiaries and/or DFU. The following study centres already participated in the MARSYAS II study, with excellent recruitment performance:

- 3. Medical Department – [redacted]
- Department of [redacted]
- [redacted]
- [redacted]

The 6 study centres in Portugal were proposed by beneficiary [redacted] as experienced and reliable.

4 of the German study centres recently participated in a clinical study on DFU[15]:

- Department of [redacted]
- Department of [redacted]
- Private wound care practice [redacted]
- Clinic for Vascular Surgery of the [redacted]

In addition, all listed potential study centres filled out a **Feasibility Questionnaire**, detailing their experience in clinical studies and to ask for the numbers of patients they could possibly recruit according to the inclusion/exclusion criteria. The questionnaire contained a short summary of the clinical study APO2FOOT, the inclusion-exclusion criteria, and the visit schedule presented in table 3. The most important questions were:

- whether the study centre is experienced in the conduct of clinical trials;
- whether the study centre is prepared to conduct the clinical trial (e.g., by having available separate refrigerators with temperature surveillance for storage of the IMP);
- How many patients with DFUs fitting our inclusion criteria the study centre could recruit per year.

All listed study centres completed our Feasibility Questionnaire. The most important results are the following:

- 81% of the study centres do possess experience in the conduct of clinical studies (=have already participated in one or more clinical studies.
- 62% of the PIs possess a valid GCP certificate.
- 52% of the study centres have a study coordinator available (who helps in the organisation of patient meeting schedules which leads to a better recruitment performance of the study centre).

[15] Dardari et al., 2024, doi: 10.1056/EVIDoa2400171

- 76% of the study centres do possess a separate refrigerator (+2 - + 8 °C) for clinical studies.
- 76% of the study centres do have a temperature surveillance system for the refrigerator.
- All study centres together indicated that they treat 648 DFU patients per year;
- Of which (all study centres together) 191 patients would fit our inclusion criteria;
- And that they could possibly recruit (all study centres together) 138 patients for the APO2FOOT study.

Remarks to the results of the Feasibility Questionnaire:

- According to our experience and evidence from literature (e.g. in the LeucoPatch study: 595 patients included in the run-in phase, 326 failed, the majority due to change of ulcer size; less than half (269 patients) could be included into the clinical study)[16], a recruitment capacity of 138 patients per year is very optimistic. We prefer to estimate recruitment capacity conservatively. However, the numbers estimated by the study centres reflect their intentions and are a good sign for the APO2FOOT study.
- Concerning missing refrigerators and GCP-certificates: The APO2FOOT project will provide refrigerators with temperature surveillance systems to all study centres lacking these indispensable items. Furthermore, we will provide a GCP course to all PIs lacking a GCP certificate and a refresher course to all PIs (GCP certificates are required to be renewed every 2 years (see workplan T2.6).
- Study centres lacking clinical study experience will be especially coached. APO2FOOT study-related trainings during study site initiation will be conducted more thoroughly. Additionally, these study centres will be supported in the preparation of the study set-up at the site (e.g., by writing of GCP-compliant SOPs; see workplan T2.6).

3.3. Please describe what additional supply (e.g. an electronic device for remote data capture, a specific instrument for administering the investigational product, etc.) is necessary to carry out the required study procedures and how this supply will be made available to the clinical sites

Medical System Platform technology to objectify TBI and toe systolic pressure for inclusion and exclusion criteria for APO2FOOT

The problem: clinical studies in DFU usually use the ankle brachial index (ABI) as an inclusion criterium, e.g. ABI of ≥0.6 in the Kerecis fish skin-study[17]. The ABI is the ratio of the systolic blood pressure in the ankle to the systolic blood pressure in the upper arm. The problem with the ABI is that DFU patients sometimes have artificially high ABI values due to medial sclerosis. This means that the arteries in the leg are calcified and do not permit compression, which is necessary for the measurement of the blood pressure.

Our solution to this problem: Instead of relying on the ABI alone we will also measure toe brachial index (TBI), and the pulse wave index (PWI). With the help of our SAB, we have identified a device, [redacted], that employs a different measurement method. Oscillometry us used instead of the usually applied Doppler-sonography. Angiography measurement using oscillography is considered superior to measurement using Doppler sonography, as it is independent of the examiner, automated, reproducible, and often less prone to error. Furthermore, the [redacted] device measures the systolic vascular perfusion pressure at the end of the toe with an optical sensor. All these measurements (systolic pressure at the ankles of the arms and the leg with oscillometry and at the toe with optical sensor) are performed simultaneously in 3 minutes. As this device provides the values required for the inclusion of the patients in the study in one simultaneous measurement, and to avoid measurements performed with different methods (with oscillometry or with Doppler sonography) we have decided to standardise the measurement of the vascular state of the patients in the APO2FOOT study by providing the [redacted] device to all study sites (renting).

[redacted] is ISO 13485 certified, the devices [redacted] are medical devices class IIa carrying CE marks according to Medical Device Directive (MDD). Furthermore, a Notified Body confirmation letter ([redacted]) confirms that the application to the transition from the MDD to the Medical Device Regulation has already been submitted.

[16] Game F. 2018, doi: 10.1016/S2213-8587(18)30240-7.
[17] Dardari et al., 2024, doi: 10.1056/EVIDoa2400171

3.4. Please provide plans on data management aspects

Source data documents will be archived in the individual study centres according to the local and European requirements. Study data will be entered into an electronic case report form (eCRF) at the study site. Risk-based on-site and remote monitoring will ensure data quality and consistency. The eCRF entries will have to be signed electronically by the principal investigator after each visit of each patient.
Wound photos will be taken by the study teams of the individual study centres utilising photo documentation. Clinical study sites will be trained, and photographs will be taken according to an SOP determined by [redacted]. Photos will be uploaded to a cloud provided by [redacted]. [redacted] will organise this cloud space so that appropriate naming of the photos will assure the correct assignment to the patient and the visit number and prevent mix-ups.

Wound size adjudication will be performed by a team of central wound adjudicators. This team will consist of independent professionals experienced in the treatment of DFU. The wound adjudication team needs to adjudicate the wounds in a timely manner, especially of photos taken at visit two, to assure compliance with inclusion/exclusion criteria.

3.5. Please give details on how reporting obligations (regarding study initiation, safety of study participants, ethical concerns, quality issues, integrity of data, study results) to regulatory bodies and ethics committees will be met.

The Clinical Study Application Dossier (Part 1 and Part 2) for the submission of the clinical study APO2FOOT in the EU Clinical Trial Information System (CTIS) portal will be prepared by APOSC, REG and MUW, e.g documents concerning the quality of the IMP, the Investigational Medicinal Product Dossier (IMPD) and Summary of Product Characteristics (SmPC) (see project Task 2.2; 3.2 and 4.3).
The Development Safety Update Report (DSUR; =Annual Safety Report - ASR) according to ICH E2F, which is to be submitted yearly to the regulatory authorities, will be prepared by APOSC in cooperation with [redacted] and submitted to CTIS by [redacted]. The DSURs will be submitted based on the monitoring results of [redacted] (in Germany and Austria) and [redacted] (in Portugal, Czech Republic and Italy); medical coding of the adverse events according to MedDRA will be performed by [redacted] (project Task 4.2)
SUSAR Reporting: Reporting of Suspected Unexpected Serious Adverse Reactions (SUSAR) will be performed for the sponsor by [redacted] to the Eudravigilance System (project Task 4.2).
A Safety Management Plan will be designed for the APO2FOOT study to define the processes, the communication flow and the roles for adverse event reporting, serious adverse event reporting, SUSAR reporting, serious breach reporting (according to Article 52 of Reg. 536/2014) medical monitoring and medical coding (T4.2).
Unblinding – as the clinical study APO2FOOT is open label, no measures for emergency unblinding are necessary in case of safety issues.

3.6. Please list all items of the sponsor's responsibilities (e.g. monitoring clinical sites, meeting regulatory obligations, data management, etc.) that will be supported by entities that are not part of the sponsor's organisation. Please describe how the sponsor will ensure oversight of these activities.

The following responsibilities of the sponsor will be **transferred to/supported by** other entities:

- **Preparation of clinical study documents**, such as: Statistical Analysis Plan, Communication Plan, Data Management Plan, Data Validation Plan, Safety Management Plan, Informed Consent Form (ICF), Monitoring Plan, Data Safety Management Board (DSMB)-Charter, and training manuals. – These study documents will be prepared by [redacted] in cooperation with the sponsor. (Study documents of the MARSYAS II study are available and will be used as templates.
- **Translation** of clinical study documents – from the English master document to German: will be done by [redacted]; to Czech, Italian and Portuguese: will be done by [redacted].
- Electronic case report form (**eCRF**) will be set up by [redacted] in cooperation with the sponsor.
- Trial Master File (**TMF**) will be set up and managed by [redacted] (paper TMF). After the clinical study, the TMF will be handed over to and archived by the sponsor.
- Clinical **monitoring** in Austria and Germany will be performed by [redacted]; in Czech Republic, Italy and Portugal, clinical monitoring will be performed by [redacted].

- With the same country split as above, [redacted] and [redacted] carry out site initiation **visits**, providing the study centres with study binders and training of the study team, and for close-out visits of study centres.
- With the same country split as above, [redacted] and [redacted] carry out data management within the frame of eCRF and clinical monitoring: controls of plausibility and consistency of eCRF data, eCRF queries if data are missing, medical coding of adverse events according do MedDRA.
- **Data management**: Handling of the database, database lock and handover of the data to the sponsor will be the responsibility of [redacted].
- Data management: Preparation of the Data Review Meeting will be performed by [redacted]
- **SUSAR** Reporting to Eudravigilance will be performed by [redacted]
- Preparation of the annual **Development Safety Update Report** (=pharmacovigilance reporting) will be done by [redacted] in cooperation with the sponsor
- Writing of the **Clinical Study Report** will be performed by [redacted] in cooperation with the sponsor.
- The manufacture of **labels** and secondary **packaging** will be performed by our pharma distributor [redacted].

Sponsor oversight will be insured by the following measures:

- Conduct of Good Clinical Practice (GCP) audits of the [redacted] and [redacted] by the sponsor.
- Technical agreements including responsibility lists will be concluded between the sponsor, [redacted] and [redacted]
- Bi-weekly video conferences between the sponsor, [redacted], [redacted] and [redacted] to discuss current tasks and challenges
- Monthly status reports of the clinical study APO2FOOT prepared by [redacted]

3.7. What are the plans for major study milestones and what evidence supports its feasibility?

Major Milestones: several milestones will ensure feasibility and timely implementation of our study – details are described below:

- Submission of the clinical study in CTIS (M6): preparation of the necessary document has started already.
- Approval of the clinical study by the competent authorities in 5 countries (Austria, Germany, Czech Republic, Italy, and Portugal) (M9)
- First patient in (M11)
- Signed study contracts with the first 10 participating study centres (M15)
- 50% of patients recruited (M32)
- Last patient out (M50)
- Results of Futility Analysis by DSMB (M52)

Schedule for the conduct of the clinical study APO2FOOT – see also main document Section 3.

Gantt chart of the APO2FOOT study

	Month	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32	34	36	38	40	42	44	46	48	50	52	54	56	58	60
T4.1	Clinical study managegent																														
T4.2	Data protection and management																														
T4.3	Submission of clinical study application																														
T4.4	Clinical study initiation																														
T4.5	Conduct the clinical study																														
T4.6	Analysis and reporting																														

M6-M9: Since enforcement of the Clinical Trial Regulation EU 536/2014, the period for validation, evaluation of and decision on the clinical study submission has been limited to 60-106 days. Hence, the study authorisation process may at most take up to 3 months.

M10-M19: We plan 10 months for the conclusion of study contracts with study centres, which are not partners in the project (subcontractors). Some of the study centres are small and it would mean too much effort for them to participate as beneficiaries. The conclusion of the contracts with the study centres will take some time, but we are positive to finish this task in time because we are already in contact with our study centres, they are familiar with our planned clinical study, and they have replied to our Feasibility Questionnaire. This is the first part of Clinical study initiation, which also includes, contract negotiation with clinical study centres and site visits to prove GCP conformity.

M10 – M50: We plan 34 months (nearly 3 years) for recruitment and conducting the trial. Since we have planned a 5-7 week screening phase + a 12-week treatment phase, we can recruit patients till month 42 so that the last patient can finish the follow-up period in M50. The study design includes a **follow-up period** of 3 months. The main purpose of this period is to check whether wounds that have closed during the treatment period re-open. Wound assessment

and wound measurement (in case that there still is a wound) will continue during this period, and the patients will by physically examined (including laboratory tests at the final visit of the follow-up period).
M50 – M60: After the last patient has completed the follow-up period, the clinical study will be unblinded to the sponsor (unblinding will be performed by [redacted]), and the study results will be analysed according to the Statistical Analysis Plan. The data will be prepared by [redacted] for the DSMB which will then perform the Futility Analysis (M52). In case of a positive Futility Analysis, the clinical study will be continued into Phase III (financed by APOSC); in case of a negative Futility Analysis, the results will be posted, and a detailed CSR will be written (until M60).

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